

Preharvest Modulation of Postharvest Fruit and Vegetable Quality

Edited by

Mohammed Wasim Siddiqui



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AND VEGETABLE
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MOHAMMED WASIM SIDDIQUI



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*For
my wife Dr. Fozia Homa
who deserves all the dedications*

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LIST OF CONTRIBUTORS

Sajid Ali

Postharvest Research and Training Centre, Institute of Horticultural Sciences,
University of Agriculture, Faisalabad, Pakistan

Emilio Alvarez-Parrilla

Universidad Autónoma de Ciudad Juárez, Instituto de Ciencias Biomédicas, Anillo
envolvente PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, México

Ram Asrey

Indian Agricultural Research Institute, New Delhi, Delhi, India

Minu B. Balkhi

GHHS Mirgund, J&K Govt., Srinagar, Jammu and Kashmir, India

Kalyan Barman

Banaras Hindu University, Varanasi, Uttar Pradesh, India

Isabella M. Brasil[†]

Federal University of Ceará, Fortaleza, Ceará, Brazil

Arpita Das

Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Laura A. de la Rosam

Universidad Autónoma de Ciudad Juárez, Chihuahua City, Chihuahua, Mexico

Elazar Fallik

Agricultural Research Organization, Volcani Center, Rishon LeZiyyon, Israel

Gustavo A. González-Aguilar

Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en
Alimentación y Desarrollo, A.C., Hermosillo, Sonora, México

Chafik Hdider

National Agricultural Research Institute of Tunisia, Tunis, Tunisia

Riadh Ilahy

National Agricultural Research Institute of Tunisia, Tunis, Tunisia

Zoran Ilic

University of Pristina, Lešak, Serbia

Sanu Jacob

Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, Kerala, India

[†]Deceased

John Jifon

Tshwane University of Technology, Pretoria, Gauteng, South Africa; Texas A&M AgriLife Research, Vegetable and Fruit Improvement Center, Texas A&M University System, Weslaco, TX, United States

M. Kalmesh

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Nouri Khamassy

National Agricultural Research Institute of Tunisia, Tunis, Tunisia

Ahmad S. Khan

Postharvest Research and Training Centre, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan

Endrit Kullaj

Agricultural University of Tirana, Tirana, Albania

Ranjeet Kumar

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Satyendra Kumar

Central Soil Salinity Research Institute, Karnal, Haryana, India

Kiran Kumari

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Samrat Laha

Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Isabel Lara

University of Lleida, Lleida, Spain

Marcello Salvatore Lenucci

University of Salento, Lecce, Italy

Sanchita Mandal

Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Khalid Z. Masoodi

Division of Plant Biotechnology, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

Nirmal K. Meena

Indian Agricultural Research Institute, New Delhi, Delhi, India

Saba Mir

Division of Plant Biotechnology, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

Jesús O. Moreno-Escamilla

Universidad Autónoma de Ciudad Juárez, Instituto de Ciencias Biomédicas, Anillo envolvente PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, México

Vishal Nath

National Research Centre on Litchi, Muzaffarpur, Bihar, India

C. Nithya

India Agricultural Research Institute (IARI), New Delhi, Delhi, India

José A. Núñez-Gastélum

Universidad Autónoma de Ciudad Juárez, Instituto de Ciencias Biomédicas, Anillo envolvente PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, México

Sukanta Pal

Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

K. Prasad

Indian Agricultural Research Institute, New Delhi, Delhi, India

Joaquín Rodrigo-García

Universidad Autónoma de Ciudad Juárez, Instituto de Ciencias Biomédicas, Anillo envolvente PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, México

Tamoghna Saha

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Vijay R. Sanikommu

Central Institute for Arid Horticulture, Bikaner, Rajasthan, India

Farheena Shah

Division of Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

Ram R. Sharma

Indian Agricultural Research Institute, New Delhi, Delhi, India

Swati Sharma

National Research Centre on Litchi, Muzaffarpur, Bihar, India

Mohammed Siddiqui

University of Agriculture, Faisalabad, Pakistan

Mohammed Wasim Siddiqui

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Maneesh P. Singh

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Dharini Sivakumar

Tshwane University of Technology, Pretoria, Gauteng, South Africa; Texas A&M AgriLife Research, Vegetable and Fruit Improvement Center, Texas A&M University System, Weslaco, TX, United States

Imen Tlili

National Agricultural Research Institute of Tunisia, Tunis, Tunisia

Ramanuj Vishwakarma

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Shabir H. Wani

Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, Wadura, Sopore, Jammu and Kashmir, India;
Michigan State University, East Lansing, MI, United States

Sajad M. Zargar

Division of Plant Biotechnology, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

ABOUT THE EDITOR

MOHAMMED WASIM SIDDIQUI

Dr. Mohammed Wasim Siddiqui is an Assistant Professor and Scientist in the Department of Food Science and Postharvest Technology, Bihar Agricultural University, Sabour, India and author or coauthor of more than 35 journal articles, more than 40 book chapters, and several conference papers. He has 18 books to his credit published by Elsevier, USA, CRC Press, USA, Springer, USA, and Apple Academic Press, USA.



He is the Founder Editor-in-Chief of two book series entitled *Postharvest Biology and Technology* and *Innovations in Horticultural Science* being published from Apple Academic Press, New Jersey, USA, where he is a Senior Acquisitions Editor for Horticultural Science as well. He also established an international peer reviewed journal *Journal of Postharvest Technology*. Dr. Siddiqui has been serving as an editorial board member and active reviewer of several international journals including *Horticulture Research (Nature Publishing Group)*, *Postharvest Biology and Technology (Elsevier)*, *PLoS One (PLOS)*, *LWT-Food Science and Technology (Elsevier)*, *Food Science and Nutrition (Wiley)*, *Journal of Plant Growth Regulation (Springer)*, *Acta Physiologiae Plantarum (Springer)*, *Journal of Food Science and Technology (Springer)*, *Indian Journal of Agricultural Science (ICAR)* as so on.

Dr. Siddiqui has received numerous awards and fellowships in recognition of research and teaching achievements. Recently, he is conferred with the Glory of India Award-2017, Best Researcher Award-2016, Best Citizens of India Award-2016, Bharat Jyoti Award-2016, Outstanding Researcher Award-2016, Best Young Researcher Award-2015, Young Scientist Award-2015, and the Young Achiever Award-2014 for the outstanding contribution in research and teaching from several organizations of national and international repute. He was also awarded Maulana Azad National Fellowship Award from the University Grants Commission, New Delhi, India. He is an Honorary Board Member and Life Time Author Society

for Advancement of Human and Nature (SADHNA), Nauni, Himachal Pradesh, India. He has been an active member of organizing committee of several national and international seminars/conferences/summits. He is one of key members in establishing the World Food Preservation Center (WFPC), LLC, USA. Presently, he is an active associate and supporter of WFPC, LLC, USA. Considering his outstanding contribution in science and technology, his biography has been published in *Asia Pacific Who's Who*, *Famous Nation: India's Who's Who*, *The Honored Best Citizens of India*, and *Emerald Who's Who in Asia*.

Dr. Siddiqui acquired BSc (Agriculture) degree from Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. He received the MSc (Horticulture) and PhD. (Horticulture) degrees from Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, India with specialization in the Postharvest Biotechnology. He has received several grants from various funding agencies to carry out his research projects. He is dynamically indulged in teaching (graduate and doctorate students) and research, and he has proved himself as an active scientist in the area of Postharvest Biotechnology.

PREFACE

Maintaining postharvest quality of fruits and vegetables has become increasingly complicated due to growing global fresh produce trade, consumer fondness for variety, increasing awareness of valuable nutritional properties of fruits and vegetables, and price best for supplying off-season high-quality fresh produce.

Most of the developing countries have, however, been losing up to 30%–40% of the value of their fruit and vegetables due to inadequate postharvest handling and facing huge monetary losses. On the other hand, international markets usually reject shipments of fruit and vegetables having banned pesticidal residues beyond permissible limits. Appropriate approaches and technologies are needed to reduce postharvest losses in quantity and quality, as well as assure food safety between produce harvest and consumption.

Among the several factors responsible for overall quality of produce, about 70% comes from preharvest conditions while only 30% postharvest factors affect the quality. In fact, with the very best of postharvest knowledge and technologies available, the best that can be achieved is a reduction in the products' deterioration rate while their normal developmental stage, such as maturation, ripening, and senescence. Therefore, it is very important to understand what preharvest factors influence the many important harvest quality attributes affecting postharvest deterioration and subsequently, the consumers' decision to purchase the product in the market.

Although, substantial preharvest research has been carried out to preserve the quality of fresh horticultural produce, however, unfortunately, available information has not been summarized so far in a book and periodicals. With 16 comprehensive chapters written by a team of experts belonging to developed and developing world, the book *Preharvest Modulation of Postharvest Fruit and Vegetable Quality* is a maiden and unique addition to maintain/modify the postharvest quality of fresh produce in terms of safety and nutrition.

The editor is confident that this book will prove to be a unique reference work in the field of postharvest produce quality maintenance. The information can be used to extend the shelf life by retaining nutritional and cosmetic appeals of fresh fruits and vegetables. The editor would appreciate receiving new information and comments to assist in the future development of the next edition.

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CHAPTER 1

Postharvest Quality of Fruits and Vegetables: An Overview

Isabella M. Brasil^{*,†}, Mohammed Siddiqui^{**}

^{*}Federal University of Ceará, Fortaleza, Ceará, Brazil

^{**}University of Agriculture, Faisalabad, Pakistan

1 INTRODUCTION

The harvested products are metabolically active, undergoing ripening and senescence processes that must be controlled to prolong postharvest quality. Inadequate management of these processes can result in major losses in nutritional and quality attributes, outbreaks of food-borne pathogens and financial loss for all players along the supply chain, from growers to consumers. Fresh fruit and vegetables are perishable living products that require coordinated activity by growers, storage operators, processors, and retailers to maintain quality and reduce food loss and waste. Also, they are a major source of essential vitamins and minerals, such as vitamin A, vitamin C, and potassium, needed for human well-being (Mahajan, 2016). Several studies are conducted to understand the effects and the results of quality during postharvest, in relation to time and storage temperature among other factors. With the progress of food globalization, the amount of agricultural products that are traded internationally has been increasing. At the same time, the distance transported and the duration have been extended (Fahmy and Nakano, 2016).

Postharvest environmental conditions, in particular temperature, have a major impact on the visual, compositional, and eating quality of fruit and vegetables. Temperature is, in fact, the component of the postharvest environment that has the greatest impact on the quality of fresh fruits and vegetable. A good temperature management is the most important and simplest procedure for delaying product deterioration. Optimum preservation of fruit and vegetable quality can only be achieved when the product is promptly cooled to its optimum temperature as soon as possible after harvest. In general, the lower the storage temperatures within the limits acceptable for each type of commodity, the longer the storage life. For each horticultural commodity there is assumed to be an optimal

[†]Deceased

postharvest storage temperature at which the rate of product deterioration is minimized. Storage of fruits and vegetables at their optimum temperature retards aging, softening, textural and color changes, as well as slowing undesirable metabolic changes, moisture loss, and losses due to pathogen invasion (Nunes, 2008).

There is a lack of information on the effect of storage temperature on volatile organic compounds, as well as on concentration of compounds and on the chemical identity (Spadafora et al., 2016). Fresh produce attributes (appearance, texture, flavor, and nutritional value) have been traditional quality criteria, but increased safety (chemical, toxicological, and microbial) and traceability are important for all the role players along the supply chain, from the farm to consumers (Mahajan, 2016). Temperature management is one of the most important factors affecting the quality of fresh produce. There is an optimum storage temperature for all products. There are many references, which outline the optimum temperature for a range of fresh products. The ideal temperature often depends on the geographic origin of the product. Tropical plants have evolved in warmer climates and therefore cannot tolerate low temperatures during storage. Plants from tropical origins must be stored above 12°C. This is in contrast to plants, which have evolved in temperate, cooler climates, which can be stored at 0°C (Kader, 2002).

Many factors can contribute to the preservation or decay of fruit and vegetables like ripening fruits with ethylene gas, temperature and humidity, packaging. This chapter summarizes studies and results about postharvest quality and sensory attributes of fruits and vegetables.

2 POSTHARVEST QUALITY

Quality can be defined as the “fitness for the purpose.” The “optimal” quality of a product is related to a determined development or ripening degree, where the composition or combination of physical attributes and chemical components has maximum acceptance by consumers. A primordial principle in maintenance of quality characteristics and the extension of shelf life is to understand that different parts of fruits and vegetables are still alive, not only when it is still attached to the mother plant, but also postharvest (Chitarra and Alves, 2001).

Quality evaluation must be realized in all segments because the field, until commercialization, to have knowledge of the real product value and its conservation capacity based on preestablished standards. Consumers may

not accept a product when it does not have the requirements or desired quality attributes that may cause major impact on the commercialization chain, especially exportation. The quality of a product can be evaluated through a sensory panel or with the use of instruments, being destructive or not (Chitarra and Alves, 2001).

Quality requirements can be grouped in three categories: sensorial, nutritional, and safety, and should be considered together to satisfy consumers' needs and public health. According to Kader and Rolle (2004) "the relative importance given to a specific quality attribute varies in accordance with the commodity concerned and with the individual (producer, consumer, and handler) or market concerned with quality assessment. To producers, high yields, good appearance, ease of harvest, and the ability to withstand long-distance shipping to markets are important quality attributes. Appearance, firmness, and shelf life are important from the point of view of wholesale and retail marketers. Consumers, on the other hand, judge the quality of fresh fruits, ornamentals, and vegetables based on appearance (including 'freshness') at the time of initial purchase. Subsequent purchases depend upon the consumer's satisfaction in terms of flavor (eating) quality of the edible part of produce" (Table 1.1).

Table 1.1 Quality attributes for fruits and vegetables

Major factors	Components
Sensorial	—
Appearance	Size: dimensions, weight, volume Shape: uniformity, longitudinal \times transversal diameter Color: intensity, uniformity Brightness: external appearance Defects: externals and internals (morphological, physics \times mechanics, physiologic, pathologic, entomologic)
Texture	Firmness, hardness, softness, fragility, succulence, lumpiness, resistance, fibrousness
"Flavor" (taste and aroma)	Sweetness, acidity, astringency, sourness, aroma, strange flavors and odors
Yield	Ratio between peel:pulp:seed
Nutritive value	Carbohydrates, proteins, lipids, vitamins, minerals
Safety	Natural toxic substances, contaminants (waste and metals), mycotoxins, microbiological contamination

Source: Chitarra, A.B., Alves, R.E., 2001. Tecnologia Pós Colheita para Frutas Tropicais. Frutal/Sindifruta, Fortaleza.

2.1 Quality Factors

2.1.1 Appearance

Appearance is the most important quality factor regarding product commercialization (Chitarra and Chitarra, 2005). These may include size, shape, color, gloss, and freedom from defects and decay. Defects can originate before harvest because of damage by insects, diseases, birds, and hail; chemical injuries; and various blemishes (such as scars, scabs, russetting, rind staining). Postharvest defects may be morphological, physical, physiological, or pathological (Kader and Rolle, 2004).

2.1.2 Textural

Textural includes firmness, crispness, juiciness, mealiness, and toughness, depending on the commodity. Textural quality of horticultural crops is not only important for their eating and cooking quality, but also for their shipping ability. Soft fruits cannot be shipped over long distances without substantial losses due to physical injuries. In many cases, the shipment of soft fruits necessitates that they be harvested at less than ideal maturity, from the flavor quality standpoint (Kader and Rolle, 2004).

2.1.3 Flavor

Flavor includes sweetness, sourness (acidity), astringency, bitterness, aroma, and off-flavors. Flavor quality involves perception of the tastes and aromas of many compounds. An objective analytical determination of critical components must be coupled with subjective evaluations by a taste panel to yield useful and meaningful information about the flavor quality of fresh fruits and vegetables. This approach can be used to define a minimum level of acceptability. To assess consumer preference for the flavor of a given commodity, large-scale testing of a representative sample of consumers is required (Kader and Rolle, 2004).

2.1.4 Nutritional

Nutritional quality is the quality factor counted in the fruits and vegetables supply chain (Chitarra and Chitarra, 2005). Fresh fruits and vegetables play a significant role in human nutrition, especially as sources of vitamins (vitamin C, vitamin A, vitamin B, thiamine, niacin), minerals, and dietary fiber. Other constituents of fresh fruits and vegetables that may lower the risk of cancer and other diseases include carotenoids, flavonoids, isoflavones, phytosterols, and other phytochemicals (phytonutrients) (Kader and Rolle, 2004).

2.1.5 Safety

Safety is the most desirable quality factor and fruits and vegetables should be free from any chemical substance that can cause injury to human health (Chitarra and Chitarra, 2005).

Significant postharvest losses occur during the supply chain of fresh produce. Postharvest decay is one of the main factors that determines losses and compromises the quality of fruit and vegetables. Traditionally, postharvest decay control is achieved using chemical fungicides; however, the important concerns relating to environmental and human health require the development of novel methods for the control of postharvest decay. Furthermore, the consumer demand and the purchasing power are higher for fresh produce that is free from pesticide application (Mari et al., 2016).

Postharvest biological control agents as a viable alternative to the use of synthetic chemicals have been the focus of considerable research for the last 30 years by many scientists and several commercial companies worldwide. Several antagonists of postharvest pathogens have been identified and tested in the laboratory, semicommercial, and commercial settings and were developed into commercial products. The discovery and development of these antagonists into a product followed a paradigm in which a single antagonist isolated from one commodity was also expected to be effective on other commodities that vary in their genetic background, physiology, postharvest handling, and susceptibility to pathogens. In most cases, product development was successfully achieved, but their full commercial potential was not realized. The low success rate of postharvest biocontrol products has been attributed to several problems, including difficulties in mass production and formulation of the antagonist, the physiological status of the harvested commodity and its susceptibility to specific pathogens. All these factors play a major role in the reduced and inconsistent performance of the biocontrol product when used under commercial conditions (Droby et al., 2016).

The preservation of fruit and vegetable quality during postharvest life, due to its economic impact and also human health, is essential for the food industry. The increasing demand for consumption of fresh fruits and vegetables, along with restrictions on the use of synthetic chemicals to minimize postharvest losses, has encouraged scientific research to develop new technologies based on natural molecules, such as salicylic acid. Salicylic acid as a safe signaling molecule can be used as a preharvest and postharvest strategy, which has high commercial potential for enhancing nutritional quality along with extension of the shelf life of fruits and vegetables. Salicylic acid could have potential postharvest application for reducing chilling injury

and decay, delaying ripening, and enhancing the health benefits of fruit and vegetable consumption by increasing the antioxidant capacity (Aghdam et al., 2016).

More than one third of harvested fruit and vegetables are lost and do not reach the customers mainly due to postharvest decay. During the last decade, several postharvest fungicides have been excluded from the market, or their allowed residues have been significantly decreased. Therefore, there is growing interest in ecofriendly and safe alternatives to synthetic fungicides. Induced resistance has gained increasing attention as a sustainable strategy to manage postharvest decay of fruit and vegetables. Their natural resistance can be increased by various means, such as biocontrol agents or their secreted elicitors. Alternatively, physical means, such as UV-C, ozone, and heat treatment, can prime plant resistance through abiotic stress. Moreover, various defense-related phytohormones, biological elicitors, nonorganic elicitors, and volatile organic compounds have been shown to induce plant resistance. Essential oils, such as *Lippia sidoides* essential oil, basil (*Ocimum micrathum*) essential oil, and lemon grass (*Cymbopogon citratus*) essential oil are being studied as an alternative control for postharvest diseases in melons, pineapples, and peaches. During the last decades, new technologies have enabled the evaluation of gene expression, such as quantitative real-time PCR and the most recent next-generation sequencing, and thus the quantification of physiological changes, which have revealed new knowledge about preharvest- and postharvest-induced resistance in response to various treatments. These techniques allow optimization of postharvest application of the control means, although these data cannot disregard the evaluation of *in vivo* effectiveness. The elicitation of host defenses prevents the appearance of resistant isolates of pathogens. Induced resistance can lead to increased levels of phenolic compounds in the plant tissues, which often have antioxidant properties that are highly beneficial to humans. Moreover, induced resistance preserves the natural microflora, which is rich in potential biocontrol agents, and which provides a combined approach in the control of postharvest decay that is sustainable and safe for both growers and consumers (Oster, 2011; Romanazzi et al., 2016).

2.2 Postharvest Factors That Influence Quality

Generally, factors that influence fruit and vegetable quality are combined in two categories: intrinsic and extrinsic. Intrinsic factors are related to inherent characteristics, such as genetic factors (species, cultivars, etc.), maturity stage in harvest, and susceptibility to physiological disorders. Extrinsic factors are

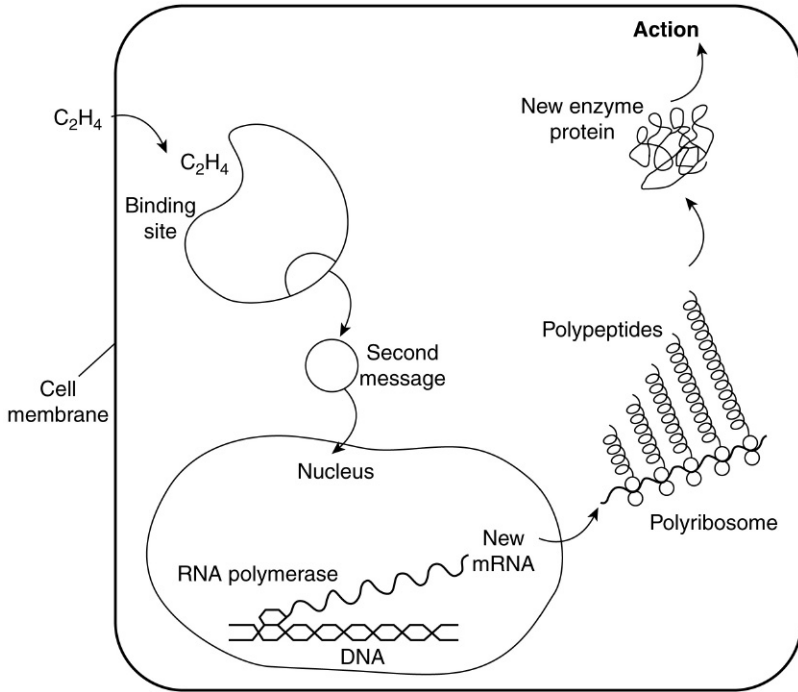


Figure 1.1 Mechanism of ethylene action. (From Kader, A.A. (Ed.), 2002. *Postharvest Technology of Horticultural Crops*, third ed. University of California, Richmond, CA).

related to development's ambient and technologies, such as handling (harvest, package, transportation, storage, and commercialization), technological (control of temperature, relative humidity, irradiation, chemical treatments) (Chitarra and Chitarra, 2005).

2.2.1 Ethylene Commercial Use

Vegetable tissues' physiological responses generally are initiated by self-catalytic ethylene production at a specific stage in plant development. These responses can also be initiated by exogenous ethylene supplementation before its self-catalytic increase. Ripening involves a lot of biochemical reactions and physiological changes that might occur simultaneously or sequentially in an independent or dependent manner. Ethylene application might affect few or all physiological changes that will reflect on fruit and vegetable quality (Chitarra and Chitarra, 2005). Fig. 1.1 shows the ethylene's mechanism of action.

Ethylene-releasing chemicals (Fig. 1.2) are approved for a variety of uses of interest to postharvest technologists (Kader, 2002).

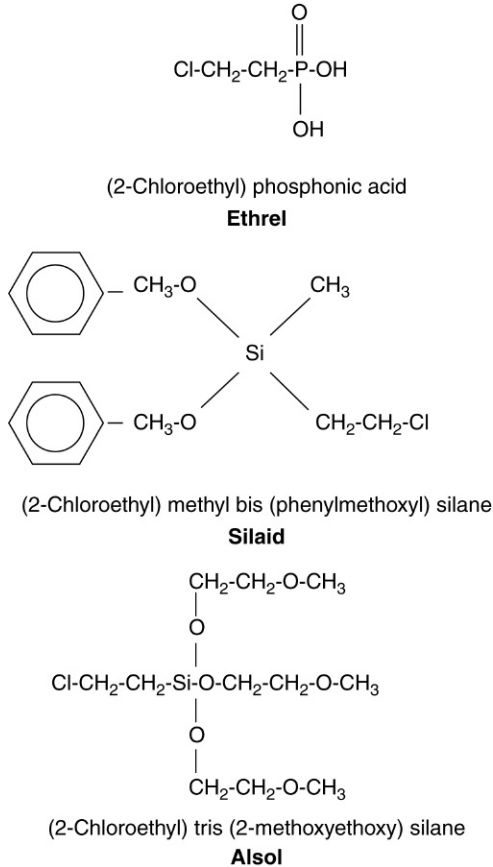


Figure 1.2 Molecules of ethephon, silaid, and alsol, three ethylene-releasing chemicals. (From Kader, A.A. (Ed.), 2002. *Postharvest Technology of Horticultural Crops*, third ed. University of California, Richmond, CA).

2.2.2 Use of 1-MCP as an Ethylene Inhibitor

1-Methylcyclopropene (1-MCP) is an ethylene-competitive inhibitor because it can interact irreversibly with ethylene fruit receptors thereby avoiding its physiological stimulus. Different factors might affect treatments with 1-MCP in vegetables:

- The MCP gas concentration needed to saturate receptors and compete with ethylene.
- Treatment application duration so 1-MCP can penetrate vegetable tissues.
- Ideal temperature so treatment might be effective in a particular time frame.
- Product's maturation as 1-MCP is not effective in advanced maturation (Chitarra and Chitarra, 2005).

The main effect of exposure of climacteric fruits to 1-MCP is to delay the natural increases in respiration and ethylene production rates during ripening, thereby delaying ripening and ripening related processes, such as softening, color change, starch breakdown (Li et al., 2016a).

Nonetheless, although nonclimacteric fruits do not exhibit any clear increases in ethylene production rates during ripening, in certain cases their exposure to exogenously applied ethylene may stimulate certain ripening-related processes, such as degreening of citrus fruit (Li et al., 2016a).

2.2.3 Temperature

Cooling the products is still one of the most often used ways to maintain quality of postharvest products because it reduces respiratory rates, slows metabolic activity, and inhibits the microbiological development (Chitarra and Chitarra, 2005).

2.2.4 Relative Humidity

Fruits and vegetables should be stored in high relative humidity conditions so the moisture loss will be minimized, but it cannot be so high that it creates condensation that might increase microbiological development (Chitarra and Chitarra, 2005).

2.2.5 Growth Regulators

Substances, such as auxins, gibberellins, cytokinins, abscisic acid, polyamides, jasmonates, and calcium, might be used in postharvest mainly for their synergistic action antagonist or ethylene inhibitor regulating ripening and senescence (Chitarra and Chitarra, 2005).

2.2.6 Atmosphere Gases Concentration

Perishables, such as fruits and vegetables, can have shelf life extended by modifying the gas composition in the atmosphere (Chitarra and Chitarra, 2005). Oxygen reduction and carbon dioxide increase in the package atmosphere for fresh fruit and vegetables and can reduce respiration rates and inhibit microbiological and insect development (Fellows, 2006).

2.2.7 Irradiation

Fruit and vegetable postharvest irradiation is done mainly to reduce or retard damage caused by diseases or insects, acting as a fungicide or insecticide. It also can be used as a preservation method, extending shelf life by slowing up budding in some products. Irradiation has some inconveniences

because, depending on radiation dosage, it might create darkening, softening and flavor loss (Chitarra and Chitarra, 2005).

Some fruits and vegetables, such as strawberries and tomatoes, might be irradiated to double or triplicate their shelf life when storage is at 10°C. A combination of irradiation and modified atmosphere packages has a synergistic effect and, as a result, a lower irradiation dose is needed to reach the same final effect (Fellows, 2006).

One important factor is that fruits and vegetables have to be mature before irradiation because this process inhibits maturation (Fellows, 2006).

3 APPLE (*Malus domestica*)

The harvest quality of apples, including appearance, taste, texture, safety, and nutritional value, declines due to continuing respiration, ethylene production, and the occurrence of postharvest diseases. Quality cannot be improved, but it can be largely maintained during storage. Generally, the main features of quality maintenance during storage are the successful control of skin background color and weight loss, high retention of flesh firmness, and retention of soluble solids and acid to give the desired sugar to acid ratio. Therefore, an effective storage method should prevent all of the previously mentioned quality changes (Li and Li, 2008).

No one single factor can provide assurance of apple postharvest quality, but many factors (i.e., harvesting at optimum maturity, careful handling to prevent fruit injury, rapid cooling after harvest, and storage at lowest safe temperature, controlled atmosphere storage) must be considered as part of the entire harvest–postharvest system (Swezey et al., 2000).

Usually, apples are stored in refrigerated (−1 to −3°C) warehouses within 1–2 days after harvest and the temperature of the apples is lowered to −1 to 5°C after 3–5 days. The relative humidity in refrigerated warehouses should be controlled at 90%–95% during storage. This method of storage can keep apples fresh for more than 6 months (Li and Li, 2008).

The treatment of “Granny Smith” apples with 1-MCP can extend the storage time in standard NA storage for at least 3 months without significantly losing freshness even 2 weeks after removal from cold storage, and is more effective in preserving sensory attributes related to apple freshness when compared with the DPA treatment (Tomic et al., 2016).

Róth et al. (2007) studied apple cv. Jonagold stored in the air and under controlled atmosphere conditions (1% of O₂ and 2.5% of CO₂). There was a considerable softening during storage in air and shelf life, but not under

controlled atmosphere conditions. Aroma profiles of air-stored and controlled atmosphere apple converge during shelf-life conditions.

[Khorshidi et al. \(2010\)](#) showed that storage with 0°C for apple cv. “Red Delicious” could maintain better product quality compared to storage at 5 and 12°C.

According to [Blažek et al.](#), cited by [Goliáš et al. \(2008\)](#), the ideal apple for long-term storage combines higher skin thickness and toughness with a lower production of ethylene, which slows the softening of fruit.

4 BANANA (*Musa spp.*)

Banana is a tropical fruit widely accepted by people worldwide ([Yuan et al., 2017](#)). Banana is one of the fruits most consumed in the world, being produced in most tropical countries. It is classified as an easily perishable fruit, with shelf life under refrigeration up to 3 weeks. This high perishability, compared with other fruit, is due to the fruit’s high respiration rates ([Borges et al., 2006](#)).

Banana fruit is very susceptible to both abrasion and impact injury. The major marketing problem of exporting bananas is mechanical injury, which shows itself as black sunken areas on the skin after ripening. So during harvesting, care should be taken not to let the bunch fall on the ground and get damaged. Other concerns are diseases, particularly crown rot, which may affect the whole carton and promote uneven fruit ripening ([Hailu et al., 2013](#)).

Bananas are subject to chilling injury at temperatures well above freezing. At the temperature of 12°C or below, the green fruit develops a dull, gray skin color, starch is no longer converted to sugar, and they subsequently fail to ripen properly ([Thompson, 2011](#)). To avoid this problem bananas are shipped at 13–14°C ([Thompson, 2011](#)).

According to [Hailu et al. \(2013\)](#), a huge postharvest loss of the banana has been reported in Africa and these postharvest losses are incurred at different stages from harvesting to the final consumption. Banana production in Mauritius is subjected to serious postharvest losses, mainly due to harvesting at the improper maturity stage, poor handling, and storage practices and postharvest diseases ([Ramma et al., 1999](#)).

[Ambuco et al. \(2013\)](#) presented results that show that banana variety, cultural practices, and harvest season affect the banana quality attributes at harvest and affect the eating quality of the fruits.

Banana chemical composition is critical for its organoleptic properties and nutritional value. The principal metabolites responsible for postharvest

senescence of bananas were valine, alanine, aspartic acid, choline, acetate, glucose, malic acid, gallic acid, and dopamine (Yuan et al., 2017).

Banana preservation under refrigeration might be increased to up to 4 months with the use of controlled atmosphere. It consists in elevated carbon dioxide concentration and decreased oxygen concentration. In this situation, respiratory rates are significantly reduced. For bananas, 7%–10% carbon dioxide and 1.5%–2.5% oxygen are recommended. However, this technique is not widely used because of its high cost (Borges et al., 2006).

When bananas are harvested close to their complete physiological development, they ripen in a nonuniform way. To homogenize the whole batch and provide faster ripening, a controlled environment might be used. The use of ripening rooms with the application of maturation gas activators, such as ethylene or acetylene combined with cooling from 12 to 18°C, is a common practice among big producers (Botrel et al., 2001).

1-MCP has obvious effects on delaying the ripening of harvested banana fruit. However, improper concentration, treatment time, and handling methods could affect normal ripening, yellowing, softening, and formation of volatiles, which are important factors of banana fruit quality (Zhu et al., 2015).

Zhu et al. (2015) studied the effect of a combination of low concentration ethephon (ethylene-releasing substance) with 1-MCP on the ripening of banana fruit and its physiological and biochemical changes. The results showed that a combination of 50 $\mu\text{L/L}$ ethephon with 400 nL/L 1-MCP for 16 h was the most suitable treatment, and significantly delayed the ripening and maintained the commodity value of harvested banana fruit without detrimentally affecting normal ripening after ripening acceleration treatment. This treatment effectively delayed and decreased respiration rate and ethylene production, inhibited the activity of pectin lyase, pectin methylesterase, cellulase, and polygalacturonase, and delayed the peak activity of ACC synthase and ACC oxidase. It also delayed the formation of volatile compounds, but did not detrimentally affect the amount of volatiles, especially the esters. The combined treatment significantly delayed the ripening and prolonged the shelf life of banana fruit with normal coloring and volatile development, which effectively maintained the commercial value of banana fruit.

Pongprasert and Srilaong (2014) studied a different way to apply 1-MCP to bananas: microbubbles. Banana fruits were immersed in 500 nL/L of aqueous 1-MCP microbubbles (1-MCP-MBs) or fumigated with 500 nL/L 1-MCP, then stored at 25°C for 8 days. 1-MCP-MBs were more effective

in delaying postharvest ripening than conventional 1-MCP fumigation. 1-MCP-MBs reduced the respiration rate and ethylene production compared to the control and 1-MCP fumigated fruit. Moreover, 1-MCP-MBs delayed yellowing and maintained firmness of banana fruit during storage.

Alencar et al. (2013) showed that treatment with ozonized water is an alternative for postharvest management of bananas because it retards the evolution of fungal infections, reduces the fresh matter loss, and retards the decrease of total soluble solids (TSS), as well as presents good sensorial acceptance.

5 CASHEW APPLE (*Anacardium occidentale* L.)

Several factors affect postharvest quality of fresh cashew apple, including use of improper harvest containers; delays between harvest and cooling; absence or ineffective use of rapid cooling, poorly designed packaging; temperature fluctuations during transport; high temperatures (above 20°C) during retail (Berry and Sargent, 2011).

Cashew fruit is not susceptible to chilling injury. Cashew apples are very susceptible to postharvest infection, especially from *Rhizopus*, *Colletotrichum*, and *Penicillium* (Berry and Sargent, 2011). They are highly susceptible to physical injury, which leads to microbial spoilage during harvest, transportation, and storage. Yeasts and molds are the primary and secondary invaders, respectively, responsible for the spoilage of the cashew apple. The insect maggots and noninsect pests are also responsible for the spoilage of fallen apple in the field along with the soil microbes (Salman Subiki et al., 2016).

Washing cashew apples removes soil and reduces temperature by removing field heat. High ambient temperatures at harvest lead to more intense metabolic activity, and consequently faster senescence (Berry and Sargent, 2011).

Moura et al. cited by Souza (2016) evaluated the postharvest quality of apples of cashew varieties CCP 76, END 183, and END 189, and reported that orange-colored peduncles should be stored at 3°C for a postharvest life of 20 days, while red-colored ones should be stored at 5°C for 15 days.

Morais et al. (2002) studied apples or false fruits from early dwarf cashew clones CCP-76, END-157, END-183, and END-189 that were stored for 25 days under refrigeration associated to modified atmosphere with the aim of evaluating the effect of the conditions presently adopted (5°C and

85%–90% relative humidity) for conservation, storage, and commercialization of fresh cashew apples. With the use of refrigeration and modified atmosphere, it was possible to attain postharvest storage life of 10 days for clone END 189, 15 days for clone END 157, and up to 25 days for clones CCP 76 and END 183. It was found that 5°C was not an adequate temperature to store cashew apples with deeper color (END 157 and END 189) than the control (CCP 76), as it caused loss of anthocyanins starting from the 10th day of storage. Weight loss was significantly reduced by the use of modified atmosphere, favoring the appearance of the cashew apples for market, independent of the clone.

Salman Subiki et al. (2016) studied cashew apple hardening pretreatments and found that 4.5% CaCl₂ was most effective in extending shelf life up to 18 days without fruit spoilage, with retention of color, texture, fruit weight, ascorbic acid, titratable acidity, tannins content, and other fruit quality attributes as against 12 and 6 days in untreated control fruits stored at low temperature (2 ± 1°C, 90%–95% relative humidity) and at room temperature conditions, respectively.

6 KIWI (*Actinidia deliciosa*)

Kiwifruit has long been called “the king of fruits” due to several compounds with functional properties, such as high vitamin C content, carotenoids, polyphenols, and balanced composition of minerals, dietary fiber, and other metabolites beneficial to human health, making this fruit very popular (Stonehouse et al., 2013).

Postharvest decay of kiwifruit due to infections by fungal pathogens, however, results in significant losses. Gray mold caused by *Botrytis cinerea* is the most important postharvest disease of kiwifruit (Minas et al., 2010), while blue mold caused by *Penicillium expansum* is another disease on kiwifruit (Neri et al., 2010). Kiwifruits have limited shelf life under ambient conditions. Controlling the atmosphere or using normal refrigeration helps to keep these fruits stored for long periods, but it still requires high care during harvest and postharvest handling because kiwifruits have high sensitivity to ethylene. Kiwi brushing for the market is one of the many small injuries that can occur triggering ethylene biosynthesis hastening the ripening process. Also bruising, occurring during harvest and postharvest practices, including the shipping, hastens kiwi ripening, increasing ethylene production and fruit temperature play an important role in symptom appearance. Water loss is another common problem during long-term storage or transport,

since kiwi pericarp is very permeable to water mass transfer. About 0.5% water loss increases cell wall enzyme activity and a further increase of water loss accelerates respiration and ethylene production, together with the loss of volatiles. Thus, before symptoms (shriveling, shrinking) become visible, minimal water loss seriously alters the fruit texture, first with cell wall degradation. In kiwifruit, the softening process occurs prior to the respiratory climacteric and production of ethylene, which means at stage 2 when net starch degradation occurs, pectin deesterifies and solubilizes, and pectin methylesterase activity increases (Taglienti et al., 2009).

Hayward kiwifruit treated with ethylene or 1-MCP was compared with nontreated fruit in a number of reports. Jhalegar et al. (2011) found that many concentrations of 1-MCP delayed the ripening of kiwifruits, but 2 $\mu\text{L/L}$ concentrations was the most effective in doing so. Fruits treated with 1-MCP at 2 $\mu\text{L/L}$ started ripening after 12 days of storage whereas untreated fruits started ripening even on the sixth day. Polygalacturonase and lipoxygenase enzyme activities were lesser in 1-MCP-treated fruits than control. 1-MCP-treated fruits respired less and evolved lesser ethylene. Tang et al. (2015) studied the use of antagonistic yeast (*Candida diversa*) with harpin (a hypersensitive response elicitor) treatment to control postharvest decay of kiwifruit and concluded its potential as an effective method to prevent infection by *B. cinerea* and *P. expansum*. Krupa et al. (2011) showed an increase in soluble solid content and a decrease in titratable acid and skin firmness, and the health characteristics (ascorbic acid content, total phenolic compounds, and antioxidant activity) of hardy kiwifruit collected at the soluble solid content 8%–10% during cold storage. Within 7–42 days of storage, fruit showed significant similarities in all examined physicochemical characteristics with the exception of titratable acid in relation to vine ripe fruit. Hardy kiwifruits, without 1-MCP treatment, showed increases in both respiration and ethylene production rates during fruit storage. The 1-MCP treatment remarkably inhibited fruit ripening by reducing respiration and ethylene production. Fruits with the 1-MCP treatment could be stored for up to 5 weeks by maintaining higher fruit firmness, ascorbic acid, and total phenolic contents compared to the control (Lim et al., 2016).

7 MANGO (*Mangifera indica* L.)

Mango, a tropical fruit of great economic importance, is generally harvested green and then commercialized after a period of storage. Unfortunately, the final quality of mango batches is highly heterogeneous, in fruit size as well

as in gustatory quality and postharvest behavior (Léuchadel and Joas, 2007). Maturity of mango could be predicted by measuring size, color, and firmness (Jha et al., 2006).

Mango fruit being climacteric have a short shelf life, and coating is considered as one of the most popular techniques to prolong its shelf life. Coatings based on starch, olive oil, beeswax, and sodium benzoate have been evaluated with reference to the shelf life and quality of mango (Langra and Samar Bahisht Chaunsa) fruit harvested at the hard green stage of maturity. The fruit was stored at various temperatures until they ripened. The fruit was analyzed for quality parameters at the harvest stage, at the time of ripening of control, and at the ripened stage, it indicated that every coating had a significant impact on the quality and shelf life of the fruit in most of the cases under the limit of $P < 0.05$. The weight loss and waste percent were the lowest, and the shelf life was the longest in beeswax coating, whereas the quality was best in the case of starch-based coating as compared with others (Bibi and Baloch, 2012).

According to Paull and Chen (2014), skin coloration, size, shape for variety, appearance, freedom from defects and decay, absence of fiber in the flesh, and absence of turpentine-like flavors are the most common quality parameters for mango. Wilted, grayish discoloration and pitting are undesirable. Some fruit varieties, such as “Hayden,” have pinhead-size black spotting that is not regarded as a defect.

Storage at 10–13°C with 85%–90% relative humidity should give a shelf life of 14–28 days for mature green fruit, depending on variety. Ripe fruit can be stored at 7–8°C. Diseases are the principal factor limiting storage life. Optimum ripening temperature is 20–23°C (68.0–73.4°F) for best appearance, palatability, and decay control (Paull and Chen, 2014).

Different cultivars show various responses to controlled atmosphere. The optimum storage atmospheres for prolonging storage and/or shipping are 3%–6% O₂ + 3%–10% CO₂ at 7–9°C with 90% relative humidity (Paull and Chen, 2014). Ripening delays are minor and may not be economic in all situations. Polyethylene or other film bags with and without an ethylene absorber give some delay in ripening. However, some bags lead to off-flavor and abnormal skin coloration (Paull and Chen, 2014).

Neves et al. (2008) observed for uncooled cv. Tommy Atkins and Haden, the lesser concentration of ethylene in the packing (cardboard and 0.06 mm of thickness LDPE film), the biggest pulp firmness, the biggest containment in the advance, and the reduction of soluble solids and acidity, respectively, as well as the best maintenance of ascorbic acid contents, had been detected in the fruits conditioned in packages containing ethylene’s adsorption system.

Chilling susceptibility varies with cultivar; “Hayden” and “Keitt” are particularly susceptible. Most cultivars show injury below 10°C, especially if fruit have just reached maturity. Tolerance to chilling increases during ripening (Paull and Chen, 2014).

Islam et al. (2013) studied the use of gibberellic acid in mangoes during storage and showed that some physicochemical properties, namely, physiological weight loss, moisture content, pulp pH, TSS, sugar (total, reducing and nonreducing), were rapidly increased from untreated mangoes. Gibberellic acid solution at 400 ppm showed better performance in delaying the changes in physicochemical properties and extended shelf life.

Ozonized water can be used as an alternative to chlorine sanitizer without causing damage to mango, cv. Palmer fruit or inducing a decrease in the various compounds and the treatment using ozonized water was efficient for maintaining fruit without microorganisms, preventing the reduction of quality and avoiding the generation of organic waste (Monaco et al., 2016).

González-Aguilar et al. (2007) showed that UV-C treatment can be a good alternative to increase the shelf life in optimal conditions of mango “Haden.” Singh et al. (2012) concluded that ethrel 750 ppm was found to be a suitable treatment in improving physical–chemical traits, that is, ripening, storage, quality, and shelf life for commercial purpose in mango cv. Amrapali.

8 MELON (*Cucumis melo*)

Table 1.2 shows storage conditions and postharvest useful life of some melons.

Tomaz et al. (2009) evaluated shelf life of five yellow melon hybrids (AF-7100, AF-1498, AF-5107, AF-4945, and AF-1805) stored at cold room at

Table 1.2 Storage conditions and postharvest useful life of some melons

Melon	Storage temperature (°C)	Storage relative humidity (%)	Postharvest useful life (days)
Canary	10–12	85–90	30
	6–9	85–90	16–20
Santa Claus	15–16	75–80	40
Black Tendral	15–16	75–80	40
Galia	7–8	90–95	28
Orange Flesh	6–8	90–95	28
Cantaloupe	3–5	85–90	30

Source: Filgueiras, H.A.C., et al., 2000. Colheita e manuseio pós colheita. In: Alves, R.E. (Ed.), Melão: Pós Colheita. Embrapa Informação Tecnológica, Brasília.

10 ± 1°C and 90 ± 2% of relative humidity for 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 days. Higher fruit pulp firmness was observed in the hybrid AF-7100, followed by the hybrid AF-5107. There was an increment in the percentage of mass loss during the storage. It was not observed that any damage on the external and internal appearances until 42 and 28 days of storage, respectively.

Fresh-cut “Orange Flesh” melons were stored for 8 days under refrigeration (6 ± 1°C–90 ± 5% relative humidity) and modified atmosphere (passive and active: 5% O₂ + 5% CO₂ and 2% O₂ + 10% CO₂). The variables pH, titratable acidity, soluble solids, and acetaldehyde were not affected, differently, for the atmospheres studied. An increase in CO₂ concentration occurred over the storage period. Active modified atmosphere (2% O₂ + 10% CO₂) was the most effective in controlling the CO₂ production until the fourth day of storage (Vilas Boas et al., 2004).

It is recommended that the use of fungicides in melon postharvest is only when absolutely necessary and in accordance with local legislation, being aware of residual levels. Overall fungicides are necessary only to avoid rot on peduncle region (Filgueiras et al., 2000).

9 ORANGE (*Citrus* × *sinensis*)

One of the most problematic postharvest diseases in oranges is green mold, caused by *Penicillium digitatum*. This pathogen is of main concern, as it is responsible for 90% of oranges deterioration during the storage period, resulting in serious economic losses. Application of fungicides is the main method carried out to control postharvest diseases of oranges (Li et al., 2016a). Li et al. (2016b) applied a combination of *Hanseniaspora uvarum* Y3 with phosphatidylcholine in oranges to study the biocontrol of postharvest green mold. Their results indicated that phosphatidylcholine at 1.5% w/v significantly enhanced the biocontrol activity of *H. uvarum* Y3 against postharvest green mold of oranges and influenced *H. uvarum* Y3 to increase rapidly in fruit wounds. Application of *H. uvarum* Y3 alone or combined with phosphatidylcholine significantly inhibited spore germination and mycelial development in orange wounds and had no influence on storage quality parameters. Postharvest treatment of *H. uvarum* Y3 combined with 1.5% phosphatidylcholine significantly reduced weight loss in comparison with the control treatment. Thus, *H. uvarum* Y3 in combination with phosphatidylcholine (1.5% w/v) may be a potential biocontrol method against postharvest green mold of oranges.

Lafuente et al. (2014) evaluated ethylene conditioning treatment in stored mature sweet oranges, Navelate and Lane Late cultivars, showing

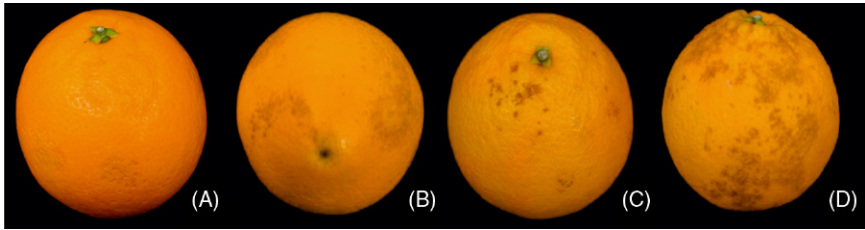


Figure 1.3 Oranges showing nonchilling peel pitting (A), chilling injury (CI) (B), and stem end chilling injury (SECI), when the disorder begins (C), and after it extends through the fruit surface (D).

this treatment as a useful and feasible tool to extend the postharvest life of nonchilling peel pitting and chilling injury-sensitive citrus cultivars. Their results indicated that ethylene conditioning had neither a deleterious effect on internal and external fruit quality nor on the concentration of phenolic or flavonoids (Fig. 1.3).

Aloui et al. (2015) showed that sodium alginate (NaAlg) and locust bean gum (LBG) coatings enriched with *Wickerhamomyces anomalus* yeast were not only effective at reducing weight loss and maintaining firmness of “Valencia” oranges during storage, but also at controlling green mold in inoculated fruits by more than 73% after 13 days. These results and the Qualified Presumption of Safety status recently obtained by European Food Safety Authority for the use of *W. anomalus* as a novel microorganism in food preservation, demonstrate the potential application of these bioactive coatings as effective and promising alternatives to synthetic antifungal agents for maintaining quality attributes and controlling green mold of “Valencia” oranges.

10 PINEAPPLE (*Ananas comosus* L.)

Belongs to the family Bromeliaceae and is one of the most important commercial fruits of the world. The pineapple fruits are normally eaten fresh or as fresh pineapple juice. Pineapple fruits are an excellent source of vitamins and minerals and supply arrays of color, flavor, and texture to the pleasure of eating (Othman, 2011).

The effects of UV-C radiation on keeping the quality of fresh-cut pineapples (*Ananas comosus* L. Merr. cv. Comte de Paris) were investigated by Pan and Zu (2012). The UV-C radiation device was homemade. The UV-C lamps were located 20 cm above and below the radiation vessel. The UV-C radiation doses selected for this experiment were 4.5 KJ/m² on each side of the produce. The samples were divided into six subgroups and radiated for 0, 60, and 90 s, respectively. Radiation on the products was carried out

in the fresh-cut preparation room at 10°C to avoid a temperature increase between the two banks of lamps during the treatment. After the radiation, the fresh-cut pineapples were packed in a tray combined with polyethylene packaging film and stored at 10°C.

Production color is probably the most important attribute that determines the overall quality as it affects consumer perception. UV-C radiation had a noticeable influence on the browning of the fresh-cut pineapples. The browning development of the fresh-cut pineapples without radiation was slower than that with radiation. Especially, since the seventh day, the browning development of the fresh-cut pineapples with radiation accelerated. Extension of exposure time resulted in increasing the browning in the fresh-cut pineapples (Fig. 1.4).

In regard to the influence of radiation on the reducing sugar of fresh-cut pineapples, they've concluded that the firmness of the fresh-cut pineapples showed a decreased trend, but the slices of the control group decreased faster compared with that in the samples with radiation (Fig. 1.5).

The slices treated and untreated with UV-C showed an upward trend after the first drop in the TSS. A significant difference in the TSS was observed between the radiated and unirradiated slices.

UV-C radiation significantly inhibited the decrease in the firmness, TSS, and reducing sugar and the increasing rate of titratable acidity in the fresh-cut

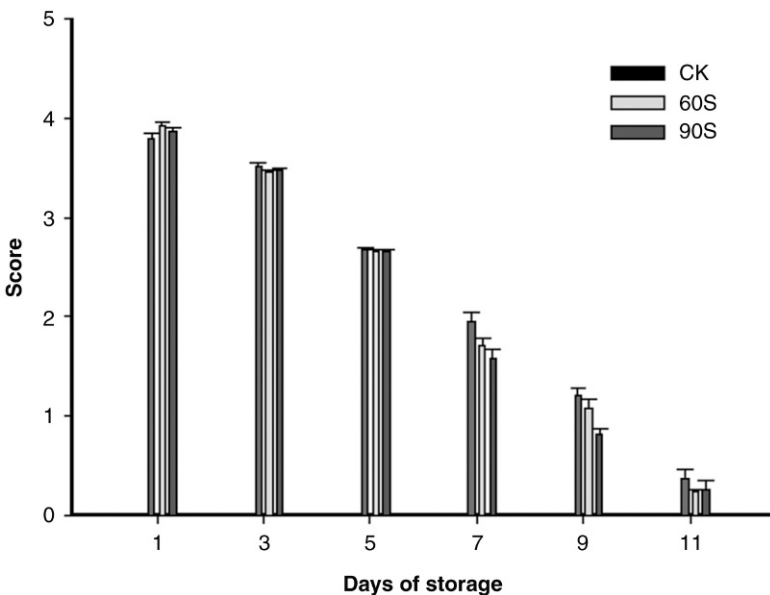


Figure 1.4 The effect of UV-C radiation for 60S and 90S on the browning of fresh-cut pineapples. (From Pan, Y.G., Zu, H., 2012. Effect of UV-C radiation on the quality of fresh-cut pineapples. *Proc. Eng.* 37, 113–119).

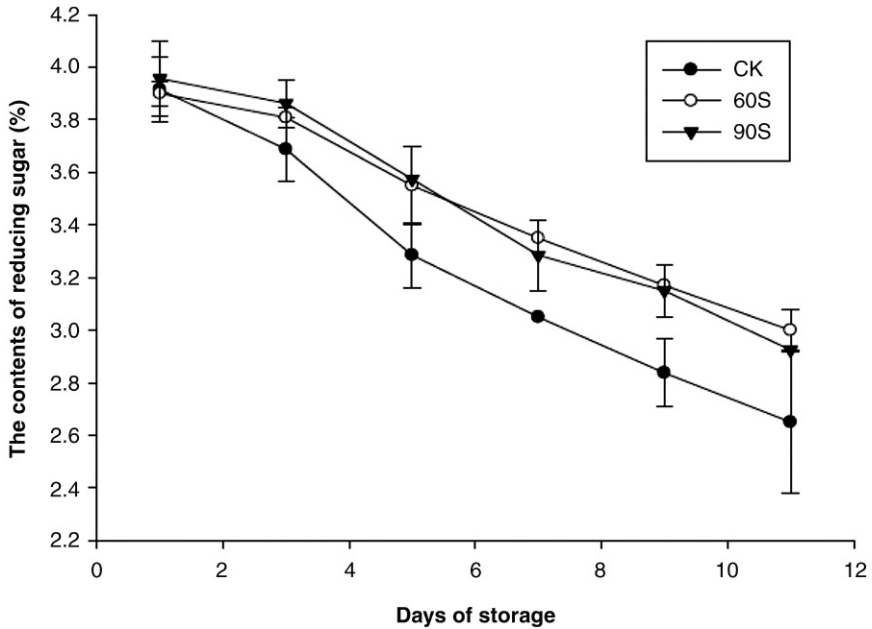


Figure 1.5 The effect of UV-C radiation for 60S and 90S on the contents of reducing sugar in the fresh-cut pineapples. (From Pan, Y.G., Zu, H., 2012. Effect of UV-C radiation on the quality of fresh-cut pineapples. *Proc. Eng.* 37, 113–119).

pineapples. And at the same time, no statistical differences were noted when the slices treated for 60S and 90S were compared ($P < 0.05$). However, UV-C radiation tremendously decreased the content of vitamin C in the fresh-cut pineapples. Likewise, no significant differences were noted when the slices treated for 60S and 90S were compared ($P < 0.01$). Meanwhile, UV-C radiation induced browning throughout the storage period, and the extension of exposure time resulted in increased browning in the fresh-cut pineapples.

11 STRAWBERRY (*Fragaria* × *ananassa*)

Demands of strawberries from the supermarket require high-quality products with continuous supply (Riswahadi, 2015). Strawberry is a product appreciated by consumers due to the organoleptic characteristics. However, strawberry fruits are highly perishable after harvest, so alternative measures to prolong the shelf life of fruits are necessary (Andrade Júnior, 2016). The influence of colored light-quality selective plastic films (red, yellow, green, blue, and white) on the content of anthocyanin, the activities of the related enzymes, and the transcripts of the flavonoid gene was studied in developing strawberry fruit by Miao et al. (2016).

As anthocyanins are one of the principle bioactive components of strawberries, food scientists have conducted comprehensive analyses to quantify and characterize them. Miao et al. (2016) have studied the effects on anthocyanin content, enzymes, and the expression of flavonoid genes were investigated after colored light-quality selective plastic films were used to cover strawberry fruits. To study the transcriptional profiles of flavonoid genes using qPCR, the levels of 12 structural genes leading to flavonoid biosynthesis in strawberries were investigated from the white to red stages after treatment with films of different colors. Quantificated qPCR, was from total RNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method designed for samples rich in polyphenols and polysaccharides. Five micrograms of total RNA were used to synthesize the first-strand of cDNA with TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China) in a final 20 μ l reaction mixture according to the manufacturer's instructions. The real-time quantitative PCR (qPCR) mixture (20 μ l in total volume) included 10 μ l of SYBR Fast Start Essential DNA Green Master Mix (Roche, Switzerland), 0.5 μ l of each primer (10 μ M), and 8 μ l of diluted cDNA. Subsequently, the qPCR was performed on a LightCycler 96 real-time PCR instrument (Roche, Switzerland), initiated at 95°C for 10 min, followed by 45 cycles at 95°C for 10 s, 50–60°C for 10 s, and 72°C for 30 s. Melting curve analysis was performed at 95°C for 10 s, 65°C for 60 s, and 97°C for 1 s. All the qPCR reactions were normalized using the CT value corresponding to the actin gene (FaActin) through the $2^{-\Delta CT}$ method (Fig. 1.6).

This study found that colored light-quality selective plastic films, especially red and yellow films, can affect anthocyanin content in strawberries by altering related enzymes, the flavonoid pathway and transcription factor genes. Treatment with red and yellow light-quality selective plastic films might be useful as a supplemental cultivation practice for developmental strawberry fruit to improve anthocyanin content (Miao et al., 2016).

12 TOMATOES (*Solanum lycopersicum*)

Tomato easily deteriorates postharvest, even under refrigeration; because of this, the shelf life is an important quality trait for many fruit, including tomatoes. To Yang (2011) fast determination of growing stages and harvest time of fruits and vegetables is necessary to implement robotic operation for horticulture automation. His study showed the spectral characteristics of a representative of tomatoes at different growing stages (Fig. 1.7).

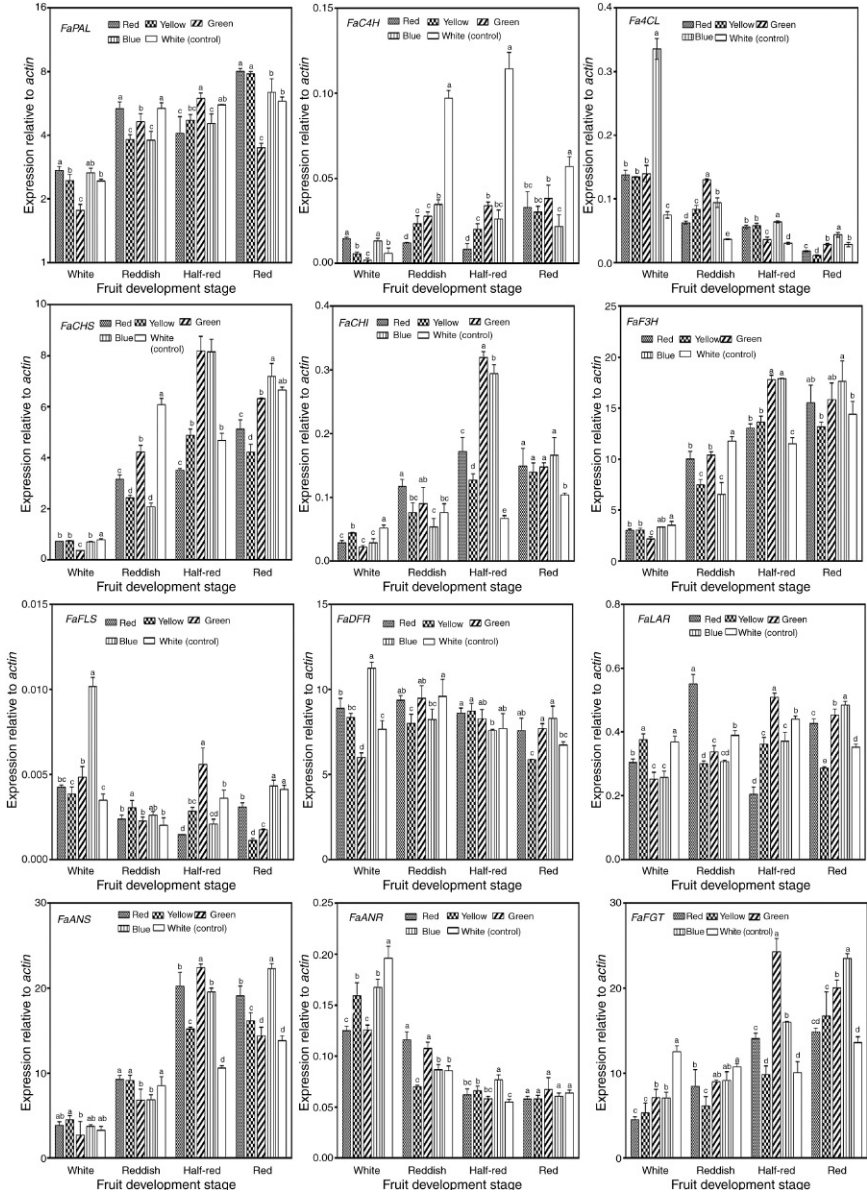


Figure 1.6 The effect of red, yellow, green, blue, and white (as the control) film treatments on the expression of flavonoid biosynthetic genes in strawberries during fruit development. Each value is shown by means \pm SD. Different letters on the bars indicate treatments were significantly different at $P < 0.05$. (From Miao, L., et al., 2016. Colored light-quality selective plastic films affect anthocyanin content, enzyme activities, and the expression of flavonoid genes in strawberry (*Fragaria \times ananassa*) fruit. *Food Chem.* 207, 93–100).

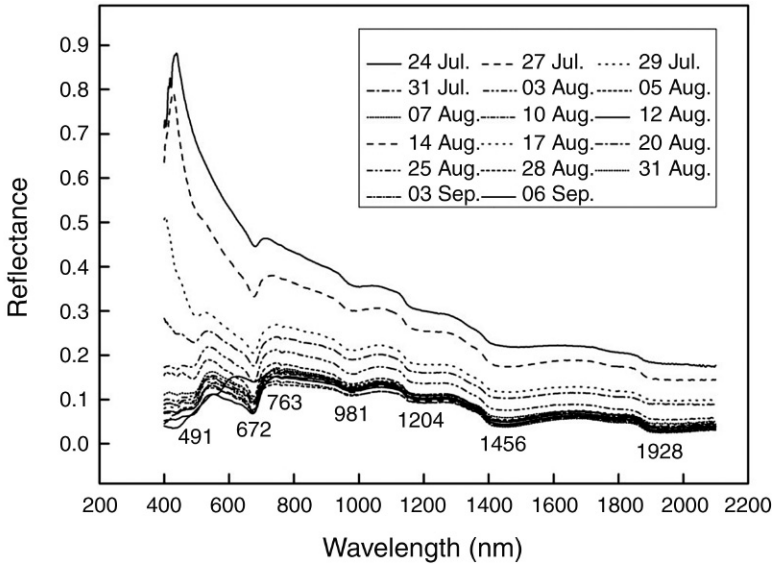
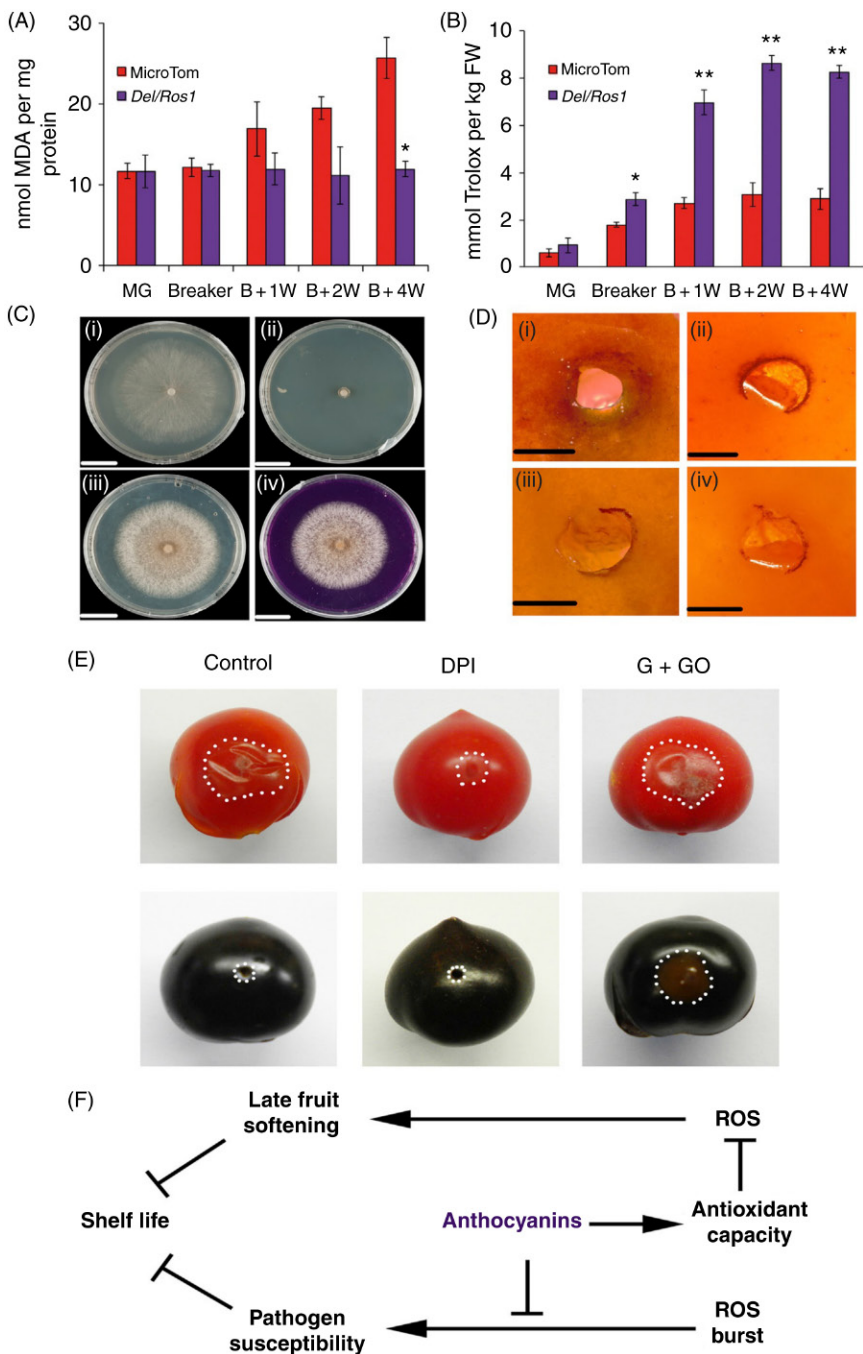


Figure 1.7 Spectral characteristics of tomatoes at different growing stages. (From Yang, H., 2011. Remote sensing technique for predicting harvest time of tomatoes. *Proc. Environ. Sci.* 10, 666–671).

In another study, Zhang et al. (2013) report that enrichment of anthocyanin, a natural pigment in tomatoes, can significantly extend shelf life. Processes late in ripening are suppressed by anthocyanin accumulation, and susceptibility to *B. cinerea*, one of the most important postharvest pathogens, is reduced in purple tomato fruit. Fig. 1.8 shows the “Extended Shelf Life in Purple Tomatoes is Associated With Their High Antioxidant Capacity.”

stages; (C) addition of juice from either red or purple tomatoes to the growth medium had no effect on growth of *B. cinerea*. PDA medium (i), PDA with 15 mg/L Triademinol (an inhibitor of fungal growth) (ii), PDA supplemented with 50% red juice (iii), and PDA supplemented with 50% purple juice (iv) are shown. Pictures were taken 3 days after plate inoculation. Scale bars represent 2 cm; (D) 3,30-diaminobenzidine (DAB) staining of hydrogen peroxide produced 24 h after inoculation of *B. cinerea*: red (i) and purple (ii) fruits stained with DAB, 24 h after inoculation, wound only red (iii), and purple (iv) fruit stained 24 h after wounding. Scale bars represent 1 mm; (E) the levels of ROS in red and purple tomatoes were altered by infiltration of a water control, 10 mM diphenyleneiodonium chloride (DPI, ROS inhibitor), or 50 units/mL glucose oxidase plus 1% glucose (G + GO, ROS inducer). Fruits were wounded and infiltrated 1 h prior to *B. cinerea* inoculation. Pictures were taken 3 dpi. White dotted lines represent lesion margin. All scale bars represent 2 cm; (F) model for the mechanism of shelf life extension in purple, high-anthocyanin tomatoes. (From Zhang, Y., et al., 2013. Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. *Curr. Biol.* 23, 1094–1100).



◀ **Figure 1.8 Tomato characteristics.** (A) Malondialdehyde levels in pericarp of red and purple MicroTom fruit during ripening. Error bars show the SEM ($n = 3$). * $P < 0.05$ compared with WT, red fruit at same stage; (B) Trolox equivalent total antioxidant capacity (TEAC) of water extracts from red and purple tomatoes during ripening. Error bars show the SEM ($n = 3$). * $P < 0.05$ and ** $P < 0.01$ in comparison to WT, red fruit at the same

13 BROCCOLI (*Brassica oleracea* var. *italica*)

Broccoli is considered a vegetable with the highest nutritional value per edible unit weight. Harvested broccoli comprises a mass of immature floral buds (florets) and thick, fleshy flower branchlets or stalks attached to the central plant axis (collectively, the head). Fresh broccoli has a storage life of 2–3 days in air at 20°C being very perishable. As broccoli deteriorates, the degradation of chlorophyll in the sepals influences the change in color of head (green to yellow), cells lose turgor, and tissues become flaccid, and cell necrosis develops during advanced stages of senescence. Broccoli branchlets undergo major losses of sugars, organic acids, and proteins within the first 6 h of harvest, which is followed by increases in the free amino acid pools (especially the amides glutamine and asparagine), and ammonia accumulation. Another characteristic of postharvest broccoli senescence is loss of membrane fatty acids. The respiration (carbon dioxide production) of broccoli florets declines rapidly during the first 12 h after harvest (Eason et al., 2007). Moreover, after harvest, broccoli heads trigger a quick senescence and cause the product deterioration, visible by the degreening of sepals. It is known that the cellular changes during senescence are induced by sugar starvation. Buchanan-Wollaston et al. (2005) has explained a common mechanism that regulates metabolic processes during sugar starvation and senescence. Also, in many cases it was shown that postharvest storage in light has also been associated with an increase of sugars and the consequent delay of senescence (Noichinda et al., 2007; Toledo et al., 2003; Zhan et al., 2014a). Such effect was also described in broccoli, in which the storage under light during postharvest retards the chlorophyll degradation and extends the product shelf life (Büchert et al., 2011; Kasim and Kasim, 2007; Zhan et al., 2012, 2014b). Zhan et al. (2012) studied the effect of light exposure and low temperature in broccoli, concluded that a combination of 24 $\mu\text{mol}/\text{m}_2/\text{s}$ intensity light exposure with 7°C storage temperature maintained quality and extended shelf life of fresh-cut broccoli. Hasperué et al. (2015) found that florets from lateral inflorescences were more perishable than fresh-cut broccoli obtained from primary heads. Terminal florets retained higher chlorophyll levels and showed delayed yellowing. Already at harvest primary-broccoli showed lower respiration rate. Florets from terminal heads showed lower weight and sugar loss during storage and maintained higher visual quality throughout the storage period at 4°C. The inflorescence type also had large impact in the initial level of antioxidants as well as in their metabolism during storage (Fig. 1.9).

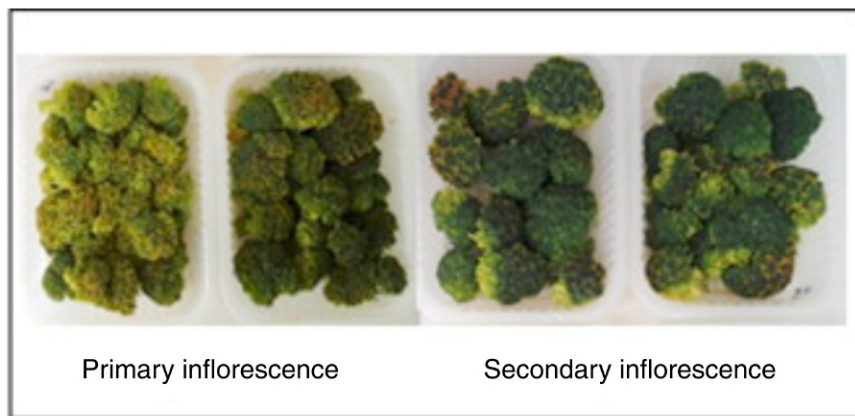


Figure 1.9 Appearance of fresh-cut broccoli prepared from primary and secondary inflorescences stored for 14 days at 4°C.

Renumarn et al. determined the effects of vapor heat treatments (VHT) at 90°C on microbial population and sensory quality of fresh-cut broccoli florets during storage. The florets were treated with VHT for various lengths of time for 0 (control), 15, 30, and 45 s and immediately cooled with water. After cooling, the florets were spin-dried, placed in PVC boxes, and stored at 4°C. At initial day of storage, VHT for 15 s reduced *Escherichia coli* by 0.51 log CFU/g but was not enough to reduce *Salmonella* spp., total bacteria and yeast and mold population. The sensory quality of the florets at this period was acceptable. With long exposure time to VHT resulted in increasing microbial populations and had significant effects on the changes in unacceptable by panelists in the fresh-cut broccoli florets during storage at 4°C. These results suggest that VHT for 15 s may be poor to control microbial population but this treatment could be accepted by panelists during 3 days of storage.

14 CARROTS (*Daucus carota*)

Carrots are one of the potential horticultural commodities that have the opportunity to be expanded following the increasing of domestic and export demand. Carrots contain nutrients that are needed by the body, especially vitamins and minerals. Carrots have beta-carotene as an important chemical compound to form vitamin A or provitamin A.

The carrots that have been harvested still need further treatment before they reach the consumer. Postharvest management includes activities, such

as cleaning, washing, sorting, grading, packing, storage, transportation, and marketing. Like other vegetable commodities, the carrot is easily damaged because after harvest, this plant still does respiration. The damage that occurs during postharvest is unavoidable. Therefore, possible efforts can be made by giving carrots proper packaging. Good packaging protects the wrapped product from damage, weight losses, and firmness changes during transportation time. Damage can be caused by the physiological process, mechanical and biological factors (Albaar et al., 2016). They researched phases to determine the best outer packaging used for carrot transportation. Initially, the carrot is selected and cleaned, then weighed for 10 kg per each plastic baskets, wooden crates, and plastic bag packaging. The transport puts each package on a table to vibrate with a frequency that corresponds to the condition of the road traveled.

Measuring the mechanical level of damage done after simulation of transportation shows the number of broken carrots in each packaging. Fig. 1.10 shows the highest level of damage after 1 h transported for plastic bag packaging is 30.4%, whereas wooden crates were 23.3%, and the plastic basket was 19.3%. Meanwhile, at 2 h, the plastic bag has the highest damage at about 48.6%.

Carrots transported for 1 h in a wooden crate had weight losses about 1.3%, a plastic bucket 1.2%, and a plastic bag 0.9%. For 2 h, the wooden crate had weight losses 2.0%, while the plastic bucket had 1.9%, and the plastic bag had 1.7%. Weight losses of carrots are caused by the loss of water due to the transpiration process during transportation. The weight losses of each package are not significantly seen (Fig. 1.11).

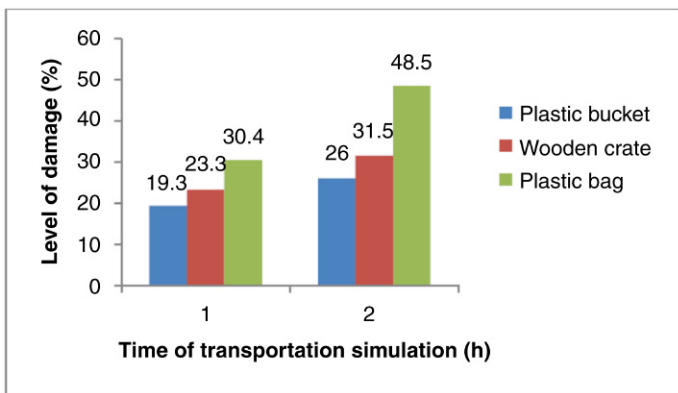


Figure 1.10 Level of carrot damage after the simulation of transportation in 1 and 2 h. (From Albaar, N., Budiastira, I.W., Hariyadi, Y., 2016. Influence of secondary packaging on quality of carrots during transportation. *Agric. Agric. Sci. Proc.* 9, 348–352).

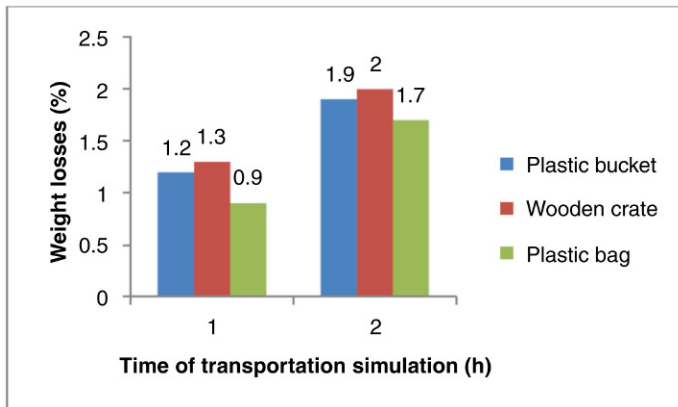


Figure 1.11 Weight losses of carrot in 1 and 2 h transportation. (From Albaar, N., Budiastara, I.W., Hariyadi, Y., 2016. Influence of secondary packaging on quality of carrots during transportation. *Agric. Agric. Sci. Proc.* 9, 348–352).

In the simulation of transportation for 1 h, the highest change of firmness occurred on wooden crate (2 N), while for plastic bucket and plastic bag each is 1.5 and 0.5 N. Likewise, the change of carrot firmness in 2 h showed that for wooden crate 3 N, plastic bucket 2 N, and plastic bag 1.5 N. Change of firmness is caused by the change of cell composition as the effect of turgor cell that made the vegetable became softer. Change of firmness during the simulation of transportation in each packaging is not significantly seen. Low firmness occurs only in a small number (Fig. 1.12).

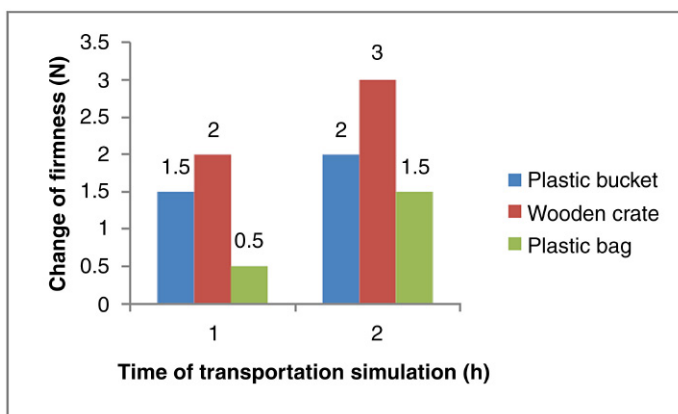


Figure 1.12 Firmness of carrot after 1 and 2 h transportation. (From Albaar, N., Budiastara, I.W., Hariyadi, Y., 2016. Influence of secondary packaging on quality of carrots during transportation. *Agric. Agric. Sci. Proc.* 9, 348–352).

The highest level of damage is found on the plastic packaging for transport of 1 and 2 h (30.4 and 48.5%), while the highest of weight losses occurred on wooden crates for 1 and 2 h of transport, and the highest change of firmness is in wooden crate packaging 1 and 2 h (2 and 3 N). The best secondary packaging for transport is by using plastic bags (Albaar et al., 2016).

15 CAULIFLOWER (*Brassica oleracea* L.)

Cauliflower is a popular vegetable mainly sold fresh, although there has been increasing interest in commercialization as a minimally processed or frozen product in recent years (Sanz-Cervera et al., 2007). Tissue browning is the main postharvest problem of fresh cauliflower, along with floret opening, stem firmness loss, and undesirable odor development, which directly decreases shelf life and consumer purchase. Several techniques have been used to extend the shelf life of cauliflower, including packaging (Dhall et al., 2010), hurdle technology with different concentration and combination of salt, potassium metabisulfite, and citric acid after blanching (Barwal et al., 2005), and combinations of different methods, such as ultraviolet light and hydrogen peroxide (Hadjok et al., 2008). Zhan et al. (2014b) showed that light exposure retarded cauliflower head tissue browning by inhibiting PPO and POD browning enzyme activity, meanwhile preserving total phenols and vitamin C contents during cool storage in comparison with darkness. The cauliflower heads were still marketable after 7 days storage with the largest fresh weight loss being 1.8%. Boumail et al. (2016) showed that cauliflowers treated with coatings with incorporation of essential oils had good antimicrobial effect and allowed to inhibit *Listeria innocua* after 7 days of storage at 4°C, with minor changes in respiration rate and no differences were visually observed on cauliflowers (Fig. 1.13).

Tawema et al. (2016) demonstrated that the application of low UV-C or gamma radiation on fresh-cut cauliflower samples, followed by the application of small amounts of natural antimicrobial formulations could help to control food-borne pathogens and extend the shelf life of cauliflower samples. This procedure reduced the negative effects of each individual method used in combined treatments on the microbial safety of vegetables, and prevents interferences between the applied methods. Gamma irradiation at 1 kGy combined with spraying antimicrobial formulations containing oregano or lemongrass essential oil plus citrus extract and lactic acid was the most suitable treatment to inhibit the growth of pathogenic bacteria, yeasts, and molds on fresh-cut cauliflowers.

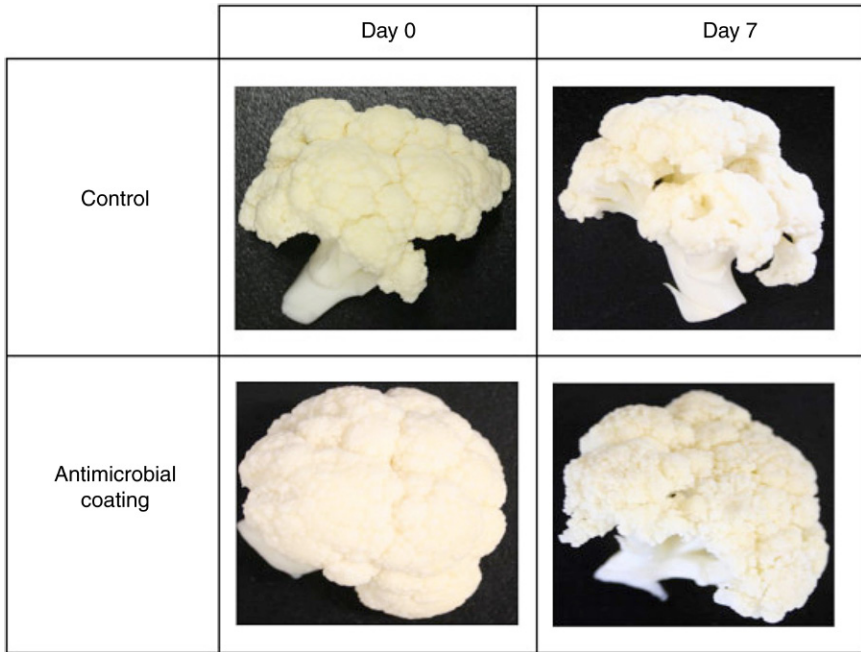


Figure 1.13 Appearance of cauliflowers treated with antimicrobial coating after storage.

16 CILANTRO (*Coriandrum sativum*)

Studies on whole/bunched cilantro indicated that it has a moderately high respiration rate ($15\text{--}20 \mu\text{L CO}_2/\text{g/h}$ at 5°C) and requires 0°C storage to maintain quality and shelf life (Luo et al., 2004).

Fresh herbs respond positively to reduced O_2 and increased CO_2 concentrations. The benefit of high CO_2 atmospheres to the shelf life of herbs can be illustrated with data of cilantro. Cilantro stored in air at 0°C has good visual quality for 18–22 days. Good visual quality can be maintained only 14 days at 5°C and 7 days at 7.5°C . A high CO_2 atmosphere (5%–10%) can double the shelf life of cilantro stored at 7.5°C , principally by maintaining the leaves green. However, a 10% CO_2 atmosphere may eventually cause damage to the leaves (Kader, 2002).

Package film oxygen transmission rate had a significant impact on the gas composition of the packages of fresh-cut cilantro leaves, and consequently affected the quality and microbiology of fresh-cut cilantro leaves. With the tested package configurations, packages prepared with 3500 and 6200 oxygen transition rates films maintained acceptable quality of fresh-cut cilantro leaves. Other treatments either had rapidly depleted O_2 and accumulated

CO₂ resulting in the development of an off-odor and loss quality (1700 oxygen transition rate film bags) or failed to maintain the moisture and color of the fresh-cut cilantro leaves (perforated bags) (Luo et al., 2004).

In a study with fresh-cut cilantro, results indicated that 1-MCP significantly ($P < 0.0001$) delayed the decrease in O₂ and accumulation of CO₂ partial pressures in the headspace of sample packages. Acidified sodium chlorite application significantly reduced initial coliform/*E. coli* counts ($P < 0.001$), and reduced decay rate at the end of storage ($P < 0.05$). A combination treatment of 1-MCP and acidified sodium chlorite, followed by acidified sodium chlorite treatment alone, maintained the lowest decay rates and the highest overall quality scores at the end of storage (Kim et al., 2007).

17 CUCUMBER (*Cucumis sativus*)

Cucumbers are member of the Cucurbitaceae family, along with melons, squashes, and many other horticulturally important species. The fruit are borne on indeterminate, tendril-bearing vines of subtropical and tropical origin (Robinson and Decker-Walters, 1997). A popular vegetable worldwide, low temperature is an effective way to maintain its quality during postharvest processes (Yang et al., 2011). The major important attributes of quality that determine consumer preference and purchasing decision is the external appearance of cucumber (*C. sativus* L.), such as color and texture (Bari and Khan, 2015).

Cucumbers are harvested at a range of developmental stages, depending on the intended use. The time from planting until the beginning of harvest generally ranges between 55 and 60 days, depending on the cultivar and growing conditions. Cucumber fruit should be harvested at an immature stage, near full size but before the seeds are fully enlarged and become hard. They have a short shelf life (<14 days) and their quality is highly reduced after harvesting because they lose too much moisture, exposed to damage and bruising, losing the good appearance during handling and storage process (Bahnasawy and Khater, 2014).

One of the main objectives of postharvest technology in fruits and vegetables is to delay senescence symptoms. Changes in peel color are part of the ripening and the natural senescence process of horticultural commodities, such as a cucumber that has its rate associated with keeping quality of products (Funamoto et al., 2002; Schouten et al., 2002). Guo et al. (2016) found 5% effectiveness of H₂O₂ in the reduction of *Salmonella enterica* serovar *Choleraesuis* in fresh-cut cucumber slices, and demonstrated that

even though after treatment, if not stored properly, contaminated cucumber slices can still have potentials to cause serovar *Choleraesuis* outbreaks.

The effects on the quality of fresh cucumber (*C. sativas* L.) treated with ionizing, nonionizing radiations and their combined treatments was studied by Bari and Khan (2015). They showed that microwave-generated nonionizing radiation along with gamma ionizing radiation both could be used combined to retain the quality of fresh cucumbers and also to reduce the storage loss to a minimum level if stored at 25°C for the period of 15 days. Hence, this combined approach may reduce the gamma radiation dose. However, further detail studies would be required with commercial microwave generator.

Changes of postharvest quality in “Bagdadagi” cucumber (*C. sativus* L.) by storage temperature because they suffer chilling injury, and generally should not be stored long term below 7–10°C (Choi, 2015) (Fig. 1.14).

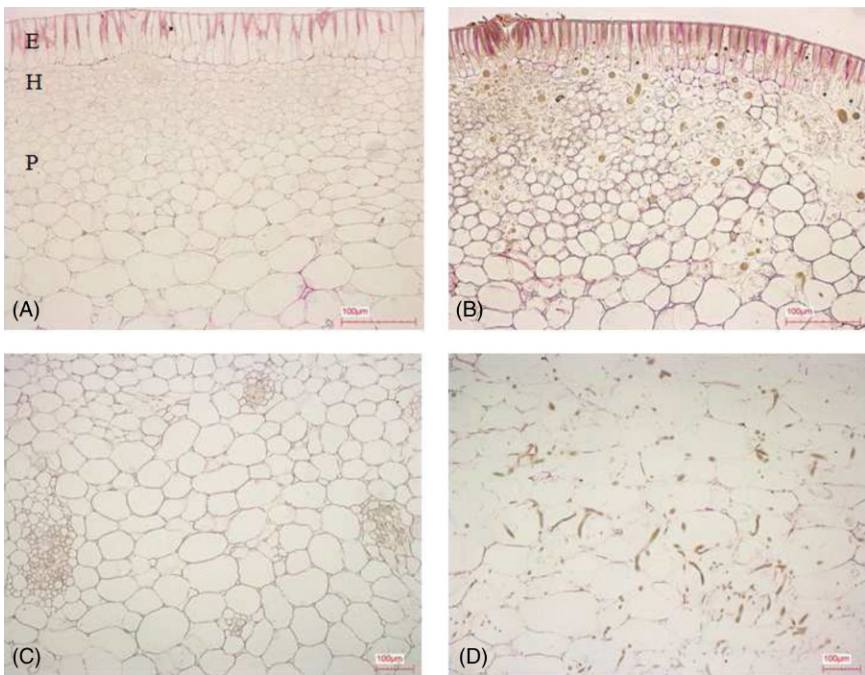


Figure 1.14 Light microscopic images of transversal sections of sound cucumber stored at 10°C and chilling-injured cucumber stored at 0°C for 14 days in storage. (A) transversal section image (×100) of sound cucumber including epidermis (E), hypodermis (H), and parenchyma (P) tissue; (B) transversal section image (×100) of chilling-injured cucumber; (C) transversal section image (×200) of parenchyma tissue of sound cucumber; and (D) transversal section image (×200) of parenchyma tissue of chilling-injured cucumber. (From Choi, J.W., 2015. Changes of postharvest quality in ‘Bagdadagi’ cucumber (*Cucumis sativus* L.) by storage temperature. *J. Food Nutr. Sci.* 3, 143–147).

It was concluded that storage at 10°C was selected as an optimal temperature of Bagdadagi cucumber for maintaining storage life up to 20 days.

18 SPINACH (*Spinacia oleracea*)

Spinach is an important dietary leafy vegetable with high vitamin and mineral contents. Leaves must be green and turgid to be accepted by consumers. However, these attributes of acceptance are quickly lost during postharvest handling because of senescence, especially under nonrefrigerated and/or dark conditions. Chlorophyll, protein, and antioxidant losses are characteristic changes that take place during leaf senescence, and these changes are under environmental and hormonal control (Ferrante and Francini, 2006).

Spinach is an annual, cool season, green, leafy vegetable that is rich in health-promoting compounds, such as vitamin A and C, and flavonoids. Spinach has a relatively high respiration and water-loss rates, and is prone to tissue decay, microbial growth, and loss of nutrients, all of which lead to a low storage potential. Cold storage at ~7.5°C with a high relative humidity (RH) (>90%) can significantly improve the shelf life of spinach (Conte et al., 2008). Grozeff et al. (2010) suggested that a single treatment with 1.0 µL/L 1-MCP for 6 h can be used to extend the postharvest storage life of spinach, and to maintain a better nutritional value (e.g., higher vitamin and protein contents) and appearance (greenness).

Kandasamy et al. studied the effect of root drench preharvest treatment of spinach with *Ascopyllum nodosum* extract (ANE) on postharvest storage and quality. Treatment of spinach with ANE improved the quality of fresh-cut spinach. The treatment significantly reduced the loss of fresh weight and improved visual quality and turgor over the period of storage. ANE-treatment reduced lipid peroxidation in cut spinach leaves during storage suggesting the ANE-reduced oxidative stress in spinach. The results suggest the potential of ANE to improve shelf life of spinach. Huang et al. (2012) proposed a new sanitizing method consisting of an application of aerosolized lactic acid (LA) plus allyl isothiocyanate (AIT) on fresh baby spinach to control *E. coli* O157:H7 during refrigeration storage. Their results highlighted a potential application of aerosolized LA + AIT as a novel processing technology for leafy greens with minimal impact on their appearance. Washing with 3% H₂O₂ resulted in an initial reduction of about 1.6 log CFU/g of *E. coli* O157:H7 on spinach. AIT showed a high antibacterial efficacy against *E. coli* O157:H7 and the addition of LA in AIT enhanced the antimicrobial efficacy of AIT during refrigeration storage.

19 CONCLUSIONS

Postharvest achievements have occurred in high-speed nondestructive segregation systems; novel packaging; storage and transport systems; pests and disease control for market access; senescence control; supply chain optimization; and track and trace systems to ensure delivery of premium quality products to discriminating markets. Future success will only occur with further R&D devoted to understanding the genetic and molecular basis of quality traits including stress resistance; resistance to postharvest diseases and pests; integrating available technologies (bio-, info-, and nanotechnology) through a systems biology approach to overcome postharvest food quality and safety issues, as well as to enhance specific health conferring components in fresh and processed products; use of robotics for harvesting, packing, and handling of individual through bulk items; managing logistics and supply chains effectively and efficiently; using bioregulators and/or biostimulants to manage productivity and quality; and to understand and hence manipulate the underlying metabolic systems controlling physiological and biochemical systems regulating product deterioration and senescence.

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CHAPTER 2

Fruit Maturity, Harvesting, and Quality Standards

K. Prasad*, Sanu Jacob**, Mohammed Wasim Siddiqui†

*Indian Agricultural Research Institute, New Delhi, Delhi, India

**Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, Kerala, India

†Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

1 INTRODUCTION

It is estimated that a total of more than 500 million metric tons of fresh fruits and nuts are produced annually in the world, representing a large, highly important, and growing industry (FAO, 2015). India produces 88.8 million metric tons of fruits, and 168.3 million metric tons of vegetables, fruits, and vegetables occupies 90% of total horticulture production (NHB, 2015), up to 15% of which is lost at postharvest stage (ICAR-CIPHET, 2015). Judging the maturity, knowing the internal characteristics and proper method and stage of harvesting the horticultural commodities not only retain the internal quality of these commodities, but also lead to reduced postharvest losses. Deviation of postharvest quality of fruit and vegetables due to internal and external factors lead to rejection of export commodities (APEDA, 2015). This chapter puts light on the importance of fruit, vegetables, and horticultural commodities in the human diet and the ways to retain the postharvest quality of these commodities through harvesting and postharvest handling techniques.

Fruits and vegetables occupy a significant proportion and position in the human diet. The word “fruit” originates from the Latin word *fructus*, which means “enjoyment of produce or harvest.” Generally, a fruit is a seed-bearing structure found in angiosperms formed from the ovary after flower fertilization. The word “fruit” can be synonymously used to botanically describe a vegetable. The origin of the word “vegetable” comes from the Latin word *vegetabilis*, which means “growing or flourishing.” In common language, fruit and vegetable normally means the fleshy seed-associated structures of a plant that is either sweet, sour, or bland, and edible in the raw and processed forms. They can be obtained from various parts of a plant, such as fruits (avocado, cucumber, eggplant, pumpkin, etc.), seeds (beans, peas,

etc.), flowers (broccoli, cauliflower, artichoke, etc.), leaves (cabbage, lettuce, spinach, etc.), stems and shoots (asparagus, leek, chive, etc.), tubers (potato, ginger root, etc.), bulbs (garlic, onion, etc.), and roots (carrot, turnip, radish, etc.) (Wills et al., 2007; Yahiya, 2011).

Fruits and vegetables are considered health capsules as they are known to possess rich sources of carbohydrates, minerals, vitamins, dietary fibers, and antioxidants. Carbohydrates (banana, dates, jackfruit, etc.), proteins (nuts, fig, dried apricot, etc.), and fats (avocado, egg fruit, dried nuts, etc.) are said to be the main energy sources, while minerals like potassium (banana, apricot, guava, avocado, etc.), phosphorus (avocado, jackfruit, kiwi, litchi, etc.), calcium (fig, lemon, olives, orange, etc.), iron (avocado, fig, amaranth, tamarind, etc.), magnesium (all green-color fruits and vegetables), and other trace elements are also found in the majority of fruits and vegetables. Most of the fruits are predominant in minerals like potassium and phosphorus while vegetables are considered rich in calcium and phosphorus. Major vitamins found in fruits and vegetables include vitamin A (mango, papaya, carrot, sweet potato, etc.), vitamin C (citrus fruits, kiwifruit, cabbage, broccoli, etc.), folic acid (spinach, broccoli, cabbage, etc.), niacin (pineapple, peach, peas, etc.), riboflavin (banana, dates, pumpkin, etc.), and thiamine (avocado, breadfruit, asparagus, etc.). The dietary fibers found in fruits and vegetables have several direct and indirect advantages. They consist of cellulose, hemicellulose, and pectin substance, which play an important role in relieving constipation, incidence of cardiovascular diseases and colon cancer. Fruits and vegetables have been strongly associated with reduced risk for some forms of cancer, heart disease, stroke, and other chronic diseases due to inherent antioxidant properties. Antioxidant functions by neutralizing small, cell-damaging molecules known as free radicals that are produced by the body as waste products. Antioxidants have oxygen radical absorbance capacity (ORAC) to neutralize these free radicals obtained from body metabolism (Chadha, 2001; Khader, 2013; Wills et al., 2007).

Some of the common antioxidants found in different fruits and vegetables are given in Table 2.1.

Quality attributes of fresh fruits and vegetables include appearance, texture, flavor, nutritional, and safety factors. Appearance factors include size, shape, color, and freedom from defects and decay. Texture includes firmness, crispiness, and juiciness. The flavor component incorporates sweetness, sourness, astringency, bitterness, aroma, and off-flavor. Another important attribute that influences the quality of fruits and vegetables is their safety aspect, which includes freedom from pesticide residues, mycotoxins produced

Table 2.1 Antioxidants with respective colors in fruits and vegetables

Antioxidants	Colors	Examples
Anthocyanins	Red	Berries, cherries, strawberries, cranberries, red apples, beets, red cabbage, kidney beans
Lycopene	Red	Tomato, watermelon, pink grape fruit
β -Carotene	Yellow-orange	Carrot, mango, papaya
Bioflavonoids	Yellow-orange	Citrus
Allicin	White	Garlic, chives, leeks, onions
Indoles and sulfaforaphanes	White	Cauliflower
Indoles	Green	Broccoli, cabbage, Brussels sprouts
Lutein and zeaxanthin	Green	Kale, peas, spinach, kiwi
Anthocyanins and phenolics	Blue-purple	Berries, dried plums, raisins, eggplant, plums, purple grapes, black currants

by certain fungi, presence of heavy metals, and microbial contamination (Doerflinger et al., 2015; Kader, 2002; Saraswati et al., 2012).

Although some preharvest factors, like growing horticulture crop varieties in accordance to the region, do affect the yield and quality of harvested crop (Prasad et al., 2016a) or selection of variety resistant to particular postharvest problem, may combat the incidence of postharvest quality loss in some cases (Prasad and Sharma, 2016). But postharvest factors are critical and decisive compared to preharvest factors for ultimately retaining the postharvest quality when it reaches the consumer. This postharvest quality retainment is not possible without the proper harvesting and handling practices. The role of maturity judgment at harvest and postharvest handling of any fruits and vegetables can be decisive in determining their quality. A good quality in a fruit or a vegetable can be expected when its harvesting is done at the right stage of maturity. Maturity is a stage where full development of a plant organ (fruit or vegetable) occurs, culminating its growth while on tree and/or facilitating the act of reproduction in it (Kader, 2002; Saraswati et al., 2012; Verma and Joshi, 2000).

2 DETERMINATION OF MATURITY INDICES

Maturity at harvest is a key factor that determines the storage life and final fruit quality (Fig. 2.1). Immature fruits when harvested will give a poor-quality and uneven ripening. On the other hand, delayed harvesting of fruits

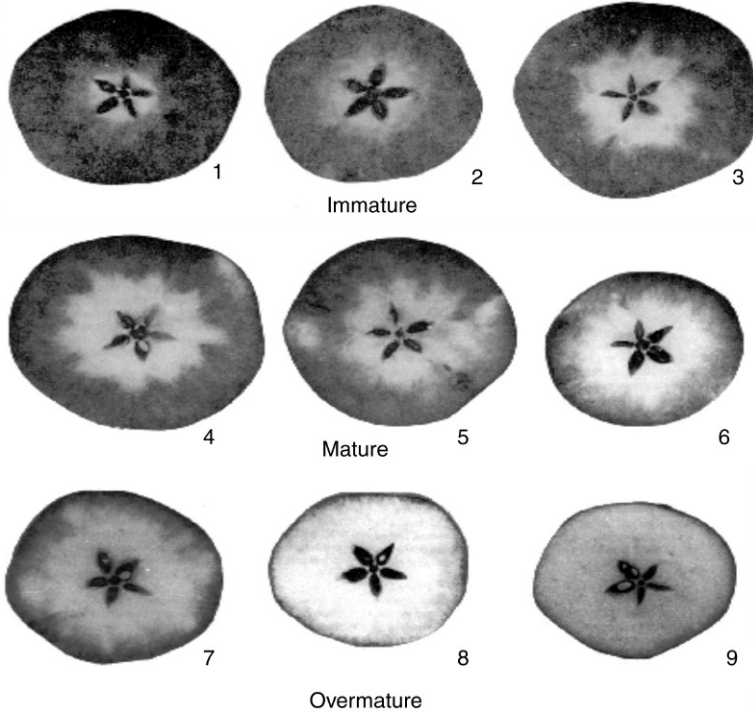


Figure 2.1 Maturity on harvest. (From <http://www.omafra.gov.on.ca/english/crops/facts/00-025.htm>).

and vegetables can increase higher susceptibility to decay, resulting in higher postharvest loss. Therefore, the growers have to be careful while picking mature fruit at the time of harvest. Any deviation in picking optimum mature fruit could prove detrimental to fruit quality during its shelf life. Picking over mature fruit can induce early softening with not much resistance to mechanical handling or damage, which can shorten the shelf life of fruit (Doerflinger et al., 2015; Rathore et al., 2012). However, such fruit will have an excellent flavor and juicy texture. Fruits harvested at immature stage are often incapable of ripening, retaining a hard texture, low flavors, and are susceptible to internal breakdown and wooliness under prolonged cold storage conditions (Doerflinger et al., 2015; Herremans et al., 2014; Saraswati et al., 2012).

There are certain guiding principles that have to be followed while harvesting a fruit or vegetable:

1. Harvesting should be done at a stage during the peak growth conditions of fruits or vegetables.

2. Harvesting should coincide with the fruit's acceptable flavor or appearance.
3. Harvesting should be made at a fruit size required by the market.
4. Harvesting should ensure least mechanical damage thereby enhancing adequate shelf life of fruit.

Maturity indices are important for deciding when a fruit or vegetable should be harvested. This helps in creating marketing flexibility as well as providing acceptable eating quality to the consumers (Doerflinger et al., 2015; Thompson, 1996). On the basis of maturity, fruits and vegetable can be categorized into:

1. *Physiological maturity*: is a stage of maturity where a fruit or vegetable reaches a sufficient stage of development while attached to a tree or plant. Most of the horticultural produces are harvested at physiological maturation state.
2. *Horticultural maturity*: is a stage of maturity where a fruit or vegetable possesses the necessary characteristics as desired by the consumers. For example, sprouts, tender beans, okra, salad crops, are harvested during their early phase, while seeds or nut crops are harvested in their late phase of crop growth as preferred by the consumers.

Most of the horticultural food products follow a pattern starting from bud initiation stage to development stage and live until their death. The development stage, which is longer in duration, comprises growth, maturation (physiological maturity), ripening, and senescence (Doerflinger et al., 2015; Thompson, 1996) (Fig. 2.2).

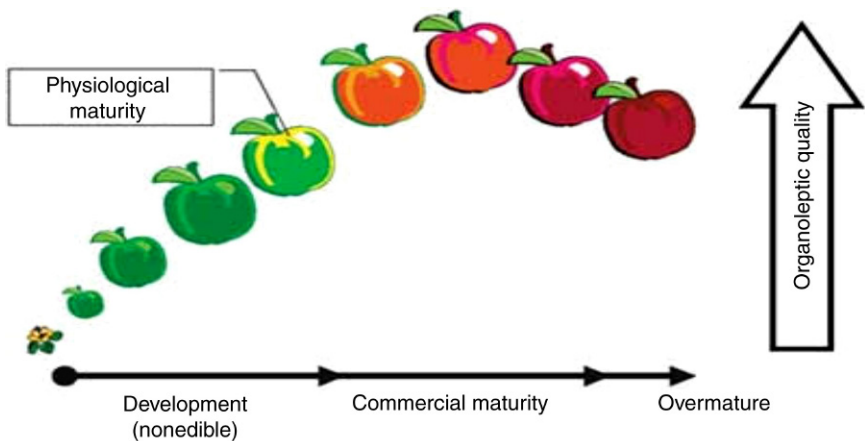


Figure 2.2 *Development stages of fruit.* (From <http://www.fao.org/docrep/008/y4893e/y4893e04.htm>).

Table 2.2 Maturity indices for selected fruits and vegetables

Maturity indices	Fruits/vegetables
Nondestructive methods	
1. Calendar date	All fruits
2. DFFM	All fruits and radish
3. Mean heat units	Apple, pear, grape, mango, litchi, and many vegetables
4. T-stage	Apple
5. Size	All fruits, beans, carrot, cucumber, cherry, asparagus, and cauliflower
6. Surface morphology	Grape (cuticle formation), banana, and litchi
7. Specific gravity	Cherries and mango
8. Fruit retention strength	Apple
9. Color	
(Surface)	All fruits, tomato, and muskmelon
(Seed)	Apple and pears
(Flesh)	Mango, papaya, watermelon, and muskmelon
Destructive methods	
10. TSS	All fruits, tomatoes, and melons
11. Firmness	Pome and stone fruits, beans, lettuce, and muskmelon
12. Juice content	Citrus fruits
13. Acidity and sugar/acid ratio	Pomegranate, citrus fruits, papaya, and kiwifruit
14. TSS/acid ratio	Grape and citrus fruits
15. Sugars	Pome, stone fruits, and grape
16. Astringency (tannin)	Persimmon and dates
17. Oil content	Avocado
18. Respiration and ethylene evolution rates	Apple and pears

DFFM, Day from full bloom; TSS, total soluble solids.

There are different methods of determining maturity indices in fruits and vegetables (Table 2.2). Most of the indices vary according to the fruit type (Thompson, 1996). The common methods of determining the maturity of a fruit are:

1. *Visual method*: this method largely depends on the outward appearance of the fruit, like skin color, size (mass and volume), presence of dry mature leaves, fullness of fruit, drying of plant body.
2. *Physical method*: firmness, specific gravity, ease of separation of abscission, etc.
3. *Chemical method*: total soluble solids (TSS), titratable acidity (TA), TSS:TA ratio, starch content, and so on.

4. *Computation method*: days from flower bloom or bud initiation stage until optimum fruit development.
5. *Physiological method*: respiration, aroma development, and so on.

The aforementioned maturity indices are also a measure of fruit quality, which can be broadly grouped into destructive and nondestructive methods.

2.1 Destructive Methods

These are methods used to determine the maturity of fruits and vegetables by destructive analysis. These include testing their TA, sugar content, juice content, starch content (Kader, 2002; Thompson, 1996; Tijskens et al., 2007).

2.1.1 Sugar

In climacteric fruit, carbohydrates are accumulated during maturation in the form of starch. As the fruit ripens starch is broken down to sugars. In nonclimacteric fruits sugars tend to be accumulated during maturation. The contents of sugar can be described as a reliable index of maturity of fruits. Usually the soluble solids, which are in the largest quantity in fruit juice, are sugars, so measuring the soluble material in fruit juice can usually give a reliable measure of its sugar content. This is done either with a suitable Brix hydrometer or in a refractometer, which provide quick and easy methods for ripeness assessment. In a German study conducted in the Bodensee area it has been shown that harvest index based on sugar content in conjunction with fruit hardness and starch color reaction of a given apple variety can be used to determine the optimal time of harvest because it was not affected by soil type, fertigation, or climate.

2.1.2 Acidity

The acidity of many types of fruit changes during maturation and ripening. In citrus and many other fruits, acidity is reduced progressively as the fruit matures on the tree. Taking samples of these fruit, extracting the juice and titrating it against a standard alkaline solution gives a measure that can be related to optimum time of harvest. It is important to measure acidity by titration and not the pH of the fruit because of the considerable buffering capacity in fruit juices. Normally, acidity is not taken as a measurement of fruit maturity by itself. It is usually related to soluble solids, giving what is termed the Brix:acid ratio.

2.1.3 Starch Content

Starch is a polysaccharide present in plants, fruits, vegetables, and so on. During growth, sugar formed in the leaves is transferred to the fruit where

it is stored in the form of starch. As the fruit matures the starch degrades to simple sugars. So measurement of starch content either by colorimeter or its reaction with iodine solution may help to determine the optimum date of picking. In a starch-iodine test, reaction of iodine with starch on the cut surface of fruit gives a dark bluish black color and is used as an indication of starch content, thus maturity. By allocating the values at each distinct stage of starch disappearance provide proper quantification of the starch content of fruits and vegetables. This creates a starch index chart. Most cultivars have a characteristic pattern of starch disappearance so these charts are often cultivar specific, hence the starch-iodine value at any optimum harvest date can vary between cultivars.

2.1.4 Oil Content

Oil content increases with the onset of maturity, which can be used as a reliable index for the determination of maturity of fruits with high fat content like avocado, which should not be less than 8% by weight excluding the skin and seed.

2.2 Nondestructive Methods

These are methods used with nondestructive analysis like skin color, optical methods, firmness, size, aroma, leaf changes (Kader, 2002; Rathore et al., 2012; Thompson, 1996).

2.2.1 Skin Color

This factor is commonly used in fruits and vegetables, where skin color changes as the fruit ripens or matures. But in some fruits and vegetables, perceptible color changes may not occur during maturation. Color changes may occur on particular cultivars but not on others. In some tree fruit, skin color may be partly dependent on the position of fruit on the tree or the weather conditions during production, which may confound its use as a maturity measurement. Assessment of harvest maturity by skin color changes usually depends on the judgment of the harvester, but color charts are used for some cultivars of apple, tomato, peach, chilli, pepper, and so on.

2.2.2 Optical Methods

Fruit maturity can be measured using light transmission properties. When the fruit or vegetable ripen, the internal optical density decreases due to the conversion of bound water into free water in tissue that changes light-scattering properties. So the technique of light transmittance, which was

initially developed for tomatoes, is now being used for intact pome fruits after incorporating certain modifications, and considered as a better indicator of maturity.

2.2.3 Firmness

During maturation fruit may change in texture, especially during ripening, and they may rapidly become softer. Excessive moisture loss may also affect the texture of crops. By touching the fruit or vegetable these textural changes can be detected easily and the harvester may be able to squeeze the fruit and judge whether to harvest it. Mechanical devices, such as penetrometer, tenderometer, pressure testers, are also used to determine fruit firmness.

2.2.4 Size

Fruit size and weight have been found to be poor measures of fruit maturity. The changes in crop size as it is growing are frequently used to determine when it should be harvested. In the case of fruits this may be related to the requirements of market and the fruit may not be physiologically mature at this stage. However, in some vegetable crops fruit/root size can be used as a reliable index of maturity.

2.2.5 Aroma

As the fruits or vegetables ripen they synthesize volatile chemicals. These may give the fruit its characteristic odor and can be used as a maturity index. These odors may only be detectable to human senses when a fruit is completely ripe and therefore have limited use in commercial situations.

2.2.6 Leaf Changes

This characteristic is used in both fruits and vegetables to determine when they should be harvested. In root crops, like carrots, radishes, the condition of the leaves can indicate the condition of the crop below ground. In case of potatoes, optimum harvest time is after the death of leaves and stems. In certain cases where the leaves do not senesce naturally they may be cut off or removed chemically to produce tubers with firmer and stronger skins when they are harvested.

2.3 Measuring Maturity: Destructive Versus Nondestructive Methods

Most conventional techniques to assess maturity in fruits and vegetables employ destructive methods, wherein few fruits are randomly selected before

harvest. These fruits are subjected to destructive analysis to measure their physical, chemical, and mechanical properties, which are later corroborated with the whole batch of fruit. Such measurements are not only cumbersome and slow, but also not representative when considering the wide variation of fruits within a harvesting batch. For example, firmness of a fruit is commonly measured by means of a penetrometer. The penetrometer test stimulates the mastication of fruit tissue inside the mouth, and also incorporates several mechanical properties, like elastic, shear, and rupture properties of the fruit tissue (Nicolai et al., 2006). Being destructive, it cannot be applied to all fruit but only to a small sample of them. If the sample has not been selected randomly then it will not be representative of the batch. As a consequence the results generated from such tests cannot be representative of the whole sampling batch firmness-based grading. On the other hand, nondestructive methods can be applied on a whole batch before putting it on the market. This method is advantageous as they can be repeatedly used for quick assessment of horticultural produce on individual basis. The gathered information from such measurements can be extensively used to study the variability of fruits in a batch (Ashtiani et al., 2016; Tijssens et al., 2007). A nondestructive measurement that could be used as a reliable maturity index would greatly improve the management of fruit supply chain. In recent years, nondestructive methods, like optical, X-ray, ultrasonic, near infrared (NIR), magnetic resonance imaging (MRI) (Herremans et al., 2014; Jarolmasjed et al., 2016; Vangdal et al., 2010), in combination with information technology, have gained momentum for online grading and screening of quality profiles in fruits and vegetables at quick succession (Herremans et al., 2014; Jarolmasjed et al., 2016).

3 HARVESTING METHODS

Harvesting is an important operation in horticultural crop production and any insufficiency during this time may lead to the loss of a whole year's work. Harvesting with improper methods results in the damage of crops by bruising, which can be caused by compression (due to overfilling of boxes or in bulky stores), impact (due to dropping of crop or from something hitting the crop), or vibration (due to loose packing during transportation). So during harvesting, factors like the delicacy of crop, maturity criteria, time, method of harvesting, mode of packaging and transportation, economy of the operations, and the need for harvesting method to fulfill the market requirement, should be taken into consideration (Rathore et al., 2012). The common harvesting methods for fruits and vegetables are given in Fig. 2.3A–B.

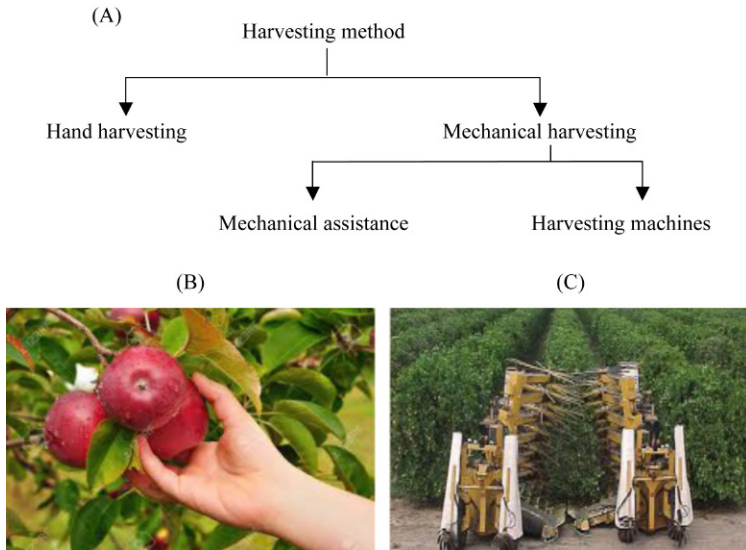


Figure 2.3 (A) Different harvesting methods, (B) hand harvesting, and (C) machine harvesting. (From <http://www.123rf.com/stock-photo/harvesting.html>).

In addition to harvesting methods, the time of harvest plays a crucial role in maintaining the best quality of crop during the course of postharvest handling and storage.

3.1 Hand Harvesting

Harvesting operations are frequently carried out in traditional ways by hand. This method is less expensive, low damage to the commodity, and the harvest rate can be increased, but harvesting of small size fruits or from thorny plants are the major obstacles. This method is more time consuming.

3.2 Mechanical Harvesting

To speed up the harvesting and field-handling operations, machines and chemical sprays are used. Mechanical harvesting is of two types.

Mechanical assistance: In this method, mechanical assistance is used by hand pickers on ladders and worker positioners (tree towers and platforms), and self-propelled carts (to reduce nonharvest time by assisting with materials handling and movement of the workers and to increase productivity during harvesting time by providing optimum working conditions and by making detachment of the fruits easier).

Harvesting machines: Mechanical harvesting devices are used to employ direct contact methods, such as combing, cutting, pulling, and snapping, twisting, stripping, and compacting.

4 POSTHARVEST HANDLING OPERATIONS

Being living organs, fruits and vegetables continue to respire even after harvesting when they have a limited source of food reserves. In addition to degradation of respiratory substrates, a number of changes in taste, color, flavor, texture, and appearance take place in the harvested commodities, which makes them unacceptable for consumption by consumers if these are not handled properly. The improper temperatures may lead to the disturbance in the normal metabolism of the harvested organs. The higher temperatures may increase manifolds in the rate of metabolic activities, thereby reducing the shelf life, while much lower temperatures may lead to the freezing or chilling injury in the harvested commodities. Mechanically damaged fruits and vegetables during harvesting are very much prone to fungal decay during the course of storage. Already infected fruits or vegetables may spread the disease to the adjacent stored commodity if not sorted out prior to the storage (Rathore et al., 2012; Thompson, 1996). Postharvest treatments retain the external quality of fruits and ensure the better price and returns to the farmers (Prasad et al., 2016b; Thompson, 1996).

It is well established that the quality of the harvested commodities cannot be improved further but it can be retained until consumption if the rate of metabolic activities are reduced by adopting the appropriate postharvest treatments (Prasad et al., 2016b) and handling operations as are given in Fig. 2.4A–B.

4.1 Precooling

Precooling (prompt cooling after harvest) is important for most of the fruits and vegetables because they may deteriorate in as much as 1 h. In addition to removal of field heat from commodities, precooling also reduces bruise damage from vibration during transit. Cooling requirement for a crop varies with the air temperature during harvesting, stage of maturity, and nature of crop.

The rate of precooling depends on the following:

- The difference in temperature between the crop and cooling medium.
- Accessibility of the cooling medium to the crop.
- The nature of the cooling medium.
- The velocity of the cooling medium.
- Rate of transfer of heat from the crop to the cooling medium.

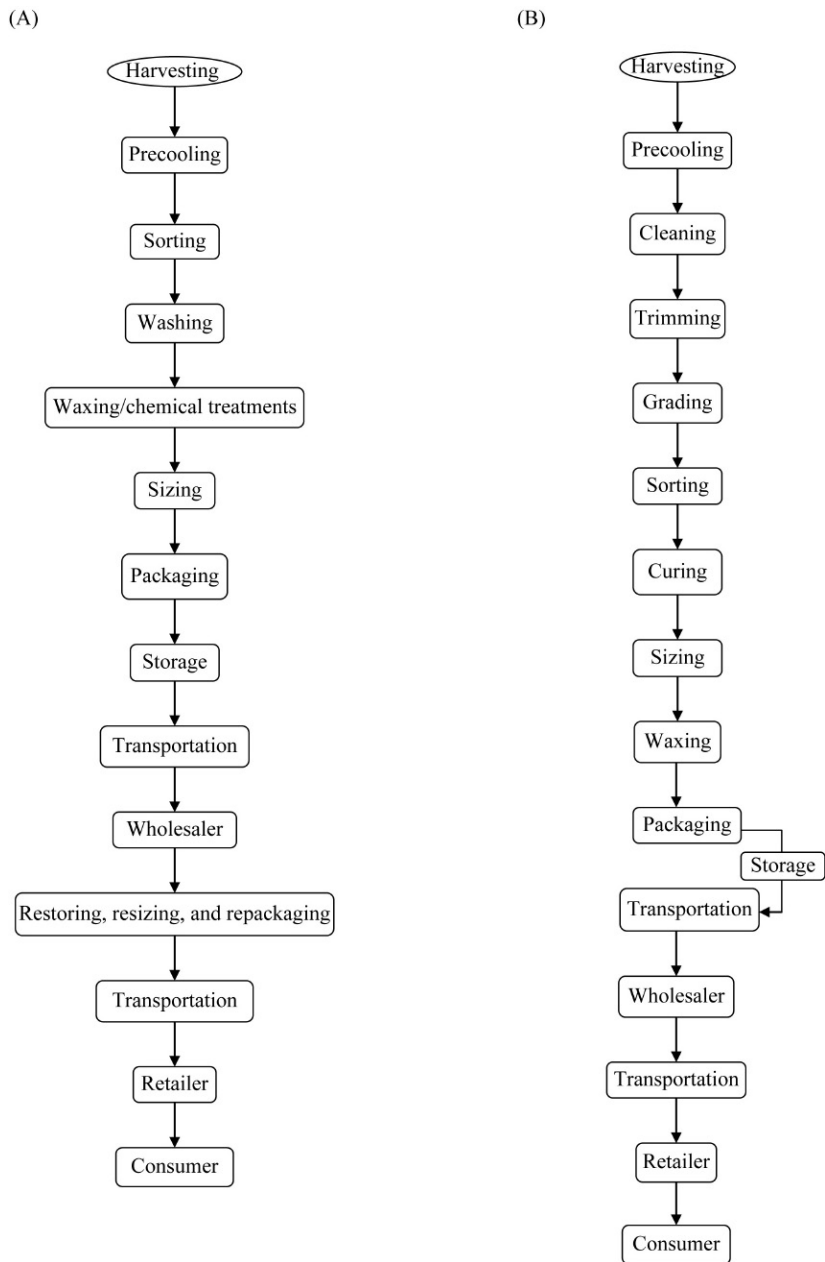


Figure 2.4 Postharvest handling operations of (A) fruits and (B) vegetables.

Table 2.3 List of commodity-wise cooling methods

Cooling method	Commodity
Room cooling	All fruits and vegetables
Forced air cooling (pressure cooling)	Fruits and fruit-type vegetables, tubers, and cauliflower
Hydrocooling	Stem, leafy vegetables, some fruits, and fruit-type vegetables
Package icing	Roots, stem, some flower-type vegetables, green onions, and Brussels sprouts
Vacuum cooling	Some stem, leafy, and flower-type vegetables
Transit cooling	
Mechanical refrigeration	All fruits and vegetables
Top icing and channel icing	Some roots, stems, leafy vegetables, and cantaloupes

There are many methods of precooling, namely, cold air (room cooling, forced air cooling), cold water (hydrocooling), direct contact with ice (contact icing), evaporation of water from the produce (evaporative cooling, vacuum cooling), and the combination of vacuum and hydrocooling (hydrovac cooling). Some chemicals (nutrients/growth regulators/fungicides) can also be mixed with water used in hydrocooling to prolong the shelf life by improving the nutrient status of the crop and preventing the spread of postharvest diseases. The different methods of cooling are given commodity-wise in [Table 2.3](#).

4.2 Washing, Cleaning, and Trimming

Before fresh fruits and vegetables are marketed, various amounts of cleaning are necessary, which typically involves the removal of soil dust, adhering debris, insects, and spray residues. Chlorine in fresh water is often used as disinfectant to wash the commodity. Some fungicides like diphenylamine (0.1%–0.25%) or ethoxyquin (0.2%–0.5%) may be used as postharvest dip to control an important disorder of apples known as superficial scald. For cleaning of some fruit-type vegetables (melons, brinjal, tomatoes, cucumber) they should be wiped with damp cloth. Many vegetables need trimming, cutting, and removal of unsightly leaves or other vegetative parts.

4.3 Sorting, Grading, and Sizing

Sorting is done by hand to remove the fruits that are unsuitable for market or storage due to damage by insects, diseases, or mechanical injuries. The remainder crop product is separated into two or more grades on the basis of the surface color, shape, or visible defects. For example, in an apple-packing

house in India three grades, namely, extra fancy, fancy, and standard, may be packed for marketing. The fourth “cull” grade is meant for processing. After sorting and grading, sizing is done either by hand or machine. The machine sizes work on two basic principles: weight and diameter. Sizing on the basis of fruit shape and size is most effective for spherical (oranges, tomato, certain apple cultivars), and elongated (delicious apples and European pears or of nonuniform shape) commodities, respectively. Today, a number of private India companies are exclusively engaged to manufacture the most sophisticated grading–packing line machines, which also have the facility of washing, waxing, and drying in addition to sizing.

4.4 Curing

Curing is an effective operation that reduces the water loss during storage from hardy vegetables, namely, onion, garlic, sweet potato, and other tropical root vegetables. The curing methods employed for root crops are entirely different than that of the bulbous crops (onion and garlic). The curing of root and tuber crops develops periderms over cut, broken, or skinned surfaces wound restoration. It helps in the healing of harvest injuries, reduces loss of water, and prevents the infection by decay pathogens. The optimum conditions for curing root, tuber, and bulb vegetables are given in [Table 2.4](#).

4.5 Irradiation

Ionizing radiation can be applied to fresh fruits and vegetables to control microorganisms and inhibit or prevent cell reproduction and some chemical changes. It can be applied by exposing the crop to radioisotopes (normally in the form of gamma rays but X-rays can also be used) and from machines that produce a high-energy electron beam. Radiation doses are measured in Grays (Gy).

Table 2.4 The optimum conditions for curing of some vegetables

Commodity	Temp. (°C)	RH	Curing time (days)
Irish potato	13–17	>85	7–15
Sweet potato	27–33	>90	5–7
Yam	32–40	>90	1–4
Taro	30–35	>95	4–7
Cassava	30–35	>80	4–7
Onion and garlic	35–45	60–75	0.5–1

4.5.1 Uses of Irradiation

Irradiation can be used to suppress sprouting in potatoes. Sparrow and Christensen showed that irradiated tubers did not sprout during 15 months storage at 4.4°C.

A dosage rate of 100 Gy was found to be adequate, but lower rates of about 35 Gy can be used to delay sprouting without the side effects, which were observed on tubers exposed to higher doses as reported by Burton in the year 1957. A combination of hot water treatment (55°C for 5 min) followed by 30 Gy irradiation was found to be the best treatment in terms of shelf-life extension and the quality of mangoes as reported by Upadhyay in the year 1993. After this treatment mangoes had a storage life of 38 days (at 15°C), 28% rotting and no irradiation injury. Irradiation can also be used to control postharvest diseases of fresh fruit and vegetables.

Irradiation can be used as a postharvest treatment to disinfect fruit insects; 300 Gy was shown to control mango seed weevil but more work is needed; 150 Gy was shown to control 11 species of Tephritid fruit fly and 75 Gy prevents the adults emerging from the fruit as reported by Heather in the year 1993. Doses of irradiation in excess of 600 Gy caused lenticel spotting, surface discoloration and retardation of ripening of Kensington Pride mangoes, but irradiation at this level contributed only minor improvements in disease control. However, irradiation followed immediately by hot benomyl treatment—controlled anthracnose and stem-end rot during storage at 20°C for 15 days. Maxie in the year 1971 reported results on disease control and other effects of irradiation in different fruits and vegetables are given in [Table 2.5](#).

4.6 Hot Water Treatment

Crops may be immersed in hot water before storage or marketing to control diseases. A common disease of fruit, which can be successfully controlled in this way, is anthracnose, caused by infections of the fungus *Colletotrichum* spp. Postharvest treatment with fungicide is generally reported to be ineffective in controlling the disease, but immersing them in hot water, preferably containing an appropriate fungicide, can give good control. Crucifix in the year 1989 recommended 53°C for 5 min and Carlos in the year 1990, 51–55°C for 30 min. In some cases it has been shown that there is an interaction between water temperature and fungicide, whereby at lower temperatures there a greater need to add fungicide as is reported by Muirhead in the year 1976.

Good control of both soft brown rot caused by *Hendersonia creberrima* and anthracnose rot caused by *Colletotrichum gloeosporioides* in Kent mangoes

Table 2.5 Effects of postharvest irradiation on some fruits and vegetables

Crop	Minimum dose required (Gy)	Maximum dose tolerated (Gy)
For postharvest disease control		
Apple	150	100–150
Apricot, peach, nectarine	200	50–100
Avocado	—	15
Lemon	150–200	25
Orange	200	200
Strawberry	200	200
Grapes	—	25–50
Tomato	300	100–150
Inhibition of growth, sprouting, or ripening		
Asparagus	5–10	15
Banana	30–35	50
Mushroom	200	100
Pear	250	100
Potato	8–15	20

was achieved by dipping them in water at 40°C for 5 min followed by a 30-s dip in 3000 ppm flusilazole plus 1000 ppm prochloraz.

Skin damage of mango fruits was reported to occur in some cases after hot water treatment. Cooke and Johnson in the year 1993, showed that this injury only occurred after rain and was caused by fruit being brushed and polished. There was no damage to nonbrushed fruit. If the fruit that have been harvested after rain are not treated until 48 h after harvest then this eliminates the damage caused by brushing, but may have implications in disease control.

Using hot water treatment for disinfestations can cause injury to the fruit, such as increased weight loss and the treatment may accelerate color development as reported by Jacobi in the year 1993. They observed little fruit injury in mangoes exposed to 46°C for 75 min and in a comparison between hot water treatment, vapor heat treatment, and untreated fruit the time to fruit softening was 3.6, 3.3, and 4.1 days, respectively.

Hot water treatment has been applied to sweet potatoes. Scriven in the year 1988 showed that there was a significant delay in disease development on roots that had been exposed to 90°C for 2 s, 80°C for 2, 4, or 10 s, 70°C for 10 s, and 40°C for 2 min. A 3-min immersion of blueberries in water at 46–55°C reduced subsequent decay by as much as 90%; 40°C was ineffective and 55°C injured the fruit as reported by Burton in the year 1974.

4.7 Waxing

Quality retention is a major consideration in modern fresh-fruit marketing systems. Predominant methods used to preserve fresh-fruit quality during handling and subsequent marketing include controlled atmosphere (CA) or modified atmosphere technique in conjunctions with refrigerated storage. Similar results can be achieved by the applications of waxes on the surfaces of fruits. Waxes are esters of higher fatty acid with monohydric alcohols and hydrocarbons and some free fatty acids. But coating applied to the surface of fruit are commonly used waxes whether any component is actually a wax. Waxing generally reduces the respirations and transpiration rates, but other chemicals, such as fungicides, growth regulators, preservatives, can also be incorporated especially for reducing microbial spoilage, sprout inhibition. However, it should be remembered that waxing does not improve the quality of any inferior horticulture product but it can be a beneficial adjunct to good handling (Kumar and Kapur, 2016; Raghav et al., 2016).

The principal advantages of wax applications are:

- Improved appearance of fruit.
- Reduced moisture losses and retards wilting and shriveling during storage of fruits.
- Less spoilage especially due to chilling injury and browning.
- Creates diffusion barrier as a result of which it reduces the availability of O_2 to the tissues thereby reducing respiration rate.
- Protects fruits from microbiological infections.
- Considered a cost-effective substitute in the reduction of spoilage when refrigerated storage is unaffordable.
- Wax coating are used as carriers for sprout inhibitors, growth regulators, and preservatives.

The majority of quality contributing factors as affected by wax application include reduction in the physiological loss and weight (PLW), delay in respiration rate, reduction in postharvest spoilage, and maintenance of improved quality of commodity intended for storage to increase the shelf life.

The principal disadvantage of wax coating is the development of off-flavor if not applied properly. Adverse flavor changes have been attributed to inhibition of O_2 and CO_2 exchange, thus resulting in anaerobic respiration and elevated ethanol and acetaldehyde contents.

Types of waxes:

1. *Solvent waxes*: These waxes widely used in citrus are composed of 70%–80% aliphatic hydrocarbon and up to 25% aromatic hydrocarbons and

solvents, such as acetone, ethyl acetate. The solvent will contain either a synthetic resin or a natural wood resin plus one or more plasticizers.

2. *Water waxes*: These are a second major type. The most extensively used being resin solution waxes and emulsion waxes. Resin solution waxes are simply solutions of one or more alkali-soluble resin or resin-like materials, such as shellac, natural gums, or wood resin. Emulsion waxes are composed of a natural wax, such as carnauba or paraffin or synthetic wax, such as polyethylene emulsion.
3. *Paste or oil waxes*: These are mainly composed of paraffins that are different in melting point and blended to give a desired viscosity. These waxes are often used on vegetables.

Categories of wax according to use:

1. Storage wax: When fruit is not to be marketed immediately.
2. Pack-out wax: When fruits are to be marketed immediately.
3. High-shine wax: For giving a very high grace on market demand.

List of waxes used commercially:

1. Paraffin wax
2. Carnauba wax
3. Bee wax
4. Microcrystalline waxes (complexes of 5-hydrocarbon having branched chain)
5. Shellac
6. Wood resins
7. Polyethylene (oxidized polyethylene wax or hydrocarbon wax)

4.8 Packaging and Storage

Fruits and vegetables retain their physiological functions, such as respiration and transpiration after harvesting and remain fresh only as long as its normal metabolism continues. So the packaging materials should be suitable for retaining these functions. Proper or scientific packaging of fresh fruits and vegetables reduces the wastage of commodities by protecting them from mechanical damage, pilferage, dirt, moisture loss, and other undesirable physiological changes and pathological deterioration during the course of storage, transportation, and subsequent marketing.

For providing uniform quality to packed produce, the commodity should be carefully supervised and sorted prior to packaging. Packaging cannot improve the quality, but it certainly helps in maintaining it as it protects produce against the hazards of journey. Striking developments have occurred in the field of packaging of horticultural produce, and the gunny

Table 2.6 New packaging materials

Packaging material	Specialty
CFB boxes	These are light in weight, easy to handle, hygienic, and recyclable. These can be turned water resistant by the use of a suitable adhesive or wax coating or a plastic film.
Combination boxes	These are made with plywood and CFB, and give a high stack-load capacity.
Corrugated polypropylene board boxes	These are light in weight, hygienic, water resistant, sturdy, and have a light bursting strength. They are useful in the multitrip packaging.
Plastic trays or crates	These are hygienic, light in weight, sturdy, and recyclable and used in the multitrip packaging.
Plastic woven sacks	These bags are made of high-density polyethylene or polypropylene, light in weight, and can be reused. They are used for packaging hard fruits to transport them over short distances.
Molded pulp trays or thermoformed plastic trays	These trays have the facility of cavities to hold individual apple fruit, which prevents the fruit from rubbing against each other that often leads to bruising or surface cracks.
Stretch wrapping	It is used for retail marketing of fresh produce in the form of cling plastic films for stretch wrapping.
Modified atmosphere packaging	In this packaging, the internal atmosphere can be manipulated with a combination of certain gases and selection of suitable packaging material.

CFB, Corrugated fiber board.

bags, grasses, and stem leaves used so far for packaging are now being replaced by a variety of containers, such as wooden boxes, baskets woven from bamboo or twigs, hessian sack/jute bags, plastic punnets, and corrugated fiber board (CFB) boxes. Due to problems of poor dimensional stability as well as stacking strength with baskets and sack bags and heaviness and adverse effects of deforestation on ecological balance by using wooden boxes, some alternative eco-friendly packaging materials have been designed to replace them (Table 2.6).

A number of storage techniques (ground storage, ambient storage, refrigerated storage, air-cooled storage, zero-energy storage, modified atmospheric storage, hypobaric storage, and controlled atmosphere storage) are being used for fruits and vegetables depending on the nature of the commodity and the storage period intended (Kader, 2002; Rathore et al., 2012; Thompson, 1996).

4.9 Labeling

The organization of the market for fresh fruits and vegetables includes labeling the products in accordance with the product-specific requirements. Labeling enables buyers to obtain adequate and correct information about the quality and size of their purchases. The country of origin (i.e., cultivation) of fresh fruits and vegetables must always be indicated. The requirements for labeling are a part of the European Commission's regulations on sales and quality requirements for shop-keeping. Fresh fruits and vegetables have product-specific requirements for labeling.

Labeling requirements include the sales and distribution chain from packaging to retail sale. When products are sold as loose goods, the required information must be visible on a sales sign. The requirements do not apply when a producer sells their produce directly to the consumer, for example, directly at the farm or by allowing berry picking on a farm.

Mandatory labels must be made clearly and indelibly on the same side of the package to ensure their visibility from the outside. Packaging may contain additional information as long as it is not misleading. Prepacked items for retail sale are considered packaged products if the packaging contains more than one item. In these cases the packaging must be properly labeled. For single-packed items in plastic the labeling on the box are adequate.

The country of origin is compulsory information and it must be indicated in the packaging even when the country of origin is the same as the country of the packager. The country of origin can be identified by its name or adjective, for example, Finland, Finnish.

In addition, some vegetables have labeling requirements concerning variety and size of produce. Variety must be indicated for oranges, apples, pears, and grapes. Consumer packages that are not weighed at the time of the purchase must include information about the contained amount, for example, the net weight (Kader, 2002; Rathore et al., 2012; Thompson, 1996).

5 QUALITY STANDARDS FOR PRODUCT ACCEPTANCE

Quality is one of the major positioning tools of the producer for marketability, profitability, and for consumer satisfaction. Quality stands for the rated ability of a product, fruit, or vegetable, or products, thereof, or a brand to perform functions. It is the combination of attributes or characteristics of a product that have significance in determining the degree of acceptability of the product to a user. Industry, on the other hand, defines quality as a measure of purity strength, flavor, size, maturity, workmanship, and conditions or

any other distinctive attribute or characteristic of the product. Thus, it is an overall measure of the product's behavior in the public or market or is a measure reflecting the product's standings on durability, reliability, precision, ease of operation, acceptability, performance with respect to valued attributes, and so on. Some of these attributes can be measured objectively and combined using a set of important weights into an index of quality. From a marketing point of view, however, quality is better measured in terms of the buyer's perceptions of quality who can rate a given set of varieties of fruits/vegetables or their products on a quality scale (APEDA, 2015; Jaeger et al., 2016).

5.1 Quality Standards

There are different standards employed to control the quality of fresh and processed fruits and vegetables. These are summarized in Table 2.7.

5.2 Methods for Determining Quality

The quality assurance manager performs a number of tests to ascertain the quality of fresh and processed products. The details are provided in Table 2.8.

Table 2.7 Different quality standards

Name of standard	Features
(a) Legal standards	<ul style="list-style-type: none"> • Mandatory standard established by federal state or municipal agencies • Set up by law or represented by appropriate act • Concerned with freedom from adulteration and proper quality control measures, that is, insects, molds, yeasts, pesticides, maximum limit of preservative, and food without contamination
(b) Company or voluntary label standards	<ul style="list-style-type: none"> • Established by food industry • Represents consumers' image and becomes trademark of symbol of product quality
(c) Industry standards	<ul style="list-style-type: none"> • Are used by private firms or supermarkets • Established by an organized group for any given commodity • Becomes effective by pressure from market organization or specific commodity group where legal standards are not involved
(d) Consumer or grade standards	<ul style="list-style-type: none"> • Represents consumer's requirement of a grade/standard product • Based on experience in use by the industry for consumers

Table 2.8 Types of methods for determining quality

Types of method	Quality attributes
(a) Physical method	<ul style="list-style-type: none"> • Size, texture, color, consistency, headspace, fill and drained weight, vacuum, container, symmetry, defects, viscosity
(b) Chemical method	<ul style="list-style-type: none"> • Enzyme, moisture, fiber, pH, acidity, protein, specific gravity, fat/oil, carbohydrate, ash, mineral, vitamins, sugars, tannins, alcohols
(c) Microbiological adulteration and contamination	<ul style="list-style-type: none"> • Mold, insect fragments, insect, excreta, foreign material • Differentiation between cell types/tissue/microorganisms • Determination of microbial count spoilage detection in the fresh and processed products, microorganisms causing spoilage/fermentation

Another type of quality determination method includes subjective method and objective method.

5.2.1 Subjective Versus Objective Methods

Subjective methods use human sense organs to evaluate the quality of fruits and vegetables. These are based on the opinion of investigators. The past training experience of an individual's power or perception is used for evaluating quality.

Objective methods are based on scientific tests. No human perception is involved in this. For example, an instrument is used to provide a special color value based on the amount of light reflected off the commodity surface or the light reflected through the commodity. The quality parameters that can be determined using these methods include appearance, color, shape and size, firmness, olfactory (smell), and absence from defects (Jaeger et al., 2016; Kader, 2002; Rathore et al., 2012).

5.2.1.1 Appearance

The visual appearance of fresh fruits and vegetables is one of the first and foremost quality determinants made by the buyer, whether the wholesaler, retailer, or consumer. Often the appearance of the fresh horticultural commodity is the most critical factor in the initial purchase while the subsequent purchases may be more related to its texture and flavor.

5.2.1.2 Color

Color is perceived when light that is reflected off the commodity's surface falls on the eye's retina. There is no color without light. The color perception depends on the type and intensity of light, chemical and physical

characteristics of the commodity, and the person's ability to characterize or differentiate various colors, or the color preferences of the individual.

5.2.1.3 Shape and Size

Uniform and characteristic shape is an important quality parameter or trait. Misshapen or deformed products may be more susceptible to mechanical injury and are generally avoided by consumers. An example where shape is important is broccoli. For the fresh market, compact broccoli florets are desirable while for fresh-cut space between the clusters of florets it is important to allow for cutting without injury. The size of product can also be important depending on its intended use. Consumers tend to associate large size with higher quality and view larger fruits as more natural.

A subjective evaluation of size and shape can be conducted on incoming products once the desirable and undesirable characteristics are determined. Size and shape charts, sizing rings, and callipers are available for various commodities and weight is a fairly accurate measure of product size. The percentage of product that does not meet the desired characteristics can be recorded (Jaeger et al., 2016).

5.2.1.4 Firmness

Firmness or the degree of softness or crispness is often measured by using an instrument for objectivity measure of firmness. Subjective measure of firmness with figures can be used as a useful check for quick measure of gross differences in firmness, particularly of soft products. There are several firmness testers available:

1. *Magness-Taylor pressure tester*: slide rule type, spring-loaded penetrometer
2. *Effegi-fruit penetrometer*: handheld probe with pressure designated gauge
3. *Effegi penetrometer*: mounted on a drill-press stand
4. *UC fruit firmness tester*: Ametek penetrometer mounted on a drill-press stand
5. *Deformation tester*: determines deformation force for soft fruits, such as tomatoes, papayas, and pears

Recommended tip sizes for firmness instruments are given in [Table 2.9](#).

Proper units for firmness may be used because it is inappropriate to use the term “pressure” in association with firmness measurement using the devices enlisted earlier, while pound force or kilogram force are preferred in the industry, Newton (N) is the required unit for scientific writing. The conversion factors are as follows:

Pound force (lbf) * 4.448 = Newton (N)

Kilogram force (kgf) * 9.807 = Newton (N)

Table 2.9 Tip sizes of firmness measuring equipments

Tip size	Commodities
11 mm (7/16 in.)	Apple
8 mm (5/16 in.)	Apricot, avocado, kiwifruit, mango, nectarine, papaya, peach, pear, persimmon, plum
3 mm (2/16 in.)	Cherry, grape, strawberry
1.5 mm (1/16 in.)	Olive

5.2.1.5 Absence of Defects

The product may be evaluated for the presence or absence of defects. The level of tolerance for each type of defect, such as cuts, bruises, disease, low temperature injury, and physiological disorders, should be determined. During quality evaluation, the percentage of fruit with each class of defect can be determined as a guide to overall product quality. A scoring system, namely, no defect = 1, slight = 2, moderate = 3, severe = 4, and extreme = 5 can be used to describe the incidence and extent of defects on acceptability level.

5.2.1.6 Further Objective Tests

Other objective tests include TSS, TA, respiratory gas analysis, nutritional quality, and eating quality (Kader, 2002; Rathore et al., 2012; Thompson, 1996).

5.2.1.6.1 Total soluble solids or soluble solid content (SSC) Carbohydrates from where fruit sugars are derived are the most abundant and widely distributed food component of fruits and vegetables. The structural frame work, texture, taste, and food value of a fresh fruit can be related to its carbohydrate content. Chemically, carbohydrates are hydrates of carbon having a general formula as $C(H_2O)_n$. They exist in different forms, namely, monosaccharide, oligosaccharides, and polysaccharides. Sucrose, glucose, and fructose are the primary sugars present in fruits and their relative proportions vary among fruits or vegetables. Such variations influence the taste because fructose is sweeter than sucrose and sucrose is sweeter than glucose. For judging the optimum maturity of a fruit or vegetable at harvest, the amount of TSS present in the extracted fruit juice sample is considered a reliable index. The TSS of a fruit indicates the amount of sugars present in the sample. This can be determined quickly with the help of a refractometer (Fig. 2.5). The TSS measurement by itself is destructive in nature as fruits are cut open and crushed to extract its juice to be put on refractometer. The instrument works on the principle of refractive index of a given sample and



Figure 2.5 Hand refractometer. (From <http://www.china-total.com/Product/meter/Traditional-Refractometer/OTR-series-hand-held-refractometer.htm>).

is usually expressed as °Brix. A higher Brix value indicates a higher degree of fruit ripeness, which is indicative of its relative sweetness in terms of sucrose content. Refractometers can be categorized according to their °Brix range, namely, 0–32, 28–62, and 56–92 of which 0–32°Brix refractometer is usually used for maturity determination in fruits or vegetables.

5.2.1.6.2 Titratable acidity (TA) Organic acids are intermediate products of metabolism. In Krebs (TCA) cycle, the oxidation of organic acids in living cells of fruit provides energy required for the maintenance of cell integrity. Most of the fresh fruits are acidic in nature with a pH range of 3–5. Together with sugars, the acidity determines the quality and taste of fruits. Total TA of specific organic acids and their relative quantities along with other factors influence the buffering system and pH of fruit or vegetable. The organic acid content of almost all fruits and vegetables changes during maturation and ripening. Acidity of a fruit progressively decreases as the fruit matures on

the tree. This can be determined by taking random samples of fruit before harvest, extracting their juice, and titrating it against a standard alkaline solution (NaOH) of known normality, with determination of titration end point using phenolphthalein ($C_{20}H_{14}O_4$) indicator. Total TA refers to the amount of acidity present in free + combined state. Free acidity denotes the amount of acidity present in free state only while actual acidity refers to the amount of (H^+) ion concentration present. It is important to measure acidity by titration than pH of the fruit because of the considerable buffering capacity in fruit juices. Normally, acidity is not taken as a measurement of fruit maturity indices by itself, but usually is related to TSS to TA ratio. In some fruits one or more acids may be present in relatively higher amount than other acids and accordingly these acids are referred as predominant acids respective to this fruit. For example, citric acid is a predominant acid in fruits like citrus and mango, malic acid is predominant acid in apples, cherries, plums, tartaric acid is predominant in grapes, quinic acid predominant in kiwifruits, oxalic acid is predominant in carambola, and so on. The TA value is expressed as equivalence of any of these organic acids (citric, malic, tartaric, etc.).

$$TA = \frac{\text{mL of NaOH} \times N\text{-NaOH} \times \text{acid meq. factor} \times 100}{\text{mL juice titrated}}$$

Use the acid milliequivalent factor for the predominant organic acid in the commodity, as given in [Table 2.10](#).

5.2.1.6.3 Nutritional quality Fresh fruits and vegetables play a very significant role in human nutrition, especially as sources of vitamins and dietary fiber. Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by physical damage, extended storage, higher temperatures, low relative humidity, and chilling injury of chilling sensitive commodities.

5.2.1.6.4 Eating quality Eating quality includes sweetness, sourness, astringency, bitterness, aroma, and off-flavors. The flavor quality involves the

Table 2.10 Milliequivalent for various organic acids

Acids	Formula wt.	Eq. wt.	Acid meq. factor	Commodities
Citric	192.12	64.04	0.064	Berries, citrus, pineapple
Malic	134.09	67.05	0.067	Apple, apricot, cherry, plum, peach
Tartaric	150.08	75.04	0.075	Grape

perception of the tastes and aromas of many compounds. Objective analytical determination of critical components must be coupled with subjective evaluations by a taste panel to yield useful and meaningful information about flavor quality of fresh fruits and vegetables. This approach can be used to define a minimum level of acceptability. To find out consumer preferences for flavor of a given commodity, large-scale testing by a representative sample of the consumers is required.

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CHAPTER 3

Effect of Elicitors in the Nutritional and Sensorial Quality of Fruits and Vegetables

Jesús O. Moreno-Escamilla*, Emilio Alvarez-Parrilla*,

Laura A. de la Rosa*, José A. Núñez-Gastélum*,

Gustavo A. González-Aguilar**, Joaquín Rodrigo-García*

*Universidad Autónoma de Ciudad Juárez, Instituto de Ciencias Biomédicas, Anillo envolvente PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, México

**Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, México

ABBREVIATIONS

ANS	Anthocyanidin synthase
4-CL	4-Coumaroyl CoA-ligase
C3H	Cinnamate 3-hydroxylase
C4H	Cinnamate 4-hydroxylase
CHI	Chalcone isomerase
CHS	Chalcone synthase
CHYB	β -Ring hydroxylase
CHYE	ϵ -Ring hydroxylase
COMT	Caffeic acid O-methyltransferase
DFR	Dihydroflavonol 4-reductase
DMPBQ	2,3-Dimethyl-5-phytyl-1,4-benzoquinone
F3H	Flavanone 3'-hydroxylase
F5H	Ferulic acid 5-hydroxylase
FLS	Flavonol synthase
GDP-D-Man	Guanosine diphosphate mannose
GDP-L-Gal	Guanosine diphosphate galactose
GTP-ME	GTP-mannose-3,5-epimerase
HGA	Homogentisic acid
HPP	<i>p</i> -Hydroxyphenylpyruvate
HPPD	HPP dioxygenase
HPT	Homogentisate phytyltransferase
HST	Homogentisate solanyltransferase
LAR	Leucoanthocyanidin reductase
LCYB	Lycopene β -cyclase
LCYE	Lycopene ϵ -cyclase
L-Gal1P	L-Galactose 1 phosphate
L-Gal1PP	L-Galactose-1-P phosphatase
L-GalDH	L-Galactose dehydrogenase

L-GalGT	L-Galactose guanyltransferase
L-GalL	L-Galactonolactone
L-GalLDH	L-Galactonolactone dehydrogenase
MPBQ	2-Methyl-6-phytyl-1,4-benzoquinone
MPBQMT	MPBQ methyltransferase
MSBQ	2-Methyl-6-solanyl-1,4-benzoquinone
NSY	Neoxanthin synthase
PAL	Phenylalanine ammonia lyase
PDS	Phytoene desaturase
SS	Stilbene synthase
γ-TMT	Gamma tocopherol methyltransferase
UPGT	UDP glucose-flavonoid-O-transferase L
VDE	Violaxanthin deepoxidase
ZDS	ζ-Carotene desaturase
ZEP	Zeaxanthin epoxidase

1 INTRODUCTION

Phytochemical compounds are secondary plant metabolites that present some beneficial effects on consumers' health further than those attributed to macronutrients and micronutrients (Saxena et al., 2013). There are several compounds found in different foods that can be classified as phytochemicals, such as carotenoids, tocopherols, phenolic compounds, and chlorophyll and each one of them can show a benefit in the consumer's health as show in Table 3.1 (Cora and German, 2000). Carotenoids and chlorophyll are phytochemicals responsible for the color of many fruits and vegetables. The carotenoids present in different fruits and vegetables, providing 70%–90% of carotenoid consumption in developed countries (Bramley et al., 2000). These compounds are responsible for the different colors that we see in the fruits and vegetables; they provide colors from yellow to red. Chlorophyll is another phytochemical that has the ability to provide color in several plants and it is responsible for the green color in a wide number of fruits and vegetables (Tanaka et al., 2008). Study results suggest that the consumption of vegetables rich in chlorophyll can prevent colon cancer and their extracts have antimutagenic activity (Ferruzzi and Blakeslee, 2007). Vitamin C is present in a wide variety of fruits and vegetables. Citrus fruit and their juices are rich sources of vitamin C, however, other fruits, such as kiwis are considered a fruit with one of the highest ascorbic acid concentrations (Padayatty et al., 2003). Tocopherols are a family of eight structurally related compounds, isolated from plants, fruits, and vegetables (α , γ , δ -tocopherol and α , γ , δ -tocotrienol). α -Tocopherol is the most important form of vitamin E that possesses the highest antioxidant activity (DellaPenna, 2005).

Table 3.1 Phytochemicals found in different food and their benefits on health

Phytochemicals	Food with this phytochemical	Possible benefit effect on health	References
Vitamin E	Cereals, nuts (almonds, walnut, hazelnut), seeds, legumes, oils, spinach, broccoli, avocado, asparagus	Reduce LDL oxidation, certain types of cancer, neurological diseases	McVean and Liebler (1997); Bramley et al. (2000)
Vitamin C	Papaya, pepper, broccoli, citric, peach, cranberry, pea, pineapple, kiwi, cauliflower, cabbage	Protect LDL and plasma lipids, neuronal maturation, immune system strengthening	Davey et al. (2000); Harrison and May (2009)
Carotenoids	Carrot, pumpkin, sweet potato, spinach, lettuce, mango, watermelon, pea, pepper, papaya, tomato	Cancer disease probability, cardiovascular, protect ocular system, skin damage	Palace et al. (1999); Stahl et al. (2000); Giovannucci (2002)
Chlorophyll	Avocado, lettuce, pepper, peas, apple (shell), kiwi, pistachio	Antimutagenic activity, reduce colon cancer probability	Ferruzzi and Blakeslee (2007)
Flavonols	Orange, apple, cranberry, raspberry, cherry, lemons, blackberry, grapes, strawberry	Anticarcinogenic effects, prevent viral diseases, reduce the hypertension, antialergic effect	Harborne and Williams (2000)
Anthocyanin	Gooseberry, apples, cranberry, nectarine, peach, purple onion, eggplant, red and green pear, strawberry	Reduce oxidative enzymes activity, cardiovascular diseases	Bridle and Timberlake (1997)

Several studies show some of the health actions of phenols intake, such as cardiovascular protection, and their ability to reduce the prevalence of different illnesses, such as Alzheimer's disease and cancer. These health-related properties have been related to their antioxidant activity (Ruiz-García and Gómez-Plaza, 2013). Phenols are found in most fruits and vegetables, however, among the richest fruits in phytochemicals, are berries, grapes, mangoes, oranges, apples, lettuce, and nuts (Złotek et al., 2014). There have been reported more than 8000 phenolic structures, which can be classified as flavonoids and nonflavonoids, according to their structure. Flavonoids, a special group of polyphenols, are pigments responsible for the color and flavor of many fruits, vegetables, flowers, nuts, and seeds (Harborne and Williams, 2000). Anthocyanins are directly responsible for the color of grapes and young wines, whereas the astringency and structure of wines are related to catechins, such as catechin and epicatechin (Kallithraka et al., 1997), and proanthocyanidins (condensed tannins) (Sun et al., 2013), while flavonols contribute to bitterness (Kong et al., 2003).

It is commonly known that there are different factors, including plant genotype and growing conditions, that can have an effect on the content of phytochemicals, specifically those with antioxidant activity (Złotek et al., 2014). There are different strategies to improve the quality of fresh vegetables, among them: genetic modification (GM), agronomic manipulation, and elicitation. The last one has become the most widely accepted safe strategy to increase the concentration of bioactive compounds, in response to the lack of consumer acceptance of GM foods (Schreiner, 2005). Elicitation has gained popularity because it not only produces a resistance of plants to abiotic stress factors, it also increases the quality of food by enhancing the production of phytochemicals (Martínez-Ballesta et al., 2007).

2 ELICITOR CLASSIFICATION AND THEIR EFFECT IN PLANTS

An elicitor is defined as a compound that, in small concentrations, can activate different plant responses, such as endogenous protection responses, including the production of different secondary metabolites (Namdeo, 2007). Elicitors can be classified according to their nature, as biotic and abiotic; and according to their origin, as exogenous and endogenous (Fig. 3.1). Biotic elicitors have biological origin derived from a pathogenic organism or from the plant itself as a consequence of defense mechanisms (Vasconsuelo and Boland, 2007). While abiotic elicitors have no biological origin, they are grouped as physical factors and chemical compounds. There is a wide range

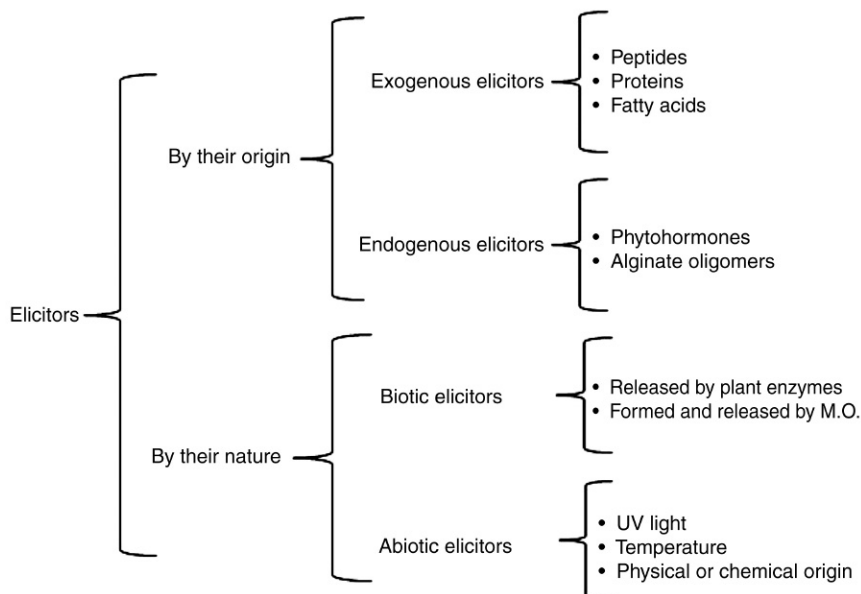


Figure 3.1 Elicitor's classification by their nature and their origin.

of physical abiotic elicitors, salinity (the use of salt), wounding, chilling injury, high and low temperature, UV light exposure, among others. Chemical abiotic elicitors may be compounds from the chemical synthesis origin of other compounds with chemical origins, as an example, mineral salts (calcium) and gaseous toxins (Ramakrishna and Ravishankar, 2011).

Endogenous elicitors can be produced during aging processes and plant degradation; they include reactive oxygen species (ROS), oligosaccharide, and protein fragments (Pearce et al., 2001), also substances generated inside the plant cell, such as hormones (e.g., jasmonic acid, salicylic acid), galacturonide, and alginate oligomer (Namdeo, 2007). Exogenous elicitors are substances totally unrelated to the composition of plants. They are anabolic products of the pathogen that triggers defense responses of the plant; they may be constituents of outer membrane, cell wall, or can be excretions (Weinberger and Friedlander, 2000).

The treatment of plants with elicitors or attacked by pathogen caused by a cascade of defense reactions, this reactions includes accumulation of a range of plant-defensive secondary metabolites in intact plants (Angelova et al., 2006). During the interaction with an elicitor the plant activates chain responses as described in Fig. 3.2. This is due to several reasons; one of them is that application of these compounds can trigger a series of chemical

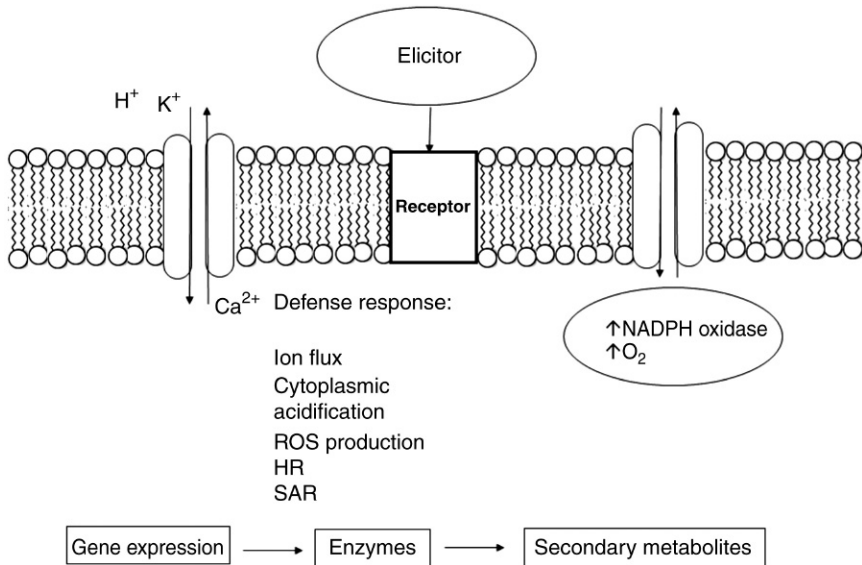


Figure 3.2 Responses triggered by the interaction of a plant with an elicitor (Baenas et al., 2014).

reactions and responses. Among them, some important responses include raising the ion flux, rapid protein phosphorylation and protein kinase activation, release of Ca²⁺, cytoplasmic acidification activation of NADPH, production of ROS leading to cell death [(hypersensitive responses (HR)], and systemic acquired resistance (SAR) response (Livaja et al., 2008; Radman et al., 2003). Among these protective mechanism it can be cited the activation of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH PX) (Bestwick et al., 2001). HR is activated by the direct interaction of the plant and the pathogen, and it consists in the rapid development of cell death and immediately surrounding the infected area (Morel and Dangl, 1997). SAR response is a distinct signal transduction pathway that plays an important role in the defense mechanisms of plants against pathogens. This response can be activated after the first direct interaction with the pathogen, establishing this secondary immune action, which is long lasting and more effective against pathogens and its subsequent attacks (McDowell and Dangl, 2000). SAR is biologically triggered by both nonvirulent and virulent pathogens that cause cell death (as a result of the HR or disease symptoms). Therefore there might be a correlation between cell death and the establishment of SAR. However, if the tissue inoculated by a necrotizing pathogen is

removed before the onset of macroscopic cell death, SAR is still observed in systemic inoculated tissues (Morel and Dangl, 1997). It acts as a modulator of other disease resistant mechanisms activating enzymes responsible for the synthesis of certain compounds with the ability to protect the plant from the stress, acting like a plant “immune system” (Ryals et al., 1996). Plants have different ways to protect themselves for an attack, one of them is the production of secondary metabolites, which compounds the ability to act as antibiotics and gives the plant other characteristics that prevent plant infections. These metabolites are synthesized and released when infection of fruit and vegetables take place, and phenolic compounds are the most commonly induced (Haslam, 1988).

3 PATHWAYS ACTIVATED BY ELICITORS

3.1 Phenylpropanoid Synthesis Pathway

The phenylpropanoid pathway is the first step by which plants synthesize all phenolic compounds. Several studies have established that interactions between an elicitor and a plant can trigger the activity of enzymes responsible for the synthesis of phenolic compounds, which is produced by increasing the activity of this enzyme; this effect is greater when the elicitors were pre-harvest added (Li et al., 2012; Xiang-Hong et al., 2010). Phenylalanine ammonia lyase (PAL) is a key enzyme in the synthesis of phenolic compounds, responsible for the transformation of phenylalanine into cinnamic acid, the first precursor of phenolic compounds. This activation could be related with the activation of SAR by the increasing of salicylic acid, which is part of the phenylpropanoid metabolism (Kessmann et al., 1994). Fig. 3.3 depicts the synthetic pathway of phenolic acids. The initial three steps of the pathway, catalyzed by PAL, cinnamate 4-hydroxylase (C4H), and 4-coumaroyl CoA-ligase (4CL) to form cinnamate, *p*-coumarate, and *p*-coumaroyl-CoA, respectively, are the main reactions that lead to the production of the other metabolites. 4-Coumaroyl-CoA represents the most important step of the phenylpropanoids biosynthesis in plants, which is the direct precursor for flavonoid and lignin biosynthesis (Dixon and Paiva, 1995).

A considerable number of elicitors have shown the ability to activate PAL. Methyl jasmonate has been able to activate PAL in sweet cherry (Yao and Tian, 2005), guava fruits (González-Aguilar et al., 2004), and carrots (Basilio Heredia and Cisneros-Zevallos, 2009). In other studies, PAL was activated by abiotic or biotic elicitors. Among them harpin (a protein from plant-pathogenic organisms, such as *Erwinia amylovora*, *Pseudomonas*, and

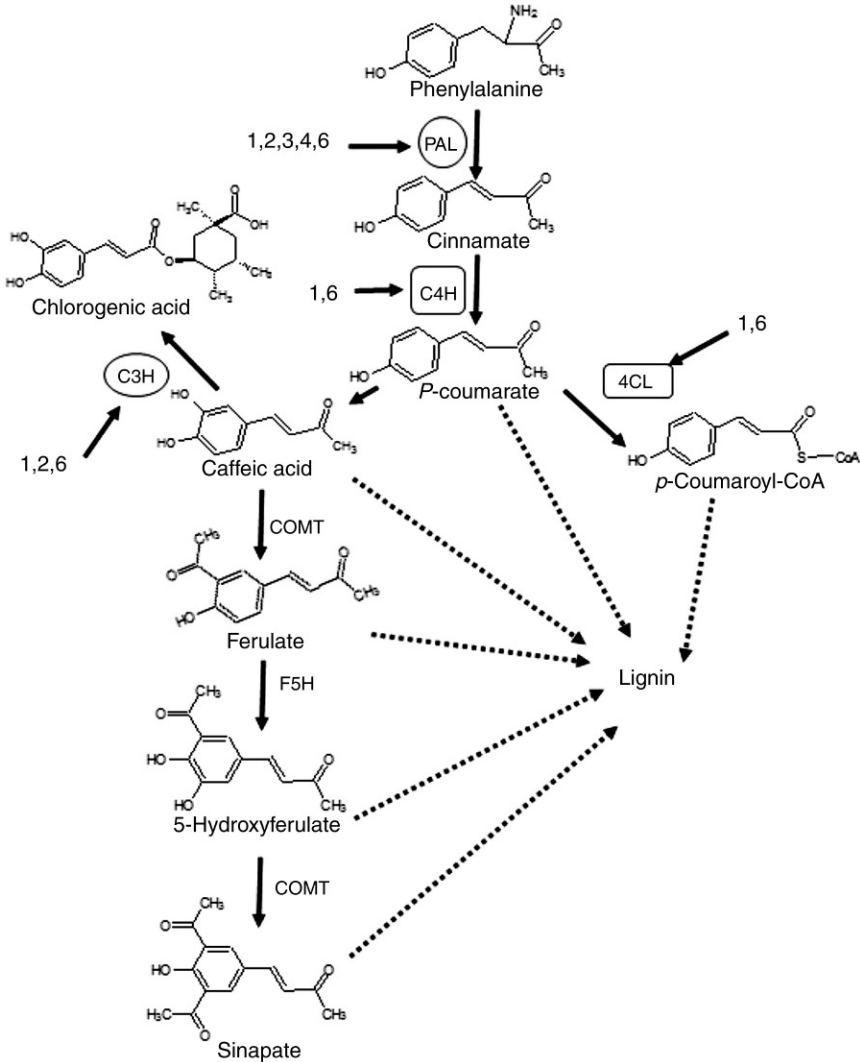


Figure 3.3 Activation of enzymes associated to phenolic acids synthesis pathway by elicitors (Dixon and Paiva, 1995). Oval: Strong evidence of activation; rectangle: scarce of evidence. Elicitors classification (1) UV light; (2) harpin protein; (3) chitosan; (4) methyl jasmonate; (5) salt; (6) others.

Ralstonia) has been reported to stimulate PAL in peaches protein by triggering the SAR by the salicylic acid stimulation ending with the activation of phenylpropanoid metabolism (Li et al., 2012). Studies show that it can also induce the activation of antioxidant endogenous proteins by activating the hypersensitive response probably by the production of ROS after

the interaction with harpin protein (Bestwick et al., 2001). Other studies have shown the activation of PAL by UV irradiation in Vitaceae leaves (Fritzscheier and Kindl, 1981), chitosan in banana (Romanazzi et al., 2002), and salt stress in hairy roots (Yan et al., 2006). As a consequence an increase in phenolic content was observed in all these products due to the activation of this enzyme. Even though PAL activation by elicitors has been described, information on the effect of these elicitors on the activity of other enzymes involved in the synthesis of phenolic acids is scarce.

Elicitation of lettuce with different physical environmental stressors showed different responses. In this instance, chilling stress produced lower phenolic concentration (i.e., lower PAL activity) compared to high luminance, while low temperatures induced a higher expression of PAL enzyme (Oh et al., 2009a). It has been described that cinnamate 3-hydroxylase (C3H) is activated after exposure to UV light (Fritzscheier and Kindl, 1981) and fungus (Bolwell et al., 1985) in beans. Due to this enzymatic activation, the synthesis of quinic acid (a precursor of chlorogenic acid) increased in carrot (Kühnl et al., 1987), resulting in an increase of chlorogenic acid content. In the case of the other two enzymes caffeic acid O-methyltransferase (COMT) and ferulic acid 5-hydroxylase (F5H), there is no evidence that demonstrates an activation mediated by an elicitor. The response of plant tissue to abiotic stress varies widely in respect to cultivar, maturation, cultivar practices, and so on. These results show that an abiotic stress will induce in different extents the secondary metabolism of plant tissue. However, more research is needed to better understand and control the secondary metabolism of plants.

The last part of the phenylpropanoid metabolism involves the synthesis of flavonoids (Fig. 3.4). This synthetic pathway proceeds through interaction of *p*-coumaroyl-CoA with malonyl-CoA. This complex can take two different directions: if stilbene synthase (SS) is activated, resveratrol and other stilbenes are produced; however, activation of chalcone synthase (CHS) triggers the synthesis of flavonoids and anthocyanins. Activation of the first pathway has been reported in pine (Preisig-Müller et al., 1999) exposed to UV light, and bacterial elicitation on grapevines (Borie et al., 2004). Chalcone synthase activation has been studied in the presence of biotic elicitors. Fungal and yeast elicitors have been reported to activate this enzyme in beans (Lawton et al., 2005), and in alfalfa (Junghans et al., 1993). UV light exposure increased the activity of this enzyme in *Petunia* flowers (Koes et al., 1989) while methyl jasmonate activated it in white spruces (Richard et al., 2000).

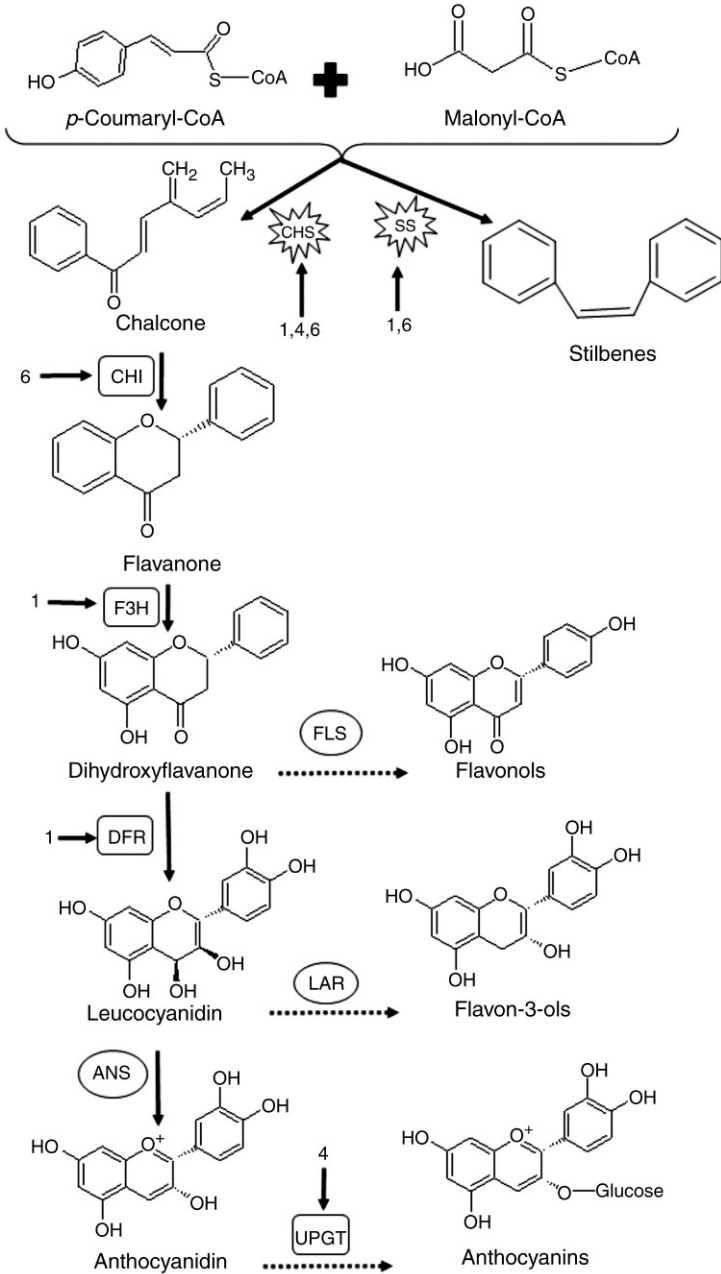


Figure 3.4 Activation of enzymes associated flavonoids synthesis pathway by elicitors (Ruiz-García and Gómez-Plaza, 2013). Explosion: Evidence of activation; rectangle: lack of evidence; oval: no evidence of activation. Elicitor classification (1) UV light; (2) harpin protein; (3) chitosan; (4) methyl jasmonate; (5) salt; (6) others.

Few elicitors have been tested for their effect on the next enzyme in the synthesis of flavonoids: chalcone isomerase (CHI). Results have shown that *Agrobacterium rhizogenes* increased the activity of this enzyme in *Glycyrrhiza uralensis* (Hai-Chao et al., 2009), while ozone showed the same pattern in beans (Paolacci et al., 2001). There is scarce information on the activation of the rest of enzymes associated to flavonoids synthesis, even though an increase on flavonoids and anthocyanins concentration have been reported in carrots and grapes (Rajendran et al., 1994; Zhang et al., 2002). Methyl jasmonate activated UPGT (UDP glucose-flavonoid-O-transferase L; responsible for the synthesis of anthocyanins) in grapevines (Belhadj et al., 2008), and UV light triggered the activity of flavanone 3-hydroxylase (F3H) and dihydroflavonol 4-reductase (DFR) in carrots (Hirner et al., 2001). The lack of information in the behavior of this enzyme opens the opportunity to investigate the possible effects of elicitors on the rest of the enzymes, besides PAL, to get a better understanding in how the mechanism works that modulates the activation of the phenylpropanoid metabolism and with this the increase of phenolic compounds.

3.2 Vitamin C Synthesis Pathway

Vitamin C synthetic pathway in plants (Fig. 3.5) starts with guanosine diphosphate mannose (GDP-D-mannose). GTP-ME (GTP-mannose-3,5-epimerase) converts GDP-D-mannose to GDP-L-galactose (guanosine diphosphate galactose). The enzyme GDP-L-galactose phosphorylase, which promotes GDP-L-galactose to L-galactose-1-P, has only been identified very recently; this step appears to have a major role in the regulation of L-ascorbate synthesis. L-Galactose-1-P phosphatase, encodes a specific L-galactose-1-P phosphatase to the hydrolysis of L-galactose-1-P to the formation of L-galactose. The next step in the biosynthesis of vitamin C is the L-galactose dehydrogenase, which oxidizes L-galactose to produce L-galactono-1-4-lactone, which in presence of the enzyme L-GalLDH (galactonolactone dehydrogenase) is oxidized into formation of L-ascorbate. There is information that demonstrates that biotic or abiotic elicitors can increase vitamin C content in plant tissues. Jasmonic acid increased vitamin C in lettuce (Złotek et al., 2014); salicylic acid and chitosan in broccoli could be linked to the indirect activation of vitamin C by the production of carbohydrates, such as sucrose and glucose, key factors in the biosynthetic pathway for L-ascorbate that involves several enzymatic steps from D-glucose (Pérez-Balibrea et al., 2011). Temperature-induced vitamin C increases in lentil sprouts (Świeca et al., 2014). There is a lack

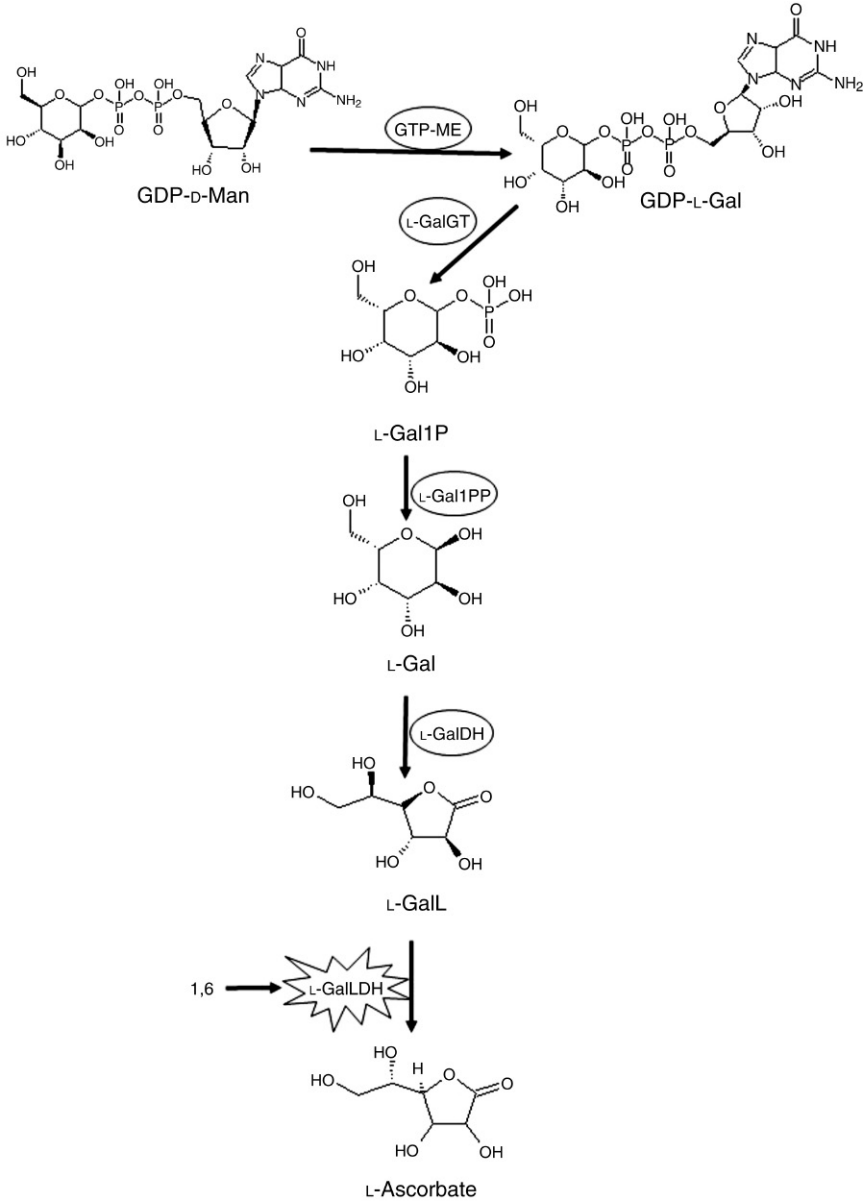


Figure 3.5 Activation of enzymes associated to vitamin C synthesis pathway by elicitors (Giovannoni, 2007). Explosion: Evidence of activation; rectangle: lack of evidence; oval: no evidence of activation. Elicitor classification (1) UV light; (2) harpin protein; (3) chitosan; (4) methyl jasmonate; (5) salt; (6) others.

of information on the activity of elicitors on the activation of the enzymes responsible of the synthesis of vitamin C on vegetable tissue. Although there is evidence for the increase of ascorbic acid during elicitation, the L-GalLDH, which is the last enzyme involved in synthesis of vitamin C, is the only one with evidence of its activation. There is evidence for activation of this protein in, for instance, wheat leaves (Bartoli et al., 2005) and lettuce (Oh et al., 2009b). This enzyme has been activated by drought stress by increasing its expression and that can result in the rise of the activity of this enzyme.

3.3 Vitamin E Synthesis Pathway

Tocopherol synthesis is depicted in Fig. 3.6. The first step in tocopherol synthesis involves the production of the aromatic head group, HGA (homogentisic acid) from *p*-hydroxyphenylpyruvic acid (HPP) by the enzyme *p*-hydroxyphenylpyruvic acid dioxygenase (HPPD) (Schulz et al., 1993). This is a complex enzymatic reaction that catalyzes the addition of two oxygen molecules, a decarboxylation and rearrangement of the side chain of HPP. HGA is then subject to prenylation with phytyl diphosphate or geranylgeranyl diphosphate to yield 2-methyl-6-phytylplastoquinol (MPBQ). MPBQ is the first intermediate in the synthesis of all tocopherols. The next synthetic step involves ring methylations and cyclization. MPBQ methyltransferase (MPBQMT) adds a second methyl group to MPBQ to form 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ); and tocopherol cyclase converts MPBQ and DMPBQ to δ - and γ -tocopherols. Finally, gamma tocopherol methyltransferase (γ -TMT) adds a methyl group to the sixth position of the chromanol ring converting δ - and γ -tocopherol to β - and α -tocopherol, respectively.

There are few reports about the effect of elicitors on the activation of enzymes from the synthetic pathway of tocopherols. TC is activated in tobacco exposed to drought stress (Liu et al., 2008). Homogentisate phytyltransferase (HPT) was overexpressed in light stressed *Arabidopsis* plants (Collakova and DellaPenna, 2003). Salicylic acid and methyl jasmonate are reported as activators of gamma tocopherol methyl transferase (γ -TMT) in *Moringa oleifera* (Saini et al., 2014), also in a recent research it was shown that an increase in this enzyme is stressed by withholding water from lettuce, and this provokes a stress and with this trigger the expression of this protein (Oh et al., 2009b). More studies are necessary to better understand the effect of elicitors on the synthesis of these compounds.

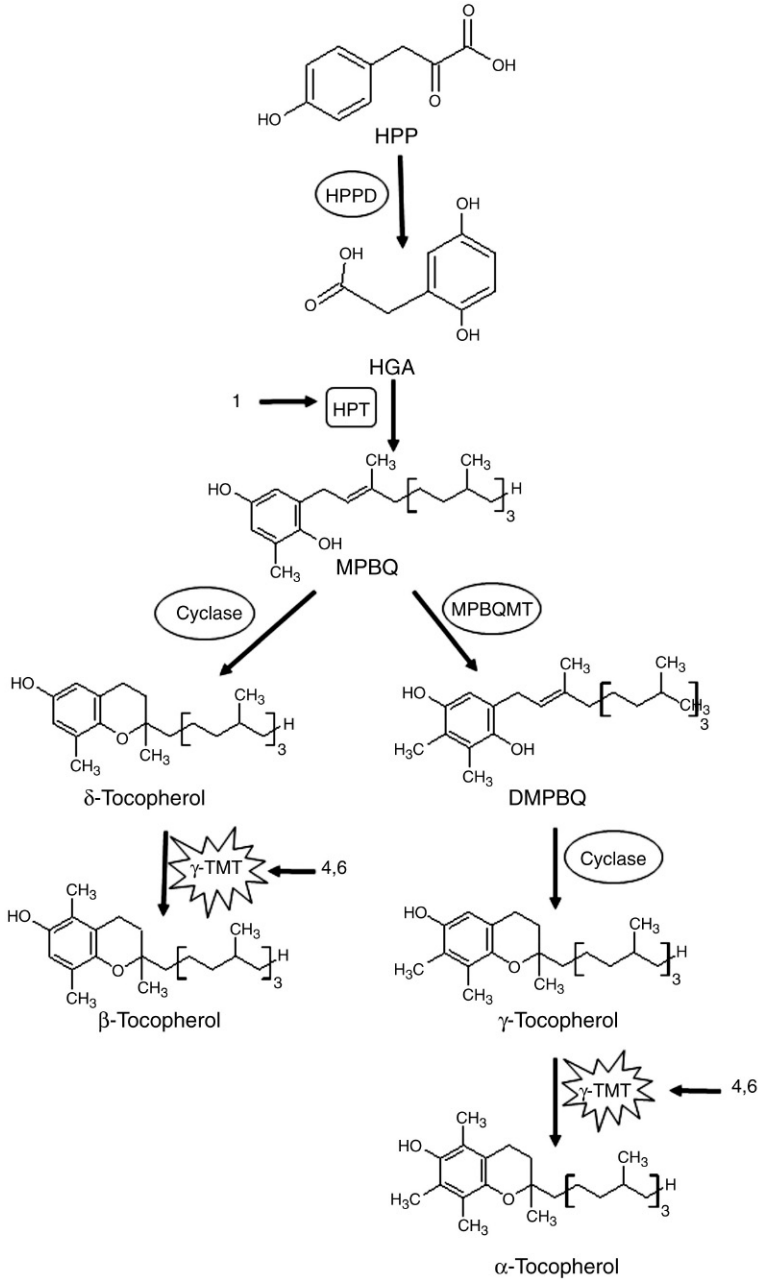


Figure 3.6 Activation of enzymes associated to tocopherols synthesis pathway by elicitors. *Explosion*: Evidence of activation; *rectangle*: lack of evidence; *oval*: no evidence of activation. Elicitor classification (1) UV light; (2) harpin protein; (3) chitosan; (4) methyl jasmonate; (5) salt; (6) others.

3.4 Carotenoids Synthesis Pathway

There are several enzymes involved in the biosynthesis of carotenoids, as shown in Fig. 3.7. Carotenoid biosynthesis starts from a C5 isoprene unit, involving several modifications of the molecule up to the formation of

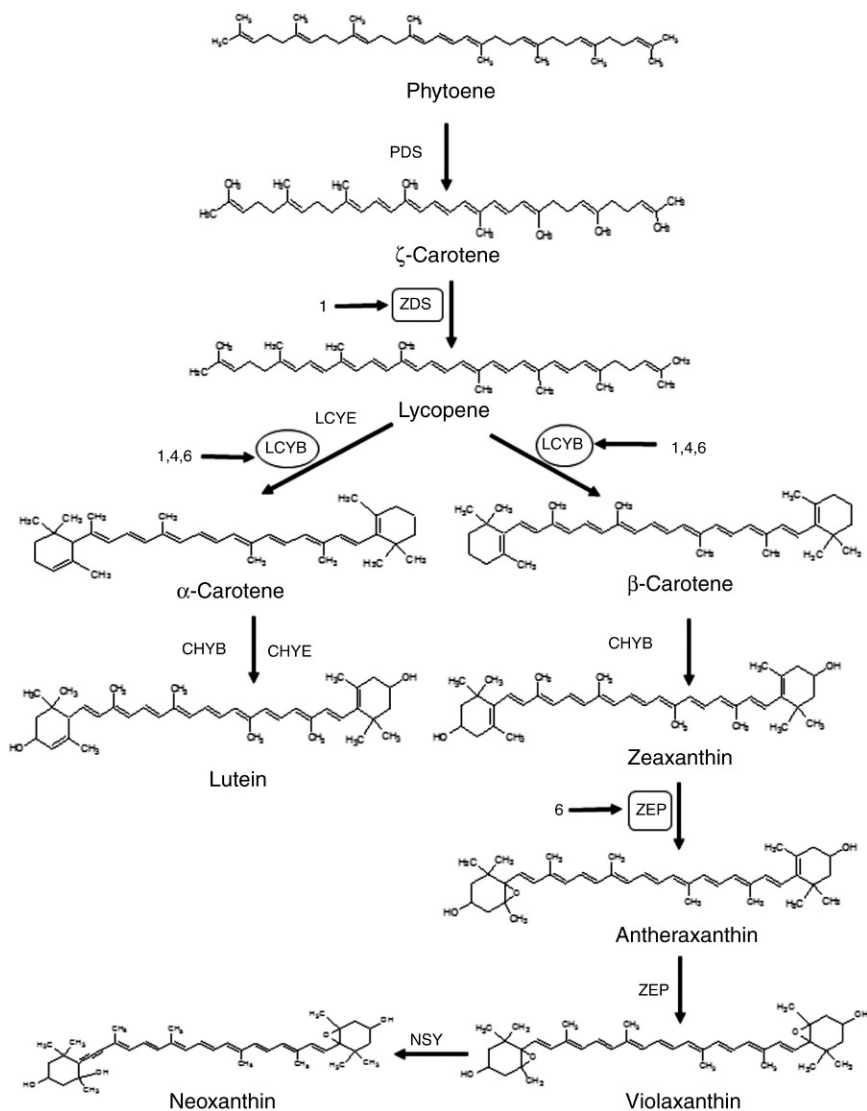


Figure 3.7 Activation of enzymes associated to carotenoids synthesis pathway by elicitors (Tanaka et al., 2008). .Oval: Strong evidence of activation; rectangle: scarce evidence of activation. Elicitors classification (1) UV light; (2) harpin protein; (3) chitosan; (4) methyl jasmonate; (5) salt; (6) others.

phytoene, the first C40 carotenoid. Conjugated double bonds are subsequently added by two structurally similar enzymes, phytoene desaturase (PDS) and ζ -carotene desaturase (ZDS). These desaturation reactions yield the intermediates ζ -carotene and lycopene, containing 5 and 11 conjugated double bonds, respectively. During the desaturation steps, several reaction intermediates with a *cis*-configuration are produced. The cyclization of lycopene is a branch point in the pathway, catalyzed by lycopene β -cyclase (LCYB) and lycopene ϵ -cyclase (LCYE). Hydroxylation of the β - and ϵ -rings is catalyzed by β -hydroxylase (CHYB) and ϵ -hydroxylase (CHYE), respectively. Epoxidation at positions C5, C6, C5', and C6' of the δ -ring of zeaxanthin, catalyzed by zeaxanthin epoxidase (ZEP), yields violaxanthin. Violaxanthin is converted to neoxanthin by neoxanthin synthase (NSY).

There is evidence that salicylic acid (SA) and methyl jasmonate (MJ) activate the lycopene β -cyclase in *Moringa* (Saini et al., 2014). Chitosan, methyl jasmonate and yeast elicitors also increased the production of β -carotene in *Cleome rosea* Vahl (Silva da Rocha et al., 2013), nevertheless the concentration of this compounds and the carotenoids synthetize depends on the plant and the elicitor used. UV-treated tomatoes showed higher lycopene and β -carotene, compared to control. All this information could suggest that lycopene β -cyclase is the most activated enzyme being because this enzyme has to be activated to transform the lycopene into these metabolites, this the reason why β -carotene is the major carotenoid molecule found in the pigmented foods treated with elicitors (Tanaka et al., 2008). *Xanthophyllomyces dendrorhous* treated with fungal elicitors, showed an increase in total carotenoids specially of astaxanthin by the trigger of HR with the production of ROS during the elicitation, this could be by an activation of β -carotene hydroxylase with also an activation of β -carotene ketolase leading to an increase of this carotenoid (Wang et al., 2006).

4 SENSORY QUALITY AFFECTED BY ELICITATION

Sensory quality is a very important factor to consider in fruit and vegetable production. There are several characteristics that provide information to determine the ideal stage of consumption. Biotic and abiotic elicitors are well known to affect many indicators, such as color, texture, and flavor (Kleinhenz et al., 2003). The induction of the activity of certain enzymes during elicitation may produce darkening of the fruits and vegetables tissue. There are a few reports about the effect of elicitors in plants, nevertheless studies showed that there are changes in sensory quality characteristics after

the use of elicitation and this effect depends on the elicitor used and the plant that is elicited. Some research showed that production of phenolic compounds during plant stress have an impact in the bitterness and astringency (Lea, 1992), furthermore, anthocyanins, chlorophyll, and carotenoids have been reported to show an important role in the color of fruits and vegetables (Horbowicz et al., 2008; Lesschaeve and Noble, 2005), consequently the increase in this phytochemicals may exert some effect in color quality. In the case of tocopherols, there is a need to be careful in the activation of this pathway because studies demonstrate that high concentrations of this molecules provoke the prooxidation and darkness of the plant and with this a chance in sensory quality (Warner, 2005), jasmonic acid, and abscisic acid have shown to increase the firmness of treated lettuce's compared with control, probably due to the increase of lignin by using phenolic compounds to reinforce the wall for protection during the elicitation (Mandal and Mitra, 2007; Złotek et al., 2014). However, aroma, flavor, crispiness, and appearance did not change after treatment with the elicitors. Chitosan has been used in wine production as a fungicide and the results suggest that they make an alteration in the wine profile; a change in aroma, color, and flavor was produced in the sample elicited with this compound (Vitalini et al., 2014). Methyl jasmonate has also been used in the production of wine, observing a 10% increase in color when MJ was used in the grape clusters, compared to control samples (Ruiz-García et al., 2012). Ethylene has been reported to modify the sensory quality in carrots, observing an increase in bitter, sweet, earthy, and green flavor after 3 weeks of treatment (Seljåsen et al., 2001).

5 CONCLUSIONS

Several elicitors activate different enzymes in the biosynthetic pathways of secondary metabolites. Methyl jasmonate triggers the activation of PAL, CHS, UPGT, LCYB, γ -TMT, luminescence stress activates enzymes like the L-GalLDH, LCYB, PAL, CHS, SS, HPT, F3H, DFR, 4CL, C3H, and C4H and harpin protein activates PAL and C3H. Therefore, elicitors appear to be a good strategy to provide an approach to obtain fruits and vegetables with higher phytochemical content and better quality. However, more research is needed to better understand the effect of these elicitors in the different phytochemical synthesis pathways in order to be able to increase the health-related properties of fruit and vegetable products without decreasing the sensory properties of these products.

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CHAPTER 4

The Fruit Cuticle: Actively Tuning Postharvest Quality

Isabel Lara

University of Lleida, Lleida, Spain

(...) interdum massam pulposam et succulentam, in medio baccarum et pomorum ita dispositam, ut haec media thalami production ab externa pulpa, sub cuticula deprehensa, consistentia et faeps etiam colore differat; interdum membranam, in interna fructus parite extensam

—**Christian Gottlieb Ludwig (1757)**

The colonization of dry land by higher plants was rendered possible by the development of an outer barrier, which protected their aerial parts from dehydration due to the very different water potential inside the plant in comparison with that in the new environmental conditions (Riederer, 1991). This mainly lipophilic barrier is known as the cuticle, which covers and waterproofs all the nonwoody aerial organs of the plant, including leaves, young branches, flowers, sprouts, and fruits.

The plant cuticle is composed chiefly of a cutin matrix embedded and covered with waxes. While cutin is a polyester constituted mainly of hydroxylated and epoxy-hydroxylated C₁₆ and C₁₈ fatty acid derivatives, arranged in a net-like structure by means of numerous cross-links established among the hydroxyl moieties, the wax component of cuticles contains primarily long- and very long-chain *n*-alkanes, fatty alcohols, fatty acids, aldehydes, and ketones, together with a considerable amount of triterpenoids. Variable quantities of phytosterols and phenolics have been also described in some cases as prominent components of cuticular waxes, which confer the surrounded plant organs particular mechanical and physical properties. Additionally, some plant cuticles contain reportedly substantial amounts of cutan, an extremely insoluble, nonhydrolyzable polymer remaining after cutin isolation. The chemical nature of cutan is still unclear, but it apparently consists largely of unsaturated polymethylenic chains assembled by means of ether bonds (Villena et al., 1999). Traditionally, the cuticle has been regarded simply as a lipidic cover that wraps the surface of outer epidermal cell walls, and whose relationship to the outermost layers of fruit is limited

to their neighboring location. However, recent findings suggest that the cuticle should be understood rather as a lipidized, chemically and structurally heterogeneous region of epidermal cell walls (Fernández et al., 2016; Guzmán et al., 2014).

Excellent recent reviews exist of the state-of-the-art knowledge on the biosynthesis of cutin and wax components of plant cuticles (Kunst and Samuels, 2003, 2009; Samuels et al., 2008), as well as on cuticle permeability (Kerstiens, 2006) and mechanical properties (Domínguez et al., 2011a,b; Heredia, 2003). These aspects, not being the direct motivation of this chapter, will be therefore not covered, and the reader is referred to those previous works for further information.

If we focus specifically on fruit cuticles, we will find that many gaps remain to be elucidated as to the composition and biosynthesis of cuticle in these organs. Published information on cuticle biosynthesis in these commodities has been scarce, and a survey of available data on this topic has been recently the object of a particular review (Lara et al., 2015). Indeed, fruit cuticles have historically received considerably less research focus than those of vegetative tissues. Although many studies have been devoted to the plant cuticle, mostly taking leaves as model systems, considerably less research efforts have been invested in fruit, and hence several aspects of cuticle formation in these organs still remain poorly understood, in spite of the economic relevance of these commodities. This circumstance may have arisen from the fact that leaves, for instance, are easier to work with, and the isolation of the cuticular membrane is thus much more straightforward than when using fruits as the starting material, which may contain high levels of interfering compounds difficult to deal with. There has also been the common idea that fruit cuticle composition could be inferred from that of the leaves. Two recent reviews have attempted to shed some light on the specificities of fruit cuticles (Lara et al., 2015; Martin and Rose, 2014), revealing important differences in comparison to other plant organs, as well as among fruit species and cultivars.

1 A BRIEF OVERVIEW OF FRUIT CUTICLES

1.1 General Features of Fruit Cuticle Composition

Substantial compositional differences have been reported in many cases for fruit in comparison with leaf cuticles, hence showing that the idea that information obtained for a given organ can be generalized for the rest

of the plant is erroneous and should be ruled out. Such differences have been observed both for the total load and for the specific profile of cutin and wax compounds in fruit species such as apple (*Malus × domestica* Borkh.) (Espellie et al., 1979), citrus (*Citrus* sp.) (Baker et al., 1975; Espellie et al., 1980), grape (*Vitis vinifera* L.) (Radler, 1965, 1970), tomato (*Solanum lycopersicon* L.) (Buxdorf et al., 2014; Petit et al., 2014; Vogg et al., 2004), olive (*Olea europaea* L.) (Huang et al., 2015), and different small berries (Järvinen et al., 2010).

In tomatoes, for instance, remarkably higher total cutin and wax loads have been reported in fruits in comparison with leaves (Buxdorf et al., 2014; Petit et al., 2014; Vogg et al., 2004). These differences are not quantitative uniquely, but also qualitative, the main compositional dissimilarities observed being the much lower abundance (around one-third) of branched alkanes and the higher amount of alcohols and fatty acids in fruit in comparison with leaves. Similarly, regarding cutin monomers, substantial differences in the amount and types of hydroxy- and dicarboxylic acids were found in the cited studies. In contrast, for “Arbequina” olives similar or even higher wax coverage was found in leaves than in fruit, but significant differences were observed as to the average chain length (ACL) of the aliphatic components in each case: C_{26} for fruit and C_{30} for leaves, which may account for the very different cuticular water permeances of each of both organs (approximately fourfold lower in leaves than in fruit) (Huang et al., 2015). These examples illustrate the profound dissimilarities among fruit species, not only regarding the profile of compounds present, but also concerning the patterns and change dynamics across the organs of the plant.

In spite of species-to-species and cultivar-to-cultivar variability, triterpenes and *n*-alkanes are in general prominent cuticular wax components of fruit cuticles in all the species for which this information exists. Usually, for a given fruit species, either triterpenoid alcohols (frequently amyrins) or triterpenoid acids (mostly oleanolic and/or ursolic acids) predominate within cuticular wax triterpenes. Similarly, the most commonly found *n*-alkanes in fruit cuticles are either *n*-nonacosane (C_{29}) or *n*-hentriacontane (C_{31}). As to cutin composition, there seems to exist likewise some grouping according to the main type of cutin monomers detected, C_{16} and C_{18} fatty acid derivatives being the most frequently identified compounds. Some examples of commonly found components of waxes and cutin monomers in fruit cuticles are shown (Fig. 4.1). No apparent relationship seems to exist between the predominance of a particular compound or compound type and either fruit type (berry, drupe, hesperidium, etaerio, syncarp, pome) or ripening pattern

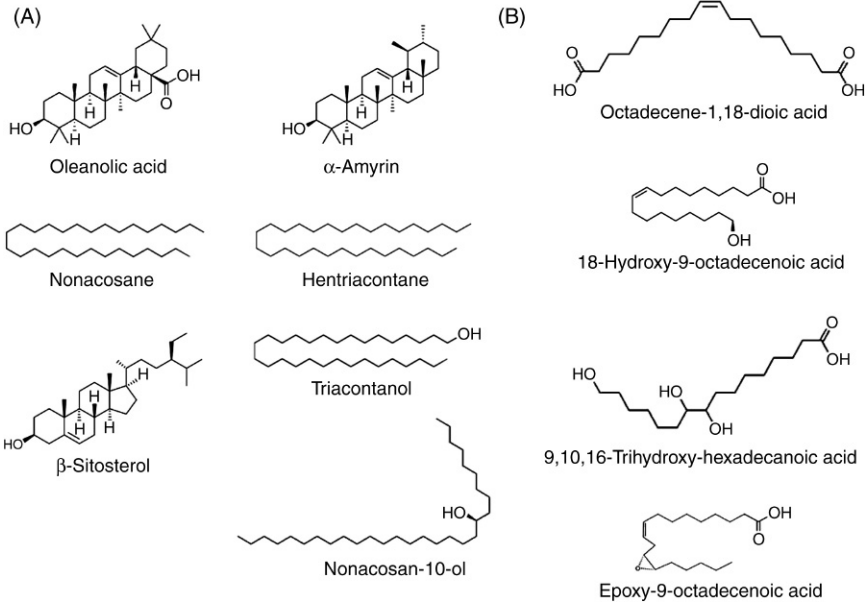


Figure 4.1 Wax (A) and cutin monomers (B) commonly found in fruit cuticles of species for which this information has been reported. (Reviewed in [Lara et al., 2014, 2015](#)).

(climacteric vs. nonclimacteric), but it clearly does within taxonomic families (reviewed in [Lara et al., 2015](#)). For example, *n*-nonacosane (C_{29}) and triterpenoid acids predominate within the botanical family of Rosaceae, whereas and *n*-hentriacontane (C_{31}) and triterpenols are quantitatively prominent within the Solanaceae family. This is very interesting, as one out of three triterpene synthases (MdOSC1, MdOSC2, and MdOSC3) recently isolated in “Royal Gala” apples and shown to possess triterpene synthase activity ([Brendolise et al., 2011](#)), namely MdOCS1, was shown to express preferentially in the fruit skin, and to produce α - and β -amyrin in a 5:1 ratio, α -amyrin accounting for >80% of the total product. This observation clearly connects with the abundant experimental evidence that the main component of the triterpene fraction in waxes of apple fruit cuticles is ursolic acid, an α -amyrin derivative. Therefore, a wide research field opens ahead, to investigate the molecular basis underlying metabolic differences between fruits in which the triterpenoid fraction of cuticular waxes is dominated by triterpenoid acids and those in which triterpenols are prevailing. Similar mechanisms may operate that explain the reasons for the apparent grouping between predominant C_{29} and C_{31} hydrocarbons in cuticular waxes, or between C_{16} and C_{18} monomers in cutin among botanical families of fruit commodities.

Even so, not only cuticle composition, but also structure and links among its different components must be a central feature of cuticle functionality, as experimental evidence indicates that cuticle structure and networks are also a key feature underlying and fine-tuning its functionality. This is evident, for example, from the observation that the simultaneous overexpression in *Arabidopsis* plants of a glycerol-3-phosphate acyltransferase and a fatty acid ω -hydroxylase, both required for cutin assembly, resulted in increased water permeability of cuticles even though wax accumulation was unchanged and cutin load was increased (Li et al., 2007). More recently, the application of solid-state ^{13}C nuclear magnetic resonance (NMR) techniques to the study of tomato fruit cuticles has demonstrated that dewaxed inner and outer epidermis cuticles display divergent composition and developmental changes (Chatterjee et al., 2016). Results suggest that close chain stacking and hydrogen bonding interactions would favor the formation of hydrophobically associated barriers against water diffusion, whereas branched components would have the potential to establish a network of cross-linked structures and/or linkages to the epidermal cell wall polysaccharides.

1.2 Is There a Relationship of Cuticle Composition to Fruit Type?

Hence, for a better comprehension of the influence of cuticle on fruit quality and storage/shelf life potential, it would be necessary to have a wide knowledge on its composition and structure as the essential preliminary information. Yet this information is available for a handful of species uniquely, a complete overview of cuticle components being available in only a few cases (Lara et al., 2015). Furthermore, fruit cuticles display considerable variability among species and even cultivars within the same species, and thus their characterization will require that these research efforts be done on a case-by-case basis. For example, when we compared the fruit cuticle composition of “October Sun” and “Jesca,” two peach (*Prunus persica* L.) cultivars displaying, respectively, a melting and a nonmelting softening pattern (Belge et al., 2014a), we found remarkable differences in major components of the cuticular wax fraction (Fig. 4.2A). Specifically, “October Sun” fruit had nearly 52% of triterpenoid acids over total waxes, while the content of *n*-alkanes in “Jesca” almost doubled that found in the melting-type peach (29.4% vs. 16.5%). Significant differences were found in weight loss and other quality attributes, which might relate to these cuticular composition traits. Similarly, significant dissimilarities in cuticular wax composition were detected between “Celeste” and “Somerset,” two

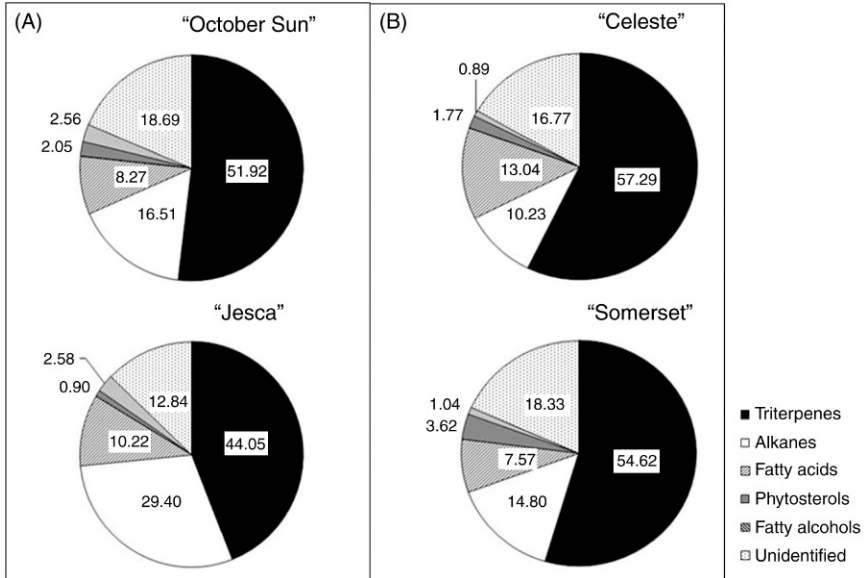


Figure 4.2 Wax compound types (percent over total waxes) identified in cuticles isolated from peach (A) and sweet cherry (B) fruit picked at a commercial harvest. (Drawn from data reported in [Belge et al., 2014a,b](#)).

sweet cherry (*Prunus avium* L.) cultivars with remarkably different postharvest performance ([Fig. 4.2B](#)).

2 IMPACT OF CUTICLE COMPOSITION AND PROPERTIES ON FRUIT QUALITY

Fruit cuticles are very relevant from a physiological point of view, and their properties modulate many attributes of economic importance. Thus, the understanding of their functions and impact on fruit quality, storage potential, and shelf life is paramount for a hypothetical optimization of postharvest procedures. Four main aspects of fruit quality have been identified on which an impact of cuticle is demonstrated or highly likely, namely water loss, susceptibility to infections, physiological alterations, and fruit firmness ([Lara et al., 2014](#)). It should be also kept in mind that cuticles are composed mostly by hydrophobic compounds, which favor the retention of pesticides. The sanitary quality of produce might be therefore compromised, as the persistence of high levels of pesticide residues may have detrimental implications for consumers' health. An exhaustive examination of all these associations would thus be of the utmost importance.

2.1 Water Loss

As the chemical nature of the main cuticular components is largely hydrophobic, protection against water loss has been considered historically the key role of fruit cuticles. Intracuticular waxes are indeed central in minimizing nonstomatal water loss across the cuticle, which originates from fruit respiration and from diffusion through the cuticular layer. Diffusion is facilitated by the existence of a water potential gradient between the internal and external sides of the fruit, which depends to a great extent on the surrounding temperature and humidity conditions. Even so, species-, cultivar-, and maturity-related differences in cuticular permeability to water exist, which are related to the genetic background, to the presence of pores and cracks, to cuticle thickness, to the chemical nature of cuticular components, and to the cross-links established among them (Gibert et al., 2010; Knoche et al., 2000; Lownds et al., 1993, 1994; Maguire et al., 1999).

Contrarily to the common idea, there is no concluding experimental proof of a major role for cuticle thickness on weight loss after fruit harvest or storage, as contradictory results have been reported on this issue. Genetic background is reportedly an important factor for the determination of this attribute; for example, profound differences in the cuticle structure and thickness of the wax layer were detected during long-term storage under ultralow oxygen (ULO) of three apple cultivars (“Elstar,” “Jonagold,” and “Jonagored”) characterized by substantially different water loss rates and diffusion properties of the cuticle. The wax layer smoothed to cover the cracks generated during storage, likely as a protection against moisture loss, and the wax penetrated into the cutin layer. The diffusion coefficient of wax was more than 100 times smaller than that of cutin.

Particular cuticle components have a role on cuticle permeability to water. Waxes have been generally reported as the major cuticular components for its function as a water-proofing barrier (Fernández et al., 2011; Kissinger et al., 2005; Nadakuduti et al., 2012), particularly the intracuticular wax layer. Long and very long-chain *n*-alkanes define the core properties of this wax fraction, apparently modulated by the presence of triterpenoids (Leide et al., 2007; Vogg et al., 2004). Reduced wax alkanes and enhanced triterpenoids would lead to an increase in the amorphous portion of the cuticular waxes thus impairing cuticle function as a barrier against water loss. In this view, the role of cutin would be limited to that of a framework into which intracuticular wax compounds could adopt a functional structure as an impermeable barrier. Accordingly, the ratio of *n*-alkanes to

isoprenoid compounds (including triterpenoids as well as sterols) was correlated inversely to dehydration in tomato (Isaacson et al., 2009) and pepper (Parsons et al., 2012) fruit. Similar observations were reported for peach (Belge et al., 2014a) and sweet cherry (Belge et al., 2014b).

Additionally, a recent work on *Arabidopsis* found unexpectedly that cuticle not only acts as a waterproofing barrier, but that it also mediates osmotic stress signaling and tolerance through the regulation of abscisic acid (ABA) biosynthesis (Wang et al., 2011b). Whether or not these connections might be also operative in fleshy fruits remains to be investigated in the future, but at any rate this finding opens an unpredicted research field on cuticle functionality.

2.2 Susceptibility to Infections and Physiological Disorders

The resistance of fruit to infections declines during maturation as well as after harvest. Although many pathogen organisms enter the fruit through microcracks, small wounds, lenticels or stomata, some of them are also capable to directly penetrate the cuticle. Cuticular properties will therefore determine, at least partially, how easily that pathogen will be able to cause a breach in this outer barrier. In contrast to dehydration, the relevance of cuticle thickness for resistance to infections has been well established in a number of fruit species, including apple (Konarska, 2012), cranberry (*Vaccinium oxycoccos* L.) (Özgen et al., 2002), stone fruit (Crisosto et al., 1997), and table grapes (Marois et al., 1986; Mlikota Gabler et al., 2003). In addition to cuticle thickness, the composition and structure of cutin, rather than those of waxes, have been suggested as good indicators of susceptibility to infections in grapes (Comménil et al., 1997; Mlikota Gabler et al., 2005), peach (Bostock et al., 1999), and tomato (Isaacson et al., 2009; Shi et al., 2013). In tomato, high susceptibility to fungal infection has been demonstrated to relate to lower contents of particular C₁₆ cutin monomers and, at early maturity stages, also of phenolic compounds, such as benzoic and *trans*-coumaric acids (Shi et al., 2013).

The skin of a fruit includes the cuticle, the epidermis, and the subepidermal tissues. This outer surface can develop cracks when submitted to a degree of strain surpassing its mechanical strength. The severity of these cracks is related to the structure of surface waxes (Faust and Shear, 1972). Depending on their extent, cracks can be detrimental for fruit appearance, and in any case they accelerate water loss rates, favor oxidative processes and thus tissue browning, and create an entry for pathogens and for the uptake of minerals. Heat-treated apples absorb less calcium than untreated fruit,

apparently owing to partial covering of surface cracks as a consequence of softening of epicuticular waxes (Lurie et al., 1996; Roy et al., 1994). Cracks or microcracks in the cuticle, in turn, give rise reportedly to other alterations, such as russetting and lenticel breakdown, or the skin spot disorder to which some apple cultivars are prone (Grimm et al., 2012). Flesh browning in cold-stored apple fruit has been found to be related to cuticle density, which may limit gas diffusion through fruit tissues hence giving rise to altered O₂ and CO₂ levels in the fruit cortex (Jobling, 2002).

Alterations in the epicuticular waxes, particularly those in their morphology, are also key for the development of other disorders, including skin discoloration in peach and nectarine (Crisosto et al., 1993), and peel pitting in “Fortune” mandarins (*Citrus reticulata* Blanco) (Vercher et al., 1994) and orange (*Citrus × sinensis* Osbeck) (Cajuste et al., 2010). In the case of rind staining of orange and mandarin fruit, though, compositional alterations in the epicuticular wax layer, rather than total wax amount or morphology, have been suggested to associate to the development of the alteration (Sala et al., 1992, Sala, 2000). Specifically, rind staining of mandarins is related to lower content of *n*-alkanes and esters, and to higher amount of ketones and fatty acids (Sala, 2000). Increased content of *n*-alkanes, in addition to morphological changes in epicuticular waxes, have also been suggested to mediate the development of chilling injury in grapefruit (*Citrus × paradisi* Macfad.) (McDonald et al., 1993).

In addition to epicuticular waxes, quantitatively minor cuticular components, such as free phenolics, have been reported to play a role on the development of particular physiological disorders. These compounds may protect apple fruit against the development of superficial scald owing to their antioxidant activity, which helps inhibiting the formation of conjugated trienes (Ju and Bramlage, 2000).

2.3 Firmness and Mechanical Support

Fruit texture depends mainly on mechanical factors, and particularly on tissue firmness. Historically, research efforts to elucidate the biochemical mechanisms of ripening-related and postharvest fruit softening have focused mainly on the modifications in cell walls. However, the cuticle also provides structural support to fruit, especially to those types lacking hard internal tissue. Cuticle composition and architecture may therefore play a substantial role in determining fruit firmness changes of fruit, and indeed the contribution of skin to the mechanical properties of fruit is being increasingly recognized (Bargel and Neinhuis, 2005; Costa, 2016). In addition,

mechanical properties of cuticle are altered according to storage conditions, such as temperature and relative humidity (Edelmann et al., 2005; Matas et al., 2005), and this may represent a capital factor in postharvest firmness changes.

Although the structure–function relationships between biomechanical attributes of fruit and cuticular composition and structural arrangements have not been intensively explored, a correlation has been found between the amount of phenolic compounds and the rigidity of the cutin matrix in fully ripe tomato fruit (López-Casado et al., 2007) whereas the polysaccharide components of the cell walls of the epidermal tissues beneath the cuticular layer would provide the elastic characteristics, such as those related to deformation or fracture, as also reported for a wide range of persimmon varieties (Tsubaki et al., 2012).

Another mechanism through which cuticular properties may partly determine fruit firmness is the modulation of cell turgor. For example, blueberries display only minor postharvest changes in cell walls, and moisture loss has been actually identified recently as the major cause of firmness changes in these fruit (Paniagua et al., 2013). Relationships between turgor loss and mechanical properties have also been found in apple and nectarine fruit (Heyes and Sealey, 1996; Lin and Pitt, 1986). Similarly, the detailed characterization of the tomato mutant DFD (*delayed fruit deterioration*), showed strongly lowered transpirational water loss and enhanced cell turgor associated to the preservation of high firmness levels and to high resistance to opportunistic pathogens in comparison to the normally softening fruit of the “Ailsa Craig” cultivar (Saladié et al., 2007). Interestingly, though, the degree of cell wall disassembly, cell-to-cell adhesion and cell wall-related gene expression were similar between the mutant and the control varieties, whereas remarkable differences in fruit cuticle composition and structure were observed.

3 DEVELOPMENT OF FRUIT CUTICLE DURING ON-PLANT RIPENING AND AFTER HARVEST

3.1 Biosynthesis of Fruit Cuticle During On-Vine Maturation and Ripening

In general, studies on cuticle deposition along maturation and ripening of fruit *in planta* have focused mainly on morphological or quantitative aspects. In most cases, an arrest in cuticle development has been found at early stages of fruit development, well before the onset of the ripening process. As this

cessation usually takes place before the cell expansion phase has finished, and hence before the final size of the fruit has been attained, the amount of cuticle per unit surface area decreases as fruit expands, leading to less thick cuticles in ripe fruit (Belding et al., 1998; Comménil et al., 1997; Dong et al., 2012; Liu et al., 2012; Rosenquist and Morrison, 1988). As fruit expansion poses appreciable strain forces on the fruit surface, this frequently results in microcracking, which can cause considerable economic losses during handling and commercialization of produce if cracks are severe enough (Becker and Knoche, 2012; Khanal et al., 2011; Knoche and Peschel, 2007a; Knoche et al., 2004; Peschel et al., 2007; Sala et al., 1992). However, even this commonly observed trend does not apply to all species that have been studied, a notorious exception being tomato fruit, for which a continuous increase in cuticular waxes and cutin monomers was reported during fruit development (Kosma et al., 2010). Likewise, cutin accumulation in oranges is reportedly synchronous with fruit expansion, while wax biosynthesis is parallel to fruit maturation (Wang et al., 2016), with a concomitant and significant upregulation of a range of genes involved in the biosynthesis of cutin, wax, and lignin taking place at later developmental stages.

As to the compositional evolution of specific cuticular components along fruit maturation, such information is available for only a handful of species, such as grape (Comménil et al., 1997), apple (Belding et al., 1998; Dong et al., 2012), sweet cherry (Peschel et al., 2007), tomato (Kosma et al., 2010), and orange (Liu et al., 2012; Wang et al., 2016). Taken together, these few reports have indicated clear interspecific differences in the time-course changes of wax and cutin constituents, and hence the unsuitability of any generalization.

From the biochemical and molecular perspectives, research efforts have been even more limited, but some studies on tomato (Isaacson et al., 2009; Leide et al., 2007; Mintz-Oron et al., 2008; Nadakuduti et al., 2012; Shi et al., 2013; Voggt et al., 2004; Yeats et al., 2012a), sweet cherry (Alkio et al., 2012), apple (Albert et al., 2013), and orange (Wang et al., 2016) have led to the identification of genes putatively related in the biosynthesis of cuticular waxes or cutin monomers in fruit surfaces during on-plant development, which should be taken as a starting point for further, more intensive research work. Such putative genes include for instance very long-chain fatty acid β -ketoacyl-CoA synthases, required for the biosynthesis of very long-chain ($>C_{30}$) *n*-alkanes and aldehydes (Voggt et al., 2004), and demonstrated to display progressively increased expression throughout in planta development of tomato fruit (Leide et al., 2007; Mintz-Oron et al., 2008;

Vogg et al., 2004), as well as additional genes involved both in wax and in cutin deposition (Albert et al., 2013; Alkio et al., 2012; Isaacson et al., 2009; Nadakuduti et al., 2012; Shi et al., 2013; Wang et al., 2016; Yeats et al., 2012b), cuticle-related transcription factors (Albert et al., 2013; Alkio et al., 2012; Shi et al., 2013; Wang et al., 2016), and cuticular lipid transporters (Albert et al., 2013; Alkio et al., 2012; Wang et al., 2016).

Given the quantitative prominence of triterpenoids in the wax fraction of fruit cuticles, research interest has also been placed on the isolation of oxidosqualene cyclases (OSC), which catalyse the cyclization of 2,3-epoxysqualene, the first committed step in triterpenoid biosynthesis (Thimmappa et al., 2014). As a result, two oxidosqualene cyclases involved in the biosynthesis of triterpene alcohols and expressed exclusively in fruit epidermis have been isolated in tomato fruit, and one of them characterized as a product-specific β -amyirin synthase (Wang et al., 2011a). Triterpene synthases have been also isolated and characterized in “Royal Gala” apple fruit, and one of them demonstrated to be primarily an α -amyirin synthase (Brendolise et al., 2011).

This overview illustrates the profound gaps in the current knowledge of almost all aspects of cuticle development in fruit commodities. However, these recent findings should provide a solid basis for further research and for a substantial widening of the current knowledge of cuticle formation in these plant organs, which is a necessary starting point for any postharvest strategy devised for the optimization of postharvest handling and storage.

3.2 Postharvest Changes in Fruit Cuticles: What Do We Know?

With the exception of the few published reports on the changes in the cuticular composition, structure, or properties in fruit stored under refrigeration (see Section 4), the information on the fate of cuticular components after harvest is virtually inexistent. However, fruit cuticles keep evolving once the produce is separated from the vine, even if not submitted to cold storage or to any postharvest treatment.

Total as well as soft epicuticular waxes were monitored during postharvest shelf life of “Navelate” orange fruit at 22°C for 3 weeks. However, although some increases were observed, they were not statistically significant (Cajuste et al., 2010). The presence of ethylene may be required for wax formation, as removing fruit from exposure to 2 μ L/L ethylene led to the development of cracks in surface waxes in comparison to those oranges held continuously under ethylene. Ethylene-treated fruit, additionally, displayed higher amounts of total and soft waxes in comparison with the

controls, although significant differences between both batches of samples were observed only 24 days after harvest. Changes in the epicuticular wax morphology of nontreated fruit were suggested to account for their increased susceptibility to peel pitting and to infection by *Penicillium digitatum*. Hence, ethylene-induced production of new waxes would be a possible protecting mechanism contributing to the lessening of the incidence of these alterations after harvest.

When the chemical composition of cuticles of “Ailsa Craig” tomato fruit harvested at the mature green (MG) and the red ripe (RR) stages and kept at 20°C for 9 days was analyzed, it was found that the total wax amount increased during postharvest ripening (604.2 vs. 910.5 $\mu\text{g}/\text{cm}^2$, respectively) (Saladié et al., 2007). Strong increases were found in *n*-alkanes and *n*-alkadienes, as well as in amyrins, although in the latter case only in absolute terms, while their percentage over total waxes remained steady at around 17.5%. Total cutin amounts per unit surface area (micrograms per square centimeter) did not differ significantly between both maturity stages considered. When the individual cutin monomers were isolated and identified, though, a significantly increased percentage of 18-hydroxy-octadecanoic acid and a decline in that of 9,10,18-trihydroxy-octadecenoic acid over total cutin monomers were observed from the MG to the RR stages. The levels of total flavonoids, as well as of the flavonoid precursor naringenin chalcone, analyzed both in isolated cuticles and in the pericarp tissue from which the cuticle and the outer epidermal layers had been removed, also showed considerable modifications between the MG and the RR stages. The flavonoids kaempferol and quercetin were found in isolated cuticles of MG fruit. At the RR stage, the levels of the precursor naringenin chalcone were roughly 300-fold higher in isolated cuticles than in the pericarp tissues, which shows tissue-specific biosynthesis or deposition of particular compounds. Some mechanical attributes of the isolated cuticles, such as extensibility, yield stress, and viscoelasticity showed significant differences according to the ripening stage, suggesting a relationship to the observed compositional changes.

Cultivar-to-cultivar variation in the compositional changes of cuticular waxes and cutin monomers after harvest has also been found. For example, we compared the modifications in the chemical composition of cuticles of “October Sun” and “Jesca” peaches, a melting- and a nonmelting cultivar, respectively, after remaining at 20°C during 5 days after harvest (Belge et al., 2014a). No significant differences were found in total cuticle amounts (micrograms per square centimeter) of “October Sun” fruit

between days 0 and 5 after harvest, whereas those in “Jesca” increased more than 25%. This increase arose from augmented yields of cuticular waxes and cutin, both in absolute and in relative (percent over total amounts) terms. For waxes, significant differences were observed for triterpenes, *n*-alkanes, and fatty acids, in quantitative terms the three main chemical families of wax compounds identified in isolated cuticles. Regarding cutin monomers, significant increases in the amount of monocarboxylic, hydroxy-, and dicarboxylic acids were also found for “Jesca,” while the levels in “October Sun” cuticles remained unchanged with respect to those at harvest.

Substantial differences in total cuticle loads and in the fate of particular cuticular components after harvest were also found between two cultivars of sweet cherry, another *Prunus* species (Belge et al., 2014b). A 70% increase in total cuticle load was observed for “Celeste” fruit after being kept at 20°C for three days after harvest, whereas no significant differences were detected for “Somerset” cherries. For “Celeste” cherries, the percentage of triterpenes and *n*-alkanes was significantly augmented, while that for fatty acids declined. On the contrary, the percentage of the main wax families remained unchanged in “Somerset” cuticles after harvest. However, since the percentage of cutin over total cuticle amount increased, active biosynthesis formation of new cutin monomers must have taken place during the experimental period.

For both peaches and sweet cherries, substantial differences in key attributes of fruit quality, such as firmness and weight loss were observed (Belge et al., 2014a,b). The question arises whether dissimilar cuticular composition may have accounted, at least partially, for the determination of these differences. Given the wide range of fruit quality traits impacted by cuticle composition, structure and/or mechanical properties, more research efforts will be needed to shed light on the influence of different factors on cuticle features and thus on fruit attributes.

4 POSTHARVEST PROCEDURES: A SUMMARY OF REPORTED EFFECTS ON FRUIT CUTICLE PROPERTIES

Postharvest handling alters fruit cuticle properties, the most conspicuous and simple procedure being fruit storage itself. Yet, postharvest management of fruit commodities is not limited to the control of temperature and humidity. Historically, many chemical and physical postharvest treatments have been assayed for their effectivity in delaying quality loss of fruit produce during storage, transportation, and commercialization. Some of them

have become a widespread practice in the postharvest handling of particular fruit species. However, the impact of these treatments on compositional or structural features of fruit cuticles has been considered in only a very few instances.

4.1 Temperature and Relative Humidity in the Storage Environment

Postharvest storage implies a strict control of environmental temperature and humidity in the storing chamber, and it is known that cuticle properties are largely influenced by these two factors (Edelmann et al., 2005; Matas et al., 2005). For example, when fruit cuticles from mature tomato fruit were examined for rheological properties, remarkable differences were observed between dry and rehydrated samples. After being dipped in distilled water, extensibility and plasticity of cuticles increased. Furthermore, the rheological properties of cuticles were also sensitive to minute temperature differences in the range from 7 to 30°C. The alterations in extensibility were unrelated to cuticle thickness or ultrastructure, and no significant differences in these properties were found between fruit cuticle and fruit skin (Edelmann et al., 2005). These data suggest that tomato fruit cuticles profoundly impact the mechanical attributes of the whole organ, which clearly points out interesting possibilities, such as an influence on postharvest changes in fruit firmness, resistance to mechanical damage, or susceptibility to biotic attack. Analogous results were obtained in a concurrent study in which the mechanical properties of tomato fruit cuticles were investigated for their dependence on temperature and relative humidity in a range of 10–45°C, and 40% to wet (immersion in aqueous solution), respectively (Matas et al., 2005). Isolated cuticles were submitted to uniaxial tension stress, in order to assess several essential mechanical properties including tensile modulus, breaking stress or maximum elongation. These tests showed that stress–strain curves of tomato fruit cuticles were biphasic when humidity values were below wet conditions, but monophasic when cuticles were wet. Whereas maximum elongation was independent of relative humidity and temperature, temperature decreased pure elastic strain and breaking stress. This response was also biphasic, consisting of two temperature-independent phases separated by a transition temperature related to the presence of a secondary phase transition in the cutin matrix of the cuticle.

No data on hypothetical modifications in chemical composition of cuticular waxes and cutin monomers in response to the different humidity

and temperature conditions applied were reported. It is thus not possible to speculate whether the observed changes in these mechanical characteristics were accompanied by or related partially to compositional alterations in fruit cuticles, or rather that they arose simply from the expectable humidity- and temperature-associated modifications in cuticle structure or physical properties. Nevertheless, these experimental data are also interesting in the light of recent findings that water loss patterns from far less studied fruit species, such as litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour.) are also biphasic over a wide range of relative humidity values (0%–80%) (Riederer et al., 2015). These two fruit species belong to the Sapindaceae family, some of whose members, among which litchi and longan, do not share the typical pericarp structure of most mature fruits, which display a unique cuticle covering their outer surface. In these fruit species, rather, the whole pericarp develops into a water loss-protective, stomatous structure, which encloses the edible aril. Both the outer and the inner surfaces of this pericarp are covered by cuticles, showing distinctive chemical composition of cuticular waxes and cutin, and representing two in-series resistances against water loss. This design exemplifies an alternative evolutionary strategy to prevent dehydration of fruits, and illustrates the complexity of water-proofing of aerial plant organs. In both fruit species, exocarp cuticles contained more waxes than endocarp cuticles, and cutin analysis revealed the presence of lignin and suberin monomers in addition to the usual C₁₆ and C₁₈ cutin components, which may reinforce the transpiration barrier function of cuticles. The mechanistic model developed also revealed that water loss-barrier properties of the exocarp cuticles depended closely on the degree of hydration of the pericarp, which in turn was determined by the external relative humidity, while those of the endocarp were not affected by this environmental factor.

4.2 Postharvest Treatments

Some published studies on changes in fruit cuticle composition in response to particular postharvest procedures are available for apple fruit. To further complicate this issue, these reports have made clear that, in addition to the already mentioned cultivar-to-cultivar variation in cuticle composition (see Section 1.2), different cultivars also seem to respond differently to the investigated postharvest factors or treatments. For instance, no changes in total wax amount or composition were found for “Sturmer” apples over 9 months storage at 3°C, while total wax amount increased in “Granny Smith” and “Dougherty” samples (Morice and Shortland, 1973). No compositional changes were observed either for “Sturmer,” but notorious modifications

took place for the other two cultivars, particularly for “Granny Smith” and especially in relation to fatty acid composition. Furthermore, total wax amount decreased in “Red Fuji” apples along storage at 0°C for up to 7 months, and wax composition was also modified (Dong et al., 2012). Decreased amounts of quantitatively prominent *n*-alkanes underlay this decline in total waxes, whereas the fate of other compounds, such as alcohols or ketones throughout storage, was found to depend on its duration.

Significant modifications in the composition of cuticular waxes were also found for sweet cherry (Belge et al., 2014b), although in this case the storage period was considerably shorter due to the limited storage potential of this produce. Fruit of the red-fleshed cultivar “Celeste” fruit were kept at 0°C for 2 weeks. The yields of cuticle per surface unit increased after being kept under refrigeration for as short as 7 and 14 days under refrigerated storage, in parallel to significant decreases in total wax yield and wax percentage over total cuticle amount. After 2 weeks at 0°C, the amount of triterpenes increased significantly with respect to that at harvest, whereas that of free fatty acids showed a sharp decline from roughly 13% at harvest to 3.4% upon removal from cold storage (Fig. 4.3A–B). For cutin composition,

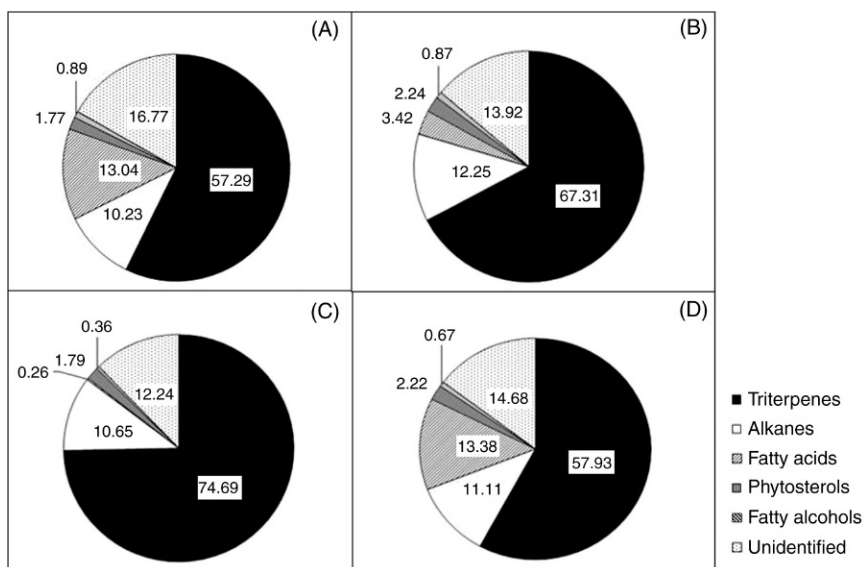


Figure 4.3 Wax compound types (percent over total waxes) identified in cuticles isolated from “Celeste” sweet cherry fruit at a commercial harvest (A), after 14 days cold storage (B), and after 14 days cold storage following CO₂ (20 kPa, 48 h) (C), and hot air (50°C, 45 min) (D) shocks. (A and B, drawn from data reported in Belge et al., 2014b; C and D, Belge et al., unpublished data).

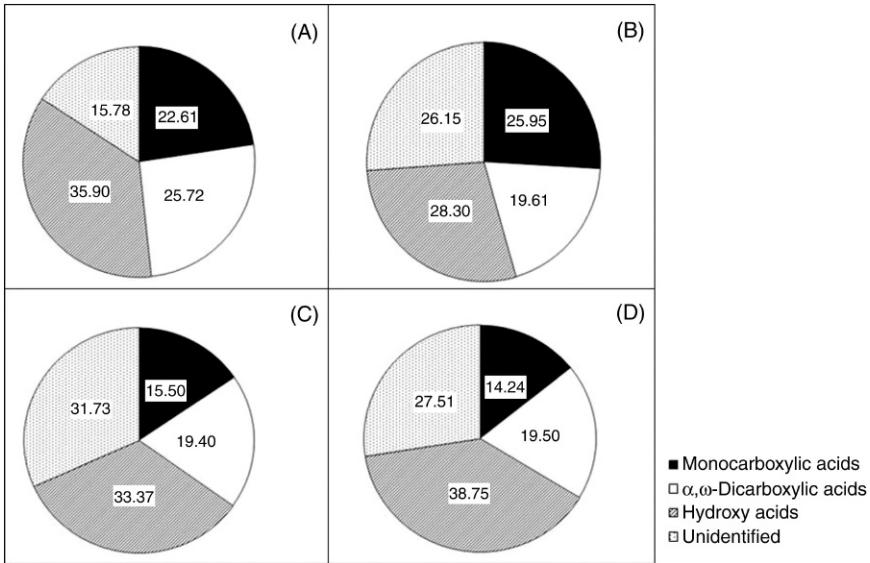


Figure 4.4 Cutin monomer types (percent over total cutin content) identified in cuticles isolated from “Celeste” sweet cherry fruit at commercial harvest (A), after 14 days cold storage (B), and after 14 days cold storage following CO₂ (20 kPa, 48 h) (C), and hot air (50°C, 45 min) (D) shocks. (A and B, drawn from data reported in [Belge et al., 2014b](#); C and D, [Belge et al., unpublished data](#)).

substantial decreases in the amount of hydroxy acids (36% at harvest to 28% after 2 weeks at 0°C) as well as in dicarboxylic acids (25.7% vs. 19.6%, in the same order) were observed (Fig. 4.4A–B). Cutin metabolism must have been profoundly affected, since the percentage of unidentified compounds augmented to more than one-quarter of total cutin after refrigerated storage. The relationships between these modifications and the changes in fruit quality are worth of a thorough examination in future works because it is highly likely that they have affected the mechanical and water-loss proofing properties of the fruit. For instance, the ratio *n*-alkane to triterpenes + phytosterols clearly decreased after cold storage, and this ratio has been suggested to relate to water loss ([Isacson et al., 2009](#); [Parsons et al., 2012](#)), as water-loss barrier properties depend largely on the structure of crystalline waxes, while triterpenes and phytosterols are amorphous waxes. Similarly, and regarding cutin monomers, hydroxy acids, and dicarboxylic acids are important for the establishment of cross-links among the monomers, hence reinforcing the cutin matrix. Since the percentage of both cutin monomer families decreased remarkably after cold storage, the properties of the cuticular layer as a whole must have been sharply altered.

4.2.1 *Controlled and Modified Atmospheres*

Modification of storage atmosphere is also a widespread postharvest procedure, particularly for commodities, such as apple or pear (*Pyrus communis* L.). It is hence reasonable to venture that such environmental conditions may alter substantially the surface properties of the fruit, particularly under very restrictive oxygen concentrations, or for very long-term storage periods, which are common for some varieties. Indeed, wax properties, structure, and chemical composition of “Elstar,” “Jonagold,” and “Jonagored” apple fruit were affected in response to controlled atmosphere under ultralow oxygen up to 9 months, as well as during the subsequent shelf-life period (Veraverbeke et al., 2001), these effects being the more conspicuous for the longest storage periods. Alkanes and esters were the most impacted wax families. Augmented contents of free fatty acids and of quantitatively important alcohols, such as nonacosan-10-ol, suggest that rates of ester hydrolysis increased over prolonged storage.

We have also studied the fate of the chemical components of fruit cuticle of peach and sweet cherry following CO₂ shocks. CO₂ shocks (10, 20, and 30 kPa) were applied at 0°C during 48 h, and cuticles analyzed after enzymatic isolation (Belge et al., unpublished data). Substantial changes were found for important wax compounds and cutin monomers after cold storage in comparison with those at harvest and with those in untreated fruit. Results for the main families of cuticular waxes (Fig. 4.3) and cutin monomers (Fig. 4.4) in “Celeste” sweet cherry submitted to 20 kPa CO₂ shocks are shown as an example. Profound modifications were observed in the amount of the different wax families identified. The most noticeable one referred to the percentage of triterpenes, which increased to roughly 75% of total waxes, while free fatty acids decreased from 13% at harvest (Fig. 4.3A) to a virtually insignificant 0.26% in treated fruit (Fig. 4.3C). Among cutin monomer families, mono- and dicarboxylic acids declined noticeably in treated fruit in comparison with amounts at harvest (Fig. 4.4A and C), while the percentage of unidentified compounds more than doubled in treated samples, which clearly indicates intense modifications in cuticle-related metabolism, deposition and structure, and hence in cuticular properties. Again, it must be stressed that the consequences of such alterations on fruit properties need to be explored in detail in order to gain insight on the actual roles of individual cuticle components on fruit quality attributes.

4.2.2 *Ethylene and Ethylene-Suppressing Treatments*

When “Red Fuji” apples were kept under cold storage at 0°C for 7 months, the amount of *n*-nonacosane, a prominent component of the

n-alkane fraction of cuticular waxes in apple fruit, declined sharply (Dong et al., 2012). However, this decrease was noticeably attenuated in fruit submitted to a postharvest treatment with 1-methylcyclopropene (1-MCP), as well as the increases in nonacosan-10-ol and nonacosan-10-one levels observed for untreated fruit. Delayed development of particular wax constituents was also observed in 1-MCP-treated “Autumn Gold” and “Royal Gala” apples during cold storage for 6 months (Curry, 2008), but most of them could not be identified unambiguously. These findings clearly suggest that wax composition of a number of apple cultivars may be an ethylene-dependent attribute. Interestingly, though, treatment of “Navelate” oranges with 2 μ L/L ethylene increased the content of epicuticular waxes and induced structural changes in surface waxes during shelf life at 22°C for to 3 weeks (Cajuste et al., 2010), which agrees with observations for apple fruit. This is remarkable, as it indicates that cuticle composition and structure of a nonclimacteric fruit species may also be under ethylene control. Since the skin of citrus fruit is usually degreened by means of an ethylene treatment, results point to a relevant role for ethylene in the development not only of color, but also of additional fruit surface attributes.

4.2.3 Miscellaneous Postharvest Treatments

To our knowledge, no published studies have addressed compositional changes in fruit cuticles in response to other postharvest treatments. In recent studies in our laboratory, we have explored the effects of some procedures, such as the application of heat shocks, calcium dips, or methyl jasmonate treatments, on cuticle composition of peach and sweet cherry, but results are currently being processed and will not be shown herein. However, preliminary data obtained for “Celeste” sweet cherry submitted to a hot air shock (50°C, 45 min) are presented (Figs. 4.3 and 4.4). While the effects of the hot air treatment on the abundance of the main families of cuticular waxes were moderate in comparison with amounts at harvest (Fig. 4.3A and D), those on the main cutin monomer classes were profound, particularly for mono- and dicarboxylic acids, which decreased substantially (Fig. 4.4A and D). Similarly to the observations for CO₂-treated fruit (Fig. 4.4C), the amount of unidentified compounds increased in comparison to that at harvest, indicative of deep alterations in cuticle biosynthetic pathways. The significance of these changes remains to be explored and fully understood.

This brief survey exemplifies the many gaps still remaining for a good comprehension of fruit cuticles. Not only chemical composition itself needs to be studied on a case-by-case basis, but also biosynthetic pathways,

deposition dynamics, relevance for particular quality attributes, as well as the impact of environmental conditions and postharvest treatments, need to be explored. This knowledge would allow the improvement of produce handling and commercialization with the aim of attaining better fruit quality and prolonged storage potential. This is a main goal to be achieved in the future, and it opens a wide research field with obvious practical applications.

5 PREHARVEST TREATMENTS: A FEASIBLE TOOL TO OPTIMIZE FRUIT CUTICLE PROPERTIES FOR IMPROVED POSTHARVEST PERFORMANCE?

So far, the reported impact of particular postharvest procedures or treatments on cuticle composition of a handful of fruit commodities has been summarized. The available information, albeit very incomplete, indicates that fruit cuticles are highly responsive to endogenous and exogenous factors. Therefore, the question arises whether particular preharvest treatments would be a feasible tool to improve postharvest performance of these commodities through the modulation of cuticular composition and/or structure. Unfortunately, the information on the influence of preharvest treatments on cuticle properties is even more limited than that on postharvest procedures. However, a few published studies exist, which are briefly described next.

Developing tomato fruit were treated at different stages from fruit set to maturity with different growth regulators, including gibberellins (GA), the cytokinin benzyladenine (BA), and forchlorfenuron (CPPU), and harvested at maturity. Gibberellins increased the amount of cuticle per unit surface area, the youngest fruit being most responsive to the treatment ([Knoche and Peschel, 2007b](#)). This is interesting, as an increase in cuticle load during fruit expansion would help resisting or mitigate the subsequent increase in elastic and plastic strain, and thus reduce the formation of microcracks on the surface of the fruit. Since the russetting disorder results from the formation of periderm to lessen the negative consequences of these microcracks, preharvest GA treatments might have the potential to alleviate two important disorders which affect importantly the surface of the fruit, and hence its appearance, an essential purchase-motivating quality attribute.

These results were corroborated in later works on “Golden Delicious” apples, a russetting-susceptible cultivar: fruit treated with GA had fewer cuticle microcracks and lower russetting incidence, and treatment effects were more marked at early stages of fruit development ([Knoche et al., 2011](#)). However, the observation that the GA treatment did not have significant

effects on deposition, strain, or rheological properties of the cuticle led the authors to suggest that GA effect was exerted rather on the epi- and hypodermal tissues beneath the cuticle, maybe through the increase of cell division and cell expansion rates resulting in more cell wall materials per unit surface area and hence in stronger mechanical support. Accordingly, orchard sprays with a mixture of BA and GA reduce russeting and skin cracking in apple fruit by maintaining a higher number of epidermal cells in comparison to unsprayed fruit (reviewed in [Ginzberg and Stern, 2016](#)). Similar experiments carried out on table grapes also demonstrated lower incidence of peel cracking in GA-treated fruit but, on the basis that late GA applications were more effective than earlier ones, it was concluded that the process was unrelated to the cell division phase ([Lichter et al., 2015](#)).

However, in addition to possible effects on cell walls of the fruit tissues located immediately below the cuticle, there is also evidence of a close association between the chemical composition of the cuticle and the resistance to cracking. This aspect has been studied in five commercial varieties of sweet cherry, and it was found that cultivars displaying significantly higher concentrations of *n*-nonacosane (C₂₉) were less susceptible to cracking than those with lower amounts ([Ríos et al., 2015](#)). Accordingly, the mechanical properties of tomato cuticle, and hence of the fruit surface, have been demonstrated to depend strongly on the relative amounts of particular components. For instance, the cutin:polysaccharide ratio underlies the viscoelastic properties, while increased stiffness and loss of extensibility are related to flavonoid accumulation and to cutin depolymerization along ripening ([Espanña et al., 2014](#)). Therefore, any preharvest treatment shown to modify the amount of these or other cuticular compounds should also have a substantial impact on the mechanical properties of the fruit. Similarly, the recent finding of a close correlation between cuticle and ABA biosynthesis ([Wang et al., 2011b](#)) opens the possibility of exploring the effects of ABA treatments on cuticle features. Furthermore, ABA has been suggested to regulate wax biosynthesis of “Newhall” orange during on-tree fruit development ([Wang et al., 2016](#)).

Based on these studies, the fruit skin appears as a tissue whose attributes could be manipulated in order to improve resistance to postharvest handling as well as to environmental stress. Indeed, the skin is the first barrier of the fruit against the surrounding conditions. A set of apple cultivars displaying a superior skin contribution to the mechanical properties of fruit could be distinguished in a recent study in which 65 accessions were screened ([Costa, 2016](#)). The question arises as to how important the compositional

and structural characteristics of the cuticular membranes of these apple varieties are in explaining these differences. These aspects will require further, intensive research. Fruit are commodities of considerable commercial value, and the efforts in this direction are likely to bring about important economic returns in the future.

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CHAPTER 5

Influence of Photoselective Shade Nettings on Postharvest Quality of Vegetables

Dharini Sivakumar^{*,**}, John Jifon^{*,**}

^{*}Tshwane University of Technology, Pretoria, Gauteng, South Africa

^{**}Texas A&M AgriLife Research, Vegetable and Fruit Improvement Center, Texas A&M University System, Weslaco, TX, United States

1 INTRODUCTION

High solar radiation, heat stress, drought stress, desiccating winds, and hailstorms are some of the major environmental limitations to optimal productivity and nutritional quality of field-grown crops. Physiological processes, such as stomatal conductance, transpiration, and photosynthesis are sensitive to these stresses. Maximum net CO₂ assimilation of most C₃ species saturates at relatively low irradiance (600–900 μmol/m²/s), which is only 30%–40% of full sunlight (1500–2000 μmol/m²/s) on a typical growing season day. The excess radiant energy predisposes plants to photo inhibition, heat stress, and stomatal closure, resulting in a reduction in net photosynthesis (P_n), the ultimate source of carbohydrate substrate for growth. Sustained high temperatures (35–40°C) as a result of high solar radiation can also impair cell division, leaf expansion, and reproductive development (Flaishmana et al., 2015). In many parts of the world, shading is a preharvest cultural practice that has been used in greenhouse and orchard systems to reduce radiation heat load, increase light-use efficiency (Baille et al., 2006; Jifon and Syvertsen, 2002, 2003; Zhang, 2006), and reduce pest and disease pressures (Glenn et al., 1999). Cohen et al. (1997) obtained similar results for citrus with reflective nets, which increased the downward scattering of the reduced direct radiation below the nets. Nonreflective (white, black, or colored) nets and reflective sprays have also been used to reduce canopy temperatures and relieve water stress (Jifon and Syvertsen, 2003; Stanhill et al., 1976). Jifon and Syvertsen (2000) observed that moderate shading with aluminized netting (Aluminet 50%, Polysack Plastic Industries, Nir Yitzhak, Israel) can reduce leaf temperature and evaporative demand, resulting in increased P_n. Increased P_n by moderate shading could therefore increase yields and

quality through increased carbohydrate supply and improved water use efficiency. Black netting is one of the most widely used shading materials; however, reflective and photosensitive nettings are increasingly being used in commercial production. In addition to reducing radiation intensity, and microclimate thermal properties, these shade nets also have the potential to modify light quality (Shahak, 2008). Nettings, regardless of color, have the potential to reduce the amount of radiation reaching the crops underneath; the higher the shade factor, the more the radiation will be blocked. Reductions in radiation resulting from netting will affect temperatures (air, plant, soil) and the relative humidity. Besides affecting the amount of radiation, nettings can also influence the direction of the radiation (Stamps, 2009) and induce penetration of the light into the inner plant canopy (Gu et al., 2002). It is documented that any shade netting can scatter radiation, especially ultraviolet light because netting is usually made with ultraviolet-resistant plastic (Wong, 1994).

Photosensitive shade netting technology is an emerging agrotechnological concept that aims at combining physical crop protection with different filtrations of solar radiation. They are based on the incorporation of various chromatic additives as well as light dispersive and reflective elements additives that selectively filter solar radiation to promote specific wavelengths of light and modern shade nets are manufactured from woven polypropylene materials or knitted polyethylene materials with different dimensions of fibers and holes to achieve specific shade levels (Arthurs et al., 2013; Milenkovic et al., 2012).

The quality of the light within the photosensitive net can make a substantial difference. “Photosensitive shade netting technology,” has been developed to not only reflect special optical properties that allow the modification of light but with the actual purpose of modifying the spectral light composition (Shahak, 2008), with different light scattering properties that modify the proportions of the R/FR wavelength ratio (Fletcher et al., 2005), providing natural light, dependent gradually on chromatic additives to the plastic, and the knitting pattern (Shahak, 2012; Tinyane et al., 2013). The major purpose for adopting the colored shade netting approach is to extend the harvest season (maturation rate), improve the yield, the product quality, and the overall agro-economic performance of agricultural crops (Shahak, 2008).

Photosensitive nets also have an ability to transform direct light into scattered light, which improves the penetration of light into the inner plant canopy, prevents burning, offers a moderate cooling effect, and influences

pest control (Shahak, 2008). Photoselective ChromatiNet was designed to enhance desirable physiological responses, such as yield, quality, and maturation rate (McElhannon, 2007). Efforts to manipulate plant morphology and physiology using photoselective nets have been ongoing for decades, especially in greenhouse environments that aim at improving yield and quality of the produce by modifying light (quality, quantity) (Milenkovic et al., 2012; Stamps, 2009). The greenhouse environments affect the thermal components of light in the infrared region, thereby enhancing desirable physiological responses, such as yield, quality, and maturation rates, plant compactness and plant morphology (Shahak et al., 2004).

In addition to its direct impact on the plants, the photoselective filtration of sunlight may also affect plant pests and diseases. It is reported that plants grown under yellow net are more susceptible to whiteflies and thrips (Shahak, 2008). Also, the use of photoselective netting is regarded as a common agrotechnological approach, was evaluated with ornamentals (Nissim-Levi et al., 2008), vegetables (Fallik et al., 2009), and fruit trees (Shahak et al., 2004). Shade nets increase light scattering but do not affect the light spectrum that has been shown to increase branching, plant compactness, and the number of flowers per plant (Nissim-Levi et al., 2008). Moreover, photoselective nettings have been shown to influence the retention of bioactive compounds in tomatoes (Selahle et al., 2014), and sweet peppers (Mashabela et al., 2015; Selahle et al., 2015) during postharvest storage. Consumer preferences are higher for fresh vegetables that are absence of decay or insect infestation or mechanical injury, excellent color, firmness and crispiness, flavor and favorable size (Maalekuu et al., 2004). Therefore, this chapter illustrates the improvement of postharvest quality and phytochemical contents in vegetables and herbs using photoselective nets to manipulate the light quality as preharvest tools during production (preharvest stage).

2 PHOTOSELECTIVE NETS

The colored shade cloths are manufactured in the following two color groups “colored-ColorNets” (red, yellow, green, and blue) and “neutral-ColorNets” (pearl, white, and gray), absorbing spectral bands shorter or longer than the visible range (Shahak, 2008). The differences between the color nets are: the blue shade cloth is designed to absorb the UV, R, and FR spectral regions, while enriching the blue spectral region, while the red shade cloth absorbs UV, B, and G and enriches the red and far-red spectral region.

The yellow shade cloth is designed to significantly reduce the UV and B; while enriching the G, Y, R, and FR wavelengths. The white shade cloth absorbs UV and enriches the B, G, Y, R, and FR wavelengths. The pearl and gray shade cloths do not enrich or absorb the different wavelengths. The pearl is designed to scatter the light to a greater extent than the other types of colored shade cloth mentioned earlier (Rajapakse and Shahak, 2007). Black nets are commonly used for crop production with a shading effect of 35%–80%. However, the commercially available black shade cloth used does not have the ability to scatter light at all (Selahle et al., 2014; Shahak, 2008) and the widely used common black nets are completely opaque and the spectral quality of radiation is not modified in any way by the net (Arthurs et al., 2013). Hence, the shading factor is almost directly proportional to the net porosity.

2.1 Impact of Photosensitive Nets on Postharvest Quality of Selected Vegetables After Storage

Green sweet peppers (HTSP-5) grown under the photosensitive pearl and red nets were shown to have higher percentage marketable fruits after postharvest storage (Mashabela et al., 2015). Whereas with tomatoes cultivar Alfa V produced under the pearl photosensitive nets showed higher marketable fruits after postharvest storage (Selahle et al., 2014). The marketability of the fruits was assessed based on the incidence of decay. With regard to the green sweet pepper, marketability of the peppers is affected by ripening. The mature green peppers can begin to ripen during storage conditions and even in retail display shelves. Partially ripened fruit (chocolate or sun tan) have lower market value than the full mature green pepper.

Decrease in color value h° corresponds to the loss of green color as fruit surface color changes from green to greenish yellow. The h° color value declined remarkably in sweet peppers grown under the commonly used black net and moderately under the yellow photosensitive nets after postharvest storage indicating the loss of green coloration and sun tanning (Mashabela et al., 2015). The green–red color coordinate, a^* value increased (positive) with ripening and with increasing red skin color of the fruit. Production of green sweet peppers (HTSP-5) under the pearl and red nets had lower a^* values and retained the lower negative skin color and expressed higher green color after postharvest storage (Mashabela et al., 2015) (Fig. 5.1). Chlorophyll pigment is responsible for the green color in green pepper. Green sweet pepper (HTSP-5) produced under the pearl and red nets, showed higher concentration of chlorophyll in the skin

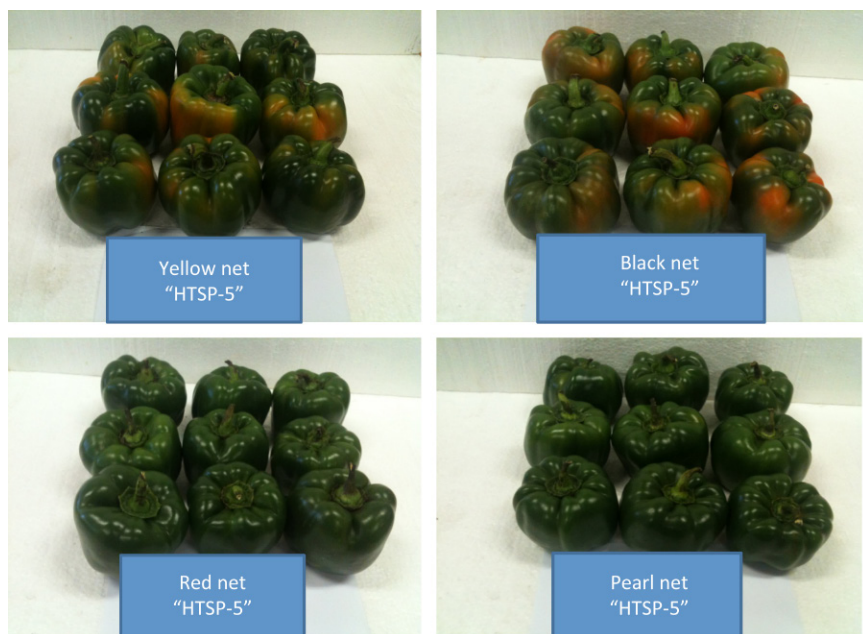


Figure 5.1 Skin color changes of green sweet pepper (HTSP-5) after postharvest storage at 7.5°C, 85% RH, after 14 days and after 5 days at the retailers shelf conditions.

after postharvest storage (Mashabela et al., 2015). Ripening of pepper fruits is linked with the biosynthesis of carotenoid and loss of chlorophyll pigments. Higher far-red/red (FR/R) radiation was reported to influence fruit ripening (Finlayson et al., 1999). Pearl photoselective nets showed higher and lower transmittance of R and FR spectral light and, exposure of green peppers to higher R/FR photon ratio during growth (Fig. 5.2) probably could have suppressed the conversion of biologically active phytochrome, which reduced the expression of genes involved in ripening related changes, including color, biosynthesis of β -carotene, and lycopene during postharvest storage. Moreover, production of yellow sweet pepper “Celaya” under the commercial black net (25% shading) significantly affected the color values h° (lower) and $chroma$ and b^* (increase) in after postharvest storage showing deeper yellow fruit surface (Selahle et al., 2015). Red color (higher a^* color value) was significantly higher in red sweet pepper “HTSP-3” fruits grown under the commercial black nets after postharvest storage further indicating the dark red skin color (Selahle et al., 2015). The higher PAR inside the nets and the higher fruit surface temperatures under the black nets (Mashabela et al., 2015; Tinyane et al., 2013) during production could have favored the

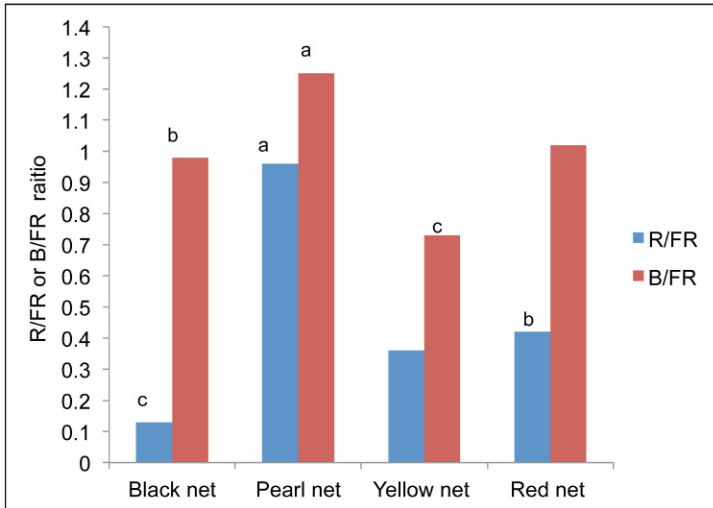


Figure 5.2 Relative transmission of light through the photosensitive nets in comparison to the widely used commercial black net with 25% shading (control) during production of green sweet pepper (HTSP-5). R, red (600–700 nm); FR, far-red (700–800 nm); R/FR, red/far-red ratio; B, blue (400–500 nm); B/FR, blue/far-red ratio. Data are means three nets type of shade net. Means in each bar within the net type with the same letter are not significantly different, $P < 0.05$.

aforementioned changes in tomatoes (Fig. 5.3) and sweet peppers (Fig. 5.4) after postharvest storage.

Tomatoes and red, yellow, and green sweet peppers grown under the photosensitive shade nets resulted in remarkable less weight loss after postharvest storage (Selahle et al., 2014). Also fruit firmness after postharvest storage is higher in tomatoes and sweet peppers produced under the photosensitive shade nets especially under the pearl nets (Mashabela et al., 2015; Selahle et al., 2014). The noted loss of firmness was due to the higher exposure of light intensity (PAR quantity) associated with the increase of fruit surface temperature (Riga et al., 2008). Green sweet peppers and tomatoes produced under the black nets showed less firmness and the yellow nets revealed moderately firm fruits after postharvest storage. Water status of tomato or sweet pepper at harvest affects the retention of firmness during postharvest storage (Saladié et al., 2013) and transpirational water loss is associated to the weight loss. Therefore, the maintenance of fruit firmness depends on reduced weight loss or with the absence of water loss. Loss of firmness during postharvest storage can related to the enzymatic changes linked with ripening (Lurie et al., 1986).

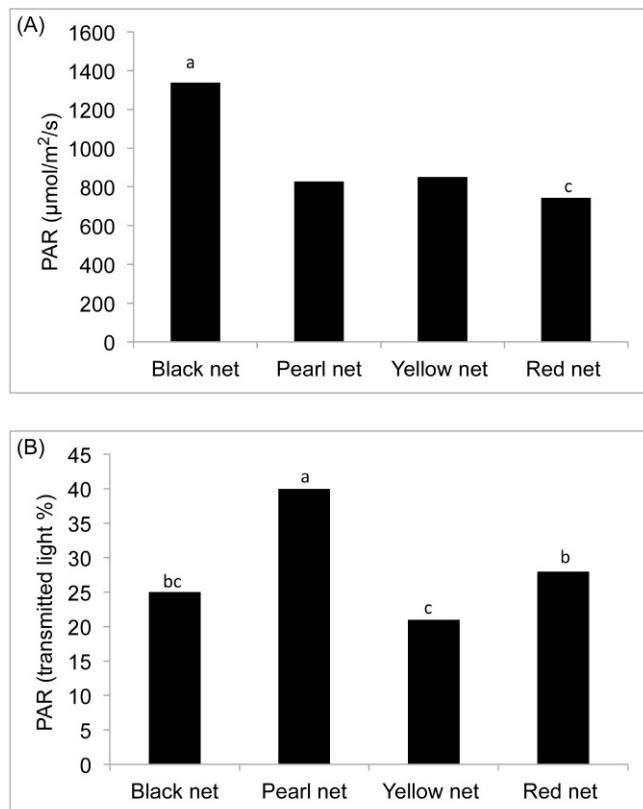
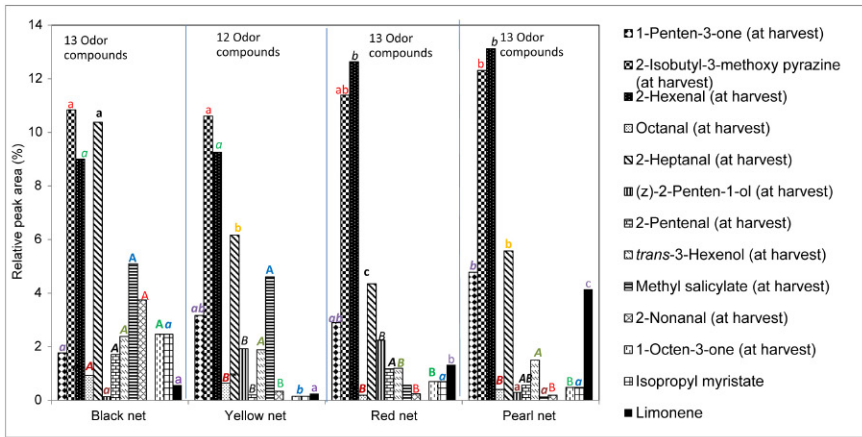


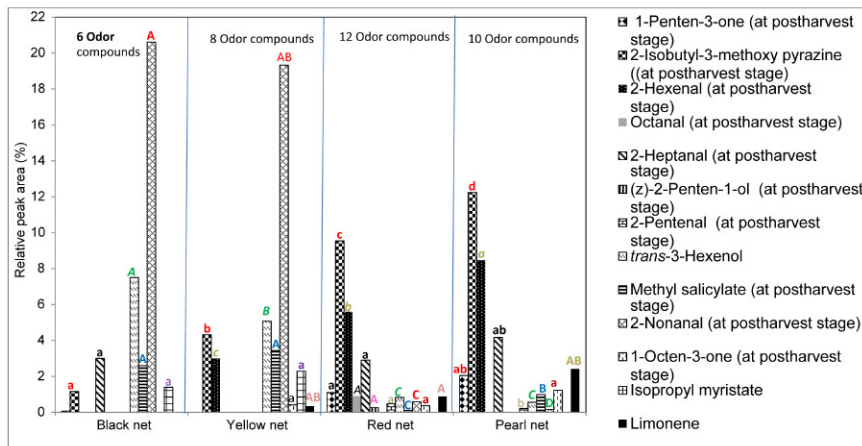
Figure 5.3 (A) Photosynthetic active radiation (PAR) measurements under different photoselective netting and the widely commercially used black net (control net) during production of tomatoes (“Alfa V” and “Irit”). (B) PAR measurements under different photoselective netting and the widely commercially used black net (control net) during production of green sweet pepper (HTSP-5). Data are means three nets type of shade net. Means in each bar within the net type with the same letter are not significantly different, $P < 0.05$.

Fruit firmness is an important retail and consumer quality attribute for many fruit crops as it indicates better texture and shelf life. Also sweet peppers yellow (Celaya) and red (HTSP-3) produced under the commercial (25% shade) black nets showed higher incidence of decay after postharvest storage (Fig. 5.5A–B).

Light intensity and temperature favors the accumulation of sugars during growth in tomatoes and sweet pepper. Generally the currently widely used commercial black nets were shown to lower the SSC/TA ratio during postharvest storage due to higher TA concentration in all



(A)



(B)

Figure 5.4 (A) Influence of photosensitive nets on the influence of odor active compounds in green sweet pepper “HTSP-5” at harvest. (B) Influence of photosensitive nets on the influence of odor active compounds in green sweet pepper “HTSP-5” at postharvest storage. Data are means 20 fruits per type of shade net. Means in each bar with the same bar within the net type with the same letter are not significantly different, $P < 0.05$.

organic acids. The tomato cultivars, AlfaV and Irit produced under the pearl photosensitive nets showed slightly higher SSC/TA ratios (Tinyane et al., 2013). Whereas in green sweet pepper (HTSP-5) after postharvest storage fruits produced under the yellow nets showed higher TSS and SSC/TA ratio (Mashabela et al., 2015). In yellow sweet pepper cultivar “Celaya” produced under the black nets and red sweet pepper

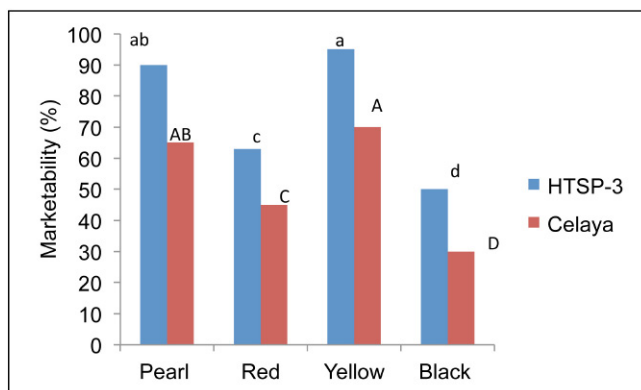


Figure 5.5 Percentage marketability of sweet pepper yellow (*Celaya*) and red (*HTSP-3*) fruit after postharvest storage (7.5°C, 85% RH, for 14 days) and thereafter, at the retailers condition (15°C for 5 days). Data are means 30 replicate bags of fruit (each bag containing 5 fruit) per nets type of shade net (each shade net type had 3 replicates). Means in each bar within the net type with the same letter are not significantly different, $P < 0.05$.

“HTSP-3” grown under the red nets showed higher SSC/TA ratio (Selahle et al., 2015). The increase of SSC/TA ratio is mainly due to the higher temperature maintained under this net slowing down the production of organic acids during fruit growth (Aldrich et al., 2010). This could be due to the direct effect of higher cumulative temperatures under the nets obtained during production. Exposing fruits to higher temperatures (36°C) especially during growth and ripening increases the SSC in tomatoes, and this is mainly due to higher carbohydrate biosynthetic enzyme activity (Walker and Ho, 1977).

2.2 Impact of Photoselective Nets on Phytochemical Contents in Selected Vegetables After Storage

Capsanthin, *cis*-capsanthin, β -carotene, and zeaxanthin are the major carotenoid compounds in peppers (Marín et al., 2004). Also, intensity of red color pigment in red sweet pepper depends on the relative composition of carotenoids, lycopene, and chlorophyll. In yellow pepper, the yellowness depends on the ratio of total chlorophyll and carotenoid concentrations (Roca and Minguez-Mosquera, 2006). The red color of sweet peppers and tomatoes plays a major role in its marketing and in the processing industry. Capsanthin, β -carotene, and lycopene contents were reported to increase with fruit ripening sweet pepper (Hallmann and Rembiałkowska, 2012) and in tomatoes (Grierson and Kader). Production of red sweet peppers

(HTSP-3), yellow sweet peppers Celaya (Selahle et al., 2015) and tomatoes (AlfaV; Irit) (Selahle et al., 2014), under the commercial black nets significantly increased the lycopene content after postharvest storage.

Lycopene biosynthesis is greatly affected by the temperature and favored at different day temperatures from 12 or 32 up to 35°C with an optimal temperature around 22–26°C (Vinson et al., 1998). The excessive sunlight was also shown to inhibit the biosynthesis of lycopene (Baqar and Lee, 1978; Roselló et al., 2011). The temperature under the black net was 30.3°C and the PAR was 1339.33 $\mu\text{mol}/\text{m}^2/\text{s}$ during tomato production in summer (October–February) (Tinyane et al., 2013) (Fig. 5.2). With respect to the sweet pepper production in summer (January–March), the air temperature was 31.7°C and the PAR was 641.5 $\mu\text{mol}/\text{m}^2/\text{s}$ (Selahle et al., 2014) (Fig. 5.3). Accumulation of β -carotenes in fruits produced under the black nets also provides photoprotection and also participates in the removal of reactive oxygen (ROS) species. However, in tomatoes and sweet peppers although the PAR was higher under the black nets than the photoselective nets, the PAR failed to increase the air temperature or the fruits surface temperature mainly due to the knitting pattern of the black nets shown in Figs. 5.3 and 5.4. Tomato cultivars AlfaV and Irit produced under the black and pearl shade net showed higher lycopene accumulation than SCX248 during postharvest storage and this implies that the observed increase could be due to the influence of the genotype on lycopene content of the fruit than the environment (Selahle et al., 2014).

Tomato cultivars, AlfaV and Irit produced under the black shade nets and the red (HTSP-3) and yellow (Celaya) sweet pepper cultivars showed significantly higher total phenolic content after postharvest storage (Selahle et al., 2014, 2015). This phenomenon could be attributed to the stress due to exposure to higher PAR (Fig. 5.4), which would have induced higher synthesis of phenolics by the activities of phenylalanine ammonia-lyase (PAL) (Macheix et al., 1990; Toor and Savage, 2006). However, it was noted that the production of green (HTSP-5), red (HTSP-3), and yellow (Celaya) sweet pepper fruits under the black nets affected the accumulation of total phenols and the concentration of the total phenols declined in these fruits after postharvest storage (Mashabela et al., 2015). Here, modification of light spectral quality was shown to influence the total phenol accumulation in green sweet pepper cultivars after postharvest storage. The reduction of R/FR ratio under the black nets during the growing season (Fig. 5.2) was reported to be responsible for the

decline in total phenolic compounds after postharvest storage (Mashabela et al., 2015). On the contrary, sweet pepper fruits produced under the pearl nets showed higher concentrations of total phenols after postharvest storage. The higher spectral R/FR ratio (Fig. 5.2) under the photoselective pearl net would have stimulated the biosynthesis higher concentration of total phenols at harvest and remained high after postharvest storage (Mashabela et al., 2015). Also the percent transmittance of PAR was higher under the photoselective nets. The higher R/FR ratio and higher percent relative transmission of PAR influenced the total phenolic compounds in red beet (Stagnari et al., 2014).

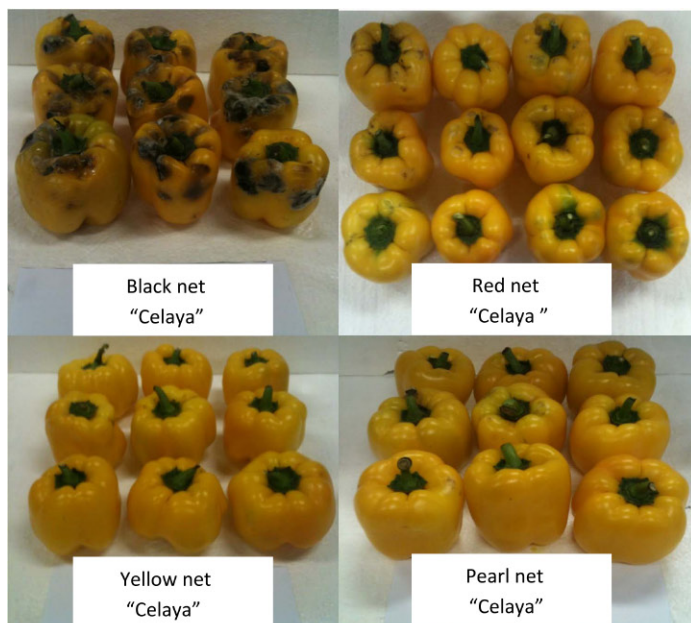
Green sweet pepper (HTSP-5) produced under the yellow nets retained higher flavonoid (quercetin) content after postharvest storage. This was associated to the observed lower PAR transmitted light effect (Fig. 5.2) shown by Mashabela et al. (2015). Higher flavonoid concentrations in baby spinach grown under low transmittance shade nets were also reported by Bergquist et al. (2007). Flavonoids provide photoprotection against light-induced oxidative damage (Materska and Perucka, 2005). Quercetin content declined remarkably with ripening after postharvest storage especially in green peppers produced under the black nets.

Tomatoes (“AlfaV” and “Irit”), sweet peppers; green peppers (HTSP-5), yellow peppers (Celaya) and red peppers (HTSP-3), produced under the pearl nets contained more ascorbic acid content at harvest and retained more after postharvest storage (Mashabela et al., 2015; Selahle et al., 2014). Blue spectral light was increased the ascorbic acid concentration in lettuce by increasing the photosynthesis accumulation of the precursors of ascorbic acid, the hexose and D-glucose sugars (Ohashi-Kaneko et al., 2007).

Higher antioxidant scavenging activity was reported during postharvest storage in cvs. AlfaV and Irit grown under black and pearl shade nets, and cv. SCX 248 grown under red shade nets (Selahle et al., 2014). This increase in antioxidant activity could be due to the accumulation of lycopene, β -carotene, and phenolic compounds during postharvest storage in cvs. AlfaV and Irit grown under the black and pearl shade nets, and cv. SCX 248 produced under the red shade nets. However, green (HTSP-5), red (HTSP-3), and yellow (Celaya) sweet peppers grown under the pearl nets revealed higher antioxidant scavenging activity at harvest (Mashabela et al., 2015; Selahle et al., 2014). Spectral quality under the pearl net improved the percentage of PAR light transmittance and the R/FR ratio (Fig. 5.2). Researchers have proven the effect of red and far-red radiation on the improvement of antioxidant activity in plants (Lee et al., 2003; Wu et al., 2007).

2.3 Odor Active Aroma Volatiles Prior to and After Postharvest Storage in Tomato and Sweet Peppers

Odor active aroma volatiles in green sweet peppers (HTSP-5) produced under different shade nettings at harvest is given in Fig. 5.6A. Comparison of volatile levels at harvest to after postharvest storage reveals many aroma volatiles are lost during postharvest storage and this could be related to fruit maturation (Luning et al., 1994) (Fig. 5.6A–B). Fruits produced under black and yellow nets retained six and eight odor active aroma volatile compounds after postharvest storage (Fig. 5.6B). However, fruits produced under the red nets retained maximum (12) odor active aroma volatiles after postharvest storage. Therefore, retention of odor active volatiles after postharvest storage in green pepper produced under different shade nets showed the following trend: photoselective red net > pearl net > yellow net > black net (commercial control) (Fig. 5.6B). Fruits under the black nets showed higher levels of 2-heptanal, 2-nonanal, 1-octen-3-one, and isopropyl myristate at harvest (Fig. 5.6A) and were probably due to the change in green color (dark green to light green) of the fruit. Changes in aroma volatile compounds with respect to maturity stages were shown by Luning et al. (1995). At harvest 2-isobutyl-3-methoxy pyrazine (nitrogen-sulfur compound) and hexanal (grassy) were found at higher levels in peppers produced under all four nets (Fig. 5.5A). Odor active compounds, 2-isobutyl-3-methoxy pyrazine and hexanal (grassy), were high in peppers produced under all four shade nets at harvest (Fig. 5.6A). During postharvest storage, fruits produced under pearl nets had higher concentrations of 2-isobutyl-3-methoxy pyrazine and hexanal (green grassy aroma), which is consistent with fruits in the green stage. A delay in fruit maturation and ripening under the pearl and red nets due to light quality could be responsible for the higher retention of green grassy odor compounds (hexanal and isobutyl-3-methoxy pyrazine) and the higher number of odor active compounds during postharvest storage. Lipoxygenase activity is known to be responsible for the biosynthesis of aroma volatile compounds C6 alcohols and aldehydes in sweet peppers (Luning et al., 1995). Decline in lipoxygenase activity can affect the biosynthesis of hexanal. However, further detailed investigations are needed to confirm the influence of light quality on the production of aroma volatiles on green sweet peppers. Isobutyl-3-methoxy pyrazine was reported to decrease in concentration with maturity (ripening) and fruit exposed to light was reported to affect accumulation of isobutyl-3-methoxy pyrazine in grape clusters (Ryona et al., 2008). During the growing seasons the PAR transmitted radiation was higher under the pearl nets, but the accumulation of isobutyl-3-methoxy pyrazine was higher during postharvest storage. Light



(A)



(B)

Figure 5.6 (A–B) Skin color changes of green sweet pepper (HTSP-3) after postharvest storage at 7.5°C, 85% RH, after 14 days and after 5 days at the retailers shelf conditions.

quality could have played a role in signaling the isobutyl-3-methoxy pyrazine accumulation. Furthermore, observed higher levels of 2-nonalnal *trans*-3-hexenol compounds after postharvest storage in green peppers produced under black and yellow nets could be due to the sweet pepper turning from green to slightly red (Luning et al., 1995). Methyl salicylate was not found in fruits produced under pearl nets after postharvest storage. The higher FR light (Fig. 5.2) could have affected the methyl salicylate (phenol derivative, green sweet aroma). Flaishmana et al. (2015) also reported that the FR light treatment suppressed the production of methyl salicylate in tomatoes and suggested it could probably be due to the conversion of Pr to biological active Pfr. Our findings also concur with the findings of Loughrin and Kasperbouer (2002) on the improvement of aroma compounds in strawberries grown over the red mulches compared to over the black mulch. Red light treatment was reported to increase the aroma components 2-methyl butanal and 3-methyl-1-butanol in tomatoes, along with a reduction of *cis*-3-hexenal. However, far-red light significantly improved the biosynthesis of *cis*-3-hexenal. Blue light treatments were reported to reduce the emission of 1-hexanol and *trans*-2-hexen-1-ol in blueberries compared to fruits under the white light (control) (Flaishmana et al., 2015).

Furthermore, red sweet peppers HTSP-3 produced under pearl photoselective nets retained the odor active aroma compounds and the sensory properties after postharvest storage. Odor active compounds (E, Z) 2,6-nonadienaldehyde and geranyl acetone were significantly higher in red peppers “HTSP-3” produced under pearl nets after postharvest storage. 3-Carene is only found in “HTSP-3” red peppers grown under the yellow nets after postharvest storage. In red sweet pepper “HTSP-3,” retention of odor active aroma volatiles in fruits produced under different colored shade nettings during postharvest storage showed the following trend: photoselective yellow > photoselective pearl > photoselective red > commercial black nets. Tomato cultivar “AlfaV” grown under the red and pearl nets had a higher number of odor active aroma compounds during postharvest storage.

The untrained taste panelist preferred red sweet pepper aroma for “HTSP-3” peppers grown under yellow and pearl nets (Selahle et al., 2015). The panelists also commented on the sweetness of peppers produced under the red net after storage. Overall acceptance for the red sweet peppers was determined by the panelist based on the red color, absence of decay or defects, and firm texture. The panelists preferred sweet peppers red “HTSP-3” and green “HTSP-5” produced under the pearl nets after postharvest storage (Selahle et al., 2015). Panelist preference was based on the firm texture

of the fruit to show preference for fruits grown under pearl nets than the yellow nets. With respect to the tomatoes, the untrained panelists preferred the fruit of “AlfaV,” grown under all photoselective nets red, pearl, or yellow nets, for its sweetness after postharvest storage (Selahle et al., 2014). The panelist also commented that growing tomato “AlfaV” under the red nets improved the characteristic tomato odor or aroma during postharvest storage (Selahle et al., 2014). Tomatoes AlfaV, Irit, produced under the pearl nets and sweet peppers red “HTSP-3” and yellow “HTSP-5” produced under the pearl and yellow nets showed higher marketability (Fig. 5.6) after postharvest storage at the retailer’s conditions.

3 CONCLUSIONS

It would be more economical to modify the light quality and microclimate (temperature) via the photoselective colored shade nettings to improve yield and the postharvest quality and phytochemical contents. Accumulation of phytochemicals during production of plants depends on many factors, such as light quality, quantity, type of varieties or cultivars, growing season, and metabolic factors. Although, the commercial black nets showed positive effects during the photoselective nets, and producing sweet peppers and tomatoes under the photoselective pearl nets improved the marketable yield, color, sensory properties, and retained higher ascorbic acid and moderate antioxidant activity. Overall, photoselective shade seem to be a cost-effective approach for manipulating crop microclimate properties that regulate not only yield, but also the retail/eating quality as well as functional or bioactive properties that are associated with human health and well-being. Improvements in the human health properties of foods, such as leafy vegetables, can also be a potent weapon in the fight against vitamin and mineral nutrient deficiency.

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CHAPTER 6

Pre- and Postharvest Treatments Affecting Flavor Quality of Fruits and Vegetables

Elazar Fallik*, Zoran Ilic**

*Agricultural Research Organization, Volcani Center, Rishon LeZiyyon, Israel

**University of Pristina, Lešak, Serbia

1 INTRODUCTION

Consumers worldwide have become more and more concerned about the quality of fresh, fresh-cut fruits, and vegetables. Product quality is a complex issue: it encompasses visual characteristics, such as size, color, shape, and defects, all encompassed in general appearance; physical and chemical properties, such as texture, and mineral and vitamin contents; and flavor and other organoleptic characteristics. Thus, fruits and vegetables are also appreciated for their beneficial health effects in humans (FAO, 2011; Havens et al., 2012; McGill et al., 2013; Wadhera et al., 2015). Once produce is harvested, post-harvest handling practices do not improve the quality attained in the field; they can only slow down the rate at which deterioration occurs. Appearance, freshness, and sensory qualities of a product can play a principal role in a consumer's decision to purchase, and can influence their perception by other senses (Peneau et al., 2006).

Flavor comprises two components: taste (sweet, sour, salty, bitter, and umami) and odor. Odors are created by volatile compounds that are perceived directly by the nose, or retronasally. Thus, total flavor perception is a function of the compositions of taste and odor compounds, and their interactions. Perceptions of what constitutes good quality vary between countries, regions, and individuals; they can be affected by culture, experience, and personal preferences. The overall quality attributes that are important to packers, transporters, and retailers are often quite different from those of consumers (Watkins and Ekman, 2005).

Although the trade ensures that consumers are presented with products of excellent appearance and at least acceptable texture, flavor is often ranked lower in importance by marketers (Watkins and Ekman, 2005). However, flavor quality is important for consumer satisfaction, and it influences further

consumption; many consumers are dissatisfied with the flavor and quality of fresh or fresh-cut products, even though they have access to a greater variety of produce than ever before (Belitz et al., 2009; Caleb et al., 2013).

Flavor depends on factors that include weather conditions, which affect growth and maturity; natural genetic variability, which results in differences in taste and smell among cultivars; and maturity during harvest and post-harvest handling (Sugiura et al., 2013). Good postharvest technology helps to maintain produce overall quality. The key to increasing consumer consumption of fresh fruits and vegetables and fresh-cut products, without loss of grower income, lies in providing produce with superior flavor that lasts during an extended shelf life.

The aim of this chapter is to summarize the most recently published information on pre- (Table 6.1) and postharvest (Table 6.2) treatments that affect flavor quality of freshly harvested and fresh-cut products.

2 FACTORS AFFECTING FLAVOR BEFORE HARVEST

Postharvest quality and shelf life of fresh produce also are determined before harvest. Factors that include weather, soil preparation and cultivation, soil type, cultivar, irrigation, fertilization practices, and crop loads affect the quality and flavor properties of harvested fresh produce.

2.1 Weather

The effects of climate change on the taste and textural attributes of foods remain largely unknown, despite much public interest. Assessing the impacts of future climate change on fruit production enables researchers to predict changes in yield (Stöckle et al., 2010) and in suitability of regions for fruit production (Sugiura and Yokozawa, 2004). Rising temperatures are already affecting phenology in many species of fruit trees; many phenological temporal trends in fruit trees were studied in regions, including Europe, North America, Asia, and the Southern Hemisphere (Fujisawa and Kobayashi, 2010; Legave et al., 2013; Menzel et al., 2006; Webb et al., 2011), and almost all of these trends are consistent with rising temperatures. In a recent report, Sugiura et al. (2013) have provided evidence that the taste and textural attributes of apples have changed because of recent global warming. Decreases in acid concentration, fruit firmness, and water core development were observed, regardless of the maturity indexes used to determine harvest date, that is, calendar date, number of days after full bloom, peel color, and starch concentration; in some cases soluble-solids concentration

Table 6.1 Summarizing the important preharvest factors that affect flavor after shelf life

Factors	Crops	Special findings	References
Weather	Apple	—	Sugiura et al. (2013)
Soil type	Melon	Heavy soil improved flavor	Bett-Garber et al. (2005)
Genetic background	Carrot	Depends on the variety	Seljasen et al. (2013)
	Banana	Depends on the variety	Castricini et al. (2015)
Mulching	Grape	Transparent mulching reduced flavor	Sen and Kesgin (2015)
Photoselective net	Pepper	Pearl net improved flavor	Selahle et al. (2015)
	Apple	Red net improved flavor	Corollaro et al. (2015)
Water	Basil	Deficit irrigation reduced flavor	Bekhradi et al. (2015)
	Pomegranate	Deficit irrigation improved flavor	Peña et al. (2013)
	Pomegranate	Deficit irrigation + vapor heat improved flavor	Peña-Estévez et al. (2015)
Fertilizer	Sapota	Vermicompost + NPK improved flavor	Patel and Naik (2010)
	Papaya	Ca-foliar application improved flavor	Madani et al. (2015)
Crop load	Apricot	10%–20% Hand thinning improved flavor	Stanley et al. (2015)
	Apple	No differences between chemical thinning and shading	Corollaro et al. (2015)
Bioregulator	Apple	AVG negatively affected flavor	Salas et al. (2011)
	Peach	No flavor differences between AVG and control	Cetinbas et al. (2012)
Greenhouse	Peach	High CO ₂ improved flavor	Xi et al. (2014)

AVG, Aminoethoxyvinylglycine; NPK, nitrogen, phosphorus, and potassium.

Table 6.2 Summarizing the important harvest and postharvest factors that affect flavor after shelf life

Factors	Crops	Special findings	References
Harvest			
Date	Blueberry	Late harvesting improved sugar and flavor	Lobos et al. (2014)
	Nectarine	Harvesting at M3 stage improved flavor	Echeverria et al. (2015)
	Pomegranate	Harvesting at H2 stage improved sweet taste	Fawole and Opara (2013)
	Nectarine	Harvesting too early improved storability but reduced flavor	Bordonaba et al. (2014)
	Acid lime	Harvesting with scissors improved marketability	Bassan et al. (2013)
Postharvest			
Temperature	Mandarin	Flavor loss increased by delaying shift from cold to warm temperature	Obenland et al. (2013)
	Tomato	High temperature storage (30°C) reduced flavor	Goren et al. (2010)
	Avocado	Ripening fruit prior to or after cold storage improved flavor	Arpaia et al. (2015)
CA	Pear	2% of O ₂ + 0.7% CO ₂ treatment had a better flavor	Rizzolo et al. (2014)
	Kiwifruit	1.5% O ₂ + 1.5% CO ₂ maintained better flavor	Latocha et al. (2014)
MAP	Sweet cherry	6.5%–7.5% O ₂ and 8.0%–10.0% CO ₂ maintained flavor	Wang et al. (2015)
	Pomegranate	16 and, especially, 20 weeks storage decreased flavor	Mayuoni-Kirshinbaum et al. (2013)
	Sweet cherry	PLA packaging improved flavor	Koutsimanis et al. (2015)
	Mushroom	PLA maintained flavor better than LDPE	Han et al. (2015)

Table 6.2 Summarizing the important harvest and postharvest factors that affect flavor after shelf life (*cont.*)

Factors	Crops	Special findings	References
Coating	Mandarin	Location and harvest time affected flavor of waxed fruit	Ummerat et al. (2015)
	Tomato	BW + 2% SBC maintained fruit flavor	Fagundes et al. (2015)
	Strawberry	Pectin, pullulan, and chitosan coating improved flavor	Treviño-Garza et al. (2015)
	Prickly pear	Flavor of red fruit coated with chitosan + 2.5% acetic acid was reduced	Ochoa-Velasco and Guerrero-Beltran (2014)
	Citrus fruit	CMC/chitosan bilayer coating maintained better flavor	Arnon et al. (2014)
	Grapes	Chitosan + UV-C maintained sensory attributes	Freitas et al. (2015)
	Jujube	Chitosan + cinnamon oil improved flavor	Xing et al. (2015)
	Strawberry	Copper-free nanochitosan induced better flavor	Eshghi et al. (2014)
	Tomato	Almond gum and gum arabic maintained flavor	Mahfoudhi et al. (2014)
Heat	Grape	HWD maintained flavor	Sabir and Sabir (2013)
	Satsuma	Hot-water shower markedly improved sensory attributes	Hong et al. (2011)
	Wolfberry	HWD + 1% chitosan coating maintained flavor	Ban et al. (2015)
	Sour cherry	HWD + <i>Aloe vera</i> coating improved sensory attributes	Ravanfar et al. (2014)

(Continued)

Table 6.2 Summarizing the important harvest and postharvest factors that affect flavor after shelf life (*cont.*)

Factors	Crops	Special findings	References
Physicochemical	Apple	1-MCP-treated fruit was preferred by consumers	Wuenschel and Heyn (2015)
	Date	Low-energy X-rays reduced flavor at high dosage	Aleid et al. (2013)
	Custard apple	X-rays at 1.5 kGy with benzyl adenine enhanced flavor	Chouksey et al. (2013)
	Blueberry	Biofumigants had negative impact on flavor	Mehra et al. (2013)
	Sweet cherry	ClO ₂ + passive atmosphere improved flavor	Colgecen and Aday (2015)
	Sweet cherry	Electrolyzed water at high concentration impaired sensory attributes	Hayta and Aday (2015)
	Shiitake	Essential oil impaired sensory attributes	Jiang et al. (2015)
	Papaya	Calcium chloride enhanced bitterness	Udomkun et al. (2014)

BW, Beeswax; CA, controlled atmosphere; CMC, carboxymethyl cellulose; HWD, hot-water dips; LPDE, low-density polyethylene; MAP, modified atmosphere packaging; 1-MCP, 1-methylcyclopropane; PLA, polylactic acid; SBC, sodium bicarbonate.

(SSC) increased. All such changes could have resulted from earlier blooming and higher temperatures during the maturation period. These findings suggest that the qualities of apples in the market are undergoing long-term changes. Climate-related factors were found to cause content changes of up to 20-fold for terpenes, 82% for total sugars, and 30%–40% for β -carotene, all of which affect sweet taste and bitter taste (Seljasen et al., 2013).

2.2 Preharvest

Soil type has been found to affect postharvest flavor attributes of fruits. Melons (*Cucumis melo*) grown in sandy loam were lower in sweet aromatics and sweet taste, and higher in moisture release and fermented flavor than were fruits grown in heavy clay soil. Fruity/melon note, sweet aromatic,

surface wetness, hardness, and moisture release attributes decreased, and fermented and sour flavor increased during storage, regardless of soil type. Clay soil appeared to have some advantages over sandy loam soil in producing cantaloupe fruits with better sensory quality attributes (Bett-Garber et al., 2005).

The genetic background of fresh-produce cultivars affects quality and flavor attributes (Corollaro et al., 2013). Cultivar differences were shown to play a crucial role in determining both the composition and concentrations of aroma volatiles; therefore, they led to different flavors (Qin et al., 2012). The genetic factor showed the highest impact on quality variables in carrots: between varieties there were 7- to 11-fold ranges in contents of terpenes, β -carotene, magnesium, iron, and phenolics, and up to 4-fold ranges in falcarindiol, bitter taste, and sweet taste (Seljasen et al., 2013). Misran et al. (2015) reported that cultivar-specific quality changes might result from preharvest application of nonchemical formulations, which might induce differing patterns of metabolite channeling and delayed fruit ripening and maturity. Observations made by Mishra and Kar (2014) indicated significant quantitative differences among cultivars in both physicochemical properties and total volatile contents, indicating the importance of cultivar in determining postharvest quality and shelf life. Mishra and Kar (2014) characterized the banana genotypes “Prata Ana,” “BRS Platina,” and “Fhia-18” at the point of harvest (green) and when ripe (stage 6 of maturity), through chemical, physical, and sensory evaluations: “Prata Ana” and “BRS Platina” were preferred and attracted stronger consumer intentions to purchase. Participants in the study preferred “Prata Ana” fruits in fingers, and “BRS Platina” and “Fhia-18” in fingers, bouquets, and bunches. However, the majority of consumers stated that they would buy “Prata Ana” bananas in bouquets and “BRS Platina” and “Fhia-18” in bunches (Castricini et al., 2015).

Mulching and covering of soil or beds have become widely used cropping practices. Rows of grapes were covered with various materials—polypropylene cross-stitch, Life Pack, Mogul, and transparent polyethylene (PE)—to compare their effects on grape quality and storability, and harvest from covered plots was delayed by one month. Harvested grapes were kept at -0.5°C for prolonged storage in sealed PE bags with sulfur dioxide pads, and all the clusters were healthy and of marketable quality after 90 days of storage. In the first year, at the end of storage, only those grapes harvested from rows covered with polypropylene cross-stitch showed fungal growth. The flavor quality scores revealed a reduced level of preference after

120 days of storage. The effects of the tested covering materials were similar regarding grape quality and storage performance, except for the transparent PE, which damaged the grapevine leaves (Sen and Kesgin, 2015).

Photoselective shade nets provide physical protection against hail and wind, and also were proved to improve plant growth, marketable yield, and product quality of various horticultural crops (Shahak, 2008). Photoselective shade nets can modify light quality by increasing the relative proportion of diffuse (scattered) light and also by absorbing various spectral bands (Shahak et al., 2008). Red and yellow peppers grown under pearl and yellow nets yielded enhanced percentages of marketable fruit after storage. Red peppers grown under the yellow net contained increased numbers of odor-active aroma compounds in the fruits, whereas black nets significantly affected synthesis of such compounds during storage. After storage, sensory analysis indicated preference for red pepper fruits from plants grown under pearl nets (Selahle et al., 2015). In addition, some significant differences among “Fuji” apples produced under various nets were reported by the trained sensory panel for 4 out of the 10 rated attributes. The nets included a neutral black net (control), and red, white, yellow, and blue photoselective hail nets. Differences were greatest between fruits from the red and yellow hail nets. Apples grown under the red hail net received higher scores for yellow color perception, sweet taste, and hardness, and a lower score for green color. The spectrum of transmitted light influenced fruit growth by affecting cell proliferation and ripening, and thereby changed the sensory perceptions of fruit appearance, taste, and texture (Corollaro et al., 2015).

Water availability and water quantity are crucial for cultivation management that aims to reduce water consumption without impairing quality and shelf life. Irrigation management can influence both yield and quality of products, both at harvest and after harvest (Yermiyahu et al., 2006). Previous studies have shown that irrigation rates can have effects on the quality of fresh vegetables (Dorais and Ehret, 2008). In fresh herbs, study of the influence of various environmental factors, such as water stress, is focused on essential oil production (Khalid, 2006). Deficit irrigation of fresh basil (*Ocimum basilicum* L.) did not improve preservation during storage, of flavor quality characteristics, such as darkening, dehydration, and aroma, compared with that resulting from irrigation control. Chlorophyll fluorescence did not show significant differences between irrigation water treatments, whereas antioxidant capacity increased when deficit irrigation was increased. These results show that basil as a fresh herb can be cultivated with less water without impairing its quality characteristics (Bekhradi et al., 2015). The influence of

sustained deficit irrigation (SDI)—at 32% of reference evapotranspiration (ET_0)—on physicochemical and sensory quality and bioactive compounds of pomegranates stored for 30, 60, or 90 days in air at 5°C, with each storage period followed by 4 days at 15°C, was studied and compared with a control—irrigated at 100% ET_0 . After long-term storage, fruits grown under SDI showed higher flavor and nutritional quality, more health attributes, and longer shelf life (up to 90 days at 5°C + 4 days at 15°C) than those irrigated at 100% ET_0 (Peña et al., 2013). Peña-Estévez et al. (2015) studied the effects on postharvest physicochemical, microbial, and flavor quality attributes, and on anthocyanin content of fresh-cut pomegranate arils throughout 18 days at 5°C of two SDI applications combined with a short vapor heating at 95°C for a few seconds, by comparison with a control. According to flavor analyses, the shelf life of arils from control and SDI-irrigated fruits became fixed after storage at 5°C for 14 and 18 days, respectively, and vapor heat treatment for 4 and 7 s, respectively, provided the best sensory quality (Peña-Estévez et al., 2015).

Fertilizer application is a very important preharvest factor that affects quality and sensory attributes of fresh produce. Among all preharvest treatments with various fertilizers, use of Vermicompost at 5 kg/tree together with sapota containing N/P/K at 400/60/300 g/tree was found to be superior with regard to extending postharvest shelf life and preserving other physicochemical parameters. Organoleptic tests showed that color and texture were more acceptable following this treatment, but flavor and taste were superior following treatment with FYM alone at 25 kg/tree (Patel and Naik, 2010). Calcium (Ca) is a major plant nutrient: it affects cell wall and plasma membrane formation and plays key roles in plant growth, and biomass production and functioning (Eryani-Raqeeb et al., 2009); it can be used to decrease fruit decay and increase firmness and shelf life. Four preharvest sprays were applied as foliar applications on papaya (*Carica papaya*) cv. “Eksotika II” plants, using three different sources of Ca—calcium chloride ($CaCl_2$), calcium nitrate [$Ca(NO_3)_2$], and calcium propionate [$Ca(C_2H_5COO)_2$]—at four concentrations (0, 60, 120, and 180 mg/L) (Madani et al., 2015). In a field trial with mature trees, using preharvest applications of Ca at 0, 4000, and 5400 mg/L, in the form of $CaCl_2$, Madani et al. (2015) showed that increasing concentrations improved fruit Ca concentration, texture, and flavor, and decreased weight loss, Mg content, and apparent disease incidence in the papaya fruits.

Tree crop load was shown to affect the firmness and flavor properties of fruits. Most studies found better-quality fruits from low crop-load

trees than from high crop-load ones (Baugher and Schupp, 2010; Henriod et al., 2011). To reduce crop load, growers may remove excess fruit by hand but, due to the cost and time this requires phytochemicals, which cause fruit drop and are widely used, followed by a hand-thinning adjustment to optimize the fruit load. Stanley et al. (2015) reported that consumers preferred apricots that were grown on trees thinned to approximately 10%–20% less than typical commercial crop loads and that were harvested in a more mature condition. Recently, Corollaro et al. (2015) demonstrated that sensory panel analysis can be used to measure the impact of new preharvest treatments that manage crop load on the quality of harvested apple fruits: “Rosy Glow” fruits that had been thinned chemically or by shading were compared, and no sensory differences between treatments were perceived by a trained sensory panel, according to quantitative descriptive profiling.

Bioregulators are used in orchards to delay fruit ripening and maturation. The effect of aminoethoxyvinylglycine (AVG) application on the quality and volatiles production of “Golden Delicious” apples was evaluated by Salas et al. (2011), who applied AVG on apple trees 4 weeks before harvesting. The apples then were stored at 8°C for 35 days, and quality parameters and volatile compounds were periodically evaluated. Salas et al. (2011) concluded that AVG application on “Golden Delicious” apples conferred a variety of benefits, such as delayed maturation, color maintenance, and reduced changes in acidity and total soluble solids. However, this treatment negatively affected production of some volatile compounds (Salas et al., 2011). Cetinbas et al. (2012) reported that preharvest AVG application to “Monroe” peaches at 150 ppm for 7 days before commercial harvest, increased financial returns to growers. However, the external appearance and flavor scores of fruits decreased, depending on the length of storage. Even though there was a positive effect of AVG treatment on external appearance, the AVG-treated fruits showed similar results to the control groups, in terms of flavor.

Greenhouses are widely used for peach cultivation in Northern China because of the protection that they provide from low temperatures and their promotion of early fruit maturation. Castilla (2013) observed that CO₂ levels in greenhouses generally increased above average values at night, but decreased below 200 ppm during the day, especially from 10:00 to 14:00; thus, low CO₂ levels may be a limiting factor for productivity of fruit trees cultivated in greenhouses. To counter such effects, CO₂ enrichment (CDE) has been used for many horticultural crops, such as strawberry, grape, tomato, and peach (Castilla, 2013). CDE-treated —with CO₂ at 360 ppm—peach

fruits were relatively well accepted by consumers compared with control fruits. A panel scored markedly higher flavor rating, sweetness, and consumer acceptance than for control fruit, for fruits that were CDE-treated during postharvest ripening, but lower sourness in CDE-treated fruits, and this correlated with changes in the abundance of flavor compounds (Xi et al., 2014).

3 HARVEST AND STAGE OF MATURITY

Harvest and postharvest handling practices do not improve the postharvest quality of fruits; they only slow deterioration and maintain fresh-produce quality. Practices, such as temperature management, controlled and modified atmosphere, coating, and physical treatments, are services for the consumer, and generally do not improve inherent quality. Nevertheless, they will determine fresh-produce flavor quality after prolonged storage and shelf life (Table 6.2).

It is well known that selection of an appropriate maturity at harvest is a key factor in determining fruit quality and consumer acceptability. However, consumers often buy for the first time according to fruit appearance, but repeated purchases are driven by expected quality factors that mainly are determined by flavor compounds and texture (Crisosto et al., 2006). In general, consumers are willing to pay more for fruits with a higher hedonic score (Delgado et al., 2013), which indicates why harvesting at the optimum ripening stage and storing the fruits under optimal temperature and duration conditions are crucial. After harvest, immature fruits are more prone to shriveling, internal breakdown, mechanical damage, and inferior quality, when they finally ripen, whereas overmature fruits are likely to become soft and mealy, and to exhibit insipid flavor. In fresh fruits and vegetables, inadequate cultural practices during the production cycle and the harvesting process can cause rupture of the peel, discoloration, and softening, which lead to reduced shelf life (Neves et al., 2015).

The effect of delayed harvest on fruit quality and storage life was measured for the late-season high-bush blueberry cultivars “Aurora,” “Elliott,” and “Liberty.” In all three cultivars, as fruit ripened there was a steady decline in fruit acidity, whereas sugar contents remained stable, indicating that the fruits were becoming sweeter. This was supported by taste panel perceptions of greater sweetness associated with later-harvested fruits (Lobos et al., 2014). Consumer satisfaction with nectarines improved significantly with advancing maturity at harvest: fruits from the third stage (M3) obtained

the highest acceptability score, which was statistically different from the scores obtained for M1 fruits, according to sweetness, hardness, juiciness, and flavor. Consumers also detected a stronger nectarine flavor in the M3 fruits than in those harvested at M1 (Echeverria et al., 2015).

The effects of harvest maturity and storage duration on pomegranate fruit-quality attributes during 6 weeks of cold storage at 5°C and 95% RH, followed by 5 days of shelf life at 20°C and 75% RH were investigated by Fawole and Opara (2013). They found that the optimal time for harvesting pomegranate was at 143 days after full bloom (DAFB, H2), when the fruit total soluble solids (TSS):TA ratio was >55, and that significantly higher ratings for sweet taste during shelf life were gained by fruits harvested at H2 than by those harvested at H1 and H3. Bordonaba et al. (2014) reported that nectarine fruits that were riper at harvest obtained better overall consumer liking scores than those harvested at earlier stages of maturity. Although harvesting at a slightly unripe stage might render fruit more resistant to postharvest handling, this practice might have a negative effect on the flavor quality of the fruit.

Bassan et al. (2013) found that harvesting of acid limes cv. “Tahiti” with scissors led to greater conservation of fresh mass, color, and peel chlorophyll content of fruit than with the use of a hook. The levels of soluble solids, titratable acidity (TA), and ascorbic acid were not influenced by the harvesting system, but fruits harvested with a hook had a higher percentage of oleocellosis and a 30% reduction in marketable amounts compared with those harvested with scissors. Mechanical injuries caused during harvest also led to changes in the flavor and aroma of these fruits (Bassan et al., 2013).

Tomato fruit quality is usually perceived as a combination of some or all of: visual appearance, firmness and texture, and contents of dry matter, organoleptic compounds, and health-influencing compounds. Research on tomato fruit storage has mostly focused on shelf life, as determined according to appearance and firmness. However, taste and health-influencing compounds of fresh tomatoes are highly relevant from a consumer’s perspective. Tomatoes at three maturity stages were harvested from commercial greenhouses and transferred immediately to controlled environments that simulated differing storage, transport, and supermarket conditions. The results revealed significant changes in development of color, fruit firmness, and contents of SSC, TA, phenolics, and carotenoids as the fruits progressed from harvest to sale, and these differences were related to postharvest conditions. Fruit firmness, SSC, and total titratable acidity (TTA) of vine-ripened

red cherry tomatoes were higher by 30, 55, and 11%, respectively, than in those harvested at breakers and ripened to red (Verheul et al., 2015).

4 POSTHARVEST TREATMENTS

4.1 Temperature Management

Temperature is one of the most important factors affecting the quality of fresh produce, and there is an optimal storage temperature for each product; the ideal temperature often depends on the geographic origin of the product. Temperature management is a key tool in extending storability and shelf life of fresh-harvested produce, by slowing down both physiological and pathological deterioration.

Obenland et al. (2013) stored mandarins, cv. “W. Murcott Afourer” for either 6 weeks at continuous 5°C, or at 20°C for either 1 or 2 weeks, following 2 or 4 weeks of storage at 5°C. Sensory quality, as indicated by likeability, was maintained throughout the 6-week storage when the fruits were kept at 5°C, but rapidly declined after fruits were transferred to 20°C, and flavor loss increased as the duration of cold storage prior to the warm holding period was lengthened. SSC and TA were relatively unchanged by holding at 20°C, but concentrations of aroma volatiles, and especially alcohols and ethyl esters, were greatly enhanced and caused off-flavor. The increases in aroma volatiles concentrations were apparent within 1 day of transferring the fruits to 20°C, highlighting the need to control postharvest storage temperatures carefully. Obenland et al. (2013) compared fruits that had been held at 5, 10, or 20°C, and observed that it was only at 20°C that aroma volatiles contributing to off-flavor accumulated. Goren et al. (2010) evaluated the influence of Mediterranean “market/shelf life” temperatures of 12, 20, and 30°C on external and internal quality of tomato fruits after 5 and 9 days in storage. They observed very little change in percentages of TSS and TA in fruits kept at the three temperatures for the two storage periods. The amount of total aroma volatiles after 9 days of storage was less than that after 5 days, and the lowest amount of volatiles was found after 5 days in fruits kept at 30°C (Goren et al., 2010).

Partially ripened avocados are often held in cold storage in an attempt to enable consistent delivery of ripe fruits to food service or retail outlets. It is also common to hold fruits for a few days to several weeks, without any prior ripening, before ethylene treatment and final ripening. Arpaia et al. (2015) assessed the impacts of storage of partially ripened fruit on both quality and sensory characteristics: they harvested “Hass” avocados 6 times

at monthly intervals, ripened them to approximately 16 N firmness at 20°C and then stored them at either 1 or 5°C for 7 or 14 days. Following storage, the fruits were ripened to eating firmness and evaluated. The occurrence of fruit-quality defects was not affected by timing of ripening; sensory panelists liked fruits ripened before or after cold storage equally well, and for any given storage time there were no differences among the ripening treatments in the levels of rich, nutty, or grassy attributes that composed the fruit flavor (Arpaia et al., 2015).

4.2 Controlled Atmosphere

Controlled atmosphere (CA) storage is used to extend the storage life of fresh produce, mainly stone fruits. The term “CA” refers to the introduction of a low-oxygen (O_2) and/or high-carbon dioxide (CO_2) atmosphere into a container or an airtight cooling room. The atmosphere is “controlled” according to a sequence of measurements and corrections throughout the storage period, and the practice is used as a supplement to proper refrigerated storage and distribution. More recently, CAs have been introduced into refrigerated containers for long-distance transport by sea. The use of CA is increasing in response to improved cost effectiveness of delivering produce with extended shelf life and enhanced quality. However, this technology may affect the volatiles contents of the room atmosphere, which, in turn, may affect volatile generation in ripe fruits.

The most preferred flavor profile, that is, juicy, not grainy, sweet, aromatic, quite sour, and no more than mildly astringent, was obtained by Rizzolo et al. (2014) for untreated pears after 20 weeks of storage at -0.5°C in CA containing 2% O_2 + 0.7% CO_2 . This preference was obtained independently of time at shelf life in normal air (NA), in fruits stored in a dynamic controlled atmosphere (DCA) containing 0.8% O_2 + 0.45% CO_2 after 4 or 7 days of shelf life, as well as in pears stored in DCA at 1°C after 4 and 7 days of shelf life. In contrast, the worst sensory characteristics—grainy, not firm, not juicy, not sweet, not sour, very astringent, and not aromatic—characterized untreated pears after 28 weeks in storage at 1°C in NA (Rizzolo et al., 2014).

Latocha et al. (2014) compared changes in flavor and physicochemical characteristics of kiwi fruit (*Actinidia arguta*) and its hybrid after cold storage in air (AS) at 1°C and 85% RH with those in fruits stored in CA with low oxygen concentration (1.5% O_2 + 1.5% CO_2) over periods of 4 and 8 weeks. The flavor characteristics of fruits stored in CA and then ripened during simulated shelf life were similar to those of vine-ripe fruits.

The most significant negative change in the flavor characteristics of fruits after long-term cold storage was increased intensity of their bitter taste (Latocha et al., 2014).

4.3 Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) involves modification of the headspace gas in a package to prolong the shelf life of the product it contains. The success of MAP depends on the packer's ability to correctly prepare the product and to control the concentrations of headspace gases within desired limits. The benefits of MAP in extending storage life of fresh produce are due primarily to decreased O₂ and/or increased CO₂ concentrations, and high RH surrounding the commodity. The steady-state O₂ and CO₂ concentrations within the MAP derive from interactions among a number of factors, including gas permeability characteristics of the film, respiratory behavior of the product, and temperature of the surrounding storage environment (Costa et al., 2011).

Sweet cherries (*Prunus avium* L.) are highly perishable and have a short shelf life, even under cold-chain management. This fruit is liked by consumers because of its sweet flavor, exquisite aroma, and contents of natural healthy compounds, such as polyphenols and anthocyanins (Longobardi et al., 2013). Major postharvest quality deterioration during long-distance ocean shipping includes flavor loss, off-flavor development, skin darkening, pedicel browning, pitting, and decay. In a study by Wang et al. (2015), three MAP liners with varied gas permeability were evaluated for their effect on quality deterioration and physiological changes of cv. "Lapins" and "Skeena" during a simulated transit of 6 weeks at 0°C. Use of MAP containing 6.5%–7.5% O₂ and 8.0%–10.0% CO₂ reduced ascorbic acid loss and lipid peroxidation, maintained flavor by retarding TA acid loss and bitter taste formation, and maintained brighter color by retarding anthocyanin synthesis after 4 and 6 weeks, compared with fruits packed in a macroperforated PE liner. In contrast, use of MAP in 0.5%–1.5% O₂ and 10% CO₂ yielded greater benefits in most of the quality attributes; however, fruits exhibited anaerobic off-flavor because of significant accumulation of ethanol (Wang et al., 2015).

Pomegranates (*Punica granatum* L.) cv. "Wonderful" were packed immediately after harvest in commercial Xtend MA bags (StePac Ltd., Tefen, Israel) within 4- to 5-kg export cartons as described by Mayuoni-Kirshinbaum et al. (2013). The fruits were stored at 7°C for 4, 8, 12, 16, or 20 weeks, after which the bags were opened and the fruits were held for 3 more days under shelf-life conditions at 20°C. Flavor acceptance tests of "Wonderful"

pomegranate arils during prolonged storage under commercial MAP conditions, yielded high preference scores during the first 12 weeks of cold storage at 7°C. However, flavor quality decreased remarkably after 16 and, especially, 20 weeks. Descriptive flavor analyses by a trained sensory panel revealed that the decrease in fruit flavor preference resulted mainly from a decrease in typical pomegranate flavor and increases in “overripe” and “off-flavor” odors (Mayuoni-Kirshinbaum et al., 2013).

Current consumer demand for high-quality ready-to-eat fresh fruit in convenient biobased packaging was met by utilizing sanitized stem-free sweet cherries and a polylactic acid (PLA) cup with a peelable microperforated PLA lid. After 27 days of storage at 1°C, the PLA package maintained cherry firmness, compared with a 50% firmness reduction in the controls. Differences in cherry aroma, color, acidity, SSC, pH, and quality index were also attributed to the packaging type. A consumer flavor evaluation showed that cherries stored in PLA packages were more acceptable than those in the controls, with regard to appearance, texture, flavor, and overall perceptions (Koutsimanis et al., 2015). Sensory panel members judged that mushrooms stored in PLA and low-density polyethylene (LDPE) films at 4°C for 18 days became spoiled after 12 days. However, mushrooms stored in PLA-7.5 and PLA-15 films were still acceptable in a marketable condition, and recorded a score of good flavor at the end of storage, possibly because of the high RH inside the PLA and LDPE film packaging. The PLA-7.5 and -15 films were formed by blending PLA with tributyl citrate by adding various amounts—0, 7.5, or 15% (w/w)—of tributyl citrate to the PLA matrix (Han et al., 2015).

4.4 Coating

Edible coatings of various compositions have been tested and used to prolong storage life of fresh and fresh-cut fruit by reducing metabolic processes and retarding microbial growth. They also can form a protective barrier to reduce respiration and transpiration rates, thereby retarding senescence while preserving quality. Application of coating materials to fresh produce imparts shine and reduces water loss and shrinkage that might follow fruit washing. However, coatings can enhance development of anaerobic conditions in the fruit by restricting gas diffusion through the peel (Dhall, 2013).

In three of four harvests (H1, 2, 3, 4) at two different locations, mandarin (*Citrus reticulata*) cv. “Pixie” (P) accumulated much higher concentrations of ethanol than “Gold Nugget” (GN) after waxing followed by storage for 3 weeks at 5°C and 1 week at 20°C. Sensory panel analysis indicated that

off-flavor development during storage was more pronounced in P than in GN, as were declines in overall acceptability. Flavor in fruit from Ojai, California (H4) was less negatively impacted by storage than fruit from the San Joaquin Valley of California (H1, H2, and H3), for both varieties (Ummarat et al., 2015). Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW), and food preservatives with antifungal properties were formulated and evaluated on cherry tomatoes during cold storage at 5°C for 21 days followed by 4 days of shelf life at 20°C. HPMC–BW coatings containing sodium bicarbonate at 2% as a food additive showed potential for industrial application, including production and commercialization of organic cherry tomatoes. Peel color, ethanol, and acetaldehyde contents of the juice, sensory flavor, off-flavors, and fruit appearance were not adversely affected by application of the antifungal coatings (Fagundes et al., 2015). Edible active coatings (EACs) based on pectin, pullulan, and chitosan and incorporating sodium benzoate and potassium sorbate were employed to improve the quality and shelf life of strawberries, which were washed, disinfected, coated by dipping, packed, and stored at 4°C for 15 days. Application of EACs reduced weight loss and fruit softening and delayed alteration of color (redness) and total SSC. In contrast, pH and TA were not affected throughout storage, and ascorbic acid content was maintained in pectin–EAC–coated strawberries. Sensory quality (color, flavor, texture, and acceptance) improved, and decay rates decreased in pectin–EAC–, pullulan–EAC–, and chitosan–EAC–coated strawberries (Treviño-Garza et al., 2015). White (*Opuntia albi carpa*) and red (*Opuntia ficus indica*) prickly pears were peeled and submerged in chitosan solutions containing various concentrations of acetic acid, to obtain ready-to-eat prickly pear products. Some physicochemical and sensory characteristics were assessed during 16 days of storage at $4 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. Most of the sensory values for white prickly pear coated with chitosan containing acetic acid at 1.0% or 2.5% were higher than those obtained for uncoated fruits. Red prickly pears coated with chitosan containing 2.5% of acetic acid did not maintain their sensory quality throughout 16 days of storage (Ochoa-Velasco and Guerrero-Beltran, 2014). Arnon et al. (2014) investigated the efficacy of a newly developed polysaccharide-based edible bilayer coating comprising carboxymethyl cellulose (CMC) and chitosan, in preserving postharvest quality of various citrus fruits, including “Or” and “Mor” mandarins, “Navel” orange, and “Star Ruby” grapefruit, after simulated storage and marketing. Sensory analyses revealed that neither the CMC/chitosan bilayer coating nor the commercial wax coating had any deleterious effects

on flavor preference of “Navel” orange or “Star Ruby” grapefruit. However, application of the commercial wax and, especially, the CMC/chitosan bilayer coating, resulted in gradual decrease in flavor acceptability of “Or” and “Mor” mandarins because of increased perception of off-flavors. [Arnon et al. \(2014\)](#) concluded that the CMC/chitosan bilayer edible coating enhanced fruit gloss sufficiently, but was not effective in preventing postharvest weight loss. Furthermore, flavor quality was slightly impaired in mandarins but not in oranges and grapefruit ([Arnon et al., 2014](#)). [Freitas et al. \(2015\)](#) applied UV-C radiation at 6.0 ± 0.1 kJ/m² combined with chitosan coating, followed by incubation at 20°C for 24 h before refrigerated storage of red table grapes; the treatment increased resveratrol content, maintained sensory quality, and reduced fungal decay in comparison with control grapes. In a different investigation, [Xing et al. \(2015\)](#) incorporated cinnamon oil into a chitosan coating to evaluate the physiological attributes and preservation quality of Chinese jujube fruits during storage at 4°C for 60 days: they found that chitosan oil coating enhanced maintenance of sensory quality of the fruits. [Eshghi et al. \(2014\)](#) evaluated the effects of edible coatings, of thickness 50–110 nm, based on nanochitosan and with or without copper loading, on physicochemical and bioactive components of strawberries. They coated fresh fruits with copper-free or copper-loaded nanochitosans and stored them at $4 \pm 1^\circ\text{C}$ and 70% RH for 20 days. Sensory evaluation of the coated strawberries revealed no effect on consumer acceptability, and showed that copper-free nanochitosan yielded better results than copper-loaded nanochitosan, with regard to preserving the overall flavor and appearance ([Eshghi et al., 2014](#)).

The strawberry tree (*Arbutus unedo* L.) belongs to the family Ericacea and is native to the Mediterranean region ([Barros et al., 2010](#)). Usually its fruits are used in production of an alcoholic distillate—a very aromatic and popular drink that contains 40%–60% (v/v) of alcohol. Recently, interest in the fresh fruit for consumption has increased because of its high nutritional value ([Guerreiro et al., 2013](#)), and in testing a different type of coating materials, *A. unedo* fresh fruits were treated with alginate (AL)-based edible coatings enriched with the essential oil compounds (EOCs) eugenol (Eug) and citral (Cit). Incorporation of Cit and/or Eug into the edible alginate coatings improved the coatings in most cases: those containing AL at 1% + Eug at 0.20%, and AL at 1% + Cit 0.15% + Eug 0.10% were best for preserving sensory and nutritional attributes and reducing microbial spoilage ([Guerreiro et al., 2015](#)). [Mahfoudhi et al. \(2014\)](#) coated mature green tomato fruits with 10% almond gum extract or with 10% gum

arabic to increase their postharvest life. The results showed that the coatings significantly delayed the color changes and reduced the losses of weight, firmness, TA, ascorbic acid content and SSC, and reduced decay percentage, compared with those in uncoated control fruits. Sensory evaluation proved the efficacy of 10% almond gum and gum arabic coatings in maintaining the overall quality of tomato fruits during storage (Mahfoudhi et al., 2014).

4.5 Heat Treatments

Heat treatments appear to be among the most promising means for postharvest decay control. Such treatments may be applied to fruits and vegetables in several ways: by hot-water dips, vapor heat, hot dry air, or brief rinsing and brushing under hot water. Heat treatments also can be used to inhibit ripening processes, or to enhance resistance to chilling injury during storage, and thereby prolong storability and marketing (Fallik, 2010).

Sabir and Sabir (2013) studied berry quality, total phenolic content, sensory characteristics, and decay incidence of grapes (*Vitis vinifera*) cv. “Muskule” and “Red Globe,” as affected by prestorage hot-water immersion at 55°C for 30 min, with or without cap-stem excision treatments. Their study extended over 21 days of storage at 1°C, with 7-day intervals, and found that immersion of stem-retained grapes in hot water was the best treatment for maintenance of overall storage quality in both cultivars. According to panel scores, it was evident that hot water had a positive effect of maintaining minimally processed table grapes without altering their flavor and taste (Sabir and Sabir, 2013). Hong et al. (2011) treated greenhouse Satsuma mandarins (*Citrus unshiu* Marc.) of an early-harvesting cultivar, “Gungchun” by hot-water showering at 65°C for 10 s on a commercial scale in a packing house, stored them at 5°C for 3 weeks and then at 18°C for 1 week, to examine the potential use of hot-water treatment (HWT) as an environmentally benign method to maintain mandarin quality characteristics during postharvest storage and marketing. The respiration rates just after heat treatment or during storage were similar in both the treated and untreated fruits. The HWT also had no detrimental effects on quality attributes including pH, TA, SSC, weight loss, firmness, and peel color. Development of stem-end rot, mold decay, and black rot was lower in the heat-treated fruits than in the untreated controls. Sensory evaluation showed that HWT markedly improved fruit appearance by making the fruits cleaner and glossier. The results suggested that HWT could be applied to Satsuma mandarins as an effective pretreatment to maintain postharvest quality during storage and marketing (Hong et al., 2011).

Wolfberry (*Lycium barbarum* L.), a key medicinal plant resource also called Goji berry, is native to Southeastern Europe and Asia (Amagase and Farnsworth, 2011). It has a wide variety of biological effects, as shown in various human clinical trials and in vivo and in vitro studies. Ban et al. (2015) studied the effects of prestorage application of a 1% chitosan coating followed by a hot-water dip at 42°C for 30 min, on fresh wolfberry quality and sensory traits, and microstructure, and also on microbiological evolution of the fruits. Results showed that the combined application of heat treatment and chitosan coating of wolfberry maintained higher levels of ascorbic acid, total phenolics, and antioxidant capacity, as well as a lower microbial population, than those in control samples after storage at $2 \pm 0.5^\circ\text{C}$ and 90% RH for 28 days. Thus, integrated application of heat treatment and chitosan coating could form an important strategy to extend storage life and maintain postharvest quality of wolfberry fruits (Ban et al., 2015).

Iranian sour cherries (*Prunus cerasus*) were coated with fresh *A. vera* gel or treated with hot water at $40 \pm 2^\circ\text{C}$ for 2 min and stored at $4 \pm 1^\circ\text{C}$ for 17 days. Sensory analyses that followed both treatments revealed beneficial effects in terms of delayed dehydration and maintenance of fruit visual appearance, without any detrimental effects on taste, aroma, or flavors (Ravanfar et al., 2014)

4.6 Physicochemical Treatments

“Physicochemical” treatments include inhibition of physiological and/or pathological deterioration of fresh-harvested produce by a combination of any type of physical, chemical, or environmentally friendly chemical methods.

An alternative method of slowing ripening is treatment with 1-methylcyclopropene (1-MCP), which inhibits ethylene action (Huber, 2008). Commercial postharvest applications of 1-MCP to retain quality of “Jonagold” apples of varied harvest maturity that then were and stored in air or CA conditions were studied by Wuensche and Heyn (2015) in Southwest Germany during three consecutive seasons. Fruit quality declined with increasing storage duration, but the quality loss was much less severe under all storage conditions when fruit was treated with 1-MCP. However, consumers, regardless of age and gender, preferred 1-MCP-treated fruit stored under ultralow-oxygen conditions (Wuensche and Heyn, 2015). Canto-Pereira et al. (2014) found that both aqueous and gaseous 1-MCP formulations delayed ripening of the Guatemalan–West Indian avocado cv. “Booth 7” without significant impairment of appearance or sensory

attributes. Therefore, these treatments could be considered for postharvest application on this hybrid.

Low-energy X-ray irradiation is one possible means of controlling the quality of some fresh produce. Low-energy X-ray techniques are advantageous in comparison with existing high-energy irradiation facilities, in that they can be developed into production-line processing units, thereby significantly reducing transportation costs. The effects of X-ray irradiation as a postharvest treatment on physical, chemical, textural, and sensory attributes of Khalas dates (*Phoenix dactylifera* L.) was investigated by Aleid et al. (2013), who subjected date surfaces to low-energy X-ray irradiation at 3, 5, and 7 kGy. Although some physical and chemical properties of the dates—hardness, contents of crude protein, total fat, tannins, total fiber, and insoluble fiber—differed significantly between treatments, there was no detrimental effect on quality. However, the sensory acceptability of X-ray-irradiated dates was significantly lower at 5 and 7 kGy than that of untreated controls; irradiation at 3 kGy did not significantly change physical, chemical, or textural properties. Adoption of low-energy X-ray irradiation potentially could provide a commercial treatment for retaining quality and ensuring safety of dates (Aleid et al., 2013). Din et al. (2011) found that a radiation dose of 3 kGy of ^{60}Co gamma irradiation had a pleasant effect on organoleptic properties of dates, such as taste, texture, odor, and appearance. Chouksey et al. (2013) applied a radiation dose of 1.5 kGy along with benzyl adenine treatment at 50 ppm, and obtained enhanced shelf life in custard apple (*Annona squamosa*) fruits stored at ambient temperature for 6 days, with better pulp texture, flavor, color, and nutritional quality than in controls.

Mehra et al. (2013) evaluated four plant essential oils—cinnamon oil, linalool, *p*-cymene, and peppermint leaf oil—and the plant oil-derived biofungicides prepared from rosemary and wintergreen oils and from rosemary, clove, and thyme oils as postharvest biofumigants to control fungal decay and maintain fruit quality of blueberry fruits cv. “Tifbluerabbiteye.” They found that biofumigation had significant negative impacts on some sensory attributes, such as sourness, astringency, juiciness, bitterness, and blueberry-like flavor (Mehra et al., 2013).

Chlorine is the most commonly used sanitizing chemical in the food industry, but it reacts with organic compounds and forms carcinogenic trihalomethanes and haloacetic acids, which are harmful both to humans and to the environment (Keskinen et al., 2009). However, chlorine dioxide (ClO_2) has a lower oxidative potential, but higher oxidation capacity than chlorine, which makes it act as an effective disinfectant. Unlike chlorine, ClO_2 does

not oxidize organic matter to produce highly toxic hazardous molecules (Gómez-López et al., 2009). Moreover, the use of ClO_2 was approved by the US Food and Drug Administration (FDA) to sanitize fruits and vegetables; they specified that the residual ClO_2 concentration in foods should not exceed 3 mg/L (Colgecen and Aday, 2015). Sweet cherries treated with ClO_2 at 16 or 20 mg/L, in combination with a passive atmosphere, tended to have higher scores than treated and untreated samples, in all flavor attributes, with no off-flavor, such as those found with higher ClO_2 concentrations (Colgecen and Aday, 2015).

Electrolyzed water (EW) is an alternative to chlorine, and can be obtained electrolytically from dilute (15 g/L) NaCl solution by applying a voltage across an electrolytic cell: chloride from the solution is converted into chlorine gas, which immediately forms hypochlorite in solution (Boal, 2009). Hayta and Aday (2015) designed a study to determine the effectiveness of EW prepared with six concentrations—25, 50, 100, 200, 300, and 400 mg/L—of available free chlorine, on postharvest quality attributes of sweet cherry: free chlorine concentrations above 200 mg/L in EW had negative impacts on sensory quality (Hayta and Aday, 2015).

Shiitake mushroom (*Lentinus edodes*) has high nutritional value and contains several bioactive compounds, including polysaccharides; dietary fiber; ergosterol vitamins B1, B2, and C, folates, niacin; and minerals. Jiang et al. (2015) evaluated several naturally occurring essential oils for effectiveness in maintaining sensory quality in shiitake mushrooms after 20 days at $4 \pm 1^\circ\text{C}$. Freshly harvested mushrooms were fumigated with clove, cinnamaldehyde, or thyme oils at 5 $\mu\text{L/L}$, at 10°C for 1.5 h. Off-odor intensity in control samples significantly increased after 10 days of storage. With regard to development of the evaluated sensory attributes, mushrooms treated with cinnamaldehyde showed the lowest deterioration rate, followed by those treated with thyme and finally those treated with clove, and the control treatment (Jiang et al., 2015).

Calcium applications have been widely used to improve fresh fruit qualities by, for example, decreasing incidence of physiological disorders and mold growth, delaying membrane lipid catabolism, reducing polyphenoloxidase (PPO)-induced browning, and improving firmness by cross-linking with both cell wall and middle-lamella pectins (Alandes et al., 2009). However, Alandes et al. (2009) reported that calcium lactate (Ca-L) provided some different benefits from those of calcium chloride (CaCl_2), in that it avoided the bitterness and off-flavor associated with the chloride, and also avoided the formation of carcinogenic compounds (chloramines and

trihalomethanes) that are linked to the use of chlorine. [Martín-Diana et al. \(2007\)](#) applied calcium at three different concentrations [0.5, 1.5, and 2.5% (w/v)] to papaya (*Carica papaya*) fruits before soaking them in a 30°Bx sucrose solution and drying them at 70°C. They found that, compared with the control, the dried, calcium-treated samples exhibited significantly lower moisture content, water activity, apparent density and shrinkage, together with higher volume; their calcium applications did not obviously influence sugar content of samples, and maintained the quality of dried papaya in terms of color and texture characteristics. [Udomkun et al. \(2014\)](#) found that CaCl₂ at 2.5% (w/v), in particular, imparted undesirable bitterness to the dried papaya, whereas Ca-L at 2.5% (w/v) provided the best acceptance scores.

5 CONCLUSIONS

Preharvest factors, such as genome, weather, and growing conditions, as well as harvest maturity and postharvest factors can significantly influence fresh fruit and vegetable flavor quality. Whereas it is relatively straightforward to assess the economic benefits associated with reducing storage costs and eliminating disorders that develop during storage, it is more difficult to assess the benefits associated with improving overall quality. Flavor quality is usually considered to encompass all those characteristics of a food product that lead a consumer to be satisfied with it ([Klee, 2010](#)). However, [Peneau et al. \(2006\)](#) concluded that taste, aroma, and freshness were most frequently chosen by consumers as decisive attributes for selecting fresh produce, although the term “quality” also may be applied to subjective attributes, such as crispness, juiciness, flavor, or attractiveness ([Bicas et al., 2011](#)). Consumers have perceived significant drop-off in flavor quality over recent decades, and produce flavor has become a major cause of consumer complaints. However, a growing segment of consumers is willing to pay a premium for flavor, and many breeders are prioritizing this important quality attribute ([Kader, 2008](#)); however, breeding for better flavor presents many challenges. Traditionally, most of the effort in breeding plants has been directed toward inclusion of desirable agronomic traits, such as high yields, ease of mechanization, perfect visual appeal, plant resistance to pests and pathogens, enhanced shelf life, and other commercially important characteristics. It is unfortunate that, in parallel with the resulting development of excellent crop varieties, traits that affect the aroma and flavor of fruits and vegetables often have been lost. In addition, with increasing time between

harvest and consumption of most fruits and vegetables, there are greater losses of characteristic flavor—comprising taste and aroma—and increased development of off-flavors; postharvest life as judged by flavor and nutritional quality is shorter than that as judged by appearance and textural quality. Thus, it is essential that good flavor quality be emphasized in the future by: selecting the best-tasting genotypes for production, using an integrated crop management system, harvesting at the maturity or ripeness stage that will optimize eating quality at the time of consumption, and using postharvest handling procedures that will maintain optimal flavor and nutritional quality of fruits and vegetables (Caleb et al., 2015; Kader, 2008). Therefore, several steps should be considered; poor-flavor cultivars should be replaced with existing good-flavor cultivars, and/or new ones should be developed to provide desirable flavor and textural qualities. This can be achieved by: using consumer feedback regarding selected fresh produce, to provide data that can be used as a reference in breeding new cultivars (Zhou et al., 2015); identifying the preharvest practices that yield the best flavor quality; and identifying the postharvest practices, from the moment of harvesting until the fresh produce is consumed, that best maintain flavor quality of fruits and vegetables and their value-added products. Last, but not least, from the human health point of view, it is a fact that healthy people often eat less of a food that provides more sensory pleasure than they do of a blander version of the food.

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CHAPTER 7

Influence of Water Quality on Postharvest Fruit and Vegetable Quality

Ram Asrey*, Satyendra Kumar**, Nirmal K. Meena*

*Indian Agricultural Research Institute, New Delhi, Delhi, India

**Central Soil Salinity Research Institute, Karnal, Haryana, India

1 INTRODUCTION

Conservation of environment and water are two most challenging and imperative tasks before world leaders and researchers. These two factors will govern food, feed, fuel fodder, and social security in the 21st century (Brundtland et al., 2012). The major concern these days is how to increase food production to feed the ever-increasing population of the world and how to manage limited good-quality water to produce enough food to meet future targets. As per an estimate, an additional 1600 km³ of water are required annually to meet the millennium development goal on hunger reduction and water security (UNMSG, 2009). However, distribution of water varies spatially and temporally. Further, about 96.5% of the total water available on the Earth is saline, which is unsuitable for consumption. The remaining 3.5% is considered to be freshwater, but 67% of it is stored as glaciers (Cai and Rosegrant, 2003). According to an estimate, global consumption of water is doubling every 20 years, and if the current trend continues, the demand of freshwater is expected to increase by 56% by 2025, that is, more than the current availability. By 2025, it is estimated that about two-thirds of the world's population (about 5.5 billion people) will live in areas facing moderate to high water stress (GE04, 2007). It is also reported that about 70%–80% of the increased food requirement will have to be watered via irrigation. On the other hand, good-quality water is becoming scarce for agriculture due to increasing competition from other sectors. Under these circumstances, saline and drainage water could be judiciously used for fruit and vegetable production, which would ensure more water for other sectors. Although saline water is in use in water-scarce areas, scientific management is needed for sustainable crop production in saline environments.

In the past, maximum research and development emphasis has been given to production and productivity enhancement in biotic- and abiotic stress-prone crop-growing regions. Little attention has been placed on influence of irrigation matrix (application of poor water) on the postproduction quality and shelf life of fruit and vegetables (Wang and Frei, 2011). The quality and nutritional value of fruits is closely related to the production of a number of metabolic products, including chlorophyll, carotenoids, xanthophylls, ascorbic acid, tannins, phenolic compounds, and other biochemicals (Moretti et al., 2010). Respiration, transpiration, and the formation of other chemicals and flavor components at maturity often depend on quality of irrigation water, cultivar, and environmental and cultural factors. In this chapter, an attempt has been made to discuss quality, shelf life, and impact of poor-quality water (with a focus on saline water) application on qualitative traits of fruit and vegetables, along with management options (production practices) with the least compromise on yield potential.

2 WATER QUALITY AND CROP PRODUCE QUALITY

The quality of water refers to its suitability for a specific use. The suitability of a water supply for different uses depends upon its physical, chemical, and biological characteristics. However, mainly physical and chemical characteristics are taken into account while determining suitability of water for crop production. Sometimes, when sewage water is being used for crop production, analysis of biological characteristic of water also becomes important. It is now established fact that irrigation water quality and management significantly affects crop production. The nutrient requirement of a crop also varies with the quality of irrigation water. The magnitude of adverse effect of poor-quality irrigation water on crop production also depends on management strategies, such as how and when water is applied. Irrigation methods and frequency of irrigation would be different for different quality water for successful crop production. Furthermore, water quality also affects the performance of an irrigation system. It is therefore imperative that to know about the quality of water to be used for irrigation before deciding what management changes are needed for sustainable crop production. Presence of dissolved solid (salinity) determines whether water can be used for plant production.

For fruits and vegetable, the term *quality* is a complex perception felt by the consumer in different ways. Simply, quality can be viewed as the

absence of defects or a degree of excellence of produce from the consumer's viewpoint (Wang, 1997). Fruit and vegetable quality embraces both sensory attributes that are readily perceived by the human senses and hidden attributes, such as safety (pesticide/microbial load) and nutrition (vitamins, minerals, sugars pigments, antioxidants, etc.) that require sophisticated instrumentation to measure (Shewfelt, 1999). The quality of produce can be categorized into two groups: product- and consumer-oriented quality. Product-oriented quality has more scientific support; it is measurable and reproducible, that is, total soluble solids (TSS), acidity, and respiratory gas evolution during handling and storage. Product-oriented quality can be adjusted or manipulated up to a certain extent by pre- and postharvest treatments. While consumer-oriented quality is purely governed by the human mood, region, religion, and gender. For example, red-color onion is preferred in India, while Gulf countries prefer white onions. Women mostly prefer narrow TSS:acid ratio in fruit as compared to their male counterparts.

3 CLASSIFICATION OF SALINE WATERS FOR IRRIGATION

Suitability of poor-quality water for fruit and vegetable production depends on variety, climate, soil, and irrigation method. It is therefore suggested that water quality classification blanket for assessing suitability of water for crop production should not be followed. However, to get some idea about the quality of water in terms of total salt concentration, a classification is given in Table 7.1. This classification, given by the US Food and Agriculture Organization (FAO), can help to make a preliminary decision for the use of water for crop production. However, to develop suitable package of practices for using saline water for any crop, an adequate understanding of interaction of salt water, soils, and plants is required. The sustainability of saline-irrigated agriculture needs constant monitoring of salinity levels and farmers need to be mentally prepared to accept lower yields than usual.

4 IMPACT OF POOR WATER ON QUALITY TRAITS OF FRUIT AND VEGETABLE

4.1 Physical Traits

Information on the effect of water salinity and soil salinity on crop quality is very scant, although such effects are apparent and have been noticed under field conditions. In general, soil salinity is caused either by saline irrigation

Table 7.1 Classification of saline water

Water classes	Electrical conductivities (dS/m)	Salt concentrations (mg/L)	Types of water
Nonsaline	<0.7	<500	Drinking and irrigation water
Slightly saline	0.7–2	500–1,500	Irrigation water
Moderately saline	2–10	1,500–7,000	Primary drainage water and groundwater
Highly saline	10–25	7,000–15,000	Secondary drainage water and groundwater
Very highly saline	25–45	15,000–35,000	Very saline groundwater
Brine	>45	>35,000	Seawater

Source: The use of saline waters for crop production, Irrigation and Drainage, paper no. 48

water or by a combination of water, soil, and crop management factors. Also the soil has heavy metals in variable concentrations and there may be an increase in these elements, as a result of human interference during crop production (Oliveira et al., 2015). Growing crops in poor soil or with poor-quality water may result in a reduction in the size of produce, change in color and appearance, and change in composition of the produce. Saline water may affect the yield or quality of the produce, which is evident from the findings of various workers. Grapefruit (citrus) yield decreased with increase in chloride ion concentration because of reduction in fruit size and weight (Bielorai et al., 1983). Salinity effects on fruit quality were similar to those caused by water stress. Lurie et al. (1991) described that saline water slightly reduced the yields of pomegranate but did not affect fruit quality or storage potential. Similarly, it had no effect on bearing, but fruits had less black heart and superficial scald after storage than fruits from trees irrigated with fresh water. In taste also, pomegranate and pear fruits from trees irrigated with saline water were reported to be better than those harvested from control plants. Grape yields are markedly reduced by irrigation with saline water, but fruits were sweeter. Oron et al. (2002) reported a higher proportion of healthy pear fruits and higher overall quality under saline water condition. Being a runner-based plant, strawberry is the most salt-sensitive fruit in comprehensive salt tolerance data list of fruit crop. Marketable yield of strawberry fruits was reduced drastically as salinity increased from 0.835 to 2.24 dS/m (Suarez and Grieve, 2013).

In the case of tomatoes, it was reported that for every increase in 1.5 dS/m in mean EC_e beyond 2 dS/m, there was a 10% reduction in yield (Mizrahi and Pasternak, 1985). Tomato fruit size was also decreased when crop was irrigated with heavy metal (As, Cd, Cr, Cu, and Pb)-laced water (Psarras et al., 2014).

An increase in salinity reduced fruit size and yield in muskmelons (Meiri et al., 1981; Rhoades, 1989); however, ripening was accelerated by salinity. Tedeschi et al. (2011) reported a reduction in fruit size of Tendral melons irrigated with 1.5-dS/m saline water, but the fruit firmness was found to be unchanged. Ratio of height to width (H/W) which is generally called as fruit shape index (FSI) significantly increased with enhancing salinity of irrigation water and becomes spherical in shape (Huang et al., 2016). Roupheal et al. (2006) reported higher fruit firmness in zucchini with increasing level of salinity in irrigation water. They also pointed out that the impact of the salinity on the cell/tissue configuration is strongly dependent on the frequency and stage of crop maturity. Botia et al. (2005) applied two levels of salinity (1.3 and 6.1 dS/m) with irrigation water at early growth and flowering stage of muskmelon. They found a sharp reduction in fruit size with early-stage irrigation while commencement of late saline irrigation does not affect fruit size and total yield. Tedeschi et al. (2011) observed in a trial in melons that yield reduction was due to fruit size reduction. The maximum salt tolerance threshold was found to be 1.73 and 1.54 dS/m, respectively, for the total and marketable yield. Beyond this salt tolerance threshold limit, the total yield was reduced with per unit increase of soil salinity by 14%.

4.2 Compositional Traits

Increasing water salinity reduced crop water consumption, plant growth, and crop yield, but increased fruit quality (Damico et al., 2003). Sugar content of satsuma mandarin fruit juices increased with higher salinity level, but acidity concentration decreased (Hepaksoy, 2004). Strawberry cultivars were treated during two vegetation seasons with 0, 40, or 80 mmol NaCl/L in the nutrient solution. Salt stress increased total antioxidant capacity, ascorbic acid, anthocyanins, superoxide dismutase, and selected minerals, such as Na^+ , Cl^- , K^+ , N, P, and Zn^{2+} , as well as lipid peroxidation. Furthermore, salt stress increased the contents of free and essential amino acids (Keutgen and Pawelzik, 2009).

While comparing the salinity levels, there was an increase in TSS and tritritable acidity of melon juice without affecting juice recovery (Mendlinger, 1993). With the use of saline drainage water, muskmelon fruit quality was found to be increased in terms of TSS and mineral

(Rhoades, 1989). Application of saline water 28.8 and 20.6 t/ha, respectively, from fruiting onward increased fruit quality (TSS and maturity index) in muskmelon cultivars (Botia et al., 2005). Further, increase in salinity increased the soluble solids concentration and slightly improved the appearance of muskmelons. Mizrahi and Pasternak (1985) and Mizrahi et al. (1988) reported that the fruits from processing tomato cultivars that were exposed to various degree of salinity had higher TSS, total sugars, and improved flavor than those irrigated with normal water (control). Doganlar et al. (2010) studied the effects of salt stress on pigment and total soluble protein contents in different varieties of tomato (*Lycopersicon esculentum* Mill.). The seedlings of *L. esculentum* Hazera, Dalli Tokat, and Argy were treated with NaCl at 25, 50, 100, 125, 150, and 200 mM for 96 h with a 24-h interval. Pigment and total soluble protein contents of all tomato cultivars were significantly decreased by salt stress depending on time intervals and salt concentrations. The salinity positively affected the TSS in several fruit and vegetable crops (Rouphael et al., 2006; Tedeschi et al., 2011). In a greenhouse experiment that was carried out during the spring–summer season to determine the influence of two irrigation systems (drip- and subirrigation) and two nutrient solution concentrations (2.0 and 4.1 dS/m) on substrate electrical conductivity (EC_e), growth, yield, fruit quality (dry matter, carbohydrates, protein, and vitamin C), yield water use efficiency (WUE) and tissue mineral composition of zucchini squash (*Cucurbita pepo* L.). In the middle and at the end of the trial, plants grown with the subirrigation system resulted in a higher EC_e in the upper and lower parts of the substrate in comparison to the drip irrigation system, especially at an EC of 4.1 dS/m. At an EC of 2.0 dS/m, zucchini yield (total and marketable) was 13% lower with the subirrigation than drip irrigation systems, but offered several benefits, such as higher fruit quality (dry matter, glucose, fructose, starch, and total carbohydrates content) (Rouphael et al., 2006).

A greenhouse experiment was carried out to determine the effects of salinity and different ripening states of pepper fruits on several compounds with antioxidant properties. The effect of salinity depended on the maturity state of the peppers: it had no effect on HAA, β -carotene, or sugars, but decreased ascorbic acid and total phenolic compounds, and increased LAA and lycopene. The use of moderately saline water was beneficial when peppers were harvested in the red state, by increasing HAA and LAA in fruits, with no significant effect on other parameters (Navarro et al., 2006). Mushroom was found to be prone to heavy metal accumulation upon growing in ore

mining and processing areas. Cadmium and lead accumulation in hymenophore (H) and rest of fruit bodies (RFB) exceeded statutory limits of the European Union [Cd: 0.5 mg/kg dry weight (d.w.); Pb: 1.0 mg/kg d.w.] for edible mushrooms in most of the samples (Árvay et al., 2015). *Capsicum annuum* L. subjected to various heavy metal growing inputs (soil and water) did not show any tendency to accumulate Pb, Cr, and Ni in their edible fruits (Antonious, 2016).

The antioxidant content of plants varies considerably depending on their growing and management conditions. It has been demonstrated that antioxidants in plants are part of a complex defense mechanism against a wide range of stresses and thus, accumulate in response to these stresses. Evidently, these findings provide an opportunity to enhance the health-promoting benefits of plant-based foods by using regulated environmental stresses. However, such an approach of using environmental stresses to improve the quality of fruits and vegetables has to be considered with some caution because of their potential adverse effects on crop growth and yield.

4.3 Pathological Traits

Generally vegetables and fruits transmit a nonpathogenic epiphytic microorganism. Spoilage of fruits and vegetable can arise as a consequence of contaminated irrigation water or treating soil with organic manure, sewage sludge, and contaminated industry effluents. The potential load of microbes on fresh produce encompasses bacteria, viruses, and parasites, but bacterial contamination is frequent and very common, particularly members of the Enterobacteriaceae. *Salmonella* and *Escherichia coli* in fruit juices and sprouted seeds are of major concern. The viruses involved in outbreaks have a human reservoir (e.g., Norwalk-like and Hepatitis A) and can be associated with fruits and vegetables grown in contact with the contaminated soil and water. Protozoa (e.g., *Cryptosporidium*, *Cyclospora*, and *Giardia*) linked with outbreaks are more associated with fruits than vegetables. Viruses and protozoa are mostly associated with contaminated water and food handlers. Utilization of municipal wastewater for the irrigation of crops is also associated with internal crops quality deterioration, specifically heavy metal toxicity (Zavadil, 2009). Tedeschi et al. (2011) reported fewer rotting in saline water-fed muskmelon during storage. Vegetables are more prone to microbe infestation than fruit crops when irrigated with municipal water (Aleid et al., 2016).

4.4 Longevity (Shelf-Life) Traits

Increase in salinity of irrigation water gave a considerable reduction in the percentage of rotten fruit with a significant increase in sugar content and TSS when stored for 2 weeks at 6°C and then at 20°C for 3 more days (Bustana et al., 2005).

Storage life of strawberry and tomato found to be increased due to higher accumulation of vitamin C and phenolics by growing them in saline moisture regime (Keutgen and Pawelzik, 2007; Sgherri et al., 2007) (Tables 7.2 and 7.3).

5 MANAGEMENT OPTIONS FOR SALINE IRRIGATION WATER FOR HORTICULTURAL CROPS

Use of salt-tolerant fruit and vegetable varieties and rootstocks, water management strategies (such as use of saline waters and municipal wastewater with minimum or no soil salinity), and heavy metal toxicity hazards could

Table 7.2 Impact of salinity stress on lipid and phenolics contents of fruits and vegetables

Lipid			Phenolics		
Crops	Effects of higher salinity	References	Crops	Effects of higher salinity	References
Basil	↑↓	Said-Al Ahl et al. (2010)	Broccoli	↑	Lopez-Berenguer et al. (2009)
Chamomile	—	Baghalian et al. (2008)	Lettuce	↓	Kim et al. (2008a)
Coriander	↓	Neffati and Marzouk (2008)	Pepper	—	Navarro et al. (2006)
Moringa	—	Anwar et al. (2006)	Raspberry	↑	Neocleous and Vasilakakis (2008)
Olive	−↑↓	Mousa (2010) and Ahmad et al. (2007a)	Strawberry	↑	Keutgen and Pawelzik (2007)
			Olive	↓	Chartzoulakis (2011)

(↑) Salinity elevates phenolics and lipid; (↓) salinity decreases phenolics and lipid; (−) salinity does not influence phenolics and lipid.

Table 7.3 Impact of salinity stress on vitamin C and carotenoid contents of fruits and vegetables

Vitamin C			Carotenoid		
Crops	Effects	References	Crops	Effects	References
Broccoli	—	Lopez-Berenguer et al. (2009)	Lettuce	↑	Kim et al. (2008a)
Cucumber	—	Huang et al. (2009)	Pepper,	—↑	Navarro et al. (2006)
Pepper	↓	Navarro et al. (2006)	Tomato	↑↓—	De Pascale et al. (2001), Fanasca et al. (2007), and Kim et al. (2008b)
Tomato	—↑↓	Sgherri et al. (2007) and Kim et al. (2008b)			
Raspberry	↑	Neocleous and Vasilakakis (2008)			
Strawberry	↓	Keutgen and Pawelzik (2007)			
Passion fruit	↑	Dias et al. (2011)			

(↑) Salinity elevates vitamin C and carotenoids; (↓) salinity decreases vitamin C and carotenoid; (—) salinity does not influence vitamin C and carotenoid contents.

be sustainable and resilient strategies for crop production. Although higher salinity in general restricts crop production and yield, there are many crops that can be grown with full or compromising yield potential to a certain level by using improved farming and management practices. In this chapter, the focus is mainly on saline water use because it is mainly used for crop production as an alternative for good-quality water in water -scarce areas. Although, saline water use for irrigation leads to salt accumulation in the soil, skillful use provides irrigation for getting good crops, particularly in orchards (Hoffman et al., 1986). In addition to ensured irrigation facility, saline water irrigation results in high content of TSS, extended shelf life, and high market price of produce due to adaptation of plants to stressful growing conditions (Mizrahi and Pasternak, 1985). Management practices, such as deficit irrigation, mulching, and the use of tolerant crops and rootstocks with grafted plants are now being practiced for managing poor-quality water irrigation.

5.1 Frequency of Irrigation

High soil moisture and low salt concentration in plant root zone is needed for better plant growth because salt presence affects water availability to the plant in direct proportion. Hence, application of the proper amount of water to the plant at the appropriate time is the key for effective and safe use of saline water for irrigation and soil salinity control. The frequency of irrigation should be such that it provides water as frequently as possible to maintain soil moisture near to field capacity. Sometimes, water should be applied in excess to leach out excess salt from the plant root zone. The frequency and amount of water application vary with the crop, soil, and method of water application. However, a stress period should be imposed carefully during the crop-growing periods to get maximum economic yield for some crops. Occasional dry periods are also needed to perform agronomic practices. Thus, frequency and amount of water application should be decided in such a way that moisture content and salinity of irrigated soils is maintained as high and low, respectively, as far as is practically possible.

5.2 Irrigation Methods

The irrigation method has a significant influence on crop growth, yield, and water productivity in saline environment. The manner of water application affects soil moisture and the salt distribution pattern (Amer, 2011). The different irrigation methods employed for irrigating crops are surface (border and furrow), sprinkling, and drip irrigation. Surface or gravity-fed irrigation where a water stream is diverted to the field is good for salinity control when using saline waters and land is leveled. However, aeration and crusting problems are sometimes observed. Water application in furrows can minimize these problems, but salts tend to accumulate in the beds. If excess salt does accumulate, it is advised to follow with sprinkler and surface irrigation periodically for controlling excess salt within plant root zone (Al-Omran et al., 2005). High-frequency irrigation is required to mitigate the adverse impact of salt present in the root zone, which can be achieved by using the drip irrigation method. Irrigation with drippers can maintain constant higher matric potential in the rhizosphere by modifying salt distribution pattern (Malash et al., 2008; Meiri and Plaut 1985), controlling salinity and increasing yields (Hanson and May, 2004; Kumar et al., 2007).

Drip system provides opportunity to cultivate vegetable crops even at higher water salinity than normal conditions (Karlberga et al., 2007; Wan

et al., 2010). In general, sprinkler and drip irrigation methods both are preferred over surface irrigation methods in saline environments due to their controlled nature of water application, which allows frequent irrigation with small quantities of saline water that leads to lower salt buildup in the zone and better crop performance. However, the drip system is most suitable, but its high initial cost is a major limiting factor that limits its use to only high-value and widely spaced crops. Thus, the choice of irrigation system does not depend only on system performance, but on economics also.

5.3 Deficit Irrigation

Deficit irrigation (DI) strategies are becoming popular in the areas where water supply is limited under erratic climate situations (Feres and Soriano, 2006). Also the DI strategies offer great opportunities for saving water without compromising production. Several researchers have reported water savings from 43% to 65% under RDI strategy with a small reduction in yield, but with higher quality of produce (Mirás-Avalos et al., 2016).

In general, the fruit and vegetable yield reduced in DI system by size and weight reduction of produce, but quality parameters, such as sugars, ascorbic acid, and anthocyanin contents in fruit increased by water restrictions (Nangare et al., 2013; Rocuzzo et al., 2014). The adoption of DI improved fruit composition of orange, peach, and grape by improving key functional quality parameters and antioxidant compounds (Mirás-Avalos et al., 2016; Permanhani et al., 2016; Rocuzzo et al., 2014). Similarly, vegetable crops, such as melons, cucumber, tomato, brinjal, and spinach have shown poor-quality water use efficiency without much loss of yield, but with added produce quality.

5.4 Mulching

In areas having saline water irrigation, salts move up during the drying phase, which creates an undesirable environment for plant growth. To minimize evaporation, upward salt movement, modifying soil temperature, and improving aeration, as well as releasing nutrients in the soil profile, mulching (surface cover) with biological material or plastics could be an effective option (Abd El-Mageed et al., 2016; Ahmad et al., 2007b; Sharma et al., 2005). An effective soil cover with crop residues acts as a water vapor barrier against evaporation losses, slow surface runoff, and increase infiltration. The

reduced evaporation losses results in better soil moisture regimes and reduction in salt buildup in crop root zone (Pang et al., 2010), which are desirable for crop production in saline environments.

5.5 Selection of Salt-Tolerant Crops and Monitoring of Salinity Status

The level of salinity to which a crop can adjust is known as the tolerance limit. It is an extremely important parameter that can be used to select suitable crops for those areas where soil salinity buildup cannot be controlled to an acceptable limit. Under that situation, keeping tolerance limit in mind, an alternative crop can be selected that can produce an economic yield. A large range of salinity tolerance is available for horticultural crops that allow a greater use of moderately saline water, which was previously not to be used. The wide range of salt tolerance of horticultural crops results in increased range of acceptability of salinity of water for irrigation (Suarez and Grieve, 2013). Regular monitoring of salinity level in crop root zone is essential to make sure that salt buildup is below the threshold value. If root zone salinity reaches a harmful level, additional water has to be applied to leach salts below the root zone to reduce the harmful effect of saline water.

5.6 Use of Tolerant Rootstock–Grafted Plants

Grafting is an old propagation method used in fruit crops to maintain their genetic superiority. However, currently, it is becoming an important tool to manage biotic and abiotic stresses, such as salinity, elemental toxicity, drought, flooding, and extreme temperature stresses in fruit and vegetable crops (Antonious, 2016; Lei et al., 2014; Yahmed et al., 2015; Zrig et al., 2016). However, to achieve a desirable change in plant root system through grafting, it is essential to select a compatible rootstock (Kumar et al., 2016). Several studies have reported that the tolerant rootstock minimizes the stress by extrusion or accumulation of harmful salts or metals, increased sugar level, higher expressions of ribulose-1,5-bisphosphate (RuBP), and regulation of stomatal opening (Martínez-Ballesta et al., 2010; Strand et al., 2000; Yang et al., 2013). Application of salt-tolerant rootstocks (Na and Cl) is commercially practiced in citrus (Pérez-Clemente et al., 2012), mango, and grape crops (Upreti et al., 2016). Among vegetables, cucurbits (Yang et al., 2015), tomato, and brinjal (Kumar et al., 2016) are mechanically or manually grafted onto tolerant rootstocks for growing in stress conditions. Grafting melon

onto *Cucurbita* rootstock decreased boron and Na uptake by the grafted plants. Also grafting appears to offer an environmentally acceptable alternative to methyl bromide application in nematode attack and the cultivation of vegetables with saline water/effluents (Edelstein and Ben-Hur, 2005).

The concentration of toxic elements, such as Ni, Cd, B, Na, and Cl were found to be lower in the shoots and fruits of grafted plants (Edelstein and Ben-Hur, 2014) (Table 7.4).

Table 7.4 Tolerant rootstocks for fruits and vegetables

Crops	Rootstocks	Tolerance against	References
Tomato cv. Ikram	Unifort and Maxifort	Ni toxicity minimized	Kumar et al. (2015)
Eggplant	<i>Solanum torvum</i>	Reduced Cd concentration in fruits	Arao et al. (2008)
Melon	<i>Cucurbita</i>	Showed higher tolerance to boron concentration	Edelstein and Ben-Hur (2005)
Melon cv. Cyrano	P360	NaCl concentration	Rouphael et al. (2012)
Cucumber cv. Akito	PS1313	Salinity tolerance	Rouphael et al. (2012)
Pepper	A/A25	Salt tolerant	Penella et al. (2016)
Capsicum cv. Adige	<i>Capsicum chinense</i> Jacq. ECU-973 (12)	Higher tolerance to salt	Penella et al. (2015)
Mandarin cv. Sunburst	Cleopatra	Lower Cl ⁻ and Na ⁺	García-Sánchez et al. (2002)
Lemon	<i>Citrus macrophylla</i>	Salinity tolerance	García-Legaz et al. (1993)
Mango cv. Osteen	Gomera-1	Suitable for saline condition	Zuazo et al. (2004)
Mango	Rangpur	Higher tolerance to salt	Roy et al. (2015)
Loquat	Anger	Salinity tolerance	García-Legaz et al. (2008)
Citrus	Cleopatra	Best chloride excluder	Levy et al. (1999) and Anjum et al. (2001)
Grape	<i>Vitis berlandieri</i>	Cl and Na excluder	Fisarakis et al. (2001)
Grape	Dogridge	High salt tolerant	Saritha et al. (2016)

6 CONCLUSIONS AND FUTURE RESEARCH NEEDS

Preharvest factors are considered to be gateways of postharvest quality parameters. Like other crop production inputs (manure, fertilizer, soil, variety, rootstock, etc.), irrigation is considered one of the major factors that have a significant influence on postharvest quality and shelf life of fruits and vegetables. As a general conclusion, it should be stressed that both positive and negative effects of poor-quality water application on crop quality occurred. As these outcomes depend on numerous physiological, environmental, and experimental factors, various findings sometimes appear to be contradictory, even if the same stress types and species were investigated. In spite of this complexity, some vital findings were observed, such as higher accumulation of protein, minerals, vitamin C, lycopene, tannins, and antioxidant concentration, with a reduction in fruit size and total yield. Different crop varieties or genotype behave differently based on the level of tolerance; therefore, a better understanding of the interactions among genetic makeup, stress conditions, and crop husbandry is required.

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CHAPTER 8

Rootstocks for Improved Postharvest Quality of Fruits: Recent Advances

Endrit Kullaj

Agricultural University of Tirana, Tirana, Albania

1 INTRODUCTION

Grafting is the natural or deliberate fusion of plant parts so that vascular continuity is established between them and the resulting genetically composite organism functions as a single plant (Pina and Errea, 2005). For nonhorticulturalists, the practice of grafting represents a difficult and even mysterious technique, as frequently perceived by new students of horticulture. However, even those applying it commercially on a daily basis do not completely understand the consequences for the two plants. In fact, it is the only example of “melding” two different organisms, in some cases belonging to different genera, and obtaining one that has the best characteristics of both [modified from Boffelli and Sirtori (2006)]. For a recent review on grafting, refer to Melnyk and Meyerowitz (2015). Those interested in the history of grafting and its uses can refer to Mudge et al. (2009).

In general, influence of rootstock on postharvest quality is poorly understood. Rootstocks influence the scion development in several ways, affecting the traits of agricultural interest, such as vegetative vigor, stress tolerance, yield, fruit quality, and so forth (Gregory et al., 2013; Lee et al., 2010). Besides changing patterns of dry matter allocation (Fig. 8.1) between roots and shoots (Dayatilake and van Hooijdonk, 2015), the controlling effect of rootstock over scion is possibly due to altered root-to-shoot and/or shoot-to-root chemical signaling (Gregory et al., 2013). Studies on long-distance signaling via graft-union provide evidence for multiple types of mobile signals, such as hormones (Alonia et al., 2010; El-Sharkawy et al., 2012; Etehadnia et al., 2008; Sorce et al., 2007), proteins (Corbesier et al., 2007; Wang et al., 2012), ribonucleoprotein (Ham et al., 2009), RNAs (Harada, 2010), small RNAs (Bai et al., 2011; Brosnan et al., 2007; Kasai et al., 2011; Molnar et al., 2010; Zhang et al., 2014),



Figure 8.1 Two dendrometers installed above and below the grafting point measuring growth rate and trunk diameter variations in apple.

minerals (Ebel et al., 2000; Jiménez et al., 2007), and so forth, conferring a wide range of effects on scion development (Kumari et al., 2015) (see Section 5).

Shoot–root–shoot signaling of auxins and other hormones has been extensively studied. For instance, when dwarfing Fuji/M9 and the vigorous Fuji/MM106 were compared, indole-3-acetic acid (IAA) content was lower in the dwarfing Fuji/M9 than in the vigorous Fuji/MM106 (Song et al., 2016).

A consistent body of research has demonstrated that grafting affects key enzymes of glycolysis, such as phosphoenolpyruvate carboxylase (PEPC), enolase (ENO), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH). Activities of these enzymes were enhanced by grafting (Li et al., 2015). Other enzymes affected by rootstock are superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GR) in grapefruit (Sharma et al., 2015); or acid invertase (AI), neutral invertase (NI), and phosphate sucrose synthase (SPS) of sucrose metabolism; and fructokinase (FK), hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) of glycolysis in cucumber (Xing et al., 2015). In addition, contents of glycolysis intermediate metabolites (citric, succinic, and malic acids) were significantly higher in leaves of rootstock-grafted seedlings (Li et al., 2015).

Furthermore, leaf adenosine triphosphate (ATP) content of rootstock-grafted seedlings was relatively higher (Li et al., 2015).

Another line of research on the physiology of the rootstock–scion interaction explains some of the effects by the modified vascular system altering the xylem vessel, as in the case of grapevine (Santarosa et al., 2015).

There are many rootstock breeding efforts to improve the quality of fruits (Giovannini et al., 2013; Guajardo et al., 2015; Kocsis et al., 2014).

2 ROOTSTOCK EFFECTS ON FRUIT QUALITY

In principle, Davies et al. (2011) states that in a grafted tree there is no transmission of fruit traits characteristic of the rootstock to the fruit produced by the grafted scion; tart and astringent flavor of quince is not transmitted to pear fruits or characteristics of peach to apricot fruits. However, the same authors offer examples of such effects, such as the “black end” defect of pears and melons on disease-resistant cucurbit rootstock (Davis et al., 2008), the effect of rootstock on capsaicin of chili peppers (Yagishita et al., 1985), and rootstock effect on fruit quality of citrus (Bitters, 1961).

Nevertheless, rootstocks are decisive not only for the vigor and yield of the grafted cultivar (Avdiu et al., 2014; Domi et al., 2014; Spahiu et al., 2013a,b; Thomaj et al., 2015), but also size, acidity, color, and firmness of the fruit [adapted from López-Ortega et al. (2016)]. In our literature review we found incongruence on the effects of dwarfing rootstocks on fruit quality, which may arise from the differences in terms of species, cultivars, rootstock combination, climates, and the biological control of dwarfism (Autio et al., 1996a,b; Daugaard and Callesen, 2002; Fallahi and Kilby, 1997; Smolka et al., 2010).

2.1 Fruit Size

Size is an important commercial standard in horticulture. The effect of rootstock on fruit size is indirect, related to vigor. Thus, it is well established that trees on dwarfing rootstocks in which most of the canopy is well exposed to sunlight will produce, on average, larger fruits than those on more vigorous rootstocks that have a larger proportion of shaded canopy (Jackson, 2003). Shaded areas of tree canopy produce smaller fruits (Gyuro, 1986; Jackson et al., 1971; Jackson and Palmer, 1977; Palmer et al., 1989; Silbereisen, 1981; Tustin et al., 1989; van Oosten, 1986).

Rootstocks can influence fruit size through their effects on the number of fruits in relation to the size and photosynthetic potential of the tree, and as this ratio is increased, fruit size is reduced; hence the practice of fruit

thinning to increase the size of the remaining fruits [modified from Jackson (2003)]. Stern and Doron (2009) found that the highest cumulative yield of large pear fruit (>60 mm) was obtained from trees on *P. betulifolia*, followed by the OHF series and BP 1 (*Pyrus communis*).

Another explanation for the reduced fruit size is the decreased rootstock hydraulic conductance mechanism to impart the size-controlling behavior, as in the case of peach (DeJong et al., 2012).

There are many more authors reporting changes in fruit size affected by rootstock in apple (Hussein and McFarland, 1994), plums (Butac et al., 2015), sweet cherry (López-Ortega et al., 2016), grapevine (Nelson et al., 2016), lemon (Dubey and Sharma, 2016), and muskmelon (Javanpour et al., 2015), just to name a few.

2.2 Color

Rootstocks' influences on fruit color appear to be mainly a secondary effect of their effects on the vigor of tree growth and within-tree shade (Jackson, 2003). For instance, in apples, fruits from the outer zone of the canopy have the greatest proportion of red-colored surface (Gyuro, 1986). Less chlorophyll would be expected in shaded fruit, as chlorophyll synthesis is light induced (Raven et al., 1992; Zucker, 1972). Shading of harvested parts of horticultural plants has pronounced effects on their eating quality and storability (Kullaj, 2016). In grapes, low light intensity decreases sugar accumulation, anthocyanins, total phenols, ammonia, pH, and the concentration of proline, and increases TA and the concentration of malate and arginine (Kliewer and Lider, 1970; Smart et al., 1985). These effects are due to the effects on photochrome-regulated enzymes, for example, phenylalanine ammonia lyase or malic enzymes, which influence fruit pigmentation in plants, such as tomato (Azari et al., 2009; Kumar et al., 2015), grape berries (González et al., 2015), apple fruits (Li et al., 2013) [see also Toledo-Ortiz et al. (2010)], and so forth. In experiments comparing sun-exposed and shaded grape bunches there was a difference in the level of berry carotenoids depending on the level of light incident on a grape cluster (Bindon, 2004; Bureau et al., 1998, 2000; Oliveira et al., 2004; Razungles et al., 1998).

Reduced canopy volume with dwarfing rootstocks, exposes more fruits to extreme levels of sun exposure, increasing the degradation of carotenoids and C¹³-norisoprenoids (a group of potent aroma compounds) (Baumes et al., 2002; Bindon, 2004; Bureau et al., 1998, 2000; Düring and Davtyan, 2002; Downey et al., 2004; Marais, 1992; Razungles et al., 1998; Ristic et al., 2007; Steel and Keller, 2000; Tevini and Teramura, 1989).

In general, shaded fruits are less prone to russet and cracking than exposed fruits, and it might be expected that fruits from trees on the most dwarfing rootstocks would suffer more from these problems, but results are inconsistent (van Oosten, 1986).

Several authors have found that rootstock affected appearance and quality (Butac et al., 2015; Machado et al., 2015).

2.3 Sugar and Acid Content

In general, with the increase of rootstock vigor, the percentage of soluble solids declines (Autio et al., 1996b; Ogata et al., 1986; Sansavini et al., 1986). Stern and Doron (2009) found changes in highest soluble solids content (SSC) values at harvest were obtained in “Coscia” pear fruit grown on quince EMA (*Cydonia oblonga*), compared to values for BP 1 (*P. communis*) and *P. betulifolia* fruit. Dubey and Sharma (2016) found that rootstocks with improved TSS and acid content in the fruit juice of lemon. Improved TSS has been found in watermelon grafted on squash (Villocino and Quevedo, 2013) and muskmelon on cucurbit (Javanpour et al., 2015).

Rootstocks in grapevine could be improved to affect different quality parameters of grape juice, such as concentration of sugar, total acids, tartaric acid, malic acid, ratio of tartaric to malic acid (beta-ratio), concentration of yeast, assimilable nitrogen, and pH value (Asiia and Michlovský, 2015; Théral et al., 2015).

2.4 Nutrients

Studies in grapevine have shown that rootstocks influence the level of nutrients in the scion cultivar after grafting (Bavaresco et al., 2003; Fardossi et al., 1995; Fisarakis et al., 2004; Nikolaou et al., 2000; García et al., 2001) highlighting the scope for selection of better rootstock for sustainable nutrition management (Somkuwar et al., 2015). Other studies with grapevine (Hanana et al., 2015; Somkuwar et al., 2015), lemon (Dubey and Sharma, 2016), sweet cherry (Küçükyumuk, 2015; Stachowiak et al., 2015), apple (Fallahi et al., 2012), pear (Ikinci et al., 2014), almond (Zrig et al., 2016), melon (Neocleous, 2015), and tomato (Riga, 2015) concluded that certain rootstock uptake and utilize more nutrients than others.

Changes in the physical characteristics of the root system, such as lateral and vertical development, depending on the rootstock, results in enhanced or reduced uptake of water and minerals (Heo, 1991; Jang, 1992). In many cases this is one of the reasons for the widespread use of rootstocks to overcome salinity (Lee et al., 2010).

Others studies demonstrated that higher salt tolerance of grafted plants is closely associated with increased translocation of K^+ , Ca^{2+} , or Mg^{2+} to the leaves (Moya et al., 1999).

2.5 Phytochemicals

Reduction of scion vigor by the rootstocks improves color development, that is, concentration of anthocyanin in the most highly colored skin areas, that is also a function of the light intensity under which the fruit is grown (Jackson et al., 1971). Rootstock affects anthocyanin concentration as in grapevine (Stevens et al., 2015; Nelson et al., 2016) and almond (Zrig et al., 2016). In grapes, low light intensity decreases anthocyanins (Kliewer and Lider, 1970; Smart et al., 1985). A number of enzymes involved in anthocyanin synthesis, including dihydro flavonal reductase (DFR) and phenylalanine ammonia lyase (PAL) correlate to light levels (Ju et al., 1997; Lister et al., 1996).

There is abundant literature on the biochemistry of grafting produced by studies aiming to explain the incompatibility between rootstock and scion [for reviews, refer to Andrews and Serrano Marquez (1993)]. There is a relationship between rootstock vigor and phenolic contents as it has been found in apple (Yildirim et al., 2015), grapevine (Asiia and Michlovský, 2015; Michlovský and Khafizova, 2015), and mango (Vishambhar et al., 2016).

Phenols, high flavonoids, anthocyanins, or their derivatives been found responsible in grapevine (Mng'omba et al., 2008; Stino et al., 2011) for graft incompatibility. Phenol is known to inhibit callus proliferation and disrupt cell chemical reaction or functions and is therefore implicated in graft incompatibility (Stino et al., 2011). Phenolic compounds have been frequently measured in grapevine to evaluate the effects of rootstocks on the wine.

Rootstocks improve tolerance to various abiotic stresses by scavenging or detoxifying reactive oxygen species (ROS) by producing different types of antioxidants (Liu et al., 2007). The latest are health-promoting phytochemicals and their accumulation increases the health value of fruits (Nora et al., 2012), sometimes at the cost of taste (Wang and Frei, 2011). This is achieved by obtaining higher activities of antioxidative enzymes and higher contents of nonenzymatic antioxidants.

3 ROOTSTOCK EFFECTS ON FRUIT MATURITY AND STORAGE

Fruiting precocity is associated with dwarfing rootstocks, and delay in fruiting with vigorous rootstocks (Davies et al., 2011). Besides allowing high-density plantings, dwarfing rootstocks induce precocious flowering, which

enables earlier fruit production [modified from Knäbel et al. (2015)]. Butac et al. (2015) found that rootstock affected bearing earliness in plums. Similarly, Fioravanço (2015) found changes in the earliness index of several apple cultivars grafted onto different rootstocks.

4 ROOTSTOCK INFLUENCE ON INCIDENCE AND SEVERITY OF POSTHARVEST DISEASE

Marques (2002) demonstrated that rootstock can also influence the incidence and severity of postharvest disease in avocado by increasing flesh Ca and B, as well as skin Ca, and reducing flesh and skin N. Calcium has a fundamental role in firmness, the physical component of texture because it is involved in the synthesis of cell walls, by binding with the polygalacturonic acid in middle lamellae, forming pectates that separate new divided cells and enabling a higher cohesion between them (Taiz and Zeiger, 2010). Cross-bonding of cell wall pectates by Ca^{2+} is directly related to fruit firmness.

Grafting of watermelon is an effective tool to overcome continuous cropping obstacles and control soilborne diseases (Lee, 1994; Miguel et al., 2004; Paroussi et al., 2007; Roupheal et al., 2010; Yetisir et al., 2003). Another grafted vegetable, eggplant, has a series of rootstocks, each conferring resistance to various diseases and other resistances. *Solanum melongena* lines and hybrids resist *Fusarium* and bacterial wilt (Monma et al., 1997; Yoshida et al., 2004a,b), controls *Phomopsis* blight, confers windfall resistance (MeiXiu et al., 2001), as well as variation of fruit epidermis and flesh firmness (Suzuki et al., 2004). *Solanum integrifolium* resists *Fusarium* and bacterial wilt (Mochizuki and Yamakawa, 1979a; Yoshida et al., 2004a). Hybrids of *S. integrifolium* × *S. melongena* demonstrate resistances from both parents (Mian et al., 1995; Sakai, 1984). *Solanum torvum* and *Solanum silymbriifolium* are resistant to bacterial wilt (Mian et al., 1995).

Some of the effects of rootstocks on the quality of horticultural products are summarized in Table 8.1.

5 COMMUNICATION OF GRAFTED PLANTS' GENOMES

Despite the wide use of grafting in agriculture, very little is known about the molecular mechanism of rootstock regulation of scion's phenotypes (Kumari et al., 2015). The effect of grafting on the genetic integrity of the scion has been an issue in biology and horticulture since antiquity (Mudge et al., 2009). From a genetic perspective, grafting involves the creation of a compound genetic system by uniting two (or more) distinct genotypes,

Table 8.1 Rootstock effect on the quality of horticultural products

Effects	Mechanisms		Species	References
	Indirect	Direct		
Fruit size	Size and photosynthetic potential of the tree	—	Apple pear on <i>Pyrus betulifolia</i>	Jackson (2003) and Stern and Doron (2009) DeJong et al. (2012)
	—	Rootstock hydraulic conductance	Peach	
Color	Reduced chlorophyll synthesis by shade	—	Grapes	Kliewer and Lider (1970) and Smart et al. (1985) Azari et al. (2009) and Kumar et al. (2015)
	Reduced pigmentation	—	Tomatoes	
Sugars and acids	Reduced SSC due to vigor	—	Grapes	González et al. (2015) Li et al. (2013) Stern and Doron (2009) Dubey and Sharma (2016) Villocino and Quevedo (2013) Javanpour et al. (2015)
	—	Improved TSS	Apples	
	—	—	Pears	
	—	—	Lemon	
	—	Total acids	Watermelon on squash	Villocino and Quevedo (2013)
	—	—	Muskmelon on cucurbit	Javanpour et al. (2015)
	—	—	Grapevine	Asiia and Michlovský (2015) and Téthäl et al. (2015)

Nutrients	Root system development and its enhanced uptake	—	Grapevine Lemon Sweet cherry Apple Pear Almond Melon Tomato Grapes	Hanana et al. (2015) and Somkuwar et al. (2015) Dubey and Sharma (2016) Küçükyumuk (2015) and Stachowiak et al. (2015) Fallahi et al. (2012) Ikinici et al. (2014) Zrig et al. (2016) Neocleous (2015) Riga (2015) Kliewer and Lider (1970) and Smart et al. (1985) Butac et al. (2015) Fioravanço (2015)
Phytochemicals	Low light intensity decreases anthocyanins	—	Plums Apple	
Maturity and storage	Precocious flowering	—	—	
Postharvest disease	Increasing flesh and/or skin Ca and B	—	—	
	Control of soilborne diseases	—	Eggplant	Monma et al. (1997), Yoshida et al. (2004a,b), MeiXiu et al. (2001), Suzuki et al. (2004), Mochizuki and Yamakawa (1979a), and Yoshida et al. (2004a)

SSC, soluble solids content; TSS, total soluble solids.

each of which maintains its own genetic identity throughout the life of the grafted plant (Mudge et al., 2009). Same authors exemplify this theory with the fact that the scion of a reflowering rose grafted on a white rose stock will continue to produce red roses rather than pink (hybrid) roses. However, controversial claims of graft “hybridization” have persisted, and new information on gene silencing caused by the transmission of RNA across the graft union suggests that grafting could have genetic consequences (Mudge et al., 2009).

The first evidence of a phenotype mixture, at least, is the *graft chimera*, known since the 16th century (Mudge et al., 2009) with the best-known example of *Laburnocytisus adammi*, developed in 1825, and perpetuated by grafting until today. These mixtures of two genetic tissues or genotypes have been explained as a special type of genetic mosaic (Marcotrigiano and Gradziel, 1997) where the lineage of genetically dissimilar apical cells continues into developing plant organs. Explanation of graft chimeras as mixtures of tissues with genetically preserved identities refuted the contention that grafting induced genetic change at the cellular level. The concept of chimeral engineering involving graft insertion of epidermal cells of an insect-resistant genotype was then suggested in Solanaceae (tomato and nightshade) and Brassicaceae (Goffreda et al., 1990; Lindsay et al., 1995).

In the last 2 decades, there is abundant literature supporting *graft hybridization*, either on changes on fruit morphology after grafting, particularly with *Capsicum annum* cultivars (Hirata, 1986; Kashara et al., 1973; Ohta, 1991; Taller et al., 1998; Yagishita et al., 1985) or the generation of cytoplasmic male sterility in petunia (Edwardson and Corbett, 1961; Frankel, 1956), sugarbeet (Curtis, 1967), and alfalfa (Thompson and Axtell, 1978). Besides this evidence, there was no support by modern molecular biology, although various authors have offered explanations (Liu, 2006; Ohta, 1991; Taller et al., 1998). As a mechanism of graft hybridization, Liu (2006) hypothesizes that stock mRNA molecules are being transferred to the scion, then reverse transcribed into cDNA that can be integrated into the genome of the scions germ cells, embryonic cells, as well as the somatic cells of juvenile plants. Another plausible mechanism for some phenomena associated with graft-induced variation are RNA-mediated gene silencing, which applies to grafting. Accumulation of double-stranded RNA (dsRNA) activates a homology-dependent mechanism that cleaves the dsRNA into 21–25 basepair fragments, known as small interfering RNAs (siRNAs) (Mudge et al., 2009). These are utilized to direct the sequence-specific degradation of mRNA or to suppress transcription via DNA methylation (Baulcombe, 2005). The silencing signal can

be propagated through the phloem, so that gene silencing occurs elsewhere in the plant (Tournier et al., 2006). Brosnan et al. (2007) is confident that with the wealth of tools available for studying RNA-mediated gene silencing, this system could be employed to carry out a rigorous reexamination of graft-induced variation.

Another useful and more recent approach to understand the genes involved in the effect of the rootstock is gene expression studies. Differences in gene expression between the organs of the scion and rootstock, from numerous functional categories related to stress responses have been found in *Arabidopsis* (Kumari et al., 2015), sour cherry (Prassinos et al., 2009), apple (Jensen et al., 2010), and grapevine (Berdeja et al., 2015; Cookson and Ollat, 2013; Cookson et al., 2014).

Recently, the analysis of grafting has been extended to proteomics (Song et al., 2014). Most recent research (Lewsey et al., 2016) shows that genetic information is actually flowing from rootstock to scion, but it is not DNA—the two grafted plants keep their original genomes—instead, epigenetic information is being communicated within the plant. In epigenetics, chemical markers act on existing genes in a plant's DNA to turn genes on or off (gene silencing), a process called DNA methylation, in which small RNAs (sRNAs) contribute to transport across grafted plants from the shoots to the roots (Melnyk and Meyerowitz, 2015), transmitting the epigenetic equivalent of alleles, called epialleles. In DNA methylation, molecular markers bind along the top of DNA to block the cell's machinery from reading or expressing the genes under the molecular markers (Melnyk and Meyerowitz, 2015). This determines how a plant reacts to different soils, climates, and disease, and in the future breeding activities could exploit epigenetic information to improve crops and yields.

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CHAPTER 9

Preharvest Sprays Affecting Shelf Life and Storage Potential of Fruits

Ahmad S. Khan, Sajid Ali

Postharvest Research and Training Centre, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan

1 INTRODUCTION

The condition of the fruit at the time of harvest is very important for its performance during the postharvest storage. This involves a suitable maturity stage along with some other related aspects, such as the nutritional status and resistance/tolerance against particular storage pathogens. To reduce these problems during postharvest handling, preharvest sprays with certain compounds/chemicals have now become an extensively used practice throughout the world (Lara, 2013). Since a very large number of postharvest alterations may arise from the mineral deficiencies or preharvest infections with different storage pathogens; hence, several preharvest sprays are aimed at supplementing the anticipated fruits with higher contents of that specific mineral and antipathogenic elements needed to enhance their storage potential (Manganaris et al., 2006; Marzouk and Kassem, 2011; Qin et al., 2010; Vangdal et al., 2008; Yao and Tian, 2005).

Postharvest quality of fruits can also be manipulated by preharvest applications of different mineral elements, plant growth regulators, fungicides, or natural antagonists (Domínguez et al., 2012; Zhu et al., 2016a; Zoffoli et al., 2009). Although some of their common effects are generally recognizable for the given preharvest sprays, some variability may exist across different fruit species and/or even among different cultivars of the same fruit species (Lara, 2013). It means that it is essential to confirm the suitability and to optimize the possible application protocols on a fruit-by-fruit basis (Lara, 2013). On the other hand, it is also possible that the anticipated beneficial effects on the specific fruit may be counteracted by a number of detrimental influences in case of inappropriate doses of different preharvest sprays (Ernani et al., 2008; Koutinas et al., 2010; Xie et al., 2003). Moreover, most of the published studies on the impact of different preharvest sprays

only focused on the postharvest quality of fruits (Lara, 2013). However, the influence of different preharvest sprays on shelf life, storage potential, decay, and certain physiological disorders has been generally overlooked, and still need further studies in order to ensure the optimal quality of fresh fruits reaching consumers around the globe. Hence, this chapter describes the published literature on the influence of different commonly used preharvest sprays, which affect the shelf life and storage potential of various fruit crops in the world.

2 MINERAL ELEMENTS

2.1 Boron

Boron (B) is one of the essential micronutrients needed for normal growth of plant. Deficiency of B is a worldwide problem responsible for the reduced productivity of plants that ultimately reduces the crop yield and plant bearing age (Nable et al., 1997). Besides using B as a common plant nutrient, it is also used in the form of preharvest sprays to reduce postharvest decay and to extend storage life of fruit (Qin et al., 2010). The pre-harvest spray of B strongly inhibited browning related disorders of “Conference” pears in controlled atmosphere conditions kept for 4 months under cold storage. B application also reduced the membrane electrolyte leakage and increased ascorbic acid contents and antioxidant activities, which protected pear fruit tissues from internal browning (Xuan et al., 2001). The preharvest spray of B is very effective against different storage pathogens, such as *Botrytis*, brown and blue rot. The effects of preharvest spray of 8% B in the form of boric acid has been reported to reduce the incidence of brown rot and maintained the membrane integrity of “Chandler” strawberry fruit (Singh et al., 2007). Moreover, B application also exhibited higher SSC and TA along with reduced postharvest decay for 5 days under cold storage. Similarly, preharvest foliar application of borax reduced weight loss and brown rot incidence in peach cv. “Andross” during cold storage (Thomidis and Exadaktylou, 2010). Exogenous application of 1% B maintained the membrane integrity and reduced *Botrytis cinerea* infection, ultimately resulted in enhanced storage life of “Thompson Seedless” table grapes for 30 days (Table 9.1) (Qin et al., 2010).

2.2 Calcium

Calcium (Ca) is an essential nutrient required for growth of plants. It also works as secondary messenger. Fruit plants absorb Ca in the form of divalent

Table 9.1 Effects of preharvest sprays of different mineral nutrients on storage potential and shelf life of fruits

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Boron	Grapes	“Thompson Seedless”	1% $K_2B_4O_7 \cdot 4H_2O$	At harvest	5 days at 23°C or 30 days at 0°C	Reduced incidence of <i>Botrytis cinerea</i>	Qin et al. (2010)
	Pear	“Conference”	0.250 kg/ha B	8 WBH	4 months at -0.5°C with 5% CO ₂ + 2% O ₂	Inhibited internal browning	Xuan et al. (2001)
Calcium	Apple	“Golden Smoothie”	0.15% Ca ⁺² (STOPIT)	10 and 60 DAFB	6 months at 0.5°C under ULO	Increased fruit firmness	Benavides et al. (2001)
		“Liivi,”“Kuldren-ett,”“Krameri,” Tuviõun,” “Talvenauding,” “Tellissaare”	660 L/ha Ca(NO ₃) ₂	3 times during summer	4–7 months at 2–5°C and 80%–85% RH	Reduced bitter pit incidence	Moor et al. (2005)
	Apricot	“Canino”	2% calcium nitrate + 4 mM Put	After fruit setting	4 weeks at 0°C and 90%–95% RH	Reduced respiration, decay, weight loss, higher firmness, TA, TSS, vitamin C, total sugars, and phenols	El-Wahab (2015)

(Continued)

Table 9.1 Effects of preharvest sprays of different mineral nutrients on storage potential and shelf life of fruits (*cont.*)

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Calcium	Banana	“Cavendish Grande Naine,” “Flhorban920”	5 g/L CaCO ₃	2 and 3 months BH	14°C until fruit began to soften	Increased green life over control	Tixier et al. (2010)
	Blueberry	“O’Neal,” “Bluecrop”	0.06 kg/m ² CaSO ₄	At harvest	23 days at 2°C	Reduced weight loss and softening	Angeletti et al. (2010)
	Fig	“Poona Fig”	2% CaCl ₂ BH + 50 ppm kinetin and 0.1% CBZ	20 DBH	4 days at 24–28°C with 76% RH	Slowed increase in SSC, sugars and reduced acidity degradation	Kurubar et al. (2013)
	Grapes	“Thompson seedless”	1 g/L CaCl ₂	Pea and veraison stages	7 days at 20 ± 2°C, 75%–80% RH	Increased firmness, berry adherence strength and decreased unmarketable fruits	Marzouk and Kassem (2011)
	Kiwifruit	“Hayward”	1% CaCl ₂ 4 times during fruiting + pruning	93, 108, 123, and 138 DAPF	42 weeks at 0°C with 95% RH	Increased firmness, SSC, and fruit calcium contents	Gerasopoulos and Drogoudi (2005)
		“Hayward”	0.75 and 1.5% CaCl ₂	93, 118, and 143 DAPF	18 weeks at 0°C + 5 weeks at 20°C with 95% RH	Increased firmness, TA and decreased SSC	Gerasopoulos et al. (1996)

	Mango	“Dashehari”	0.6% CaCl ₂	20 and 10 DBH	10 days at 35 ± 3°C and 65 ± 5% RH	Delayed ripening and increased marketable fruits	Singh et al. (1993)
	Nectarine	“Caldesi 2000”	12 mg CaCl ₂ L ⁻¹	4 or 8 weeks BCH	6 weeks at 0°C with 95% RH + 5 days at 20°C	Increased cell wall and pectin calcium contents firmness and overall quality	Manganaris et al. (2006)
	Peach	“Andross”	0.12% calcium	10 sprays during fruit season	2 or 4 weeks at 0°C and 95% RH	Increased fruit calcium contents and decreased brown rot development	Manganaris et al. (2005)
	Pear	“Conference”	2 kg/ha CaCl ₂	6 weeks AFB + 1 week BCH	150 days at 0°C + 2 days at room temperature	Better color, higher firmness, reduced internal browning, and increased organic acids	Wojcik (2012)
	Strawberry	“Chandler”	2 kg Ca ha ⁻¹ and 150 g B ha ⁻¹	1st spray at petal stage and 2nd after 7 days of 1st spray	5 days at 10°C with 90% RH	Reduced membrane leakage	Singh et al. (2007)
Potassium	Mandarin	“Cadoux”	5 kg 100 L ⁻¹ KNO ₃	Flower initiation-differentiation	30 days at 4°C with 85%–95% RH	Decreased TA, increased maturity index and ascorbic acid	El-Otmani et al. (2004)

(Continued)

Table 9.1 Effects of preharvest sprays of different mineral nutrients on storage potential and shelf life of fruits (*cont.*)

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Magnesium	Nectarine	“Silver King”	0.103 mM Mg	10 days after anthesis and before manual thinning	28 days at $0 \pm 0.5^{\circ}\text{C} + 5$ days at 20°C with 90% RH	Reduced weight loss, SSC/TA ratio, ethylene production, along with enhanced SSC and TA	Serrano et al. (2004)
	Peach	“Sevilla-II”	0.103 mM Mg	10 days after anthesis and before manual thinning	28 days at $0 \pm 0.5^{\circ}\text{C} + 5$ days at 20°C with 90% RH	Reduced weight loss, SSC/TA ratio, ethylene production, along with enhanced SSC and TA	Serrano et al. (2004)
Nitrogen	Mandarin	“Cadoux”	1 and 1.6 kg 100 L^{-1} urea	Flower initiation-differentiation	30 days at 4°C with 85%–95% RH	Decreased TA, increased maturity index, ascorbic acid, and higher free amino acids	El-Otmani et al. (2004)
Nickel	Persimmon	“Saijo”	0.1% NiCl_2	12 and 42 DBH	4 days at 20°C under 60% RH	Increased ACC synthase activity and shelf life for 2 days, than control	Zheng et al. (2006)

Selenium	Peach	“Suncrest”	1 mg/L selenium	24 DBCH	46 days at 2°C + 8 days at 17°C	Increased firmness and reduced SSC	Pezzarossa et al. (2012)
	Pear	“Conference”	1 mg/L selenium	24 DBCH	46 days at 2°C + 8 days at 17°C	Increased firmness and reduced SSC	Pezzarossa et al. (2012)
	Tomato	“Provence”	1 mg/L Selenium	Fruit set	20 days at 25°C	Reduced lipid peroxidation, decay, enhanced enzymatic and nonenzymatic antioxidants	Zhu et al. (2016b)

ACC, 1-Aminocyclopropane-1-carboxylic acid; AFB, after first bloom; BCH, before commercial harvest; BH, before harvest; CBZ, carbendazim; DAFB, days after first bloom; DAPF, days after petal fall; DBCH, days before commercial harvest; DBH, days before harvest; mM, millimole; ppm, parts per million; RH, relative humidity; SSC, soluble solid contents; TA, titratable acidity; TSS, total soluble solids; ULO, ultra low oxygen; WBH, weeks before harvest.

cation (Ca^{2+}) that is needed for numerous key physiological processes related with ripening, cell wall structure, integrity of membranes, activity of certain enzymes, and signal transduction. The deficiency of Ca in fruits may result in various physiological disorders of significant economic value like fruit cracking, vitrescence, and bitter pit (White and Broadley, 2003). Hence, application of Ca has tremendous potential to delay ripening and senescence, and possesses highly beneficial influences on several other characteristics associated with the quality and postharvest storability of fruit crops (Aghdam et al., 2012). Ca treatments may also be used prior to the commercial harvest in order to ensure suitable supply of this vital mineral before the actual appearance of its deficiency symptoms. It has been reported that the uptake and movement of Ca from soil is limited; hence, direct preharvest Ca sprays on the canopies of fruit trees are preferable because this is a more effective way to enhance Ca contents in fruits (Ferguson and Boyd, 2002). However, it may not necessarily be true in all cases (Lester and Grusak, 2004). No consistent effects have been reported on the Ca contents in Ca-sprayed apple fruit (Katsurayama et al., 2011; Val et al., 2008). Moreover, the phytotoxic effects have also been reported, which specifies the necessity to optimize the treatment conditions individually for each species and/or cultivar (Cooper et al., 2007). It has also been described that chloride (Cl^-) salt is known as one of the most excessively used Ca sources for preharvest sprays. However, few studies have also been conducted with other sources of Ca/formulations regarding their potential suitability for enhancing the Ca content in fruits and/or for extension of their postharvest storage and shelf life (Antunes et al., 2005; Blanco et al., 2010; Katsurayama et al., 2011; Koutinas et al., 2010; Lester and Grusak, 2004; Moor et al., 2005; Serrano et al., 2004; Singh et al., 1993; Manganaris et al., 2005, 2006; Val and Fernández, 2011). In addition, some Ca formulations have been found to influence the efficacy of the treatments, especially about the incidence of physiological alterations and/or decay. Moreover, besides the sources/formulations; season-to-season variation in effectiveness of preharvest Ca treatments has also been occasionally found in apples (Benavides et al., 2001; Ernani et al., 2008) and kiwifruit (Koutinas et al., 2010). Preharvest Ca sprays have been mostly reported to delay fruit ripening, as indicated by the ethylene production, respiration rates and to increase fruit firmness at commercial harvest or after storage, and to reduce the postharvest decay incidence of numerous fruits. Moreover, significant enhancement in the antioxidant activities or contents of antioxidant compounds has also been reported. The enhanced antioxidants ultimately lead to increased storage life due to reduced postharvest

oxidative stress (Koutinas et al., 2010; Vangdal et al., 2008; Xie et al., 2003). Furthermore, Ca treatments have also been found to prevent the occurrence of commercially physiological and storage disorders of fruits, such as scald, bitter pit, lenticel blotch pit, internal breakdown, cork spot incidence, and postharvest cracking (Benavides et al., 2001; Blanco et al., 2010; Demirsoy and Bilgener, 1998; Dris and Niskanen, 1999; Ernani et al., 2008; Malakouti et al., 1999; Moor et al., 2005; Raese and Drake, 1993; Vangdal et al., 2008). Moreover, preharvest Ca spray treatments also helped to prevent and/or reduce internal browning and chilling injury (CI) in susceptible fruit species like kiwifruit (Gerasopoulos and Drogoudi, 2005), peach (Val and Fernández, 2011), pear (Wojcik, 2012), and rambutan (Chiradej et al., 2012). Beneficial effects of the preharvest Ca sprays on increased firmness and reduced decay incidence appeared to be quite general. However, influence of preharvest Ca sprays on other quality attributes, such as weight loss, color, soluble solid contents, and sugar:acid ratio have been reported to be erratic and even contradictory. This variability could be related to the genotypic differences among the different fruit species, cultivars, and/or used Ca concentrations/formulations (Lara, 2013).

It has been widely reported that preharvest Ca sprays lead to increase fruit firmness but the physiological and biochemical basis for the delayed softening in the treated fruits has received less attention. However, some pivotal information is available in this regard for apple, blueberry, olive, kiwifruit, nectarine, and peach. Reduced softening has been found to occur from delayed pectin-solubilization and matrixglycan breakdown in the Ca-treated fruits (Angeletti et al., 2010; Manganaris et al., 2005, 2006; Ortiz et al., 2011; Siddiqui and Bangerth, 1995a; Xie et al., 2003). Indeed, preharvest exogenous Ca applications may favor formation of the noncovalent cross links between numerous polyuronides through the Ca bridges; therefore, inhibiting the dissolution of middle-lamella and strengthening cell walls structure. Preharvest, Ca sprays often result in increased chelator soluble fraction of the pectins comprised of mainly noncovalently bound cell wall units of polyuronides, related with the better retention of the total uronic acids. In addition, the reinforcing of cell wall structure by Ca may also enhance ability to maintain firmness through the modulation of the critical cell wall, modifying enzyme activities. Similarly, Ca-treated apple and peach fruits have been found to possess lower activities of β -galactosidase (β -Gal), polygalacturonase (PG), pectinmethylesterase (PME), pectate lyase (PL), α -L-arabinofuranosidase (AFase), or β -Xylosidase (β -Xyl) enzymes (Manganaris et al., 2005, 2006; Ortiz et al., 2011; Siddiqui and Bangerth,

1995b). The effects of preharvest Ca applications on fruit/flesh firmness and cell wall composition are not only associated with its electrostatic properties, but also comprise some of the specific effects, as treatment of apple fruit with strontium chloride failed to mimic effects of a similar related source, that is, CaCl_2 on β -gal activity or cell wall properties (Siddiqui and Bangerth, 1995b). In the case of apple, the normal practice is to harvest fruit before it reaches full ripeness, which is aimed at attaining the highest firmness and extended storage potential of fruits (Lara, 2013).

The major objectives of the preharvest Ca sprays have been fundamentally focused on prevention of certain physiological alterations as well as extension of storage and shelf life of fruits as perceived by the general quality characteristics (Lara, 2013). On the other hand, very little information is available on the possible effects of Ca sprays on development of fruit aroma volatiles in response to preharvest sprays. This aspect has largely been overlooked despite the fact that it is one of the major contributors to the sensory quality and consumer's acceptance of different fruits.

2.3 Potassium

Potassium (K) is also an essential nutrient that plays an important role in growth regulation and protein synthesis, as well as opening and closing of stomata. It has been reported that preharvest K sprays led to higher rind firmness of mandarin fruit. Higher firmness is prerequisite for the extension of storage and shelf life of fruits, while other quality attributes remained unaffected (El-Hilali et al., 2004). In contrast, preharvest K application resulted in higher SSC, TA, and ascorbic acid contents, which may be due to different concentrations of K (El-Otmani et al., 2004). Moreover, K application led to reduced peroxidase activities in the treated mandarin fruit during cold storage at 4°C, proposing that the treatment prompted CI tolerance, which ultimately resulted in increased storage life (Table 9.1) (El-Otmani et al., 2004). These reports indicate tremendous potential of K for physiological storage disorders prevention and it is worthy for the further future research.

2.4 Magnesium

Magnesium (Mg) is an integral part of chlorophyll and plays an imperative role in the process of photosynthesis (Reay et al., 1998). Mg also plays an important and effective role in enhancing plant growth by increasing the activities of iron in chloroplasts (Reay et al., 1998). It has been reported that preharvest application of 2.5 mg Mg along with 4 mg Ca and 2 mg

titanium 10 days after anthesis and before manual thinning resulted in reduced weight loss and suppressed ethylene biosynthesis, as well as enhanced SSC and TA under cold storage for 28 days at 2°C in “Sevilla-II” peach and “Silver King” nectarine fruits (Table 9.1) (Serrano et al., 2004).

2.5 Nitrogen

Nitrogen (N) is a macronutrient that has been found to play vital role in plant growth. Preharvest sprays with N-containing substances have also been reported to affect the postharvest quality and storage life of numerous fruits. Preharvest application of urea and KNO_3 were found highly effective to maintain high juice contents, SSC, SSC/TA ratio, and ascorbic acid contents of mandarin fruits stored for a period of 30 days (El-Otmani et al., 2004). So, application of urea and KNO_3 could be used commercially not only to enhance quality but also extend storage life of mandarin fruits (El-Otmani et al., 2004). Similarly, table grapes treated with three preharvest sprays of *N*-(2-chloro-4-pyridyl)-*N*-phenylurea resulted in significantly higher firmness and juice content and led to reduced weight loss and high marketable fruit percentage after being kept for 1 week under ambient temperature conditions (Table 9.1) (Marzouk and Kassem, 2011).

2.6 Nickel

Nickel (Ni) as metal ion is present in Earth’s crust and has been reported as an environmental pollutant. However, it has also been identified as an inhibitor of 1-aminocyclopropane 1-carboxylic acid (ACC), the precursor of ethylene biosynthesis, due to its antisenescence properties. According to Lau and Yang (1976), Ni^{2+} reduced ethylene biosynthesis due to the replacement of Fe^{2+} in ACC synthase enzyme. The effect of preharvest Ni^{2+} application on “Saijo” persimmon regarding the postharvest storage life has been reported by Zheng et al. (2006). Preharvest 0.1% NiCl_2 spray on the persimmon before commercial harvest significantly retarded ACC accumulation, fruit softening, and ethylene production by increasing the shelf life up to 7 days. In contrast, control fruits were completely softened after 5 days of storage. Hence, exogenous application of NiCl_2 delayed the fruit softening and enhanced shelf life for 2 days, as compared to control (Table 9.1) (Zheng et al., 2006).

2.7 Selenium

It has been reported that selenium (Se) is an essential micronutrient that plays a critical role in antioxidant defense systems. Se is an essential part of

glutathione peroxidase enzyme as well as some other seleno proteins (Ellis and Salt, 2003). Although the higher plants may not need Se and these are prone to Se toxicity, still there are increasing reports that it may also possess beneficial biological effects due to its antioxidant characteristics (Stadtman, 1990; Zhu et al., 2016b). Preharvest foliar treatment of 1 mg/L sodium selenate 24 days before commercial harvest led to maintained firmness for 46 days in “Conference” pears and “Suncrest” peaches (Pezzarossa et al., 2012). Similarly, preharvest application of 1 mg/L sodium selenate at time of fruit set reduced membrane lipid peroxidation, fruit decay, and enhanced enzymatic as well as nonenzymatic antioxidants that ultimately led to prolonged shelf life of “Provence” tomato fruit for 20 days (Table 9.1) (Zhu et al., 2016b).

3 PLANT GROWTH REGULATOR SPRAYS

The preharvest sprays with different plant growth regulators could also be used with the objective of modifying fruit ripening process and/or modulating development of certain physiological mechanisms with the influence on postharvest storage potential of fruits. Although certain common effects of particular compounds on the postharvest fruit physiology may be recognized in each studied case, preharvest growth regulator applications on the postharvest storage as well as shelf life and quality of fruits show certain levels of variations across a number of same fruit types or even cultivars (Table 9.2).

3.1 Auxins

3.1.1 Naphthalene Acetic Acid

The impact of naphthalene acetic acid (NAA) and 1-MCP on the fruit drop and storage potential of “Bartlett” pear has been reported by Acuña et al. (2010). Combine application of NAA and 1-MCP was most effective against the storage diseases. Moreover, preharvest application of NAA and 1-MCP delayed fruit ripening due to reduced ethylene biosynthesis (Table 9.2) (Acuña et al., 2010).

3.1.2 2,4-Dichlorophenoxyacetic Acid

The 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin that has been historically used as one of the most common herbicides to control broadleaf weeds. At the same time, it also has good potential to enhance storage life and quality of fruits (Ferguson et al., 1982; Yuan and Carbaugh, 2007).

Table 9.2 Effects of preharvest sprays of different plant growth regulators on storage potential and shelf life of fruits

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Auxins	Grapefruit	“Marsh White”	20 mg/L 2,4-D	Color break stage	12 weeks at 15.5°C	Reduced decay, lower over ripe color index and higher puncture strength	Ferguson et al. (1982)
	Pear	“Bartlett”	50 mg/L 1-MCP + 96 g a.i. ha ⁻¹ NAA	At maturity	6 months at -1°C with 80%–95% RH followed by 24 h incubation at 20°C	Maintained fruit color, reduced ethylene production and softening	Acuña et al. (2010)
Gibberellins/cytokinins	Grapes	“Thompson seedless,” “Ruby Seedless”	40 µL/L GA ₃ + 6 µL/L CPPU	55 DAFB	60 and 90 days at 0°C + 3 days at 20°C	Increased berry pedicel thickness, cuticle, and reduced decay	Zoffoli et al. (2009)
		“Thompson seedless”	25 mg/L GA ₃ + 5 mg/L CPPU	Pea and veraison stage	7 days at 20 ± 2°C 75%–80% RH	Increased firmness and decreased percentage of unmarketable berries	Marzouk and Kassem (2011)

(Continued)

Table 9.2 Effects of preharvest sprays of different plant growth regulators on storage potential and shelf life of fruits (*cont.*)

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Abscisic acid	Kiwifruit	“Hayward”	20 ppm CPPU	15 DAFB	5 months at −0.5°C with 95% RH	Reduced softening and enhanced firmness	Costa et al. (1995)
		“Hayward”	10 and 20 ppm CPPU	20 DAFB	4 months	Higher SSC and lower flesh firmness	Costa et al. (1997)
	Mandarin	“Cadoux”	10 ppm GA ₃	White flower bud stage	30 days at 4°C with 85%–95% RH	Increased ascorbic acid	El-Otmani et al. (2004)
	Litchi	“Calcuttia”	500 µL/L ethephon and 150 or 300 mg/L ABA	28 DBH	14 days at 5°C with 90%–95% RH	Reduced pericarp browning index and increased anthocyanin contents	Singh et al. (2014)
Brassinosteroids	Sweet cherry	“Tak Danehe Mashhad”	0.75 mg/L BR	Fruit set + 10 DBH	14 days at 1°C	Increased firmness, SSC, TA ascorbic acid, maintained anthocyanins and phenolics	Roghabadi and Pakkish (2014)

ABA, Abscisic acid; a.i., active ingredients; BR, Brassinosteroids; CPPU, *N*-(2-Chloro-4-Pyridyl)-*N*-Phenylurea; 2,4-D, 2,4-dichlorophenoxyacetic acid; DAFB, days after first bloom; DBH, days before harvest; NAA, naphthaleneacetic acid; SSC, soluble solid contents; TA, titratable acidity.

Preharvest application of 20 mg/L 2,4-D along with 20 mg/L GA₃ resulted in reduced decay incidence, inhibited over ripe color development and led to significantly higher puncture strength of “Marsh White” seedless grapefruit stored at 15.5°C for 12 weeks (Table 9.2) (Ferguson et al., 1982).

3.2 Gibberellins

Gibberellic acid (GA₃) is a pentacyclic-diterpene-acid that promotes the plant cell growth and elongation. It has also been reported as a “juvenile” plant growth regulator. GA₃ has been found to delay fruit ripening and senescence along with enhanced quality characteristics (Khader, 1991; Lara, 2013). GA₃ sprays are known as a common horticultural practice in numerous production areas of the world to control the color changes and/or to delay senescence of rind in citrus fruits, or to enhance the fruit firmness in sweet cherry (Choi et al., 2002; El-Otmani et al., 2004). Several studies on the effects of preharvest GA₃ sprays have generally shown a delay in ripening of the treated climacteric and nonclimacteric fruits. Higher firmness and delayed color changes are some of the most commonly reported effects in treated fruits. Certain GA₃ treatment-related changes in the cuticular wax morphology have been reported for cactus pear fruit (Schirra et al., 1999). However, very few studies have been reported regarding the underlying biochemical mechanisms for improved firmness retention in fruits. Nevertheless, reduced PG and cellulase enzymes activities have been demonstrated for GA₃-treated sweet cherry fruit with few cultivar dependent inconsistencies (Choi et al., 2002). Preharvest GA₃ application lessened the prevalence of CI, reduced puffiness in mandarin (Garcia-Luis et al., 1985), reduced peel crinkling and discoloration of oranges (Bevington, 1973) and pitting during storage in tangerines and grapefruit (Petracek et al., 1998). Furthermore, it also reduced the seed germination in “Marsh” grapefruit (Ali-Dinar et al., 1976) and stem end breakdown in tangelo (Ismail, 1997). It has also been reported that preharvest exogenous application of GA₃ enhanced peel firmness along with increasing shelf life by lowering the decay and anthracnose incidence in “Ruby Red” grapefruit and “Fallglo” tangerines (Ritenour and Burton, 2005). Similarly, the preharvest exogenous application of GA₃ in combination with Ca before 14 days of color break delayed puffiness in “Satsuma” mandarin fruit for 21 days over tree storage (Sen et al., 2013). Similar results have been reported in “Umran” ber (jujube). Softening of ber fruit is mainly due to the cellulose enzyme activity that ultimately leads to the development of decayed spots on fruit; lowering the marketing value and cosmetic appearance. Fruit of ber cv “Umran” treated

with preharvest spray of GA_3 , $CaCl_2$, and bavistin inhibited the activity of fruit-softening enzymes and maintained higher firmness during storage (Jawandha et al., 2009). Similarly, exogenous preharvest application of GA_3 extended the storage life of peach, tangerines, persimmon, and cherry fruits (Ben-Arie et al., 1996; Ferri et al., 2004; Kappel and MacDonald, 2002; Marur et al., 1999). Likewise, the CI in plum has been reduced by the preharvest application of GA_3 under MAP storage in combination with intermittent warming. Moreover, it also reduced internal browning index of plum during storage (Table 9.2) (Wang et al., 2013).

3.3 Abscisic Acid

Abscisic acid (ABA) influences many physiological aspects of plants, such as floral induction, embryo maturation, cell elongation, and dormancy. The main function of ABA is in stress-related responses including cold, drought, pathogen attack, and UV radiation (Sharp, 2002). ABA also affects the storage potential and some quality attributes of fruits. Preharvest application of ABA at color break stage significantly increased color development by enhancing anthocyanin contents in “Calcutti” litchi fruit. Preharvest ABA treatment also maintained significantly higher anthocyanins along with individual cyanidin-3-O-rutinoside contents and better postharvest quality of litchi fruit for 14 days during storage, than control (Table 9.2) (Singh et al., 2014).

3.4 Brassinosteroids

Brassinosteroids are known as a group of steroidal plant hormones that have been found essential for the normal growth and development of plants. It has been reported that brassinolide (BL), castasterone (CS), and 24-epibrassinolide (24-EBL) are naturally occurring brassinosteroids that are considered pivotal due to their extensive distribution and strong biological activities (Bartwal et al., 2013). Brassinosteroids affect a wide range of biological processes essential for normal growth and development of plants when exogenously applied, regulate several gene expressions, and ultimately affect the critical activities of the complex metabolic pathways. Increased yield and quality has been found when plants were subjected to exogenous brassinosteroids application at appropriate growth stages (Bartwal et al., 2013). Preharvest application of brassinosteroid with 0.75 mg/L + postharvest dose of 0.2 mg/L increased fruit firmness, maintained higher anthocyanins and phenolic contents in “Tak Danehe Mashhad” sweet cherry fruit stored for 14 days (Table 9.2) (Roghabadi and Pakkish, 2014).

3.5 Forchlorfenuron

Forchlorfenuron [*N*-(2-chloro-4-pyridyl)-*N'*-phenylurea] or CPPU is a synthetic cytokinin which is normally used to increase the berry size of grapes and kiwifruit. CPPU stimulates the periclinal cell division leading to the more round and/or oval shaped berries (grapes and kiwifruit). CPPU also delays the anticipated maturity and red color development along with increase in pedicel thickness and rachis size in grapes. Besides these general effects, CPPU has also been used to extend shelf and storage life of grapes (Marzouk and Kassem, 2011; Zoffoli et al., 2009). Preharvest application of CPPU resulted in reduced weight loss, berry shattering, and unmarketable berries, along with significantly higher firmness and berry adherence strength for 7 days under ambient conditions (Table 9.2) (Marzouk and Kassem, 2011). Likewise, preharvest application of CPPU led to reduced gray mold incidence, berry shattering, and berry splitting of table grapes for 60 days during cold storage (Zoffoli et al., 2009). Similarly, preharvest applications of CPPU also led to reduced softening and better color retention in kiwifruit (Costa et al., 1995, 1997).

4 ETHYLENE-INHIBITING COMPOUNDS

Ethylene is excessively produced during ripening of climacteric fruits. Ethylene biosynthesis and production normally lead to reduced storage potential of fruits. Therefore, some ethylene-inhibiting compounds are exogenously applied to reduce its production and to increase storage life of fruits. Numerous ethylene-inhibiting compounds are available that could effectively reduce ethylene production and result in enhanced storage life (Table 9.3).

4.1 1-Methylcyclopropene

1-Methylcyclopropene (1-MCP) is a cyclopropene derivative synthetic plant growth regulator. 1-MCP is structurally related with the natural plant hormone known as ethylene. It is commercially used to downregulate the ripening of fruits (Watkins, 2006). Biosynthesis of the ethylene is also strongly influenced by 1-MCP through feedback mechanism in certain species and lower concentration of 1-MCP is active than ethylene. Preharvest application of 1-MCP delayed ripening of many fruits (Table 9.3) (Elfving et al., 2007; McArtney et al., 2009; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Preharvest application of 1-MCP maintained firmness and showed better fruit appearance by reducing the superficial scald incidence of “Law Rome” apple fruit (McArtney et al., 2008). Soft scale

Table 9.3 Effects of preharvest sprays of different ethylene-inhibiting compounds on storage potential and shelf life of fruits

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
1-MCP	Apple	“Scarletspur Delicious” and “Cameo”	125 and 250 mg/L 1-MCP	21 DBH	50 and 60 days under normal air while 120, 125, 215, and 225 days in controlled atmosphere	Reduced ethylene biosynthesis while there was no effect on SSC, TA, and texture of both cultivars	Elfving et al. (2007)
		“Honeycrisp”	3.8% 1-MCP	At maturity	5 months under air storage at 3°C plus 1 or 7 days at 21°C	Soft scald and soggy development was reduced	DeEll and Ehsani-Moghaddam (2010)
	Mango	“Carabao”	10 ppm 1-MCP	90, 100, 110 DAFI	26 days at 12.5°C	Maintained firmness and delayed ripening due to suppressed ethylene production	Israel et al. (2014)
	Pear	“Bartlett”	50 mg/L 1-MCP + 96 g a.i. ha ⁻¹ NAA	At maturity	6 months at -1°C with 80%–95% RH followed by 24 h incubation at 20°C	Maintained fruit color, reduced ethylene production and softening	Acuña et al. (2010)
	Yellow pitahaya	—	200 and 400 µg/L 1-MCP	15 days DBH	15 days at 25 ± 2°C with 75% RH	Increased ethylene sensitivity, ripening and maintained fruit texture	Serna et al. (2012)

AVG	Apple	“Lodi,” “Senshu,” “Redchief Delicious,” and “Red Fuji”	124 g/ha a.i. AVG	4 weeks before harvest BH	30 days at 4°C	Higher firmness, SSC/ TA ratio, lowers TA	Escalada and Archbold (2009)
	Banana	“Cavendish”	0.8 g/L AVG	74 and 78 days after forma- tion of last hand	30 days at 13 ± 1°C, 85%–90% RH	Lower ACC, ACC oxidase, and ethylene production rate	Van-Toan et al. (2011)
	Nectarine	“Caldesi 2000”	1.28 mM AVG	10 DBH	4 weeks at 2°C, 90%–95% RH + 1 day shelf	Higher SSC, TA, flesh color, and organoleptic quality	Torrigiani et al. (2004)
	Peach	“Royal Glory”	0.1% a.i. AVG	10 DBH	4 weeks in PE bags at 2°C with 90%–95% RH + 1 day shelf	Higher SSC, TA, flesh color, and organoleptic quality	Argiriou and Nanos (2010)
	Pear	“Camusina di Genova” and “Camusina di Bonarcado”	250 mg/L AVG	2 WBH	10 days at 18°C	Higher firmness, reduced internal browning, reduced ethylene and respi- ration rates, delayed ripening	D’Aquino et al. (2010)

(Continued)

Table 9.3 Effects of preharvest sprays of different ethylene-inhibiting compounds on storage potential and shelf life of fruits (*cont.*)

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Oxalic acid	Plum	“Black Beauty”	200 mg/L AVG	4 WBH	28 days at 0°C 90 ± 5% with RH	Reduced weight loss, higher firmness, antioxidants, and phenolics	Kucuker et al. (2015)
	Kiwifruit	“Bruno”	5 mM OA	130, 137, and 144 DAFB	15 days at 20 ± 1°C	Higher firmness, reduced lesion diameter, patulin accumulation, and defense-related enzymes	Zhu et al. (2016a)
	Peach	“Anjiry Maleki”	5 mM OA	15 DBH	28 days at 1°C with 90% RH	Reduced softening, increased enzymatic and nonenzymatic antioxidants	Razavi and Hajilou (2016)
Salicylic acid	Grapes	“Thompson Seedless”	8 mM Put + 100 mg/L SA	30–35 DAFS and 50% berry ripening	7 days at 25 ± 1°C	Shelf life was increased by due to berry adherence	Marzouk and Kassem (2011)
		Flame Seedless	1.5 mM SA	Pea and veraison stage	75 days at 3–4°C with 90%–95% RH	Maintained peel color, firmness, lower pectin methyl esterase activity, electrolyte leakage, higher anthocyanins, TSS TA, and phenolics	Champa et al. (2014)

Salicylic acid		Flame Seedless	100 ppm SA	Pea and veraison stage	30 days at 0°C and 80%–85% RH + 7 days at ambient conditions	Higher firmness, TSS, rachis condition, berry adherence strength; decreased berry shattering and weight loss	Al-Obeed (2011)
	Pineapple	“Comte de Paris”	2 mM SA	4 sprays at 15 days interval till harvest	20 days at 10°C + 2 days at 20°C	Reduced internal browning, PPO, PAL and delayed decrease of ascorbic acid contents	Lu et al. (2011)
	Sweet cherry	—	2 mM SA	3 DBH	60 days at 0°C + 15 days shelf at 25°C	Inhibited mycelial growth, higher activities of β -1,3-glucanase and PAL	Yao and Tian (2005)
	Sweet orange	“Lane Late,” “Valencia Late”	8 and 9 mM SA	10 DBH	93 days at 5°C	Higher rind puncture strength, rind tensile, firmness, SSC, TA, individual sugars, and organic acids	Ahmad et al. (2013)

(Continued)

Table 9.3 Effects of preharvest sprays of different ethylene-inhibiting compounds on storage potential and shelf life of fruits (*cont.*)

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Methyl jasmonate	Plum	“Black Beauty,” “Black Amber,” “Fortune” “Fortune”	2240 mg/L MJ	2 WBH	28 days at $0 \pm 0.5^\circ\text{C}$ with $90 \pm 5\%$ RH	Higher SSC, lower TA, firmness, phenolic and antioxidant activity	Kucuker and Ozturk (2014)
			1120 mg/L MJ	2 WBH	28 days at 0°C with $90 \pm 5\%$ RH	Reduced weight, higher firmness, total phenolics and antioxidants	Karaman et al. (2013)
		“Black Splendor” and “Royal Rosa”	0.5 mM MJ	63, 77, and 98 DAFB	50 days at 2°C , 9 days at 20°C	Reduced ethylene production, respiration rate, softening, higher APX, CAT, and POD activities	Zapata et al. (2013)
	Strawberry	“Chilean strawberry”	1 mM MJ	80% flowering, full bloom, turning fruit stage	72 h at ambient conditions	Exhibited higher firmness, anthocyanins, lignin contents, phenolics, SSC/TA ratio, and delayed decay incidence	Saavedra et al. (2016)
Polyamines	Apricot	“Canino”	4 mM Put + 2% calcium nitrate	After fruit setting	4 weeks at 0°C with 90%–95% RH	Reduced respiration rate, decay, weight loss, maintained firmness, biochemical attributes	El-Wahab (2015)

Poly- amines	Grapes	“Thompson Seedless”	8 mM Put + 100 mg/L SA	30–35 DAFS and 50% berry ripening	7 days at $25 \pm 1^\circ\text{C}$	Shelf life was increased by due to berry adherence	Marzouk and Kassem (2011)
		—	2 mM Put; 2 mM Spd	20 and 40 DBH	55 days at $1.5 \pm 1^\circ\text{C}$ with $90 \pm 5\%$ RH	Reduced weight loss, senescence, decay, softening, maintained color and firmness	Mirdehghan et al. (2013b)
		“Thompson Seedless”	8 mM Put	30–35 DAFS and 50% berry ripening	7 days at $25 \pm 1^\circ\text{C}$	Reduced berry shattering, weight loss, increased firmness, berry adherence strength and shelf life	Marzouk and Kassem (2011)
	Pistachio	—	2 mM Put; 2 mM Spd	20 and 35 DBH	45 days at $1.5 \pm 1^\circ\text{C}$ with $90 \pm 5\%$ RH	Higher color, firmness, reduced weight loss and fungal infection	Mirdehghan et al. (2013a)
	Plum	“Angelino”	2 mM Put	1 WBACH	6 weeks at $0 \pm 1^\circ\text{C}$ with $90 \pm 5\%$ RH	Delayed ripening and maintained quality	Khan et al. (2008)
		“Angelino,” “Amber Jewel,” and “Black Amber”	2 mM Put	1 WBACH	13 days at $20 \pm 1^\circ\text{C}$	Reduced respiration, ACS, ACO activities, delayed softening, and maintained quality	Khan and Singh (2010)

(Continued)

Table 9.3 Effects of preharvest sprays of different ethylene-inhibiting compounds on storage potential and shelf life of fruits (*cont.*)

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Nitric oxide	Tomato	“Punjab Ratta”	1 mM/L Put	120 and 130 DAT	26 days at 13 ± 2°C with 85%–90% RH	Reduced weight loss, decay, increased firmness and TA	Babu et al. (2014)
	Apple	“Golden Delicious”	50 μM SNP (donor of NO)	14 DBH	23 days at 18°C with 85%–90% RH	Reduced activities of ACS, ACO, and inhibited ethylene biosynthesis	Deng et al. (2013)
	Tomato	“No. 4 Zhongshu”	5 mM L-arginine (precursor of NO)	50 DBH	12 days at 25°C with 95% RH	Reduced decay and exhibited higher activities of PAL, chitinase, β-1,3-glucanase, and PPO enzymes	Zheng et al. (2011)

ACC, 1-Aminocyclopropane-1-carboxylic acid; ACO, 1-aminocyclopropane-1-carboxylate oxidase; ACS, 1-aminocyclopropane-1-carboxylate synthase; a.i., active ingredients; APX, ascorbate peroxidase; AVG, aminoethoxyvinylglycine; BH, before harvest; CAT, catalase; DAFI, days after flower induction; DAFS, days after fruit set; DBH, days before harvest; 1-MCP, 1-methylcyclopropane; mM, millimole; NO, nitric oxide; PAL, phenylalanine ammonia-lyase; PE, polyethylene; POD, peroxidase; ppm, parts per million; PPO, polyphenol oxidase; Put, putrescine; SA, salicylic acid; SNP, sodium-nitroprusside; Spd, spermidine; SSC, soluble solid contents; TA, titratable acidity; TSS, total soluble solids; WBACH, week before anticipated commercial harvest, WBH, weeks before harvest.

development and soggy breakdown in “Honeycrisp” apple was also delayed under cold storage by preharvest foliar spray of 1-MCP (DeEll and Ehsani-Moghaddam, 2010). 1-MCP maintains fruit quality and extends storage life by binding to ethylene receptors and preserving membrane integrity under extended storage in apple (Elfving et al., 2007). Pre- and postharvest application of 1-MCP at fruit setting stage reduced ethylene sensitivity of yellow pathia (*Selenicereus megalanthus* Haw) fruit. 1-MCP treated fruit showed higher firmness, enriched color development and reduced desiccation (Serna et al., 2012). Preharvest foliar application of 1-MCP (10 ppm) after 100 days of flowering delayed fruit ripening with better color development and higher firmness in “Carabao” mango fruit during postharvest storage (Israel et al., 2014). Similarly, preharvest application of 1-MCP in combination with NAA to “Bartlett” pears led to higher firmness, reduced ethylene production, and internal browning index for 4 months during cold storage (Acuña et al., 2010).

4.2 Aminoethoxyvinylglycine

Aminoethoxyvinylglycine (AVG) acts as one of the most effective competitive inhibitors in conversion of S-adenosylmethionine (SAM) to ethylene precursor, that is, ACC. Due to its capacity to block the reversibility of ethylene biosynthesis pathway, both pre- and postharvest AVG treatments have been reported as a possible way to delay ripening and to improve the storage potential of various climacteric fruits (Table 9.3). Generally, substantial delays in fruit ripening have been found to occur from preharvest sprays of AVG with pronounced decrease in the production of ethylene and higher levels of firmness in treated fruits with extended shelf life (Lara, 2013).

Preharvest spray of AVG before commercial harvest led to reduced respiration rate and higher firmness for 30 days under cold storage of “Lodi,” “Senshu,” “Redchief Delicious,” and “Red Fuji” apple fruits (Escalada and Archbold, 2009). Similarly, preharvest application of AVG 2 weeks before harvest to early ripening “Camusina di Genova” and “Camusina di Bonarcado” pears resulted in higher firmness, reduced respiration rate, ethylene production, delayed ripening, and inhibited internal browning under cold storage (D’Aquino et al., 2010). Likewise, preharvest AVG treatment prior to harvest exhibited higher firmness and significantly reduced CI in “Caldesi 2000” nectarines and “Royal Glory” peaches for 28 days under perforated polyethylene bags at 2°C conditions (Argiriou and Nanos, 2010). Preharvest application of AVG to “Cavendish Banana” on the 78th day of flower cutting showed reduced ACC, ACC oxidase, and

ethylene production, which ultimately extended its storage life for 42 days (Van-Toan et al., 2011). “Black Beauty” plums treated with preharvest AVG application exhibited reduced weight loss and higher firmness under cold storage for 28 days (Kucuker et al., 2015).

Besides extended storage and shelf life, some other practical benefits of preharvest AVG sprays have also been reported. For instance, preharvest applications of AVG have been found to improve the uniformity of maturity time at harvest in peach fruit (Cetinbas and Koyuncu, 2011). However, regardless of the general beneficial effects of preharvest AVG application on the storage potential indicated by normal standard quality attributes, it must be kept in mind that the treatment influence should be assessed in all possible means because the extension of the storability of fruits may be counteracted by certain undesirable effects on the sensory characteristics and overall consumer acceptance (Cetinbas and Koyuncu, 2011).

It has been shown that preharvest AVG application reduced the contents of bitterness contributing phenolics and enhanced the eating quality of olive fruit (Tsantili et al., 2012). Since the characteristic flavor and texture contribute to the overall consumer acceptance of fruits, the deficient development of these quality traits could result in the detrimental influences on the sensory quality. For instance, the total flavor contributing esters production from “Redchief Delicious” apple after cold storage was decreased by 44% in response to the preharvest sprays of AVG rising from the reduced supply of certain alcohol precursors for ester biosynthesis (Sigal and Archbold, 2009). Similarly, negative impact was also found regarding the biosynthesis of aroma volatiles in preharvest AVG-treated apple cv “Delbarde Estivale” fruit (Harb et al., 2011).

4.3 Oxalic Acid

Oxalic acid (OA) is an abiotic elicitor that induces systemic resistance against diseases caused by bacteria, fungi, and viruses in plants. The pre- or postharvest OA applications are effective ways to decrease disease incidence in some fruits, such as mango (Zheng et al., 2007) and pear (Tian et al., 2006) during storage. Besides postharvest applications, preharvest sprays of OA have also been found to maintain quality and to extend storage and shelf life of fruits (Table 9.3) (Zhu et al., 2016a). The preharvest OA application increased resistance against *Penicillium expansum* in the “Bruno” kiwifruit during postharvest storage. Preharvest OA-treated kiwifruit exhibited higher firmness and showed reduced lesion diameter as well as patulin accumulation during postharvest storage for 15 days (Zhu et al., 2016a). Moreover,

preharvest OA-treatment also resulted in increased activities of defense-related enzymes, such as phenyl alanine ammonia-lyase (PAL) and peroxidase, which ultimately led to enhanced storage life of kiwifruit (Zhu et al., 2016a). Similarly, preharvest application of OA resulted in reduced softening along with higher enzymatic and nonenzymatic antioxidants that ultimately enhanced storage life of “Anjiry Maleki” peach fruit up to 28 days (Razavi and Hajilou, 2016).

4.4 Salicylic Acid

Salicylic acid (SA) is an important plant hormone (Raskin, 1992) that regulates several functions in the plant metabolism systems (Popova et al., 1997). SA being an endogenous plant growth regulator from the phenolic group has been extensively used for storage/shelf life extension and quality improvement in several fruit crops (Table 9.3) (Karlidag et al., 2009; Peng and Jiang, 2006). SA also significantly influence different physiological and biochemical processes, such as fruit quality aspects, membrane permeability, and certain enzyme activities (Arberg, 1981). A single preharvest application of 2 mM SA 3 days before commercial harvest was found more effective than the postharvest treatment with the same chemical concentration as it enhanced the resistance against *Monilinia fructicola* infection of sweet cherry fruit. Moreover, preharvest SA spray also reduced the lesion diameter as compared with the postharvest applications (Yao and Tian, 2005). Preharvest SA spray enhanced the defense related enzymes activities, such as β -1,3-glucanase, peroxidase, and PAL, eventually proved a good strategy for the control of sweet cherry postharvest decay to increase its storage life (Yao and Tian, 2005). Preharvest application of 100 mg/L SA at pea and veraison stages increased firmness and berry adherence strength along with reduced weight loss for 7 days under ambient and 15 days cold storage of “Flame Seedless” grapes (Al-Obeed, 2011). Similarly, preharvest SA treatment to “Comte de Paris” pineapple delayed internal browning, reduced activities of polyphenol oxidase (PPO), and PAL enzymes under cold storage at 10°C (Lu et al., 2011). Likewise, preharvest application of SA to “Lane Late” and “Valencia Late” sweet oranges reduced rot, CI, and maintained higher rind firmness for 93 days under cold storage conditions (Ahmad et al., 2013). “Flame Seedless” grapes treated with preharvest SA spray exhibited higher anthocyanins, peel color, firmness, reduced PME activity, and membrane electrolyte leakage that ultimately increased storage life for 75 days (Champa et al., 2014).

4.5 Methyl Jasmonate

Jasmonic acid (JA) and/or its methyl ester, that is, methyl jasmonate (MeJA), has been reported to exist naturally in different plants. Both JA and MeJA have been found to act as elicitors and/or signaling agents who are involved in numerous physiological and biochemical cascades of plants (Creelman and Mullet, 1997). Most of the literature about MeJA has been shown as vital postharvest treatments particularly focused on reducing various stress-induced injuries during postharvest storage (Pena-Cortes et al., 2005; Sayyari et al., 2011).

MJ also plays various key roles in different responses to certain environmental stress conditions being involved in the signal transduction of biochemical pathways that lead to biosynthesis of the critical defense compounds, that is, phenolics and alkaloids of fruits. Accordingly, the preharvest sprays of MeJA/MJ have been found effective to induce resistance against diseases and/or to increase the certain stress responses in various types of fruits (Table 9.3). The preharvest sprays of MeJA and/or propyl dihydrojasmonate were found to induce numerous transcriptional changes in peach fruits (Ziosi et al., 2008). Observed effects included complex transcriptional changes apparently ensuing from the overlap between the ripening and certain stress responses that ultimately inhibit the biosynthesis of ethylene and eventually upregulated defense-related pathways and resulted in the increased storage potential (Ziosi et al., 2008). In relation with the postharvest quality, preharvest MeJA treatments resulted in enhanced fruit firmness and delayed color changes due to the downregulation of EXP-3 and PG genes expression (Ziosi et al., 2008). Preharvest application of MeJA 3 days before the commercial harvest was found to be quite effective than the postharvest treatment with the same chemical concentration as it enhanced the resistance against *M. fructicola* infection and activities of β -1,3-glucanase, peroxidase and PAL of sweet cherry fruit (Yao and Tian, 2005). Similarly, preharvest treatment of *Fragaria chiloensis* fruit with MeJA in combination with 2% chitosan enhanced firmness, anthocyanin contents, phenolics, and reduced decay for 72 h at 20°C storage (Saavedra et al., 2016). Likewise, “Fortune” plums sprayed with MeJA 2 weeks ahead of the commercial harvest resulted in reduced weight loss as well as higher firmness for 28 days at 0°C conditions (Karaman et al., 2013). Similarly, preharvest spray of MeJA to “Black Beauty,” “Black Amber,” and “Fortune” plum fruits effectively maintained higher firmness, phenolics, and eventually resulted in extended storage life for 28 days (Table 9.3) (Kucuker and Ozturk, 2014).

4.6 Polyamines

The polyamines (PAs) are known as positively charged aliphatic amines found in almost all living organisms, and have been reported to affect several biological processes, such as growth, development, flowering, ripening, senescence, and response to certain stress conditions under storage of fruits (Malik and Singh, 2004). PAs generally implies to spermine (SPM), putrescine (PUT), and spermidine (SPD) in combination with few other secondary conjugated products (Malmberg et al., 1998). It has been reported that PAs and ethylene biosynthesis pathways share the common precursor, that is, SAM. These cationic aliphatic amines have been used as the potential ethylene production antagonists. Hence, all the three major PAs (PUT, SPD, and SPM) found in the plants have an effect on the fruit-ripening-related events. Studies have shown that preharvest applications of PAs on the commencement of ethylene production cause extension of shelf life in various fruits (Table 9.3). However, delayed ripening as well as extended storage and shelf life depends upon the concentrations of specific applied PAs (Malik and Singh, 2004). Besides, increased storage and shelf life, preharvest PAs applications also either enhance or decrease certain quality attributes of different fruits. For instance, preharvest application of SPM increased the ascorbic acid contents in mango, whereas PUT and SPD decreased it. The general effects of preharvest PAs sprays enhanced fruit firmness and delayed color changes that ultimately resulted in enhanced storage and shelf life of mango fruit (Malik and Singh, 2004). Such dependence of fruit firmness or delayed color changes on the specific applied PAs compounds has been reported for nectarine fruit and the influence of PUT was considered strong (Torrighiani et al., 2004). In contrast to the experiments with other chemicals that generally comprise numerous applications throughout on-tree fruit development, preharvest sprays of PAs have been applied only as a single application at a specific time period before harvest. These PAs applications have been reported to delay the biosynthesis of ethylene production from the reduced S-adenosylmethionine decarboxylase (SAMDC), ACS, and/or ACO expression, ultimately leading to delayed ripening related changes with increased storage or shelf life having acceptable quality characteristics of fruits for consumers (Bregoli et al., 2002; Khan et al., 2007; Torrighiani et al., 2004). The preharvest application of PUT to “Punjab Ratta” tomatoes showed reduced decay, ripening index, weight loss, and maintained firmness under storage for 26 days (Babu et al., 2014). Similarly, preharvest PUT application to “Thompson

Seedless” grapes increased firmness and berry adherence strength, while significantly reducing the percentage of unmarketable fruit and physiological weight loss during postharvest shelf storage for 7 days at 20°C (Marzouk and Kassem, 2011). It has also been reported that the preharvest application of PUT and SPM showed good color and higher firmness, along with reduced fungal infections, weight loss and softening of “Kalleh Ghochi” and “Fandoghi” fresh pistachio stored at 1.5°C for 45 days (Mirdehghan et al., 2013a). Similarly, table grapes treated with preharvest application of PUT and SPM exhibited higher firmness and reduced fungal infections, physiological weight loss, and fruit softening for 55 days at 1.5°C storage (Table 9.3) (Mirdehghan et al., 2013b).

4.7 Nitric Oxide

Nitric oxide (NO) is a natural free-radical gas, having multifunctional signaling roles in different plants (Wendehenne et al., 2001). Formerly, NO has attracted great attention because of its potential as an environmental pollutant, but later on, it was reported to control various physiological, pathological, and developmental progressions in plants (Lamattina et al., 2003; Neil et al., 2003). Furthermore, NO also has additional evidence of antisenescence and antiripening properties in several types of fruits by decreased respiration rate, disease incidence, ethylene biosynthesis, delayed changes in peel color, and enhanced antioxidative enzymatic activities (Duan et al., 2007; Ku et al., 2000; Manjunatha et al., 2010). Preharvest spray of 50 µM sodium nitroprusside (donor of NO) 14 days before commercial harvest of “Golden Delicious” apples reduced activities of ACS and ACO enzymes, which ultimately inhibited the biosynthesis of ethylene and maintained their quality for 23 days at 18°C temperature (Deng et al., 2013). Similarly, preharvest application NO precursor, that is, L-arginine 50 days before harvest reduced decay incidence and exhibited higher activities of PAL, chitinase, β-1,3-glucanase, and PPO enzymes with extended shelf life of “No. 4 Zhongshu” tomato fruits for 12 days (Zheng et al., 2011).

5 EDIBLE COATINGS

The preharvest sprays with some suitable edible coatings, that is, *Aloe vera* gel, chitosan, oligochitosan, and sucrose have also been reported to extend the storage potential of fruits (Meng et al., 2008; Xuan et al., 2000; Yan et al., 2012). Besides the general beneficial effects on the quality, these

treatments have also been generally shown to increase the resistance against decay incidence through the modification of some defense-related enzymes activities that are associated with ascorbate peroxidase, superoxide dismutase, PPO, peroxidase, and/or PAL antioxidants in various fruits (Table 9.4) (Meng et al., 2008; Xuan et al., 2000; Yan et al., 2012).

5.1 *Aloe vera* Gel

A. vera gel is a colorless mucilaginous material obtained from the leaf parenchymatous cells of *Aloe* spp. At present, there are increasing interests in the use of *A. vera* gel in the global food industry (Eshun and He, 2004). Besides the food industry, currently *A. vera* is being used to maintain postharvest quality of fruits (Castillo et al., 2010). Preharvest application of 1:4 ratio *A. vera* was effective to reduce decay of “Prime Giant” and “Skeena” sweet cherries, “Garrofa” nectarines, and “Rich Lady” peach fruits at harvest. Preharvest *A. vera* gel spray also reduced yeast, mold, and aerobic counts, respectively during postharvest storage (Zapata et al., 2013). Similarly, “Autumn Royal” table grapes treated with 250 mL/L preharvest spray of *A. vera* before commercial harvest reduced its weight loss, respiration rate, and decay as well as mesophilic aerobics, molds, and yeasts counts for 35 days under cold storage (Table 9.4). It also maintained higher firmness and better fruit color (Castillo et al., 2010).

5.2 Chitosan

Chitosan is a deacetylated chitin and it is obtained from the outer shell of crustaceans, that is, crabs, krills, and/or shrimps (Sandford, 1989). The major advantages of chitosan application include low relative cost and that its use as a food additive has been permitted by the United States Food and Drug Administration (USFDA) (Knorr, 1986). Its use has been reported in numerous types of fruits as a postharvest application. Besides postharvest applications, it is now also being used as a preharvest spray/treatment to manage postharvest quality and storage life of fruits. Preharvest spray of 6 g/L chitosan enhanced firmness, delayed ripening, increased anthocyanins, and reduced *B. cinerea* induced decay of “Seascape” strawberry fruit for 35 days (Reddy et al., 2000). Similarly, three preharvest applications of 2% chitosan at 1 week intervals effectively reduced decay and maintained quality attributes of “Autumn Bliss” raspberries for 12 days under cold-storage (Tezotto-Uliana et al., 2014). Likewise, preharvest chitosan spray at 1 g/L along with 1×10^8 cells/mL *C. laurentii* reduced decay, enhanced antioxidant enzymes activities, such as peroxidase and PAL of “Jingxiu”

Table 9.4 Effects of preharvest sprays of different edible coatings on storage potential and shelf life of fruits

Chemical	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
<i>A. vera</i> gel	Grapes	“Autumn Royal”	250 mL/L <i>A. vera</i> gel	1 and 7 DBH	35 days at 2°C with 80%–95% RH	Reduced weight loss, fungal growth and less no of rotten berries	Castillo et al. (2010)
	Nectarine	“Garofa”	1:4 diluted <i>A. vera</i> gel	1 and 7 DBH	No storage/quality was evaluated at harvest	Do not significantly modify fruit quality, but microbial counts were significantly reduced	Zapata et al. (2013)
	Peach	“Reach Lady”	1:4 diluted <i>A. vera</i> gel	1 and 7 DBH	No storage/quality was evaluated at harvest	Do not significantly modify fruit quality, but microbial counts were significantly reduced	Zapata et al. (2013)
	Sweet cherry	“Prime Giant,” “Skeena”	1:4 diluted <i>A. vera</i> gel	1 and 7 DBH	No storage/quality was evaluated at harvest	Do not significantly modify fruit quality, but microbial counts were significantly reduced	Zapata et al. (2013)

Chitosan	Grapes	“Italia”	1% chitosan	At pea size berry	5–15 days at 0°C + 2 days shelf at 20 ± 2°C	Increased PAL activity and reduced incidence of gray mold	Romanazzi et al. (2002)
		“Jingxiu”	1 g/L chitosan	10 DBH	42 days at 0°C + 3 days at 20°C shelf conditions	Increased PPO, POD, and PAL activities while SOD activity was reduced	Meng and Tian (2009)
		“Jingxiu”	1 × 10 ⁸ cells mL ⁻¹ of <i>Cryptococcus laurentii</i> + 10g/L chitosan	10 DBH	42 days at 0°C with 90%–95% RH + 3 days shelf at 20°C	Increased SSC, TA, and reduced decay index in treated fruits	Meng et al. (2010b)
	Peach	“Florida Prince”	0.5 and 1.0% chitosan and 2 and 4% CaCl ₂ combinations	30 DBH	35 days at 0 ± 2°C with 90%–95% RH	Reduced firmness loss, higher SSC, and increased storage life	El-Badawy (2012)
	Plum	“Sanhuali”	40 mM AsA, 1.0% chitosan, and 40.0 mM AsA + 1.0% chitosan	After harvest	20 days at 5°C with 90–95% RH	Reduced color changes, higher SOD, CAT, lower ROS and MDA contents	Liu et al. (2014)
	Raspberry	“Autumn Bliss”	2% chitosan	BH at pinkish color and after harvest	15 days at 0°C	Reduced ethylene production, respiration rate, MDA contents, and post-harvest decay	Tezotto-Uliana et al. (2014)

(Continued)

Table 9.4 Effects of preharvest sprays of different edible coatings on storage potential and shelf life of fruits (*cont.*)

Chemical	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
	Strawberry	“Seascape”	6 g/L chitosan	5 and 10 DBH	28 days at 3°C	Higher anthocyanin contents and reduced decay index	Reddy et al. (2000)
Oligochitosan	Peach	“Florida Prince”	0.5 and 1.0% chitosan and 2 and 4% CaCl ₂ combinations	30 DBH	35 days at 0 ± 2°C with 90%–95% RH	Reduced firmness loss, higher SSC, and increased storage life for 35 days	El-Badawy (2012)
Biofresh	Apple	“Elstar,” “Champion”	1% Biofresh (SFAE + CMC)	1 DBP	3 months at 3–4°C with 92% RH	Retarded chlorophyll degradation rate and firmness loss	Xuan et al. (2000)

AsA, Ascorbic acid; CAT, catalase; CMC, carboxymethyl cellulose; DBH, days before harvest; DBP, days before picking; MDA, malondialdehyde; mM, millimole; PAL, phenylalanine ammonia lyase; POD, peroxidase; PPO, polyphenol oxidase; RH, relative humidity; ROS, reactive oxygen species; SFAE, sucrose fatty acid ester; SOD, superoxide dismutase; SSC, soluble solid contents; TA, titratable acidity.

table grapes during postharvest storage for 40 days (Meng and Tian, 2009). Preharvest application of 1% chitosan along with 4% calcium chloride reduced weight loss, decay incidence, and suppressed increase in SSC of “Florida Prince” peach fruits stored for 35 days (El-Badawy, 2012). It has also been reported that the preharvest spray of 1 g/L chitosan 10 days before commercial harvest resulted in reduced weight loss, decay, and higher activities of peroxidase and PAL enzymes in “Jingxiu” table grapes for 16 days at shelf conditions (Meng et al., 2008). Similarly, preharvest treatment of *F. chiloensis* fruit with 2% chitosan in combination with 1 mM MeJA showed higher firmness, anthocyanin contents, phenolics, and reduced decay for 72 h stored at 20°C storage (Saavedra et al., 2016). Moreover, integrated preharvest spray of 1×10^8 cells/mL *C. laurentii* and 10 g/L postharvest chitosan coating reduced decay incidence and maintained overall quality of “Jingxiu” table grapes for 42 days under cold and postcold shelf conditions, respectively (Meng et al., 2010b).

5.3 Oligochitosan

Oligochitosan is low molecular weight water-soluble compound that is usually obtained by the degradation of hydrolysis of chitosan (Xu et al., 2007; Yang et al., 2010). It has the ability to reduce or even completely inhibit the growth and development of different bacterial and fungal microbes. It has been shown that oligochitosan application effectively inhibited the postharvest diseases and maintained better quality in apple and pear fruits (Meng et al., 2010a). The preharvest spray of 1 g/L oligochitosan resulted in reduced disease incidence and delayed color changes as well as enhanced activities of defense enzyme, such as peroxidase and β -1,3-glucanase in “Dongzao” jujube fruit stored for 60 days (Yan et al., 2012). Similarly, preharvest spray of 1.5% oligochitosan reduced anthracnose of navel orange under ambient storage for 26 days. Moreover, oligochitosan sprayed “Navel” oranges also maintained higher activities of chitinase, peroxidase, methyl-esterase, and PAL enzymes (Deng et al., 2015).

5.4 Biofresh

Biofresh edible coatings can also be used to increase the storage and shelf life potential of fruits (Xuan et al., 2000). It contains sucrose fatty acid esters and carboxymethyl cellulose. The 1% Biofresh application 1 day before picking delayed chlorophyll degradation and maintained firmness of apple fruit for 3 months stored at 3–4°C (Xuan et al., 2000).

6 FOOD ADDITIVES

6.1 Ascorbic Acid

Ascorbic acid (AA) has been used as an antibrowning agent in many fruits as it forms the phenyl radicals and phenolics back to their precursor by reducing the oxidation reduction reaction and inhibits the formation of quinone (Kitts, 1997). Besides use of AA as an antibrowning agent, it has also been used in the form of preharvest sprays (Marzouk and Kassem, 2011). The preharvest foliar application of AA in combination with GA₃, CaCl₂, PUT, SA, and cytoflex on the seedless grapes at pea and veraison stages increased fruit quality and shelf life. Moreover, berry firmness was increased along with significantly reduced number of unmarketable fruits for 7 days under ambient storage (Table 9.5) (Marzouk and Kassem, 2011).

6.2 Hexanal

Hexanal has been approved by the USFDA as a food additive and it has an oral mammalian lethal dose (ORL-MAM LD₅₀) of 3700 mg/kg. Being a natural volatile compound with antimicrobial activity, hexanal has been found to extend shelf life of the fruit and retain their original color (Song et al., 1996). Preharvest application of 1% hexanal + postharvest treatment of 1 ppm 1-MCP to “Bing” sweet cherry enhanced color, firmness, anthocyanins, and phenolics along with higher enzymatic and nonenzymatic antioxidants for 30 days (Sharma et al., 2010). Likewise, greenhouse grown “Prunus” tomato subjected to preharvest application of hexanal before harvest showed enhanced brightness and increased firmness for 21 days (Cheema et al., 2014). Similarly, “Allahabad Safeda” guava fruits treated with preharvest application of 0.015% hexanal exhibited reduced decay incidence, PME activities, enhanced firmness, pectin, and phenol contents along with maintained organoleptic ratings for 28 days (Table 9.5) (Gill et al., 2016).

7 ANTAGONISTS/BIOCONTROL AGENTS

It has been reported that most of the postharvest diseases occur in the pack houses, but the initial infections normally begin in the fields (Palou et al., 2002). The fungus *P. digitatum* persists in the form of conidia from season to season in orchards and its infections arise when the airborne spore penetrate into rind of oranges through the injury points (Thonglem et al., 2007). Postharvest diseases and decay ultimately reduce storage

Table 9.5 Effects of preharvest sprays of different food additives, biocontrol agents, and artificial fungicides on storage potential and shelf life of fruits

Chemicals	Fruits	Cultivars	Concentrations	Time	Storage conditions	Inferences	References
Ascorbic acid	Grapes	“Thompson Seedless”	500 mg/L ascorbic acid	30–35 DAFS and 50% berry ripening	7 days at 25 ± 1°C	Reduced berry shattering, weight loss, increased firmness, berry adherence strength, and shelf life	Marzouk and Kassem (2011)
Hexanal	Guava	“Allahabad Safeda”	0.015% hexanal	2 and 4 WBH	28 days at 6–8°C	Reduced decay, pectin methyl-esterase activities, enhanced firmness, SSC, TA, and phenol contents along with maintained organoleptic quality	Gill et al. (2016)
	Sweet cherry	“Bing”	1% hexanal	7 and 15 DBH	30 days at 4°C	Enhanced color, firmness, anthocyanins, phenolics, APX, and SOD	Sharma et al. (2010)
	Tomato	“Prunus”	2 mM hexanal	7 DBH	21 days at 15°C	Enhanced brightness, increased firmness, SSC, and ascorbic acid contents	Cheema et al. (2014)
Biocontrol agents	Mandarin	“Ponkan”	1 × 10 ⁶ cells mL ⁻¹ <i>Rhodosporidium paludigenum</i>	2 DBH	20 days at 25°C	Reduced decay and enhanced defense related enzymes	Lu et al. (2013)
Artificial fungicides	Tomato	“Raf,” “Amadeo,” “Nereida”	1.5 kg ha ⁻¹ fenhexamid, pyraclostrobin, boscalid	3 times during growth season	21 days at 10°C ± 0.5 with 65% RH	Reduced postharvest decay, weight loss, respiration rate, and maintained quality	Domínguez et al. (2012)

APX, Ascorbate peroxidase; DAFS, days after fruit setting; DBH, days before harvest; mM, millimole; RH, relative humidity; SOD, superoxide dismutase; SSC, soluble solid contents; TA, titratable acidity; WBH, week before harvest.

potential of fruits, and eventually cause huge economic losses in the world. Therefore, it would be very beneficial to apply the biocontrol agents before the actual harvest in order to decrease the initial infection levels and actively subdue the pathogen during the postharvest storage periods (Tian et al., 2004). Preharvest treatments with the biocontrol agents have emerged as an effective strategy to manage postharvest decay and ultimately extend storage life of fruits (Droby et al., 2002; Ippolito and Nigro, 2000). Induction of tolerance/resistance in the harvested fruits has been found as a useful approach to stimulate the whole fruit tolerance/resistance response, ultimately leading to the reduced postharvest decays and enhanced storage life (Zheng et al., 2011). Increased resistance can also be accomplished by the preharvest application of certain biocontrol agents through production of some specific antifungal substances by the anticipated hosts that provide a long term and/or systemic resistance to a large number of broad spectrum storage pathogens of fruits (Nantawanit et al., 2010; Walling, 2001). Preharvest applications of biocontrol agents may also help to overcome numerous pre- and postharvest infections by enhancing levels of phenolic substances and certain defense associated enzymes activities. It has been observed that preharvest application of *Pichia guilliermondii* induce disease resistance in cherry tomatoes by activating its defensive enzymes, such as peroxidase, PAL, and β -1,3-glucanase (Zhao et al., 2011). Similarly, preharvest application of antagonistic yeast, that is, *Rhodosporidium paludigenum* induce resistance against the postharvest diseases of Ponkan mandarin orange due to enhanced defensive enzymes for 20 days at 25°C temperature (Table 9.5) (Lu et al., 2013).

8 ARTIFICIAL FUNGICIDES

Preharvest infections may cause postharvest disease and decay outbursts during extended storage. Therefore, presence of various fungal diseases has made the growers rely heavily on preharvest applications of artificial fungicide. Some of the fungicides, such as boscalid, fenhexamid, and pyraclostrobin have been found to be very effective to manage diseases during postharvest life that ultimately help to extend their storage potential (Domínguez et al., 2012; Hauke et al., 2004; Ziogas et al., 2003). Preharvest application of fenhexamid, or pyraclostrobin + boscalid to tomato resulted in reduced postharvest decay, weight loss, respiration rate, and maintained overall quality for 21 days (Table 9.5) (Domínguez et al., 2012).

9 CONCLUSIONS

Preharvest sprays possess good potential to modulate the postharvest quality, storage life, and marketing. Due to the variability across the species and/or cultivars, the treatment conditions should be studied and optimized precisely for each specific case. In addition, the treatment properties need to be evaluated further as a whole through paying particular consideration to eating quality attributes and storage related aspects of fruits.

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CHAPTER 10

Training and Pruning for Improved Postharvest Fruit Quality

Swati Sharma*, Kalyan Barman**, Mohammed Wasim Siddiqui†,
Vishal Nath*

*National Research Centre on Litchi, Muzaffarpur, Bihar, India

**Banaras Hindu University, Varanasi, Uttar Pradesh, India

†Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

1 INTRODUCTION

The commercially cultivated perennial fruit crops need canopy management at regular intervals to control their form and to maintain regular and prolific fruitfulness. Training and pruning processes at regular intervals are necessary to avoid difficulties during cultural operations, to minimize the insect pest and diseases infestation and to optimize the light interception throughout the canopy. This ensures the maintenance of trees and quality fruit production. Several techniques are used to modify the tree architecture by high-density planting, reduction of tree size (Singh, 2012). The maintenance of tree architecture is vital for proper light interception and distribution in the orchard, which directly affects the quality of the fruit produced.

Training operations are primarily done during the formative years of the orchard to achieve the desired shape of trees for proper interception of light and ventilation (Srivastava, 2007). However, it is essential to train old trees as well for center openings and rejuvenation purposes. Training is essentially the shaping of trees. The training system helps to attain proper light distribution, canopy porosity, ventilation to avoid high build up of relative humidity, and to reduce the competition with vegetative sinks at the time of fruiting. This also enables the development of fruits of proper color and edible quality. The knowledge of tree growth and development characteristics, branching, and fruiting are prerequisites for choosing optimal training systems. The shaping of fruit trees, namely peach trees to an open center system, apple and pear fruit trees to modified leader systems, and grapes to bower systems have been found to be most beneficial for optimum production of quality fruits. However, the training of the fruit

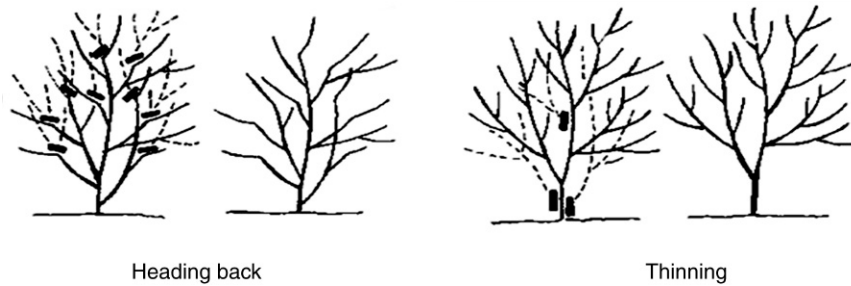


Figure 10.1 Heading back and thinning out. (From (heading back): <http://www2.ca.uky.edu/agcomm/pubs/ho/ho59/ho59.htm>; (thinning out): <http://www2.ca.uky.edu/agcomm/pubs/ho/ho59/ho59.htm>)

trees during the initial years of establishment of the orchard may result in a delay in fruiting. Proper care and attention is warranted to attain the desired shape and size of the fruit tree by training and pruning. The main procedures in training are heading back and thinning out (Fig. 10.1). In heading back, the branches are severed to achieve well-spaced scaffold branches with wide crotch angles, while in thinning out, thin-dried, diseased, or broken branches are removed.

The tree branches rising at wide angles are mechanically stronger than branches at narrow angles. The well-spaced branches have better exposure to light and air. The light availability within trees is vital for quality fruit development. The trees can be trained to multiple leader, central leader, modified leader, vertical axis, bower, hedge rows, or slender spindle forms among many training systems depending on the kind and age of the plant and needs of the grower. The chief objectives of training a fruit tree are:

1. Allowing proper sunlight interception and distribution within the canopy by presenting maximum leaf area to the sunlight.
2. Permitting proper ventilation to avoid build-up of very high relative humidity levels.
3. Maintaining the fruit trees of proper size and shape so that the preharvest cultural and fruit harvesting operations can be performed easily.
4. Ensuring a tree structure/framework, which is protected from wind damage by removing the crisscross branches and branches at narrow crotch angles.
5. Reducing the insect pest and diseases by maintaining favorable microclimate in the orchard and healthy fruit trees.
6. Early bearing and production of superior quality fruits in new plantations of fruit trees by influencing the color and size of the fruit, as well as by maintaining favorable preharvest environment.

7. Organizing the tree canopy in such a way that the fruit load can be thinned to optimum with minimal pruning operations.
8. Attaining the maximum fruit production potential of the orchard as early as possible.
9. Avoiding improper heading back to avoid unwarranted competition of fruiting branches with vegetative sinks.

Pruning is done by removing unwanted branches of fruit tree to achieve a balance between the vegetative and reproductive growth (Bal, 2010). Pruning operations ensure regular production of quality fruits. The removal of dense unproductive branches helps in lowering the foliar density and thus creates an unfavorable microclimate for insect pests and pathogenic microorganisms (Biggs, 1992). Pruning is essentially the removal of excess and undesirable branches. The temperate fruit crops, namely, apple, pear, peach, plum, grapes, subtropical deciduous fruit crops, namely, phalsa in particular, need regular annual pruning for consistent production of quality fruits year after year. Pruning is a specialized horticultural practice that is governed by various factors, like the type, age, and vigor of the fruit trees. It influences the quality of fruits by primarily altering the C:N (carbohydrate:nitrogen) balance of fruit trees. The pruning is done at different levels by retaining different number of nodes and at various times of the year for different influences. It encourages new shoot development, increases light penetration into the canopy and defers shoot senescence. The fruiting of the trees may be influenced by adequate pruning and thinning. However, different researchers have reported inconsistent influence of pruning on fruit quality, fruit size, and yield. Pruning is essential in deciduous trees like peach, which bear fruits on 1-year-old shoots only. Thus, it is required that 50% of the shoots must be pruned every year for getting a regular yield. Pruning is done by heading back or thinning out. The major objectives of pruning are:

1. Removing the crisscrossed, diseased, and/or dried branches.
2. Ensuring light penetration in the tree canopy and enhancing regular fruit bearing.
3. Increasing yield of quality fruit.
4. Managing the insect-pest and disease incidence in the orchard.

Modifications in the tree canopy by training and pruning help in orchard management and quality fruit production. Training procedures can either make use of the natural shape of the tree or modify it to Y, V, or T shape to attain the highest productivity and for easy mechanization of operations like pruning and harvesting. Bending fruit tree branches helps in promoting flowering and fruiting.

2 DIFFERENT TYPES OF TRAINING AND PRUNING SYSTEMS

The different types of training systems commonly used for shaping and forming the trees are detailed in the following sections.

2.1 Central Leader

In central leader, the main trunk develops without any disturbance. The widely spaced lateral branches are permitted to rise from the main trunk at wide crotch angles (Fig. 10.2). The natural growth of the tree is utilized and the tree is allowed to grow. The developed framework of the trees trained to central leader is strong. However, with the increase in height it becomes difficult to perform cultural operations like spraying, pruning, thinning, and harvesting from these trees. The canopy interior becomes dense and the light penetration is reduced in these areas in later years.

2.2 Open Center

In the open center system the main branch is headed back after few years of growth to allow the penetration of maximum sunlight in the canopy. Here the well-spaced branches distributed in different directions at wide crotch angles are allowed to develop (Fig. 10.3), which helps in developing a spreading framework. This system facilitates better color development of fruits, and also helps to reduce the insect pest and disease infestation and attacks. This system is commercially followed in peaches, apples, cherries, and plums. This



Figure 10.2 Central leader system of training. (From: http://aces.nmsu.edu/pubs/_h/H333/images/H333-fig4.jpg)

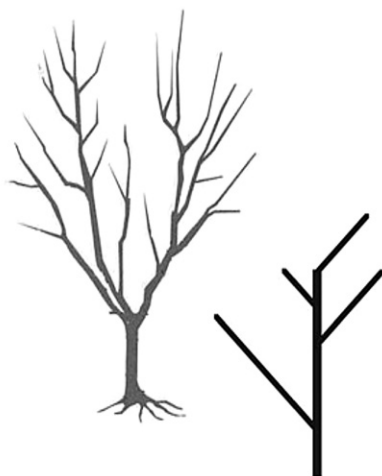


Figure 10.3 *Open center system of training.* (Available from: http://ecoursesonline.iasri.res.in/pluginfile.php/1813/mod_page/content/2/Chapter_41_3.JPG)

system is not required generally in tropical areas with plenty of sunshine and the tree framework is also weaker than the natural tree canopy structure.

2.3 Modified Leader

The main branch is allowed to grow for few years and then headed back. The other upright growing branches, crisscrossed, diseased, and weak branches are removed at regular intervals. The well-distributed lateral branches are allowed to grow (Fig. 10.4). The height of the tree is regulated by constant



Figure 10.4 *Modified leader system of training.* (Available from: <http://www.ipm.ucdavis.edu/PMG/GARDEN/IMAGES/CULTURAL/training.jpg>)

heading back of the main leader branch. The framework of the trees so developed is strong and assists in the preharvest cultural operations. Hence, this system is preferred commercially for most of the fruit trees.

2.4 Palmette Leader

Palmette leader system is comprised of alterations in the central leader training system. It enhances the sunlight penetration and distribution in the interior tree canopy. The branches growing east and west are removed, enabling the proper sunlight interception in the tree canopy. The tree has a fan-shaped look in many instances (Fig. 10.5).

2.5 Dwarf Pyramid

This training system principally maintains the height of the tree at not more than 2.0 m. The lateral branches are allowed to develop from about 30 to 35 cm above the ground (Fig. 10.6). Regular pruning is necessary to maintain the shape of the tree. The main advantage is the ease in the preharvest operations, as well as harvesting of fruits.

2.6 Slender Spindle Bush

Slender spindle bush is a variation of the dwarf pyramid training system with the main advantage of achieving early cropping. The main branch is not allowed to grow more than 2.0 m in height. The wide crotch angle branches are maintained and the lowest branch is allowed at 30 cm above the ground (Fig. 10.7).

2.7 Trellis

The trellis training system was developed by Irrigation Research Institute at Tatura, Australia in 1973. It comprises V-shaped tree rows in north–south



Figure 10.5 Palmette leader system of training. (Available from: <http://www.plantnet.com.au/media/pics/site/imagecache/1/0/104B58729DC973EDCDF7EB5C0F3CEB19.jpg>)

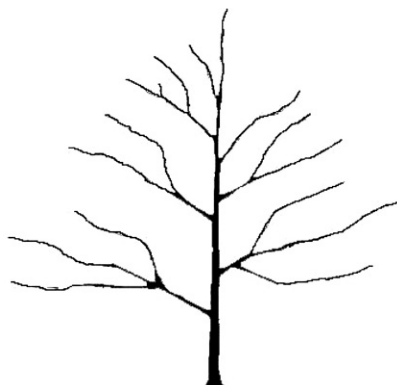


Figure 10.6 Dwarf pyramid system of training. (Available from: <http://secrets-of-self-sufficiency.com/wp-content/uploads/2011/05/Dwarf-Pyramids-apples.jpg>)

direction. Every tree is allowed to have two branches in east and west (Fig. 10.8), which aids enhanced flowering and early fruiting. It is commercially used for training peaches for obtaining early and high yields of quality fruits. The main advantage is improved light penetration and suitability to mechanical harvesting operations. However, consistent training and pruning is required to maintain the trees in the desired form.

2.8 Solen

The solen system is used mainly in vigorous tip-bearing varieties, which are grown on dwarfing rootstocks. About 10–15 fruiting branches are allowed

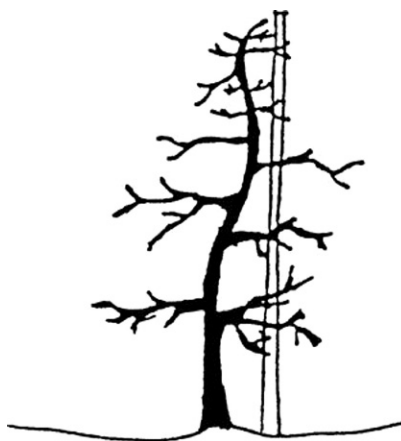


Figure 10.7 Slender spindle bush system of training. (Available from: <http://content.ces.ncsu.edu/high-density-apple-orchard-management-techniques>)

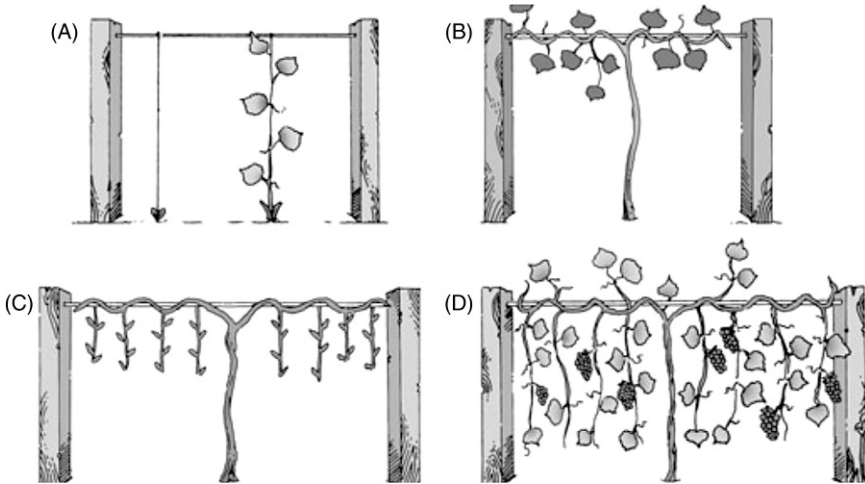


Figure 10.8 Trellis system of training. (Available from: <http://www.virtualorchard.net/idfta/cft/1998/vol31no3/hampson/Image10.gif>)

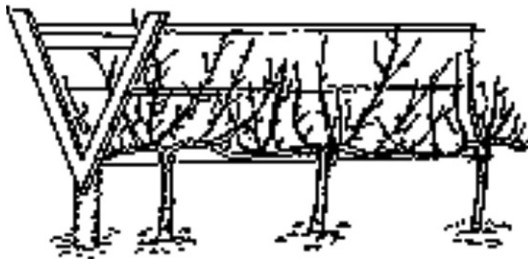


Figure 10.9 Solen system of training. (Available from: <http://deborahsilver.com/wp-content/uploads/2012/03/espalierhorzcordon31.jpg>)

to develop on the sides of the tree by bending and tying (Fig. 10.9). The tree framework assists in harvesting and pruning.

2.9 Espalier

The espalier system indicates the training of fruit trees to walls, trellises, and fences. This training system helps in saving space by growing trees with the support of a wall or fence. It is generally used for training apples and pears. The plants are pruned and tied against a support structure to control the plant growth as desired. It is attractive to the eye and also helps in saving space. It comprises a central system and paired horizontal branches, which are trained by taking support against wall or fence to get maximum sunlight exposure to harvest good-quality fruits (Fig. 10.10). This system is mostly

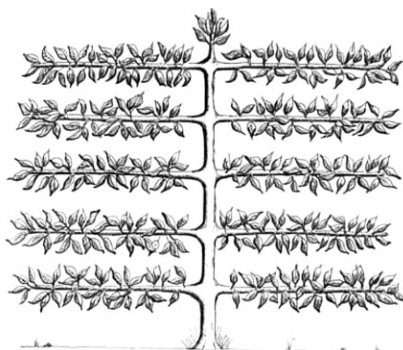


Figure 10.10 *Espalier system of training.* (Available from: <http://extension.missouri.edu/explore/images/g06090art03.jpg>)

used in European countries. The word “espalier” has its origin in the French language and means “something to rest the shoulder against.”

3 PRUNING

It is essential to prune trees at regular intervals for the uniform penetration and distribution of photosynthetically active radiation (400–700 nm). The sunlight penetration prevents the insect pest and diseases infestation, which prefer shady dense canopy conditions (Fig. 10.11). Thus, pruning trees by removal of undesirable branches, shoots, roots, or any other parts of a plant

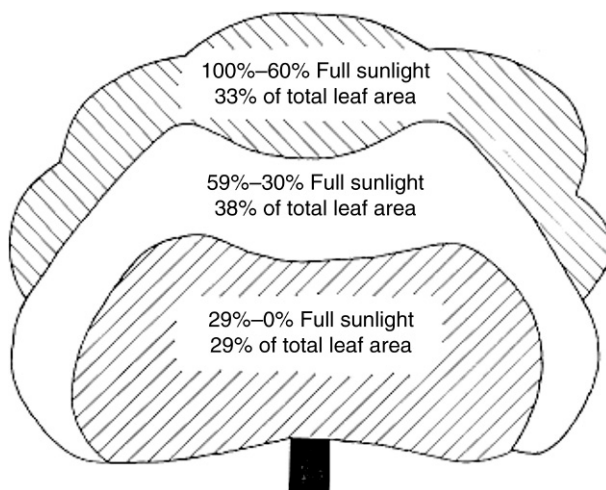


Figure 10.11 *Light penetration into the canopy of a large tree.* (From Heinicke, D.R., 1975. *High density apple orchards—planning, training and pruning*. In: *Agricultural Handbook*, vol. 458. USDA, Washington, DC., http://eap.mcgill.ca/CPTFP_7.htm)

to achieve regular fruiting and maintain the health of the trees is necessary (Costa et al., 1992). It can be done by heading back or thinning out. The time and extent of pruning depends on the age, kind, bearing habit, purpose, and resources available with the orchardist. The growth and flowering habits, like the location of fruit buds, length of the internodes, time of flowering of particular species and cultivars, must be considered while deciding the nature and degree of pruning. Pruning is a very important cultural operation because it helps in improving the tree shape, flowering time, fruiting, size, color, and quality of the fruits. It is a specialized operation and the extent of pruning is different for young and fully-grown trees. Pruning operations result in dwarfing trees, which is because of carbohydrate loss and reduced photosynthesis due to leaf removal. It also lowers the transpiration rates. Heavy pruning of trees is generally not recommended until the tree is very old and in deteriorating condition while light and moderate pruning are useful to achieve high-quality yield. The root pruning also influences dry matter partitioning to fruits (Erez et al., 1992; Ferree et al., 1992). The different training system efficiently influences the fruit quality, while the effects on regular bearing vary with the cultivar (Lauri and Grappadelli, 2014).

4 HOW DOES TRAINING AND PRUNING INFLUENCE POSTHARVEST FRUIT QUALITY?

The fruit trees, when allowed to grow naturally, develop compact, dense canopies with tall height, which hinders the preharvest operations, like thinning, spraying, and the harvesting processes. The poor penetration and distribution of photosynthetically active radiation in the interior canopy result in the production of low grade fruits of small size and poor color. Training and pruning are essential specialized agricultural operations, which not only enhance the appearance of the tree and orchard but qualitatively influence the form and function of the fruit trees. They are an effective tool in the hands of an experienced orchardist for obtaining the maximum yield of high-quality fruits. The postharvest fruit quality, such as fruit color, total soluble solids, firmness, titratable acidity, and disease occurrence are considerably influenced by the canopy architecture management.

The management of the fruit tree canopy is essential for utilizing the solar energy, land space, and nutrients effectively. Besides, the spraying of agricultural chemicals becomes easier and can be completed effectively, thus obtaining the desired beneficial results. The training and pruning operations

Table 10.1 Effect of training and pruning on postharvest quality of fruits

Reference	Treatment (various training and pruning systems and resulting change in canopy)	Fruit crop	Effects of training and pruning
Zhang et al. (2016)	Light penetration levels (20%, 20%–40%, 40%–60%, 60%–80%, and 80%–100%)	Pear cv. d’Anjou	The increase in light penetration levels enhanced soluble solids content while lowering the firmness and titratable acidity. It was concluded that the light microclimate significantly affected the fruit quality.
Bem et al. (2015)	Y-trellis and vertical shoot positioning (VSP) training systems	Grape cv. Cabernet Sauvignon	The VSP training system was reported to be best for grapes to reduce downy mildew and botrytis bunch rot occurrence.
Sabbatini et al. (2015)	Different training systems and pruning levels	Grape cv. Niagara	With the increase in number of nodes retained the cluster weight, berry weight, soluble solids decreased while yield and cluster number increased. No difference between training systems on fruit composition was noted.
Sousa and Abreu (2015)	High-density planting model systems: (2700 trees/ha, 3600 trees/ha, and 5400 trees/ha)	Pear cv. Rocha	Significant beneficial effect in fruit grades was achieved by tilt angle and pruning intensity by influencing the fruit growth. The apt arrangement of planting density, training and pruning techniques have potential for achieving higher yield, productivity and fruit quality of Rocha pears.
Vivaldi et al. (2015)	Pruning-mechanical (topping, hedging, trimming) and manual (thinning)	Olive	The mechanical pruning helps in controlling canopy size and allows mechanical harvesting. The manual pruning helps in continuous mechanical harvesting.

(Continued)

Table 10.1 Effect of training and pruning on postharvest quality of fruits (cont.)

Reference	Treatment (various training and pruning systems and resulting change in canopy)	Fruit crop	Effects of training and pruning
Xu et al. (2015)	Training systems (modified VSP, fan-shape systems)	Grapes cvs. Cabernet Sauvignon	The results showed that the training systems influence the vine vigor, yield, fatty acids composition and derived volatiles of grape berries and wines.
Lee and Skinkis (2013)	Complete cluster zone leaf removal at three preveraison stages (bloom, grain-pea size, bunch closure)	Grape cv. Pinot noir	The increase in anthocyanin accumulation can be achieved by the leaf removal around berries cluster during early season or between bloom and bunch closure period. The other fruit quality parameters were not affected significantly.
Nan et al. (2013)	Crawled cordon training (CCT) and independent long-stem pruning (ILSP)	Grape	Aroma was maximum in berries from vines trained to ILSP while CCT stabilized yield and quality.
Gorriz et al. (2012)	Unthinned (UT), hand-thinned (HT), mechanical thinning (MT), mechanical and hand-thinning (MHT)	Peach	There was no significant difference in net fruit value between HT, MT, and MHT. They suggested that mechanical thinning is a promising technique for thinning peach trees for canning industry due to the increase in work speed.
Bussi et al. (2011)	Dormant pruning intensities: light, medium, and severe	Peach cv. Alexandra	Severe pruning enhanced fruit diameter, but did not influence the soluble solids content.
Rana et al. (2011)	Timing (complete petal fall, 15 and 30 days after petal fall) and severity of summer pruning (4 nodes, 6 nodes)	Kiwifruit cv. Allison	Summer pruning at complete petal fall stage presented higher yields of high-grade fruits displaying highest fruit weight, size, total soluble solids and titratable acidity. It resulted in maximum net benefit over control.

Martin et al. (2010)	Hand-thinned and mechanically thinned	Peach	All thinning processes reduced crop load and increased fruit size. They noted that non-thinning technique resulted in the maximum economic impact.
Tworkoski and Glenn (2010)	Intensive number of pruning cuts (IP) or reduced number of pruning cuts (RP)	Peach cvs. Jersey Dawn, Redskin	Large sized fruits and increased crop load was obtained in trees with reduced pruning cuts. It was noted that reduced pruning helps to maintain marketable yield.
Mercier et al. (2008)	Combinations of irrigation and pruning treatments	Peach cv. Nectaross	Minimum brown rot incidence was noted in (Mod I + Mod P) and highest in (Conv I + Conv P). Manual pruning decreased disease sensitivity and enhanced taste by increasing total soluble solids. An increase in higher grade fruits and yield due to manual pruning, the profits were higher under modified treatments.
Tahir et al. (2007)	Pruning treatments: March, May, July, August, and September pruning treatments	Apple cv. Aroma	Thinning cut during August (5–6 weeks before harvesting) improved fruit color and decreased decay without negatively influencing tree yield.
Lombard et al. (2006)	Pruning and rest-breaking treatments	Grape cv. Sultana	The treatments enhanced budburst and long pruning was considered necessary for adequate fruit set and yield.
Cavallo et al. (2001)	Bilateral guyot, bilateral spur pruned cordon, and bilateral free cordon	Grape cv. Aglianico	Bilateral spur pruned cordon training did not enhance grape quality, while bilateral free cordon reduced the grape quality.
Ferree et al. (2001)	Mechanical root pruning	Grape cvs. Seyvalblanc and Catawba	Root pruning showed little effect on yield or juice composition.

(Continued)

Table 10.1 Effect of training and pruning on postharvest quality of fruits (*cont.*)

Reference	Treatment (various training and pruning systems and resulting change in canopy)	Fruit crop	Effects of training and pruning
Mika et al. (2001)	Different planting density (2857, 1904, 1428, 1142, 952 trees/ha) and training systems: spindle or hedgerow	Plum cv. Dąbrowicka	It was observed that dense planting suppressed tree growth, resulting in decreased yield per tree. However, yield per hectare increased. Spindle trained trees gave higher yield while the maximum yield was realized from most densely planted plots. Planting density and training system influenced the fruit quality only slightly.
Miller et al. (2001)	Summer pruning: leader and conventional pruning	Kiwifruit cv. Hayward	Leader pruning increased fruit yield by enhancing both fruit size and number of fruits per unit area. Moreover, fruits from upper canopy areas were larger in size with higher soluble solids content than fruits from lower areas of vine.
Li et al. (1994)	Long-pruning (LP) and traditional short-pruning (SP) technique	Peach	Early cropping, higher yield and best fruit quality with higher total soluble solids and enhanced color was achieved by long pruning.
Deckers and Missotten (1993)	Pruning: free spindle, classical pruning	Apple cvs. Jonagold, Schonevanboskop and Gloster	They noted that the pruning method showed significant effect on the final fruit quality in the terms of fruit color and size.
Jong et al. (1992)	Perpendicular V, parallel V, central leader, and standard open vase	Peach cv. Flavorcrest, Nectarine cv. Royal Giant, and Plum cv. Simka	The high-density planting systems enable in getting early yield in peaches, plums and nectarines.

Fallahi (1992)	Open center, parallel V, perpendicular V, parallel pars and perpendicular	Peach cvs. Florida prince and Earligrande	Highest yield was obtained in “Florida prince” trees trained to open-center system. The best quality fruits in terms of color and size were obtained in trees trained to Pars and V techniques.
Flore (1992)	Summer pruning and hedging	Stone fruit trees	It was documented that summer pruning has a varying effect on fruit quality, fruit size and yield in peach.
Hirst et al. (1992)	Palmette center leader or pyramid center leader	Apple	Best quality spurs were noted in pyramid center leader trees while the maximum mean fruit size was obtained from the palmette center leader canopy. The treatments and renewal-pruning methods improved fruit distribution within the tree, harvesting efficiency, yield and fruit size.
Lakso and Grappadelli (1992)	Canopy microclimate and time of season	Apple cv. Empire	They reported that obtaining high exposure of the spur canopy and avoiding excessive shade on spurs and shoots is vital for quality production.
Mika et al. (1992)	Severe pruning and control	Apple	Reduced number of fruit buds, fruit set and yield was recorded, may be due to lesser flower buds and reduced tree size.
Robinson (1992)	Freestanding central leaders, Y-shaped hedgerows	Apple cvs. Empire and Ace Delicious	No constant optimum angle for best fruit size was observed. However, red fruit color was best at the most vertical angle.
Raese (1992)	Summer pruning and calcium sprays	Pear cv. Anjou	Summer pruning showed higher Ca and Mg concentrations in fruit and leaves, reduced incidence of cork spot and alfalfa greening, same soluble solids content as control and enhanced fruit appearance and size.

(Continued)

Table 10.1 Effect of training and pruning on postharvest quality of fruits (*cont.*)

Reference	Treatment (various training and pruning systems and resulting change in canopy)	Fruit crop	Effects of training and pruning
Redalen (1992)	Pruning once a year, in January, April, July, and October	Apple cv. Aroma	The tendency of biennial cropping was high in unpruned trees. Although, the highest yield was obtained from unpruned trees, but only about 50% of these met the Class I standards while trees pruned in late April recorded the second highest yield and the maximum yield of Class I fruits.
Rom (1992)	Spur pruning	Apple cv. Delicious	An increase in mean fruit weight, while reduced total yield was recorded by pruning young trees and combining spur pruning and heading back.
Schupp (1992)	Root pruning at full bloom, summer pruning in mid-August, root- and summer pruning	Apple cv. McIntosh	The root pruning lowered the preharvest drop by 52%, internal ethylene content and starch index of fruit while summer-pruned trees showed higher starch index with no influence on internal ethylene content.
Tehrani (1992)	Modified leader, free palmette, and mini-tutura).	Pear cvs. Anjou, Bartlett, and Flemish Beauty	They observed that the training systems had no effect on the number of flower clusters per tree, flower density, number of florets per cluster or tree vigor.
Yunus (1992)	Various training and pruning intensities	Guava cv. JP 1	Higher yield was obtained from light training than medium training. However, no difference in the mean fruit weight and total soluble solids was recorded.

Ystaas (1992)	Summer pruning	Apple cv. Summer Red	No effect of late summer pruning on yield and fruit size was noted, soluble solids content were reduced with no constant effect on acidity. The red fruit color development improved significantly specifically from the interior canopy. Recommendation of summer pruning where light penetration in interior canopy is less was given for ensuring acceptable red color development.
Saunders et al. (1991)	Varied intensities of dormant pruning	Pear cv. Packham's Triumph	The fruit set and parthenocarpic fruits were reported to increase with pruning. However, delayed pruning did not improve fruit set.

influence the postharvest fruit quality significantly. Most significantly, the uniform distribution of photosynthetically active radiation in the canopy on the maximum leaf area is essential for the production of large size fruits with good color, which can be achieved by employing proper training and pruning operations. Another major advantage is that maintaining the trees in a proper shape presents the maximum leaf exposure to sunlight and provides proper ventilation, lowering the high humidity levels in dense canopies. This assists in the creation of unfavorable environment for the pathogenic microorganisms and insects. This goes a long way in ensuring postharvest quality for a longer period of time as many pathogenic diseases are initially picked up as latent infection from the field in preharvest stage. The reduction in postharvest diseases can save considerable fruit loss both in terms of fruit quality and quantum of production. The strong tree framework and canopy architecture averts wind damage and protects the tree thus reducing the fruit drop and bruises. This ensures high yield and quality fruit production. Precocious bearing can be achieved by planting trees in high density, training them to specialized structures, and pruning at regular intervals. Regular pruning is essential for fruit bearing in many species like peach, where only 1-year-old shoots can bear fruits. [Table 10.1](#) presents the influence of training and pruning on the postharvest quality of fruits as reported by different workers.

5 CONCLUSIONS

Training and pruning operations complement each other by principally modulating the form and function of the plant, respectively. These are essential to maintain the orchard in a healthy condition and to achieve regular production of quality fruits. The size and quality of the fruit, especially the color and physiological disorders like sunburn, are majorly affected by the shape of the tree and the amount of light penetration and distribution within the canopy. Further, the unfavorable microclimate helps in deterring the incidence of insect pest and diseases and thus helps in the maintenance of healthy fruit trees in orchards. The quality of the fruits in postharvest stage can be only as good as it was in the field at harvest time at the starting point of handling and transport. Hence, the minimization of preharvest infections and the maintenance of high-quality fruits free from sunburn and other physiological disorders help in achieving fruits of superior postharvest quality.

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CHAPTER 11

Insect Pest Management of Preharvest Vegetables for Better Postharvest Quality

Tamoghna Saha*, M. Kalmesh*, C. Nithya**, Maneesh P. Singh*, Kiran Kumari*

*Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

**India Agricultural Research Institute (IARI), New Delhi, Delhi, India

1 INTRODUCTION

Agriculture is the backbone of human society and it is very necessary for our present and future because, without good quality food, no organism on this earth can survive. Even though there is much improvement in agricultural practices, the problem of crop loss still exists. So it is important to know how to control the insect pests before harvest to control the post-harvest losses.

Insect pests are one of the major constraints to increase food production and higher agricultural productivity. On a global level, pests are reported to destroy a significant part of the agricultural harvest. A detailed study showed that crop losses due to insect pests range from 25% to more than 50%, depending on the crop and agroclimatic conditions (Oerke et al., 1999).

Among the agricultural crops vegetables plays an important role in nutrition as well as a supplier of many lifesaving compounds like vitamins, protein, and minerals. Vegetable production for processing (e.g., canning, freezing, or dehydration) is distinct from fresh market vegetable production. Whereas vegetables for processing are grown exclusively in the field, a significant quantity of the fresh market production can be in greenhouse structures (e.g., tomatoes, cucumbers, and lettuce). Many other areas of the vegetable industry include the production of vegetable transplants and vegetable seeds for commercial and home garden vegetable growers. Commercial production of vegetables is supplemented by home garden production.

The vegetables can be grouped under specific groups with similar types of insect and pest attacks, such as cole crops, solanaceous vegetables, cucurbitaceous vegetables, tuber crops, and bulbous crops like onions. The major

pests that cause significant damage to the postharvest quality of vegetables in different groups are reviewed.

2 COLE CROPS

The different cole crops grown largely include cabbage, cauliflower, knol-khol, radish, leafy mustard, Chinese cabbage, and so on. As all the crops are mainly leafy types of vegetables, these crops are almost affected by similar pests. So damage by insects reduces postharvest quality. Among the many insect pests, the following sections are major pests that reduce the postharvest quality.

2.1 Diamondback Moth *Plutella xylostella* (Lepidoptera: Yponomeutidae)

Distribution: Diamondback moth (DBM), *P. xylostella*, is an important pest of cruciferous crops and enjoys cosmopolitan distribution including India (CIE, 1967). It is found in Europe, Asia, Africa, the Americas, Australia, New Zealand, and the Hawaiian Islands. In India, DBM was first reported in Fletcher (1914).

Life cycle: adult moths are minute delicate moths with silvery patterns on its dorsal surface. They have whitish silvery marks on forewings, which look like diamond-shaped patterns when wings lie flat over the body. Females lay very tiny pinhead-size eggs under the surface of leaves in batches or singly. A female lays 18–356 eggs in her life span. The incubation period of 3–10 days depends on temperature; larval period is 8–16 days. Caterpillars of middle instars skeletonize leaves from above and the older instars from the lower side of leaves, with the upper epidermis untouched. The pupal stage is passed within a transparent, loose cocoon. The pupal stage lasts 4–5 days, with the total life cycle completed in 15–18 days. Adults survive for 3–6 days without food and 11–16 days with food (Jayarathnam, 1977).

Damage symptoms: The pest mainly feeds on cabbage and cauliflower along with many other cruciferous and some noncruciferous plants (*Amaranthus viridis*) (Vishakantaiah and Visweswara Gowda, 1975). The main damage caused by the larvae is by mining, scraping, and biting leaves. Larvae are very minute and their excreta present on the plant reduces the postharvest quality. In the case of severe infestation, the whole plant is skeletonized and large numbers of pupae and pupal cases are present on cabbage heads or cauliflower curds.

2.1.1 Management

This pest has resistance to pesticides in field conditions so it is not easy to manage, but by following different integrated pest management (IPM)

tactics, it reduces the population of this pest and gives better postharvest quality.

Cultural: Use tomato or black bold-seeded mustard as inter- and trap crop. Indian mustard attracts 80%–90% DBM moths. Use it to grow Bt-varieties.

Mechanical: Remove and destroy the stubbles after crop harvest and proper field sanitation. Heavily infected curd or heads that have a large number of pupae can be roughed out and burned. Proper washing of curd/head with water is done before marketing.

Biological: Larval stages are parasitized by a large number of hymenopteran wasps like *Voria ruratis* (Ichneumonidae), *Brachymeria excarinata*, *Tetrastichus sokolowskii* (Eulophidae), *Diadegma semiclausum*, and so on.

Chemical: Use a need-based spray of Spinosad 2.5 SC, Emamectin benzoate 0.58 G, Indoxicarb 15.8 EC, or Cartap hydrochloride 50 SP.

2.2 Cabbage White Butterfly *Pieris brassicae* (Lepidoptera: Pieridae)

Distribution: The cabbage white butterflies are cosmopolitan in distribution, found in Europe, Asia, and North Africa. In India, they are distributed all along the Himalayan region. During summer, pests breed in the hills and in winter in the plains; when winter is over they return back to the hills. They feed on all cruciferous plants and some noncruciferous plants.

Life cycle: A female butterfly after mating lays eggs on the under surface of the leaves of the host plant. Adults live for 3–12 days. Adults are pale white butterflies with a black patch on apical angle and a black spot in the middle of the forewing both sides; males have black spots only on ventral side of forewing. Eggs are laid in batches of 50–80. The incubation period varies in different seasons. Caterpillars are greenish yellow with numerous hairs and black spots and they are gregarious in nature in the beginning but later on they disperse. They voraciously feed on the leaves of the host plant and undergo five moltings to attain full-grown size. The fully-grown caterpillars measure about 5 cm in length and become greenish-yellow in color. The larval period lasts for 15–40 days. The matured caterpillars undergo pupation, which occurs rarely on the host plants but generally occurs on the stem of a nearby tree. The pupal period in different seasons lasts for 7–29 days. Generally, four generations are completed in a year.

Damage symptoms: Damage is caused by caterpillars that feed on foliage. Newly hatched caterpillars lacerate the leaf surface of the host plants and skeletonize them. The grown-up larvae feed gregariously on leaves of

cabbage and cauliflower. Large numbers of blackish excreta that are easily observed on heads and curds in the field are an important indication of pests. Irregular feeding patches present on leaves reduce the market value and postharvest quality.

2.2.1 Management

Mechanical: As the butterflies lay eggs in groups and initial instar caterpillars feed gregariously in groups, they can be collected mechanically and killed by crushing or putting in kerosenated water.

Biological: Use larval parasitoid *Apanteles glomeratus* (Braconidae) for the control of pests.

Chemical: Spraying the crop with malathion (0.05%) or diazinon (0.02%) 3 weeks before harvesting can reduce the infestation of this pest.

2.3 Cabbage Head Borer *Hellula undalis* (Lepidoptera: Pyralidae)

Distribution: Distributed worldwide, this sporadic but occasional pest is serious on crucifers. In India principal host plants are cabbage, cauliflower, and some other crucifers.

Life cycle: Caterpillars are creamy yellow with pinkish tinge and have seven purplish brown longitudinal stripes. Adult moths are slender pale yellow with gray wavy lines on the forewings. Females lay oval pinkish eggs in clusters or singly head or under the surface of leaves. The incubation period is 2–3 days, larval period 7–12 days with five larval instars and a pupal period of 6 days. The life cycle is completed in 15–25 days.

Damage symptoms: Young caterpillars mine the leaves and feed by biting and scraping, while older caterpillars feed on the underside of curd and heads and also makes a silken webbing. Caterpillars feeding on young plants frequently cause death of the plants, especially when the larvae feed on the growing point. Mature caterpillars feed on leaves as well as stems and growing points. They are often hidden behind a web of silk and masses of frass. In addition, insect feeding and the presence of caterpillars and/or their excrement reduce the market value of the produce. Frass accumulated at the entrance of the tunnel along the stems is an indication of damage. In the case of severe infestation head or curd becomes deformed.

2.3.1 Management

Cultural: Regular monitoring of young plants in the nursery and after transplant is important. Inspect crops for the presence of caterpillars and damage

symptoms. Use clean-planting materials: transplant only healthy, vigorous insect-free seedlings. Uprooting and burning of cabbage and kale stalks and crop rotation are important to reduce field populations. Indian mustard also acts as a trap crop for the pests. Some cauliflower lines like Early Kumari, 78-1S, 234-S, Sel.916, and Sel.1012 were resistant to this pest.

Mechanical: Collect and carefully destroy the larvae at the gregarious stage with leaves twice a week.

Biological: Natural enemies of the cabbage webworm include parasitic wasps (such as braconid, ichneumonid, and chalcidoid wasps). Some of the predators include: *Chrysoperla carnea*, coccinellids, king crow, common mynah, wasp, dragonfly, spider, robber fly, reduviid bug, praying mantis, fire ants, big eyed bugs (*Geocoris* sp.), pentatomid bug (*Eucanthecona furcellata*), earwigs, ground beetles, rove beetles and so on. Conservation of these natural enemies is important.

Chemical: The grown-up larvae can be controlled by application of 5% malathion dust at 37.5 kg/ha or cartap hydrochloride at 500 g/ha (CABI, 2005).

2.4 Tobacco Caterpillar *Spodoptera litura* (Lepidoptera: Noctuidae)

Distribution: This pest has ubiquitous distribution, observed in Afghanistan, Burma, China, Indonesia, Japan, Korea, Sri Lanka, Taiwan, Thailand and so on. It is a serious pest of tobacco and it also attacks cole crops, castor, cotton chili, sunflower, groundnut, pulses, amaranthus, taro, and so on. *Spodoptera litura* has been widely distributed all over India infesting different host plants.

Life cycle: The pest breeds throughout the year, although development is retarded during winter. Each female may lay 300 eggs in clusters covered with brown hair. Eggs hatch in 3–5 days. Young larvae feed gregariously initially and old larvae disperse to feed individually. Larvae become fully fed in 15–30 days and there are six larval instars in the development. Pupal period is 7–15 days and its pupation takes place in soil. Adult longevity is 7–10 days. The total life cycle is completed in 32–60 days. As many as eight overlapping generations are observed in a year. Larvae are velvety black with yellowish green dorsal strips and lateral white bands. The moths are about 22 mm in length and about 40 mm across the wings. The forewings have beautiful golden and grayish brown patterns.

Damage symptoms: The main damage is caused by the caterpillars as they feed on leaves and fresh growth. They are active at night and more serious on tobacco. The young caterpillars bite holes on leaves and the older larvae defoliate the entire foliage.

2.4.1 Management

Cultural: Clean cultivation exposes the larvae to natural enemies. Grow repellent plants, namely, osimum/basil.

Mechanical: Pheromone traps are used to predict egg laying and mass killing. Hand-picking and destruction of egg masses and early gregarious instars is practiced to control the pest.

Biological: Use parasitoids: *Trichogramma chilonis* (egg), *Tetrastichus* spp. (egg), *Telenomus* spp. (egg), *Chelonus blackburni* (egg-larval), *Carcelia* spp. (larval-pupal), *Campoletis chloridae* (larval), *Eriborus argentiopilosus* (larval), *Microplitis* sp. (larval). Predators: *Chrysoperla carnea*, coccinellids, king crow, common mynah, wasp, dragonfly, spider, robber fly, reduviid bug, praying mantis, fire ants, big-eyed bugs.

Spraying of NSKE at 4.0% at the early growth stage of the crop and SINPV at 250 LE per hectare is very effective.

Chemical: Spray chlorpyrifos 20 EC 2 L/ha or dichlorovos 76 WSC 1 L/ha.

2.5 Cabbage Aphid *Brevicoryne brassicae* (Hemiptera: Aphididae)

Distribution: The cabbage aphid is native to Europe, but now has a world-wide distribution (Kessing and Mau, 1991). In India, Lefroy and Howlett (1909) reported the species for the first time on brassica crops. *B. brassicae* is a cosmopolitan species and well distributed throughout the world (Carvalho et al., 2002).

Life cycle: Eggs: pale-yellow with greenish tinge. Nymphs: 1–1.5 mm long and yellow green with light ash gray tinge. Adults: about 2 mm in length and ash gray in color. They are active from October to April; in the mid-hills of Himachal Pradesh it appears in the last week of January with a peak during the first week of April. It reproduces through parthenogenetic vivipary, however, during severe winter, sexual reproduction may also occur. Overcrowding coupled with high temperatures and low humidity results in the appearance of alates for migration. The nymphs mature in 10–15 days and immediately start laying and there are four nymphal instars. A single female can produce 40–45 young ones. Total life cycle is completed in 10–45 days, so there are many generations in a year.

Damage symptoms: Cabbage aphids feed on the underside of the leaves and on the center of the cabbage head (Hines and Hutchison, 2013). The aphids suck the cell sap from tender leaves/shoots and induce stunted growth and poor head formation. Under severe infestation the entire plant may

dry up. When seedlings are infested they lose vigor, get distorted, and become unfit for transplanting. Aphids produce a sugary waste product called honeydew, which is fed on by ants. In turn, the ants provide protection to aphids from natural enemies. The honeydew attracts sooty mold and interferes with photosynthesis. If an attack starts at the early stage, heavy losses can occur. The cabbage aphid and green peach aphid (*Myzus persicae*) can be confused when they are both found feeding on cabbage plants. However, they have different morphological characteristics; the cabbage aphid is waxy with short cornicles and the green peach aphid lacks a waxy covering and has long cornicles (Opfer and McGrath, 2013).

2.5.1 Management

Cultural: Cabbage cultivars, like red drum head and KK cross, which are moderately resistant to this aphid, can be used.

Mechanical: Cut and destroy the infested leaves/shoots mechanically, as soon as the aphid attack appears. A spray of fine pulverized mica powder at 0.2% repels the alates.

Biological: Predators like coccinellids, syrphids, and chrysopids, and parasitoids like *Aphidius* spp. also reduce the population.

Chemical: Spray conventional insecticides like malathion (0.05%) or oxydemeton methyl (0.025%), or dimethoate (0.03%).

Some other minor pests of cole crops that reduce the postharvest quality are:

1. Cabbage semilooper, *Thysanoplusia orichalcea* (Lepidoptera: Noctuidae).
2. Cabbage leaf webber, *Crocidolomia binotalis* (Lepidoptera: Pyralidae).
3. Cabbage flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae).
4. Painted bugs, *Bagrada cruciferarum* (Hemiptera: Pentatomidae).

Most of these pests having similar types of management and proper integrated pest-management tactics that reduce the pest population.

3 SOLANACEOUS CROPS

The solanaceous family has large numbers of vegetable crops and out of these vegetables are some major vegetables of our country, as well as the world, like potato, tomato, and eggplant. The potato is called future food. As these crops have great economic importance, at the same time they are attacked by a large number of insect pests that cause crop loss either directly or indirectly. Some are major pests that cause heavy postharvest losses are described in the next sections.

3.1 Potato Tuber Moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae)

Distribution: This pest is cosmopolitan in distribution, especially in warm temperate and tropical regions where host plants are grown. A native of South America, the moth was introduced to India in 1906 with seed potatoes imported from Italy. The potato is the principal host for *Phthorimaea operculella*, but has also been reported from other solanaceae crops, namely tomato, tobacco, chili, eggplant, sugarbeet, and cape gooseberry. They also attack many weeds and wild plants (Das and Raman, 1994; Weber, 2013).

Life cycle: The female moth can lay 150–200 eggs. The eggs are laid singly or in batches on the leaves of the host plants or on exposed tubers near the eye buds and have an incubation period of 3–6 days. Eggs are oval, smooth, and yellowish, iridescent, and measure less than 1 mm in diameter. Newly emerged larvae are gray yellowish white with brown heads. The fully grown *P. operculella* larvae are about 15 mm in length. Heads are dark brown; prothoracic plates are sometimes pinkish, and the body is grayish-white or pale greenish gray. The pinacula are small, dark brown, or black; the pupa is yellowish or reddish brown, the eighth abdominal segment with spiracles on slightly raised, backward pointing spiracles; cremaster with a median, dorsal, thorn-like spine and eight slender hooks. Full-grown caterpillars come out of the tubers and pupate in silken cocoons either in dried leaves, soils, over the stored tubers or in cracks and crevices in the store. The pupal period lasts 5–9 days. Adult moths measuring about 1 cm in length when at rest, are colored pale brown with darker marbling. The wingspan is 15–17 mm. Head and thorax are pale brown, palpi curved, ascending and terminal segment is about as long as the second. The moth breeds continuously where conditions permit; up to 13 generations a year have been recorded in India (Trivedi et al., 1994).

3.1.1 Management

Cultural: The use of healthy tubers will reduce levels of field infestation. Following the proper earthing up operation, light irrigation is every 4 days and mulching with neem leaves during the last 4 weeks before harvest were the most effective treatment for PTM. Harvested potatoes should be lifted to cold stores immediately; if cold store facilities are not available, only healthy tubers should be stored.

Mechanical: Pheromone traps are used both for monitoring and controlling in the field and in storage. Under field conditions more than 20 traps/ha are required. Cover the stored tubers with 2.5 cm layer of chopped dry

leaves of lantana or eucalyptus or eupatorium below and above the potato. Clip off the leaves showing the mining of the potato tuber moth larvae and destroy them.

Biological: The parasitoids *Chelonus curvimaculatus*, *Bracon gelechia*, *Apanteles subandinus*, *Melanis* sp. *Copidosoma koehleri*, and *Diadegma molliplum* have become established in a number of countries and found to be successful biological control agents (Herman, 2008). The nematodes *Steinernema feltiae*, *S. carpocapsae*, and *Heterorhabditis heliothidis* were used to control the tuber moth. *Bacillus thuringiensis* has also been reported to suppress this pest.

Chemical: Spray the crop with chlorfenvinphos (0.4 Kg a.i./ha) or quinalphos (0.375 Kg a.i./ha) or acephate (0.5 Kg a.i./ha). In stores, dust the tubers with 5% malathion or 1.55 quinalphos dust at 125 g dust/100 Kg of potatoes. Alternatively, dip tubers before storage with 0.0028% deltamethrin.

3.2 Hadda Beetle *Epilachna vigintioctopunctata*/ *E. dodecastigma* (Coleoptera: Coccinellidae)

Distribution: Hadda beetles are universally distributed. They originate from east of Russia. Presently they occur mainly in tropical and semitropical parts of the world. Their existence is also well noticed throughout India, Pakistan, China, Japan, South East Asia, and Australia (CABI, 2010). In India, the beetle is present in higher hills and in plains of Jammu and Kashmir, Punjab, Himachal Pradesh, Uttar Pradesh, Karnataka, and Bengal and in the plains (Shankar et al., 2010). In addition to potato, the phytophagous coccinellids that feed on foliage of the solanaceae, cucurbitaceae, fabaceae, and asteraceae (Weber, 2013).

Life cycle: Two types of hadda beetles are commonly found in India: *E. vigintioctopunctata* and *E. dodecastigma*. The *E. dodecastigma* is 12 spotted and *E. vigintioctopunctata* is 28 spotted beetles. The black dark spots are present on the elytron. These two species can interbreed among themselves. Adult beetles are about 8 mm in length and 5–6 mm in breadth. *E. dodecastigma* is a copper color while the *E. vigintioctopunctata* is a deep red color. The body is hemispherical and smooth. Adults are good fliers and move from plant to plant.

After mating the females start laying eggs in March and April. A female lays about 120–180 eggs. Eggs are laid in the cluster. The eggs are cigar-shaped yellowish in color and are arranged side to side on the surface of the leaf in an erect position. The larva hatches in 3–4 days in summer months and in 4–9 days in winter. Newly hatched first instar is yellowish in color and

has six rows of long-branched spines (Tayde and Simon, 2013). The grubs are oval, fleshy, and yellow in color, bearing hairs and spines on the body surface. The grubs restrict their feeding to the epidermis of the leaves. A fully grown larva measures about 8 mm in length. The larva changes into pupa. The pupation takes place on the leaf surface or on the stem or at the base of the plants. Pupa is oval and dark in color. The pupal period lasts for 3–6 days. The life cycle is completed in 17–18 days in summer but in winter, it may prolong up to 50 days. The pest completes 7–8 generations in a year.

Damage symptoms: Both larvae and adults are destructive. Adults and grubs scrape the lower epidermis on the green tissues of leaves in a characteristic manner leaving behind stripes of uneaten areas. The leaves give a stifled appearance. In severe infestation, all leaves may be eaten off leaving only the veins intact and which ultimately dries up.

3.2.1 Management

Cultural: The beetles larvae, pupae, and eggs can be hand-collected and destroyed. Thorough irrigation of infested crop can minimize the increase in pest population.

Mechanical: Shake plants to dislodge grubs, pupae, and adults in kerosenated water early in the morning or collect them mechanically and destroy.

Biological: In India and Pakistan the hadda beetle is attacked by three predatory bugs: *Rhynocoris fuscipes* (Reduviidae), *Cantheconidea furcellat* (Pentatomidae), and *Geocoris tricolor* (Lygaeidae).

Botanicals: Use 1 L of neem oil with 60 g of soap dissolved in 1/2 L of water, dilute emulsion by adding 20 L of water, then mix about 400 g of well-crushed garlic and spray.

Chemical: The spray of carbaryl 50% WP 2 kg + wettable sulfur 2 kg or malathion 50 EC 1.5 L or azadirachtin 0.03% 2.5–5.0 L in 500–750 L of water is recommended for the control of pest.

3.3 Potato Aphid *Myzus Persicae/Macrosiphum Euphorbiae* (Hemiptera: Aphididae)

Several species of aphids feed on potatoes throughout the world; the most important are green peach aphid *M. persicae*, potato aphid *M. euphorbiae*, Buckthorn aphid *Aphis nasturtii*, and Foxglove aphid *Aulacorthum solani*; they may vector several damaging viruses in a persistent or nonpersistent manner. Virus transmission is of highest concern for growing seed potatoes, which are used to plant subsequent crops.

Distribution: The potato aphid is worldwide in distribution, in all areas in which potatoes are grown. The potato aphid attacks more than 200 plants, including vegetable and ornamental crops, as well as weeds. Cultivated food hosts include apple, bean, broccoli, cabbage, corn, eggplant, ground cherry, lettuce, mustard cabbage, papaya, pea, pepper and so on.

Life cycle: Green peach aphids arrive on potatoes in the spring from weeds and various crops where it has wintered as nymphs and adults, or from peaches and related trees where it winters as eggs. The life cycle varies considerably, depending on the presence of cold winters. Development can be rapid, often 10–12 days for a complete generation and with more than 20 annual generations reported in mild climates. The eggs measure about 0.6 mm long and 0.3 mm wide, and are elliptical in shape. Eggs initially are yellow or green, but soon turn black. Mortality in the egg stage sometimes is quite high. Nymphs initially are greenish, but soon turn yellowish, greatly resembling viviparous adults. There are four instars in aphids and the average length of life was about 23 days. The adults vary in appearance, occurring in a green or pink form. Winged adults have a black head and thorax and a yellowish-green abdomen with a large dark patch in the middle of the abdomen as viewed from above. They measure about 2 mm in length. Wingless adults are yellowish, greenish, or reddish. The cornicles are long and colored similar to the body.

Damage symptoms: Green peach aphids can build large populations on a variety of crops. Infested leaves become yellowish, wrinkled, and cupped, while, tender shoots turn yellowish and die away. They also excrete honeydew on which sooty mold develops covering affected parts with a thin superficial black coating that hinders photosynthetic activity of leaves resulting in stunted growth of plants. The potato aphid transmits potato virus Y on tobacco, but almost never on potatoes. It is also considered a poor vector of potato virus A and potato leaf roll.

3.3.1 Management

Cultural: The most important source of virus in a potato field is infected plants already in that field. Therefore, purchasing certified seed, with low or no virus infection, is the best first step in controlling aphid-related damage to potatoes.

Biological: Potato aphids are often controlled by the natural occurrence of predators, such as coccinellid larvae, predatory bugs in genera *Orius*, *Nabis*, and *Geocoris* spp., lacewings, spiders, syrphid fly larvae, flower flies, predatory gall midge larvae.

Chemical: Green peach aphids are generally controlled with application of insecticides; however, insecticide resistance has been widely documented in this species. Spray dimethoate 0.3%.

3.4 Colorado Potato Beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

Distribution: The Colorado potato beetle is native to Mexico and was first reported in Florida in 1920, but it is not often a major pest. The species has been introduced into Europe and parts of Asia. Potatoes are the preferred host for the Colorado potato beetle, but it may feed and survive on a number of other plants in the family Solanaceae, including belladonna, common nightshade, eggplant, pepper, tobacco, ground cherry, henbane, horse-nettle, thorn apple, tomato and so on.

Life cycle: The eggs are bright orange in color, about 1.7–18 mm long and 0.8 mm wide. They are usually deposited in batches of about 30 on the underside of host leaves. Under field conditions, females can lay 200–500 eggs. After 4–15 days, the eggs hatch into reddish-brown larvae with humped backs and two rows of dark brown spots on either side. Larvae progress through four distinct growth stages. Larvae bear a terminal proleg at the tip of the abdomen, as well as three pairs of thoracic legs. Upon reaching full size, each fourth instar spends an additional several days as a nonfeeding prepupa, which can be recognized by its inactivity and lighter coloration. The prepupae drop to the soil, burrow the soil to a depth 2–5 cm, and after about 2 days begin to pupate. The adults are yellowish-orange with multiple black stripes down the back with five per elytron. They are robust and oval when viewed from above. The head has a triangular black spot, and the thorax has irregular dark markings (Capinera, 2001). The Colorado potato beetle is one of the most important defoliators of potatoes. Both adults and larvae feed on leaves.

3.4.1 Management

Cultural: Colorado potato beetles can be reduced with crop rotation practices that can be easily implemented. Manipulating planting time or early planting also reduced the populations. Planting trap crops that attract beetles away from the main crop may be effective in intercepting overwintered beetles colonizing a field in the spring.

Mechanical: Digging plastic-lined trenches along a field border will intercept migrating Colorado potato beetles.

Biological: Predaceous stink bugs *Perillus bioculatus* and *Podisus maculiventris* have been shown to have significantly controlled the Colorado potato

beetle. The lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae) preys on eggs and larvae.

3.5 Tomato Fruit Borer *Helicoverpa armigera* (Lepidoptera: Noctuidae)

Distribution: This is a major pest of the tomato widely distributed in tropics, subtropics, and warmer temperate regions of the world. The pest is cosmopolitan in nature and widely distributed in India. A polyphagous pest, it infests a large number of crops like pea, beans, gram, and vegetables like okra, chili, cabbage, and cauliflower and so on.

Life cycle: A single female lays 750–1000 eggs and are laid singly, generally on leaves and flowers in the upper canopy of the plant, singly on developing immature fruit and floral buds. The incubation period of 2–6 days depends on temperature. Larval period varies 13–19 days and larvae are greenish brown with dark gray lines along the side of the body. Cannibalism is observed in larvae if there is no suitable host. Pupal period varies 8–15 days and the pest pupates inside soil. Adults are yellowish brown stout moths with grayish dark margins on wings and forewings having black spots in the middle.

Damage symptoms: The damaging stage is larvae. Young larvae of fruit borers feed on the foliage and late larval instars bore inside the fruits and one larva may damage more than four fruits, making them unfit for marketing. Such fruits are not preferred by consumers. The holes made on the fruits are circular and larvae feeding half-body remains inside the fruit and the other half-body remains outside the fruit.

3.5.1 Management

Cultural: Deep summer ploughing exposes the pupae to sunlight and predatory birds like the myna and starling bird. Use of marigold (*Tagitus erecta*) as a trap crop in bud stage also attracts away the adults from infesting tomato plants. Use certified seeds, which are free from insect and disease.

Physical: Cold storage of fruits and vegetables reduces pest infection. Damaged fruits and crop residue should be burned to avoid the carryover of pests.

Mechanical: Hand-pick larvae, use light and pheromone traps (12/ha) to monitor and mass trap adults. Collect and destroy the damaged fruits.

Biological: Use bird perches in the field to increase the activity of predatory birds. Release egg parasitoid *Trichogramma chilonis* six times at 50,000/ha/week, with the first release coinciding with the flowering time. Release the cocoon of *Campoletis chlorideae* (Ichneumonidae) for parasitizing the larvae. Use HaNPV at 250–500 LE per ha. Use Bt-formulation for spray.

Chemical: Pest population can be checked by spraying Indoxycarb 14.5SC, Novaleuron 10EC, Spinosad 4.5SC, and Flubendiamide 20 WDG.

Biologically intensive IPM or biointensive IPM (BIPM) is only a variation of the basic theme of IPM and relies on host-plant resistance, biological control, and cultural control and the use of biorational pesticides, which can be integrated with these.

3.6 Brinjal Fruit and Shoot Borer *Leucinodes orbonalis* (Lepidoptera: Pyralidae)

Distribution: Shoot and fruit borers are the most destructive pests of brinjal. Besides brinjal they also infest many solanaceous crops. In India these pests have a countrywide distribution. Besides India, they are also found in Sri Lanka, Burma, Malaysia, Congo, and South Africa.

Life cycle: The pest is active all year in places with a moderate climate. The moth is a medium-sized adult with brown and red markings on the forewings and hind wings. The female moth lays about 250 eggs singly on tender shoots and developing fruits of brinjal. Within 3–5 days larvae hatches out from the eggs. The larva enters the plant tissues immediately after its hatching and it becomes fully matured after five moltings. The fully-grown larva is stout, pink color with a brown head. The caterpillar is the most damaging stage. Fully-grown larva measures about 20 mm long and pupates in a tough silken cocoon on the plant itself. The entire life cycle is completed in 3–6 weeks. Pupates in silken cocoons, which are attached to leaves or plant surfaces but outside the infested fruit. The pupal period is 6–17 days and the whole lifecycle is completed in 20–43 days. There are five overlapping generations in their active phase in a year. During winter the larvae hibernates inside the soil.

Damage symptoms: It is the most important and destructive pest of brinjal in India it also seriously infests potatoes. Damage to the plant is caused mainly by the larvae, which bores through the terminal part of the mid-rib of large leaves and tender shoots to cause “dead hearts.” A single caterpillar may destroy as many as 4–6 fruits. Damaged fruits show circular exit holes. The entry holes on the brinjal fruit can also be seen plugged with excreta, thus making the fruits unfit for consumption and marketing.

3.6.1 Management

Cultural: Continuous cropping or ratooning may be avoided. Adjust the time of planting by transplanting up to the fourth week of June. Remove and rough out infested fruits and small plants and destroy properly. Grow

less susceptible varieties, such as pusa purple round, brinjal long green, pusa purple cluster, brinjal round white, and annamalai.

Mechanical: Carry out shoot clipping operations at weekly intervals. Collect and destroy affected shoots and fruits. Collect and destroy the pupae present on leaves and other plant parts and kill them by crushing. Keep pheromone traps at 12/ha. Cultivation of brinjal under protected cultivation (net house condition) is found to reduce the incidence of shoot and fruit borer.

Biological: In nature many parasitoids are associated with this pest, like braconids, ichneumonids. Use them as biocontrol agents.

Chemical: Soil application of Carbofuran 3 G at 30 kg/ha 10 days after transplanting is also very effective in controlling the pests. Spray Flubendiamide 20 WDG, Emamectin benzoate 5% SG, and Spinosad 4.5 SC used to check infestation.

4 CUCURBITACEOUS VEGETABLES

Cucurbits are major crops of countries having a wide variety of vegetables. Cucurbits include bottle gourd, bitter gourd, pumpkins, squashes, ridge gourd, sponge gourd, and cucumbers. There are large numbers of pests associated with cucurbits; fruit flies are major, greatly reducing the postharvest quality.

4.1 Fruit Fly *Bactrocera cucurbitae* (Diptera: Tephritidae)

Distribution: The melon fruit fly is distributed all over the world and has been recorded from East Africa, some parts of the United States, northern Australia, Taiwan, Japan, South China, Southeast Asia, and the Indian subcontinent. The most destructive fruit fly in cucurbits and other species that are found in India are *Bactrocera tau* and *Bactrocera ciliates*.

Life cycle: The melon fruit fly remains active throughout the year on one or the other host. During winter months, they hide and huddle together under dried leaves and trees. During the hot and dry season, the flies take shelter under humid and shady places and feed on honeydew aphids infesting the fruit trees. After emerging from the pupae adult flies mate at dusk and have preoviposition for a period of a few days, but it depends on temperature and weather condition. A female lays a dozen shiny white eggs just below the epicarp of fruits by using a sharp ovipositor. On average, a female lays up to 50–100 eggs. An incubation period of 1–9 days, larvae mature in 3–5 days in summer and 2–3 weeks during winter. After maturation, larvae

fall from the attacked fruit and reach to somewhat sandy soil by jumping. Jumping is possible due to folding and unfolding of the body. They pupate inside soil up to 12–20 cm depth. Pupal period is 6–9 days and prolonged in winter. There are several generations in a year.

Damage symptoms: Adult fruit flies damage the fruit where they lay their eggs, causing blemishes and discoloration. The maggots bore inside the pulp and chew the pulp making galleries and pave the way for secondary invaders (fungi/bacteria), which cause extensive rotting and dropping of fruit. They contaminate the fruit by their excreta. Damaged fruits are unfit for human consumption. Damage symptoms do not vary on different crops.

4.1.1 Management

Cultural: The most effective method in melon fruit fly management is field sanitation. Collection and destruction of attacked fruit, frequent raking of soil near vines, deep summer ploughing, and crop rotation should be followed. Grow resistant or early maturing varieties.

Physical: The sterile males are released in the fields for mating with the wild females. Sterilization is accomplished through irradiation, chemosterilization, or by genetic manipulation.

Mechanical: Bagging fruits on the tree (3–4 cm long) with two layers of paper bags at 2–3 day intervals minimizes fruit fly infestation. The fallen and infested fruits should be collected and destroyed to prevent the carryover of the pest. Monitor and control with parafferomone lures/cue-lure and protein hydrolyze traps. Frequent raking of the soil under the vines or ploughing the infested fields after the crop is harvested will help in killing the pupae.

Biological: Pupae are parasitized by *Opius fletcheri*, *O. confensatus*, and *O. insisus* (Braconidae).

Chemical: Use a bait spray containing 50 ml of Malathion 50 EC + 0.5 kg of gur or jaggary in 50 L of water per ha. It should be repeated if infestation is severe. Adult flies rest on broad leaf plants like maize, and so on, which are growing near to cucurbits also spreading.

4.2 Pumpkin Beetles *Raphidopalpa foveicollis*, *Aulacophora lewisii*, *A. cincta* (Coleoptera: Chrysomelidae)

Distribution: *R. foveicollis* is found in almost all states of India though it is more abundant in northern states in association with *A. lewisii*. In South India *A. cincta* is more common.

Life cycle: Adult beetles of *R. foveicollis* are 6–8 mm long having glistening yellowish red to yellowish brown elytra that are uniformly covered with

fine punctures. Adults of *A. cincta* are similar in size and appearance except that the color of elytra is grayish yellow to brownish gray with distinct palm margins all around. Adults of *A. lewisii* are slightly smaller (5–6 mm) with blackish blue elytra. They are active from March to October and the peak period of activity is April to June. The yellowish pink spherical eggs are laid in the soil, which turns orange after 2 days and a beetle may lay 150–300 eggs. The egg period is 5–8 days. The grubs become full-grown in 13–25 days and pupate in the soil. The prepupal period is 2–5 days. The pupal period ranges 7–17 days. Total life cycle occupies 32–65 days. In a year there may be 5–8 generations of the insect.

Damage symptoms: The beetles bite irregular holes on leaves and also feed on flowers. They prefer young seedlings and tender leaves and the damage at this stage may kill the seedlings. The root, stem, and fruits that come in contact with the soil are damaged by the grubs. Pumpkin is preferred by *R. foveicollis* and sponge gourd by *A. lewisi*. Both the species feed also on snake gourd, pumpkin, cucumber, melon, and ribbed gourd.

4.2.1 Management

Cultural: Practices like clean cultivation and early sowing will reduce pest damage. After harvesting, deep plough the field to kill the grub in the soil.

Mechanical: The larvae when found in small numbers may be hand picked and destroyed.

Chemical: Spray malathion 50 EC at 500 mL or dimethoate 30 EC 500 mL or methyl demeton 25 EC at 500 mL/ha.

5 LEGUMINOUS VEGETABLES

5.1 Pea Pod and Beans Borer *Helicoverpa armigera* (Lepidoptera: Noctuidae)

This is discussed in tomato as fruit borer.

5.2 Pea Pod Borer *Etiella zinckenella* (Lepidoptera: Phycitidae)

Distribution: This is a major and serious pest of green peas and causes considerable crop loss. It is distributed in most of the pea-growing areas of India, especially in the northern region.

Life cycle: Adult moths are grayish in color; half of the forewings are darker in color with a light blackish band on the middle of the forewing. The moths are gray with a wing expanse of about 25 mm. The forewings have dark marginal lines and orcheous scales. Moths lay eggs singly or in

small clusters. Eggs hatch in 5 days at 25°C. Caterpillars are tiny and greenish in color, but full-grown larvae are rosy with purplish tinge. The larval period is 10–27 days and the pupal stage lasts for 10–15 days. Pupation takes place in soil. The pests complete five overlapping generations and breed throughout the year.

Damage symptoms: The main damage is caused by the caterpillars. Initial instars feed on floral buds and newly formed pods. Later, bores inside the pods feed on developing seeds and reduce the postharvest quality. In many cases heavy reduction in the yield occurs.

5.2.1 Management

Cultural: Deep summer ploughing as the pupa inside the soil can be exposed to sunlight along with field sanitation should be practiced.

Chemical: At flower initiation, spray the crop with Deltamethrin at 0.0028%. Insecticides recommended for the control of *H. armigera* are also effective.

6 MISCELLANEOUS VEGETABLES PESTS

6.1 Sweet Potato Weevil *Cylas formicarius* (Coleoptera: Apionidae)

Distribution: This pest has cosmopolitan distribution (CABI/EPPO, 2004). The origin of *C. formicarius* is not definitely known, but it is thought to have originated in Africa or India. *Cylas* weevils are serious pests of sweet potatoes globally, particularly in drier agroecological zones. It is also a major pest of morning glory and other plants of the same family. Alternate hosts of sweet potato weevils are *Ipomoea* spp. weeds.

Life cycle: The adult insect is ant-like and the basic color of the insect is red. The adult female lays eggs singly in cavities excavated in vines or in storage roots, preferring the latter. The egg opening is sealed with a protective, gray fecal plug. The initial larvae tunnel is in the vine or storage root. Pupation takes place within the larval tunnels. A few days after eclosion, the adult comes out from the vine or storage root. Because the female weevil cannot dig, she finds storage roots in which to lay her eggs by entering through soil cracks. Adults of all species may be conveniently sexed by the shape of the distal antennal segment, which is filiform in males and club-like in females. The males have larger eye facets than the females. At optimal temperatures of 27–30°C, *C. formicarius* completes development in about 33 days. Adult longevity is 2.5 to 3.5 months and females lay between 100 and 250 eggs in this period. At suboptimal temperatures, growth takes longer.

Damage symptoms: Adult sweet potato weevils feed on the epidermis of vines and leaves. They also feed on the outer surfaces of storage roots, causing round feeding punctures, which can be distinguished from oviposition sites by their greater depth and the absence of a fecal plug. The developing larvae of the weevil tunnel in the vines and storage roots, causing significant damage. Frass is deposited in the tunnels. In response to damage, storage roots produce toxic terpenes, which render storage roots inedible even at low concentrations and low levels of physical damage. Feeding inside the vines causes malformation, thickening, and cracking of the affected vine (Stathers et al., 2005).

6.1.1 Management

Cultural: Cultural practices have proved to be effective against the sweet potato weevil and should be the main basis of control. Some of the cultural practices include the use of uninfected planting material, especially vine tips, crop rotation, intercropping with Proso millet (*Panicum miliaceum*) and sesame (*Sesamum indicum*), removal of volunteer plants and crop debris (sanitation), flooding the field for 24 h after completing a harvest, timely planting and prompt harvesting to avoid a dry period, removal of alternate, wild hosts, and avoiding planting of sweet potatoes in the same area for 2 successive years.

Physical: Use heat treatment and vapor heat treatment of tubers.

Mechanical: Manually collect infested tubers and vines, taking care during cultural operations because cracks present in soil leads to infestation and they should be closed during hoeing. The species-specific pheromone traps are used as monitoring and management. Many effective traps have been designed by farmers using locally available materials.

Biological: Promising biological control agents for sweet potato weevils appear to be the fungi *Beauveria bassiana* and *Metarrhizium anisopliae* and the nematodes *Heterorhabditis* spp. and *Steinernema* spp. The fungi attack and kill adult weevils, whereas the nematodes kill the larvae. Ants, spiders, carabids, and earwigs are important generalist predators that attack weevils.

Chemical: Spray 2 L of Malathion 50 EC in 625 L of water per ha.

6.2 Sweet Potato Stem Borer *Omphisa anastomasalis* (Lepidoptera: Pyralidae)

Distribution: The borer is widely distributed in the Philippines, Indonesia, India, Sri Lanka, Malaysia, Taiwan, Hawaii, and Vietnam. It also occurs in China, Japan, Cambodia, Laos, and Burma.

Life cycle: The eggs are laid individually along the underside of the leaves, along the leaf margins. Some are laid on the stem. Full-grown larvae are 30 mm long. The egg, larval, and pupal stages last an average of 55–65 days. A newly emerged larva has a brown head and a reddish or pinkish body. After a few days, it turns creamy with black markings. There are six larval instars. Before pupating, the larva makes an exit hole that is covered with the epidermis of the stem. Pupation lasts about 2 weeks and takes place in a web-covered cocoon within the tunnel. After emerging from the cocoon, the adult moth lives only 5–10 days. Most eggs are laid in the first 3 days of adult life. The adults emerge by breaking through the dry papery covering of the exit hole.

Damage symptoms: The larva bores into the main stem and sometimes penetrates the storage roots. Larval feeding causes enlargement and lignifications of the stems at the base of the plant and in the formation of hollow cavities filled with frass. Attack during the early stages of plant growth may inhibit the formation of storage roots.

6.2.1 Management

Cultural: Prevention is the best way to control the stem borer and can be accomplished by the use of healthy planting material that is free of stem borer eggs and larvae. Clean planting material can be obtained by careful selection of cuttings or by planting roots. Hilling-up often is practiced to reduce damage from sweet potato weevils. Hilling-up is effective when the holes, made to provide the adults with a means of exiting the stems, are covered with soil.

Mechanical: Destroy infested crop residues after harvesting.

Biological: Earwigs and ants may attack the larvae developing within sweet potato vines.

6.3 Spotted Bollworms of Okra *Earias vittella*, *E. insulana* (Lepidoptera: Noctuidae)

Distribution: These are widely distributed bollworms that infest the cotton, but they also infest the okra and cause considerable postharvest loss, especially in India, Pakistan, and North Africa.

Life cycle: Larvae are dull green caterpillars with bristle and blackish spots on body. Adult moths are yellowish green in color. Female moths appear during cropping season and start to lay eggs on floral buds and tender fruits singly at night. A single moth lays up to 200–400 eggs. Incubation period is 3–4 days, larval period is 10–16 days depending on the temperature passing

through six stages. Pupation takes place either on plants or on fallen leaves on the ground. The pupal period is 4–9 days, but is several weeks during the winter.

Damage symptoms: Caterpillars cause damage by feeding on florets and developing fruits. Fruits are partially eaten and one larva may damage more than a fruit. Thus, postharvest and market quality are reduced.

6.3.1 Management

Cultural: Use proper field sanitation, remove alternate hosts like cotton hol-lyhock, collect and destroy the attacked fruits. Grow resistant and tolerant cultivars.

Biological: Egg parasitoid *Trichogramma* spp. reduces the considerable population of this pest.

Chemical: Spray the crop with 250 mL of Fenvalerate 20 EC, 400 mL Deltamethrin 2.8 EC, and 200 mL of Cypermethrin 25 EC in 300 L of water per ha.

6.4 Onion Maggot *Delia antiqua* (Diptera: Anthomyiidae)

Distribution: These pests are distributed worldwide and in India cause considerable postharvest loss to the onion bulbs.

Life cycle: The flies are small and grayish but the maggot is creamy white. Females lay elongated eggs near the base of plant or on exposed bulbs. The incubation period is 2–7 days and the larval period is 2–3 weeks and when larvae become fully fed they come out from the infested bulb and pupate inside the soil. The pupal period is 2–3 weeks and depends on moisture and temperature.

Damage symptoms: After hatching, larvae bore into leaf sheath and then enter in bulbs. They feed inside the bulb by making mines and sometimes many larvae are found in a single bulb. Attacked bulbs become prone to fungal and bacterial attack. A large number of partially damaged bulbs are found that reduce the postharvest quality and marketable value. Also, this causes the rotting of bulbs in the storage.

6.4.1 Management

Cultural: Use regular racking of soil and earthing up of bulbs, which are exposed. Plants that show the symptom of damage should be roughed out. Use deep summer ploughing to expose the pupae to sunlight.

Chemical: Spray malathion 1 L/ha

7 CONCLUSIONS

Postharvest treatment is very important for facilitating national and international trade of horticultural commodities. These treatments have increased significantly over the last few years because horticultural crops help to increase trade and restrict the use of fumigants in horticultural crops. A number of deficiencies currently exist in the postharvest management and processing of fruits and vegetables in India. Action must be taken in order to upgrade systems to reduce the levels of postharvest losses in India. Presence of pests in horticultural foodstuffs has been the center of attention of many procedures and treatments to prevent continuous damage to the foodstuffs or accidental movement of pests from one area to another. The use of chemicals in postharvest management are harmful to environment as well as human body. Therefore, it is desirable to explore some alternative methods that are more environmentally safe and have no impact on human health.

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CHAPTER 12

Preharvest Approaches to Control Insect Infestation in Fruit

Ranjeet Kumar, Ramanuj Vishwakarma

Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

1 INTRODUCTION

The production of fruit is one of the important sectors of horticultural industries all over the world. This sector plays a vital role in farm income enhancement, food and nutritional securities, as well as poverty alleviation and sustainable development in developing countries. The well-strengthened horticultural sector may have a positive impact on the millennium development goals (World Bank, 2008). The major fruit, including apple, aonla, apricot, avocado, banana, beal, ber, citrus, date palm, grape, guava, jamun, litchi, mango, papaya, peach pear plum, pine apple, pomegranate, sapota, strawberry, walnut are important contributors in the national economy of countries. The incidence of pesticide residues in fruit beyond permissible limits and postharvest losses in the country is new challenges faced under trade liberation and globalization, so serious efforts are needed to eliminate such challenges.

India represents a variety of climates, from north to south and east to west ranging from temperate to tropical and subtropical, suitable for the production of a range of economically important fruit not only for domestic consumption but for regional and international trade. India produced 81,285 metric tonnes of fruit from 6982 hectares of land in the year 2014 (APEDA, 2014, <http://agriexchange.apeda.gov.in/india>).

Despite decades of advancement in science, the most common reason for postharvest losses of fruit in developing countries to be inadequate preharvest management of insect infestation and maintenance of fruit. The lack of detection technology for identification of the immature stage of insects before sorting and storage of fruit is also a major factor for deterioration of quality and shelf life of fruit. The several factors responsible for quality deterioration of highly perishable fruit and losses due to insect infestation is

higher in India as compared to remaining parts of the world. The packing, handling, wholesale distributors, and retailers may perceive quality as ease of handling, uniformity of packing, and freedom from any types of infection or infestation, as well as physiological changes. Although there is much to learn about the quality of a given temperate, subtropical, and tropical fruit in response to the postharvest environment, previous research indicated handling with common principles of good commodity management. Generally temperature, relative humidity, and modification in environment, cooling and spraying of insecticides, and fungicides after harvesting are commonly used.

To prevent postharvest losses, we must understand the biological and environmental factors that are responsible for the quality deterioration of fruit. Basically, insect pests, microorganism enzymes, temperature, relative humidity, and moisture content are deteriorating the quality of fruit and reduce the shelf life. To manage these aspects, a sound knowledge of relevant discipline is a must (Wills et al., 1981). The postharvest technology is multidisciplinary approaches and coordinating links between fruit growers and consumers or vice versa (Verma, 1995).

The first essential requirement of protection against insect pests is sustainable management during the production of fruit and wise full harvesting, grading, transportation, storage, and marketing. The storage environment plays a vital role in development of potential insect pests of harvested produces. The temperature, relative humidity, and moisture content of fruit are directly responsible for growth and development of insect pests, as well as microorganisms.

In this chapter we discuss the preharvest control measures to prevent postharvest insect infestation in fruit. Preharvest approaches may influence the development and maturation of fruit without infestation of any insects. This approach may also have a physical impact on quality of fruit and insect scarring may reduce the percentage. We are trying to cite only those insects that preharvest infestation deteriorates the quality and postharvest life of temperate, tropical, and subtropical fruit.

2 INSECTS OF IMPORTANT FRUIT CROPS

The major insects of important fruit in India, as well as the world infest fruit in the orchards resulting in a reduction in production and deterioration in quality of produce (Table 12.1). However, we discuss only those insects that provide infestation continued after harvesting of the fruit.

Table 12.1 Major insects of major fruit varieties

Tropical and subtropical fruit

	Common name	Scientific name	Order	Family	Major host	Damaging stage	Distribution	Remarks
Mango	Mango hopper	<i>Amritodus atkinsoni</i>	Hemiptera	Jassidae	Mango	Nymph and adult	All over the world	Deteriorate postharvest quality
	Mango mealy bug	<i>Drosicha mangiferae</i>	Hemiptera	Coccidae	Mango and other tropical fruit	Nymph and adult	All over the world	
	Mango stone weevil	<i>Sternochaetus mangiferae</i>	Coleoptera	Curculionidae	Mango	Grub	All over the India	
	Mango shoot borer	<i>Chlumetia transversa</i>	Lepidoptera	Noctuidae	Mango	Caterpillar	All over the India	
	Mango gall psyllid	<i>Apsylla cistella</i>	Hemiptera	Psyllidae	Mango	Nymph and adult	All over the world	
	Mango aphid	<i>Toxoptera odinae</i>	Hemiptera	Aphididae	Mango	Nymph and adult	All over India	
	Fruit borer	<i>Hyalospila leuconeurella</i>	Lepidoptera	Phycitidae	Mango	Caterpillar and adult	All over India	
	Mango stem borer	<i>Bactrocera rufomaculata</i>	Coleoptera	Cerambycidae	Mango	Grub	All over the India	
	Mango fruit fly	<i>Bactrocera dorsalis</i>	Diptera	Tephritidae	Mango and other tropical fruit	Maggots and adult	All over the world	

(Continued)

Table 12.1 Major insects of major fruit varieties (*cont.*)

Tropical and subtropical fruit

	Common name	Scientific name	Order	Family	Major host	Damaging stage	Distribution	Remarks
Guava	Bark borer	<i>Indrabela tetraonis</i>	Lepidoptera	Metarbelidae	Guava and other tropical fruit	Caterpillar	All over the India	
	Fruit borer	<i>Conogethes punctiferalis</i>	Lepidoptera	Pyraustidae	Guava	Caterpillar	All over the world	Deteriorate postharvest quality
	Guava fruit fly	<i>Dacus dorsalis</i>	Diptera	Tephritidae	Guava and other tropical fruit	Maggots and adult	All over the world	Deteriorate postharvest quality
	Mealy scale	<i>Pulvinaria psidii</i>	Hemiptera	Lycaenidae	Guava and other tropical fruit	Nymph and adult	All over the world	
Banana	Banana weevil	<i>Cosmopolites sordidus</i>	Coleoptera	Curculionidae	Banana	Adult	All over the India	
	Banana stem borer	<i>Odoiporus sordidus</i>	Coleoptera	Curculionidae	Banana	Grub	All over the India	
	Scale moth	<i>Nacoleia octasema</i>	Lepidoptera	Pyralidae	Banana	Caterpillar	All over the India	
	Banana aphid	<i>Pentalonia nigronervosa</i>	Hemiptera	Aphididae	Banana	Nymph and adult	All over the India	
	Scarring beetle							
Citrus	Citrus butterfly	<i>Papilio demoleus</i>	Lepidoptera	Papilionidae	Citrus	Caterpillar	All over the India	

	Citrus psylla	<i>Diaphorina citri</i>	Hemiptera	Psyllidae	Citrus	Nymph and adult	All over the India
	Orange borer	<i>Chelidonium cinctum</i>	Coleoptera	Cerambycidae	Orange	Grub	All over the India
	Citrus whitefly	<i>Dialeurodes citri</i>	Hemiptera	Aleyrodidae	Citrus	Nymph and adult	All over the world
	Citrus blackfly	<i>Aleurocanthus woglumi</i>	Hemiptera	Aleyrodidae	Citrus	Nymph and adult	All over the world
	Citrus red scale	<i>Aonidiella aurantii</i>	Hemiptera	Diaspididae	Citrus and other fruit	Nymph and adult	All over the world
	Cottony cushion scale	<i>Icerya purchasi</i>	Hemiptera	Margarodidae	Citrus	Nymph and adult	All over the world
	Citrus mealy bug	<i>Pseudococcus filamentosus</i>	Hemiptera	Pseudococ- cidae	Citrus and other fruit	Nymph and adult	All over the world
	Fruit sucking moth	<i>Ophideres conjuncta</i>	Lepidoptera	Noctuidae	Citrus and other fruit	Adult	All over the India
Grape	Grapevine leafhopper	<i>Erythroneura</i> sp.	Hemiptera	Cicadellidae	Grape	Nymph and adult	All over the India
	Grapevine thrips	<i>Rhipiphorothrips cruentatus</i>	Thysanoptera	Heliothripidae	Grape	Nymph and adult	All over the India
	Leaf roller	<i>Sylepta lunalis</i>	Lepidoptera	Pyralidae	Grape	Caterpillar	All over the India
	Grapevine beetle	<i>Sinoxylon anale</i>	Coleoptera	Bostrychidae	Grape	Grub and adult	All over the world
	Grapevine girdler	<i>Sthenias grisator</i>	Coleoptera	Cerambycidae	Grape and other fruit	Grub	All over the India

(Continued)

Table 12.1 Major insects of major fruit varieties (*cont.*)

Tropical and subtropical fruit

	Common name	Scientific name	Order	Family	Major host	Damaging stage	Distribution	Remarks
Ber	Ber fruit fly	<i>Carpomyia vesuviana</i>	Diptera	Tephritidae	Ber	Maggots and adult	All over the world	Deteriorate postharvest quality
	Ber beetle	<i>Adoretus pallens</i>	Coleoptera	Scarabaeidae	Ber	Grub	All over the India	
	Fruit borer	<i>Meridarches scyroides</i>	Lepidoptera	Carposinidae	Ber	Caterpillar	All over the world	Deteriorate postharvest quality
Pomegranate	Pomegranate butterfly	<i>Virachola isocrates</i>	Lepidoptera	Lycaenidae	Pomegranate	Caterpillar	All over the India	
	Fruit fly	<i>Dacus zonatus</i>	Diptera	Tephritidae	Polyphagus	Maggots and adult	All over the world	Deteriorate postharvest quality
Custard apple	Mealybug	<i>Ferrisia virgata</i>	Hemiptera	Pseudococcidae	Custard apple	Nymph and adult	All over the India	
	Fruit fly	<i>D. zonatus</i>	Diptera	Tephritidae	Polyphagus	Maggots and adult	All over the world	Deteriorate postharvest quality
Fig	Stem borer	<i>Bactocera rufomaculata</i>	Coleoptera	Cerambycidae	Fig	Grub	All over the India	
	Fruit fly	<i>Bactrocera dorsalis</i>	Diptera	Tephritidae	Polyphagus	Maggots and adult	All over the world	Deteriorate postharvest quality

Pineapple	Fruit borer	<i>Olenecamptus bilobus</i>	Coleoptera	Cerambycidae	Fig	Grub	All over the India	Deteriorate postharvest quality
	Fig midge	<i>Anjeerodiplosis peshawarensis</i>	Diptera	Cecidomyiidae	Fig	Maggots and adult	All over the India	
	Thrips	<i>Thrips tabaci</i>	Thysanoptera	Thripidae	Pineapple	Adult and nymph	All over the India	
Loquat	Slug caterpillar	<i>Parasa lepida</i>	Lepidoptera	Limacodidae	Pineapple	Caterpillar	All over the India	
	Fruit fly	<i>B. dorsalis</i>	Diptera	Tephritidae	Polyphagus	Maggots and adult	All over the world	
Date palm	Bark borer	<i>Indrabela quadrinotata</i>	Lepidoptera	Metarbelidae	Loquat and other tropical fruit	Caterpillar	All over the India	
	Date palm scale	<i>Aspidiotus destructor</i>	Hemiptera	Diaspididae	Date palm	Nymph and adult	All over the India	
	Rhinoceros beetle	<i>Oryctes rhinoceros</i>	Coleoptera	Scarabaeidae	Date palm	Grub and adult	All over the India	
Litchi	Date palm weevil	<i>Rhynchophorus ferrugineus</i>	Coleoptera	Curculionidae	Date palm	Grub and adult	All over the India	
	Litchi bug	<i>Chrysocoris stollii</i>	Hemiptera	Pentatomidae	Litchi	Nymph and adult	All over the world	
Sapota	Sapota leaf webber	<i>Nephoteryx eugraphella</i>	Lepidoptera	Pyralidae	Sapota	Caterpillar	All over the India	
Jamun	Bark borer	<i>Indrabela quadrinotata</i>	Lepidoptera	Metarbelidae	Jamun and other tropical fruit	Caterpillar	All over the India	

(Continued)

Table 12.1 Major insects of major fruit varieties (*cont.*)

Tropical and subtropical fruit

	Common name	Scientific name	Order	Family	Major host	Damaging stage	Distribution	Remarks
Jamun	Leaf miner	<i>Acrocercops phaeospora</i>	Lepidoptera	Gracillariidae	Polyphagous	Caterpillar	All over the India	
Sapota	Sapota moth	<i>Nephopteryx eugraphella</i>	Lepidoptera	Phycitidae	Sapota	Caterpillar	All over India	Deteriorate postharvest quality
	Budworm	<i>Anarsia epotias</i>	Lepidoptera	Gelechiidae	Sapota	Caterpillar	All over India	Deteriorate postharvest quality
	Fruit fly	<i>D. zonatus</i>	Diptera	Tephritidae	Polyphagous	Maggots and adult	All over the world	Deteriorate postharvest quality

Temperate fruit

Apple	San jose scale	<i>Quadraspidiotus perniciosus</i>	Hemiptera	Diaspididae	All temperate fruit	Nymph and adult	All over the world	Deteriorate postharvest quality
	Wooly apple aphid	<i>Eriosoma lanigerum</i>	Hemiptera	Aphididae	All temperate fruit	Nymph and adult	All over the world	Deteriorate postharvest quality
	Tent caterpillar	<i>Malacosoma indicum</i>	Lepidoptera	Lasiocampidae	Apple	Caterpillar	All over the world	
	Codling moth	<i>Cydia pomonella</i>	Lepidoptera	Tortricidae	Apple	Caterpillar	All over the world	Deteriorate postharvest quality

	Apple stem borer	<i>Apriona cinerea</i>	Coleoptera	Cerambycidae	Apple	Caterpillar	All over the world	Deteriorate postharvest quality
	Indian gypsy moth	<i>Lymantria obfuscat</i>	Lepidoptera	Lymantriidae	All temperate fruit	Caterpillar	All over the world	
	Apple root borer	<i>Dorysthenes hugelii</i>	Coleoptera	Cerambycidae	Apple	Grub	All over India	
Peach	Peach leaf curl aphid	<i>Brachycaudus helichrysi</i>	Hemiptera	Aphididae	Peach, Pear, Plum	Nymph and adult	All over the world	
	Peach fruit fly	<i>B. zonata</i>	Diptera	Tephritidae	Polyphagous	Maggots and adult	All over the world	
	Tent caterpillar	<i>Malacosoma indicum</i>	Lepidoptera	Lasiocampidae	Peach, Pear, Plum	Caterpillar	All over the world	
	Peach stem borer	<i>Sphenoptera lafertei</i>	Coleoptera	Buprestidae	All temperate fruit	Grub	All over India	
Pear	Stem borer	<i>Sahydrassus malabaricus</i>	Lepidoptera	Hepialidae	Pear	Caterpillar	All over India	
Cherry	Cherry stem borer	<i>Aeolesthes holosericea</i>	Coleoptera	Cerambycidae	Polyphagous	Grub	All over the world	
Walnut	Walnut weevil	<i>Alcidodes porrectirostris</i>	Coleoptera	Curculionidae	Walnut	Grub	All over India	
	Walnut beetle	<i>Bactocera horsfieldi</i>	Coleoptera	Cerambycidae	Walnut	Grub	All over India	
Almond	Almond weevil	<i>Myllocerus lactivirens</i>	Coleoptera	Curculionidae	Polyphagous	Grub	All over India	

(Continued)

Table 12.1 Major insects of major fruit varieties (*cont.*)

Tropical and subtropical fruit

	Common name	Scientific name	Order	Family	Major host	Damaging stage	Distribution	Remarks
<i>Plantation crops</i>								
Arecanut	Foliage mite	<i>Raoiella indica</i>	Acarina	Tetranychidae	Polyphagous	Nymph and adult	Arecanut growing area	
	Spindle bug	<i>Carvalhoia arecae</i>	Hemiptera	Miridae	Arecanut	Nymph and adult	Arecanut growing area	
	Root grub	<i>Leucophalis burmeisteria</i>	Coleoptera	Melolonthidae	Polyphagous	Grub and adult	Arecanut growing area	
	Inflorescence caterpillar	<i>Tirathaba mundella</i>	Lepidoptera					
	Painted bug	<i>Halyomorpha marmorea</i>	Hemiptera	Paintomidae	Polyphagous	Nymph and adult	All over the India	
	Scale insect	<i>Aonidiella orientalis</i>	Hemiptera					
Cashew nut	Stem and root borer	<i>Plocaederus ferrugineus</i>	Coleoptera	Cerambycidae	Cashew nut	Grub and adult	Cashew growing area	
	Tea mosquito bug	<i>Helopeltis antonii</i>	Hemiptera	Miridae	Polyphagous	Nymph and adult	Cashew growing area	

Coconut	Leaf miner	<i>Conopomorpha syngamma</i>	Lepidoptera	Gracillariadae	Cashew	Caterpillar	Cashew growing area
	Leaf and blossom webber	<i>Lamida monocusalis</i>					
	Nut borer	<i>Thylocoptila panrosema</i>					
	Flower thrips	<i>Rhynchothrips raoensis</i>					
	Red beetle	<i>Monolepta longitarus</i>					
	Rhinoceros beetle	<i>Oryctes rhinoceros</i>	Coleoptera	Scarabaeidae	Coconut and palm	Grub and adult	Coconut growing area
	Red palm beetle	<i>R. hynchophorus ferruginus</i>	Coleoptera	Curculionidae	Coconut and palm	Grub and adult	Coconut growing area
	Black headed caterpillar	<i>Opisina arenosella</i>	Lepidoptera	Cryptophasidae	Coconut and palm	Caterpillar	Coconut growing area
	White grub	<i>Leucopholis coneophora</i>	Coleoptera	Melolonthidae	Coconut and palm	Grub and adult	Coconut growing area
Coreid bug	<i>Paradasynus rostratus</i>	Hemiptera	Corridae	Coconut and palm	Nymph and adult	Coconut growing area	

(Continued)

Table 12.1 Major insects of major fruit varieties (*cont.*)

Tropical and subtropical fruit

	Common name	Scientific name	Order	Family	Major host	Damaging stage	Distribution	Remarks
Coconut	Scale	<i>Aspidiotus destructor</i>	Hemiptera		Coconut and palm	Nymph and adult	Coconut growing area	
	Mealy bug	<i>Pseudococcus cocotis</i>	Hemiptera	Pseudococ-codae	Coconut and palm	Nymph and adult	Coconut growing area	
	Nut borer Coconut weevil	<i>Cyclodes omma</i> <i>Diocalandra frumenti</i>	Coleoptera	Curculionidae	Coconut and palm	Grub and adult	Coconut growing area	
Cocoa	Mealy bug	<i>Planococcus lilacinus</i>	Hemiptera	Pseudococ-codae	Cocoa	Nymph and adult	Cocoa growing area	
	Aphid	<i>T. aurantii</i>	Hemiptera	Aphidiae	Cocoa	Nymph and adult	Cocoa growing area	
	Cow bug	<i>Gargara</i> sp.			Cocoa	Nymph and adult	Cocoa growing area	
	Stem borer	<i>Z. coffee</i>	Lepidoptera	Pyralidae	Cocoa	Caterpillar	Cocoa growing area	

Coffee	White stem borer	<i>Xylotrechus quadripes</i>	Coleoptera	Cerambycidae	Coffee	Grub	Coffee growing area
	Coffee berry borer	<i>Hypothenemus hampai</i>	Coleoptera	Scolytidae	Coffee	Grub and adult	Coffee growing area
	Shot hole borer	<i>Xylosandrus compactus</i>	Coleoptera	Scolytidae	Coffee	Grub and adult	Coffee growing area
	Mealy bug	<i>P. citri</i>	Hemiptera	Pseudococcidae	Coffee	Nymph and adult	Coffee growing area
	Green scale	<i>Coccus viridis</i>	Hemiptera	Coccidae	coffee	Nymph and adult	Coffee growing area
	Coffee been beetle	<i>A. fasciculatus</i>	Coleoptera	Scolytidae	Coffee	Grub and adult	Coffee growing area
	Thrips	<i>H. haemorrhoidalis</i>	Thysanoptera	Thripidae	Coffee	Nymph and adult	Coffee growing area

3 TROPICAL AND SUBTROPICAL FRUIT

3.1 Mango

After harvesting of mango some insects, for example, fruit flies, fruit borer, stone weevil, fruit sucking moth, are responsible for the deterioration of fruit quality and the reduction of shelf life (Veeresh, 1989). Among fruit flies, oriental fruit fly (*Bactrocera dorsalis* H) (Figs. 12.1–12.3) and melon fruit fly (*Bactrocera cucurbitae* C) are notorious international insects infesting mango worldwide. In Queensland *Bactrocera tryomi* (F) and *Ceratitidis capitata* F are dominant species (Jacobi and Wong, 1992). The fruit fly species have been recognized as one of serious threats to the fruit production system in the world (De Meyer et al., 2012).



Figure 12.1 Infested mango by fruit fly.

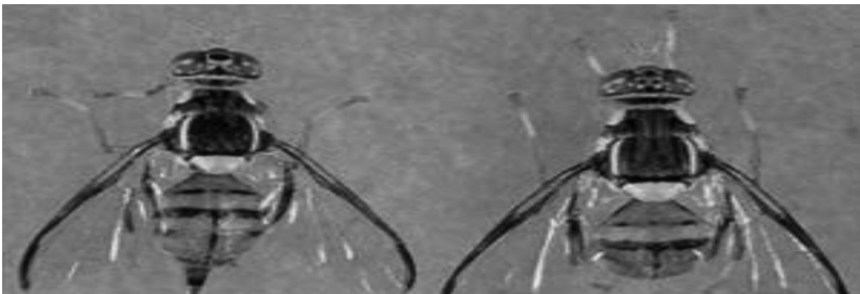


Figure 12.2 Female and male of *Bactrocera dorsalis*.

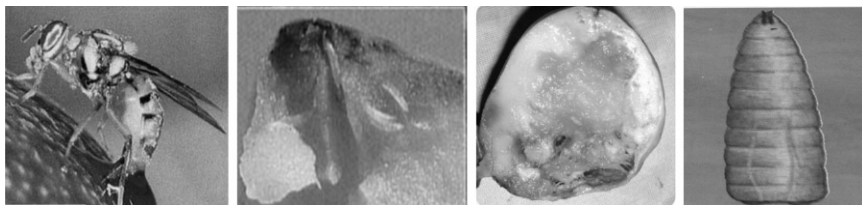


Figure 12.3 *Egg laying, Bactrocera dorsalis on mango, eggs, maggot on fruit, and pupa of Bactrocera dorsalis.*

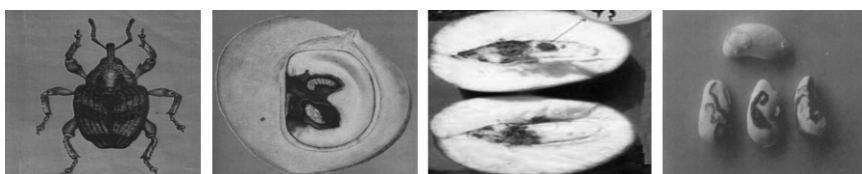


Figure 12.4 *Adult of Sternochaetus mangiferae and damaged fruit and stone.*

The stone weevil *Sternochaetus mangiferae* (Fig. 12.4) is another key pest of mango (Tandon and Shukla, 1989). It is cosmopolitan in nature and reported to occur in several countries (Tandon and Verghese, 1985). Generally it is not considered a major pest because only a small portion of few fruit are damaged by the adult weevil but they emerge from pupae by boring into the pulp of mango during storage and transportation. The economic importance has increased considerably, however in view of the quarantine restrictions imposed by the USA and other countries on mango imported from India because of potential risk of introducing this pest (Singh, 1989).

3.2 Citrus

Citrus Red Mite, *Panonychus citri* caused discoloration of fruit, which reduce marketable quality. Citrus Thrips, *Scirtothrips citri* makes circular rings on the fruit and deteriorate the quality of citrus fruit. The citrus red scale *Aonidiella aurantii* (Fig. 12.5) also damages to the fruit in the same manner. Besides fruit sucking moth (Fig. 12.6) and citrus butter fly citrus fruit is more susceptible to the sucking pests.

3.3 Ber

The ber fruit fly *Carpomyia vesuviana*, *B. dorsalis*, *Bactrocera zonata* infests the fruit at ripening stage. Their hidden infestation comes in storage and deteriorates the quality, as well as shelf life of ber. The *C. vesuviana* is a monophagous insect of ber and their severe infestation may cause 80%–90% damage to all



Figure 12.5 *Citrus fruit damaged by scale insect.*

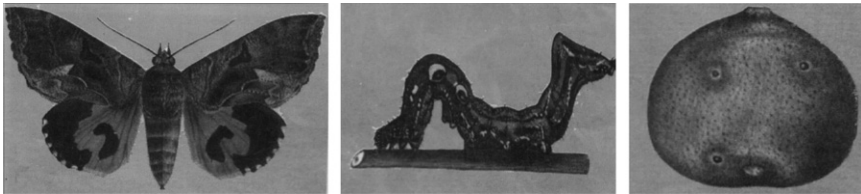


Figure 12.6 *Adult, caterpillar of fruit sucking moth, and damaged fruit of citrus.*

Ziziphus spp. and distributed all over the world (Vadivelu, 2014). *C. vesuviana* infest the developing fruit by egg laying and maggot feed on the juicy part of fruit, resulting in fruit becoming deformed and unfit for human consumption.

3.4 Litchi

The litchi fruit borer (Fig. 12.7) is one of the important insects of litchi that deteriorate the quality, as well as the shelf life of fruit. The caterpillar bore into the fruit and feed on pulp of litchi resulting in deformation in fruit size, which is the first sign of infestation. This insect is well distributed in all litchi growing areas of the world.

The litchi nut borer is polyphagous in nature and infest the litchi at the ripening stage, which causes deterioration in quality.

Litchi mite is a common pest in all litchi growing areas and their infestation started from leaves to inflorescence and young growing fruit. After infestation the peel of fruit becomes hard and deformed in shape.

3.5 Pomegranate

Pomegranate fruit borer *Virocola isocrates* (Fig. 12.8) is distributed all over India and Asia. It is the most abundant, polyphagous, and destructive pest of



Figure 12.7 Adult, caterpillar of litchi fruit borer, and damaged fruit.



Figure 12.8 Damaged fruit and caterpillar of pomegranate fruit borer.

pomegranate. The adult female lays eggs on buds, flowers, and young fruit; after emergence, the caterpillar bores into the fruit and feeds on the pulp resulting in deterioration of quality and yield, which will decline. The occurrence of this pest remains throughout year.

Pomegranate thrips, *Scirtothrips dorsalis* and *Rhipiphorothrips cruentatus* (Fig. 12.9), deteriorate the quality of fruit by sucking cell sap from the peel of fruit because they always prefer feeding on the new growth of plants.

3.6 Banana

The banana scaring beetle *Colapsis* sp. infests banana fruit since formation of inflorescence and deteriorates the quality of fruit after harvesting. The banana rust thrips *Chaetanaphothrips signipennis* is widely found in the banana-growing area of the world, however, very slight infestation has been observed in South East Asia (Shamsudin and Suphrangkasen, 1990). The fruit fly infests banana crops at maturity stage in South East Asia; the oriental

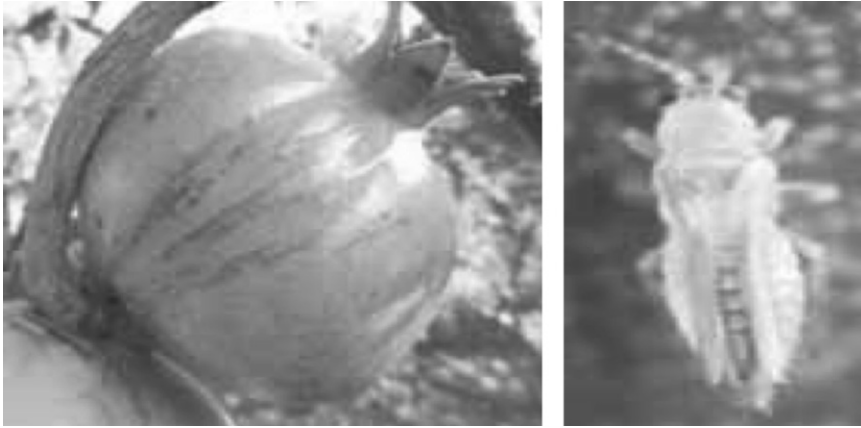


Figure 12.9 Damaged fruit and adult of pomegranate thrips.

fruit fly *Dacus dorsalis* usually infests ripening fruit, while in Queensland the banana fruit fly *Strumeta musae* and *Strumeta tryoni* infest bananas at the greening stage (Petersen and Pineze, 1982). The banana scab moth *Nacoleia octasema* infests at young emerging fruit stage, it is important in some parts of the world, particularly in Queensland and it's very difficult to manage (Stover and Simmonds, 1987). The banana moth *Opogena sacchari* produce similar symptoms in Brazil (Robinson, 1996). Several insect pests of bananas infest only their peels and cause decaying during storage (Shamsudin and Suphrangkasen, 1990).

3.7 Papaya

The papaya fruit fly *Toxotrypana curvicauda*, webworm *Homotapaipa daiera*, whitefly *Trialeuroides variabilis* and leaf hopper *Empoasca papaya* are infesting bananas before maturity and reduce the shelf life, as well as the quality of fruit (Marte, 1986). The fruit fly infests papaya at the ripening stage, resulting in rotting of fruit. Yee et al. (1970) reported oriental fruit fly *D. dorsalis* H, melon fruit fly *Dacus cucurbitae* C, and Mediterranean fruit fly *C. capitata* are common pests of papaya and their infestation occurs when fruit are near to maturity.

3.8 Guava

The quality and shelf life of harvested guava adversely affected by the fruit fly and their several species are reported from all over the world. The most commonly found species is *D. dorsalis* (Lam and Khoo, 1990) while *Dacus*

zonatus reported from India (Rana et al., 1992), Caribbean fruit fly *Anastrepha* sp. (Coledonia-Hurtado et al., 1995) and Oriental fruit fly *B. dorsalis* (Stark et al., 1994). All fruit flies infest the guava at ripening stage and after infestation many fungi and bacteria develop on it, which causes further deterioration of fruit.

Mealy bug sucking the sap from fruit of guava at ripening stage in tropical regions, resulting in deterioration of quality and deformation of fruit and such types of fruit become susceptible to secondary infection of several microorganisms (Mania, 1995).

3.9 Custard Apple

Custard apple infested by *Planococcus citri*, *Psuedococcus fragilis*, *Psuedococcus longispinus*, *Aphis gossypii*, *Heliothrips haemorrhoidalis*, *Oligonychus yothersi*, and *Tetranychus urticae*, and sometimes Mediterranean fruit fly *C. capitata*, cause damage at the ripening stage of fruit, resulting in deterioration in quality and shelf life.

3.10 Pineapple

The infestation of fruit flies *C. capitata*, *D. cucurbitae*, and *Dacus dorsalis* are very common in pineapple at maturity stage and they are responsible for the decay and deterioration of fruit quality (Armstrong et al., 1979; Seo et al., 1970). The exotic pineapple caterpillar *Thecla basilides*, *Metanasius ritchieri*, *Batrachedra methesoni*, *Paradiophorus crenatus* are causing infestation in some part of the world (Rohrbach, 1983). Generally females lay eggs on flowers and larvae feed on the developing fruit, when harvested fruit is stored the emergence of adult insects are started.

The pineapple scales *Diaspis bromeliea* infests the pineapple at harvest stage and reduces the physical appearance of fruit, as well as quality. The universally occurring pineapple mite *Steneotarsonemus ananas* infest the inflorescence and fruit resulting in quality deterioration (Rohrbach and Schmitt, 1994).

3.11 Grape

The grape is an important fruit in several parts of the world and the grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae) is a key insect pest of grapes; this insect sometimes affects the grapevine industries (Ioriatti et al., 2008). Grapefruit also is infested by the grape berry moth *Eupoecilia ambigulla* (Lepidoptera: Tortricidae), Thrips *Drepanothrips reuteri*, and *Frankliniella occidentalis* (Thysanoptera: Thripidae) and so many homopterans,

coleopterans, and hymenoperan insects (Brunelli et al., 1993; Lucchi, 1997). The grapevine moth *L. botrana* and grape berry moth *E. ambigua* are polyphagous and multivoltine insects of grape and generally completed two to three generations on grape (Ioriatti et al., 2008), their infestation started from flowering stage to ripening. Mazzoni et al. (2003) reported the infestation of leaf hopper *Jacobiasca lybica* (Homoptera: Cicadellidae) at ripening stage of grapes in Italy. The infestation of grape *Phylloxera daktulo* has been excellently controlled since the 20th century by grafting of European vines onto American rootstock.

3.12 Sapota

The sapota bud borer *Anarsia achrasella*, sapota seed borer *Trymalitis margarias*, and sapota fruit fly *B. dorsalis* deteriorate quality and shelf life of fruit. Their severe infestation becomes not fit for human consumptions.

3.13 Temperate fruit

3.13.1 Apple

The preharvest infestation of codling moth *Cydia pomonella* (Fig. 12.10) deteriorates the quality, as well as the shelf life of apples, and their hidden infestation becomes a notorious quarantine insect. Codling moth adopted well in all apple growing of the world. The complex infestation at maturity stage of the apple codling moth damaged pulp and seed, which is not fit for human consumption (Jones et al., 2008).

Apple maggots became a serious menace to fruit growers in some parts of the world and are regulated as quarantine insects. Apple maggots cause damage to fruit by egg-laying and voraciously feeding on the pulp. Their hidden infestation is very difficult to manage. In the case of severe infestation they completely destroy the fruit of apple.

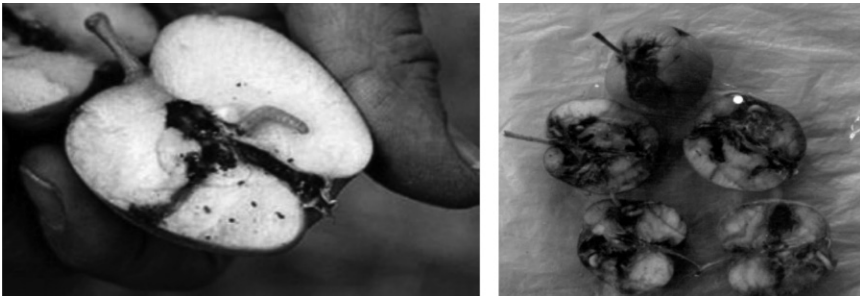


Figure 12.10 Fruit damaged by apple codling moth.

Woolly apple aphid *Eriosoma lanigerum* caused serious losses to apples by sucking the fruit sap and secreting honeydew, which attracts infection of several microorganisms. Their infestation started from the flowering time and continued till harvesting. The damaged fruit and presence of aphids on fruit are rejected in international trade. The apple mealy bug causes economic losses in same way as aphids, resulting in the deterioration of fruit quality.

The severe infestation of San Jose scale *Quadraspidiosus perniciosus* replaces the original color of fruit. San Jose scale is also capable of infesting the stem and calyx in initial stage.

3.13.2 Peach

The peach fruit fly is a notorious insect of peaches and its infestation started from the developing stage of fruit to the harvesting of fruit. It deteriorates the quality, as well as storage life of peach.

3.13.3 Walnut and Almond

The walnut weevil infests walnuts and almonds at the time of maturity and continues after harvesting. Their infestation deteriorates the quality of fruit in international trade. The codling moth, *C. pomonella* (Fig. 12.11) is the most economically important pest of walnuts, and an estimated 60% of the commercial crop in California and is susceptible to attack (ipmcenters.org, www.ipm.ucdavis.edu/PDF/PMG/pmgwalnut.pdf). This insect overwinters as a pupa in a silken cocoon and adults emerge in early spring. Females lay eggs in the spring, which then hatch and the white- to pink-colored larvae bore through the blossom end of the nut and cause damaged nuts to fall to the ground (ipmcenters.org, www.ipmcenters.org/CropProfiles/docs/cawalnuts.pdf), their eggs also infest the fruit by remaining inside at maturity stage and deteriorate the quality of fruit.



Figure 12.11 Walnut damaged fruit by codling moth.

The almond moth *Cadra cautella* infests the fruit at maturity stage and come to storage. This insect causes serious economic, as well as quality loss to fruit under storage condition. The almond moth is most abundant in temperate zones (Khare, 2006).

3.13.4 Plantation Crops

3.13.4.1 Areca Nut

Arecanut plants grow in tropical Asia Pacific region and Africa and are affected by so many insects, but spindle bug *Carvalhoia* sp., inflorescence caterpillar: *Tirathaba mundella* (Fig. 12.12) and mites deteriorate the quality, as well as shelf life of fruit. During the storage arecanut infested by the arecanut beetle: *Caccotrypes carpophagus* H. (Coleoptera: Scolytidae), coffee bean weevil: *Araecerus fasciculatus* D. (Coleoptera: Anthribidae), cigarette beetle: *Lasioderma serricornis* (F) (Coleoptera: Anobiidae) and rice moth: *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), which causes serious economic loss to fruit and also deteriorate the quality of fruit (Kumar, 2016).

3.13.4.2 Cashew Nut

The cashewnut *Anacardium occidentale* L.; Family: Anacardiaceae is a tropical evergreen plantation crop in India. The cashewnut is native to Central and South America. The tree is very attractive; it produces beautiful rose-colored scented flowers in panicles, followed by the enticing red fruit. It can grow in dry tropical conditions especially in coastal regions. The cashewnut plant is infested by several insects at different crop stages in all growing areas (Devasahayam and Nayar, 1986; Eguagie, 1972; Malipatil and Houston, 1990; Pillai et al., 1976; Xianli and Geest, 1990). The tea mosquito bug *Helopeltis antonii* infest the young growing fruit and continuing till their maturity results in deformation of fruit and the deterioration of fruit quality



Figure 12.12 Arecanut damaged fruit by caterpillar.



Figure 12.13 Cashewnut damaged fruit and inflorescence by nut borer.

(Maruthadurai et al., 2012). The apple and nut borer *Thylocoptila panrosema* (Fig. 12.13) causes serious economical losses to cashew crops by feeding on nuts and shapes the decline of yield, caterpillar bore into nuts, resulting in unfit for human consumptions. The nymph and adult of cashew thrips *Rhynchothrips raoensis* directly feed on cashew apples and fruit, resulting in quality and shelf life declining sharply. Mealy bug *Ferissia virgata* infest in the inflorescence and fruit of cashews and deteriorate quality of fruit. The storage life of cashewnuts infested by the rice weevil *Sitophilus oryzae*, red rest flour beetle *Tribolium castaneum*, coffee bean weevil: *A. fasciculatus*, and rice moth *C. cephalonica* (Kumar, 2016).

3.13.4.3 Coconut

The coconut is an important plantation crop in India and their production ranked as third in total coconut world production and it provides fruit, fiber, fuel, and timber. Coconuts are infested by numerous insects from seedling stage to harvesting and storage of fruit. Among the insects coconut plants and fruit are infested by rhinoceros beetle *Oryctes rhinoceros* (Fig. 12.14), red palm weevil *Rhynchophorus ferruginus*, black headed caterpillar *Opisina*



Figure 12.14 Coconut damaged fruit and inflorescence by rhinoceros beetle.

arenosella, mealy bug *Pseudococcus cocotis*, scale insect *Aspidiotus destructor*, and nut borer *Cyclodes omma*, which cause deterioration in quality of fruit (FAO, 2013). The rhinoceros beetle, black headed caterpillar feed on the inflorescence of plants and developing young fruit, resulting in the deformation in fruit and yield deduction. The nut borer feeds on nut by boring into them and also attracting infection of microorganisms; affected fruit are not fit for human consumption. The mealy bug and scale insects suck the sap from flower developing nuts and leaves of coconuts, resulting in the appearance of notch and spot and affect the physical appearance, as well as the quality of fruit.

3.13.4.4 Coffee

The coffee plant *Coffea* spp. are infested by several insects and mites and all part of the plants are susceptible to insect infestation, and symptoms of damage may appear from the seed bed, nursery, plantation, and in storage. Some insects affect the coffee plant seasonally while others spend several generations on it; in most of the cases the yield declines sharply while coffee berry quality is affected by coffee bean borers. The coffee plant is infested by more than 850 species of insects that feed on coffee in the world, among them 200 (23.5%) have been reported in the tropical and subtropical areas in America. The economic losses varies from country to country and species of plants

The coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) (Fig. 12.15), coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), and the root mealybugs (Pseudococcidae) are causing serious problems in all coffee-growing countries. Mealy bugs, green scale, and thrips directly deteriorate the quality of fruit by sucking the sap from developing berry, flower, and leaves (Luis et al., 2016). Coffee berry borer is a notorious insect because it affects the product to



Figure 12.15 Coffee berry damaged by coffee berry borer.

be harvested, the coffee berry. They infest green, ripe, and dry fruit or berries and usually show a hole on its apical portion. The hole is located at the center or ring of the berry and filled by excreta or emission of berry dusts. Its attack reduces the yield and adversely affects the berry quality. Their infestation also attracts the infection of so many microorganisms. The coffee borer may cause berry yield losses of 30%–35% with up to 100% perforated berries at harvest time in all over the world (Cardenas and Posada, 2001). During storage, the coffee berry is infested by rice weevil *S. oryzae*, lesser grain borer *R. dominica*, red rest flour beetle *T. castaneum*, coffee bean weevil: *A. fasciculatus*, and rice moth *C. cephalonica* (Kumar, 2016).

3.13.4.5 Cocoa

The cocoa *Theobroma cacao* plant is infested by several insects like cocoa pod borer *Conopomorpha cramerella*, stem borer *Zeuzera coffee*, mealy bug *Planococcus lilacinus*, cocoa aphid *Toxoptera aurantii*, and cow bug *Gargara* sp. (Konam et al., 2008). The cocoa pod borer infests cocoa fruit at the developing stage (Fig. 12.16), females lay eggs on the surface of fruit and caterpillar bore into the pod, due to their feeding habit pods are fully destroyed and sometimes attract the infection of fungi and bacteria, resulting in the deterioration of fruit. Cocoa mealy bug, aphid, and cow bug deteriorate the quality of fruit by sucking sap from fruit, flowers, and leaves, resulting in the deformation and reduction of yield. After harvesting and during the storage cocoa pod infested by rice weevil *S. oryzae*, lesser grain borer *R. dominica*, red rest flour beetle *T. castaneum*, coffee bean weevil: *A. fasciculatus*, and rice moth *C. cephalonica* (Kumar, 2016).

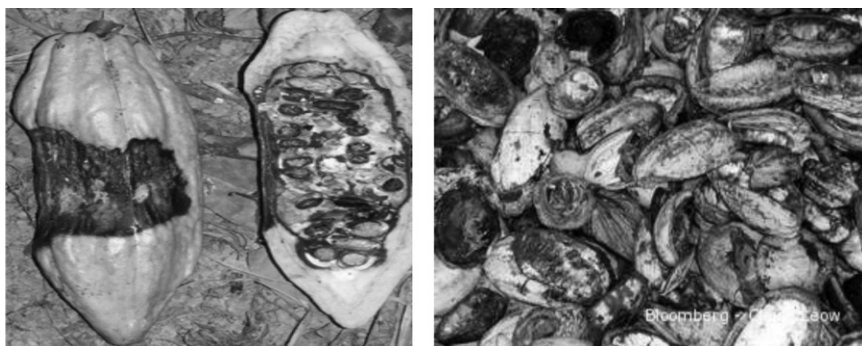


Figure 12.16 Cocoa fruit damaged by cocoa pod borer.

4 PREHARVEST APPROACHES

The preharvest management of insect infestation to maintain quality and shelf life of produce is very crucial activity in fruit ecosystems. There is little evidence in current literature that preharvest management may influence the postharvest fruit quality of temperate, tropical, and subtropical fruit. All preharvest factors are helpful to the success of handling temperate, tropical, and subtropical fruit because typically long transit times to market are required and fruit is subject to insect disinfestation treatments. Understanding the effects of the preharvest management on growth and maturation and susceptibility to insect infestation will help toxic-free and better quality of postharvest fruit. The preharvest management also helps in development of immunity in plants to escape insect infestation. Efforts of preharvest management on postharvest quality brings farmers into actively managing the quality of their product. The preharvest management component includes temperature, relative humidity, atmosphere modification, cooling, and judicious and recommended use of insecticides and wax coatings following harvest. These critical inputs are important for fruit given their long transit conditions and the potential need for postharvest insect disinfestation quarantine treatments. The preharvest management may also have a physical impact on fruit quality because they reduce the insect dispersal and infestation (Sarwar, 2013; Sarwar et al., 2014).

Several preharvest approaches will illustrate for the improvement of fruit quality and reduction in postharvest protection cost.

5 ORCHARD SANITATION

To eradicate survival stages of different insect pest field sanitation is one of the important preharvest practice under horticultural ecosystem. This technique is very effective for management of the fruit fly. In orchards weed leaves or decay fruit are a major source of insect development and survival, if we destroy these things we can reduce the subsequent insect infestation of healthy fruit. The bagging or deep burying of infested fruit or emerging adults of the fruit fly prevents the postharvest losses. However, mechanical field sanitation is recommended for large orchards. Klungness et al. (2005) reported the application of new device augmentoria, made up from screen materials that inhibit the dispersal of fruit flies and emergence of adults from fruit is placed under this tent like structure augmentoria. Field sanitation may also be achieved by ploughing sites or shredding or chopping, burning residues. In India sanitation practice helped to reduce the incidence of hopper infestation under orchards.

6 SOIL MANURING AND FERTILIZATION

The proper application of manures and fertilizer in orchards helps in strengthening of fruit plants and to resist insect infestation. [Rwomushana \(2008\)](#) reported that in well-managed orchards the population of the fruit fly is lower as compared to nutritionally deficient farms. The adoption of better soil health orchards is prerequisite for production of export quality fruit to reduce infestation of the fruit fly in African countries ([Badil et al., 2015](#)). The amount and timing of application of nitrogen and potassium is directly responsible for the quality of fruit and resistancy against insects, while calcium-based fertilizers are required during early fruit growth ([Burdon et al., 1991](#)).

7 SELECTION OF PLANTING MATERIALS

The planting materials must be free from infestation of insects. [Eaks and Jones \(1959\)](#) reported that the incidence of fruit borers in mango and citrus, fruit flies in oranges, started from planting materials.

8 PRUNING AND TRAINING

The well-known practices of pruning and thinning in orchards should be done at regular intervals to avoid old, damaged, and infested portions of plants. This practice is helpful in the destruction of any survival stage of insects. The quality of fruit in terms of their appearance depends on the skill of pruning. Early pruning just after harvesting helps to synchronize shoot growth and uniformity in flowering ([Fivas and Grove, 1998](#)). Farmers must be aware of the thinning of fruit because it is directly correlated with size and quality of fruit ([Yeshitela et al., 2003](#)).

9 HAND-PICKING OF INSECTS

Hand-picking and destruction of egg, larvae, pupa, and adult stage of usually large-sized insects at regular intervals may reduce population development of insects and the repetitive application of insecticides.

10 TREE BANDING

The banding of mango trees with sticky materials to prevent infestation of mango mealy bug and other insects are found to be highly effective: a band of some sticky materials directly painted on the stem or waxed paper strips

wound round the stem. Even two metallic cones with their mouths in opposite directions and inside painted with sticky substance could be fitted around the stem to check both ascending and descending insect movement.

11 HARVESTING TIME

To avoid infestation of fruit fly is possible by harvesting fruit at the maturity stage when fruit are not favorable for the fruit fly. Some species of fruit fly attract fruit at the ripening stage, but *B. invadens* and *C. cosyra* are still infesting green mango (Ekesi and Billah, 2006). However, early harvesting of fruit to evade infestation of the fruit fly is important to preharvest practices.

12 BAGGING OF FRUIT

Preharvest bagging of fruit reduces the infestation of the fruit fly. The bagged fruit will ripen uniformly without any infestation and this method also increases the quality, as well as shelf life of harvested fruit (Maqbool and Muhammad, 2007).

13 TRAPPING

The first known record of attraction of some adult insects to artificial light was observed and recorded by the Greek Poet Aeschylus (525–456 BC). In earlier days bonfires were used for attracting and killing insects under orchard conditions. This was followed by kerosene burning lamps and lanterns as sources of attractants. In a number of cases, these lamps or lanterns were used inside a room or other sheltered location where the moths were attracted. Recently black light trap is a popular name for UV radiant energy with the range of wavelengths from 320 to 380 nm. Incandescent light traps are used for trapping cutworm leafhoppers and biting midges in the field. Mercury light traps attract insects belonging to the family pyralidae, sphingidae, noctuidae.

Use of light traps

1. Light traps are useful in studying the seasonal incidence of insect pests and evolving forecasting models.
2. Light traps can be employed to survey the occurrence, distribution, abundance, and flight pattern of various insect pests in a given locality.
3. Light traps give an indication about brood emergence of insect pests for taking up appropriate control measures.
4. From light trap catches migration of certain insect species can be worked out.

14 SPRAYING OF FRUIT FLY BAIT

Preharvest spraying of fruit fly bait is helpful in the reduction of pest population and the production of toxic-free fruit. Organophosphate insecticides have been used to control fruit flies for the last 50 years but today they have been ineffective against this pest (Kesiser, 1968). The combination of pesticides and food bait has been found effective after spraying on nearby vegetation of orchards (Nishida et al., 1957). The female of the fruit fly needs protein for ovarian development so protein bait spray drastically reduces the female population under fruit ecosystem and such spraying may be used for a fruit fly eradication program (Roessler, 1989). Several commercial formulations of protein bait, for example, NU-lure, F-lure, Bactro lure, are available worldwide and compatible with traditional insecticide (Prokopy et al., 1992). The new protein bait formulation along with Spinosad has been found effective against the fruit fly in USA (McQuate et al., 1999). The protein bait spraying does not affect the quality of the fruit, as well as the environment and natural enemies. Sometimes the protein attractant Solulyls bait, (GF-120 fruit fly Dow Agro Science) may used for preharvest control of fruit fly. GF-120 fruit fly bait act as a substitute of normal poison bait and traditional insecticides (McQuate et al., 1999; Vargas et al., 2007).

15 MALE ANNIHILATION TECHNIQUE (MAT)

Both the notorious genus of fruit fly *Bactrocera* and *Dacus* are more attracted to methyl eugenol and Cue-lure (Hardy, 1979; White and Elson-Harris, 1992). The male annihilation technique (Koyama et al., 1984; Steiner et al., 1970; Vargas et al., 2000) is economical, ecofriendly, and socially accepted, having excellent potential to inhibit the male of fruit fly. This technique is well adopted worldwide and popularized in India also for the preharvest management of the fruit fly in horticultural ecosystem.

16 STERILE INSECT TECHNIQUE (SIT)

Several reports indicate the sterile insect technique is very effective for preharvest management of fruit flies all over the world (Cunningham et al., 1980; Koyama, 1996; Steiner et al., 1970). However, the application of both forms (male and female) are also effective practice (Vargas, 1996). The excellent benefit of this technique not only avoidance of damage by females but also avoidance of mating between sterile males and sterile females. The impact of eliminating sterile female transform into increased effects of

sterile insect techniques by enhancing mating between sterile males and wild females (McInnis et al., 1994; Rendon et al., 2004).

A new strain of melon fruit fly was developed and tested on the basis of color separation of males and females at pupal stage with the help of high-speed sorting machines (McInnis et al., 2006). In this way males were released in a orchard mated with wild females of melon fruit flies, resulting in sharp degradation of normal population (McInnis et al., 2007).

17 BIOLOGICAL CONTROL

The utilization of predator, parasite, parasitoids, and pathogens to manage insect population is one of important preharvest management approaches. The orchard ecosystem is more stable than field crop ecosystem, so the success of biological control agents (Fig. 12.17) (Table 12.2) is higher as compared to other stable systems. The conservation of natural enemies in orchard systems may significantly contribute to the management of all stages of fruit flies (Stibick, 2004). The avoidance of blanket spraying of insecticides and spot treatment will maintain abundant populations of biological control agents; these tactics are economical, stable, and safe to nontargeted organisms (Ekesi and Billah, 2006; Vayssières et al., 2009).

The successful preharvest controls of the fruit fly with the help of parasitoids reduce the cost of protection and provide toxic-free better quality of fruit. The parasitoids *Fopius arisanus* and *Braconid* species effectively suppress the population of the fruit fly below the economic threshold level (Prokopy et al., 2003; Stark et al., 2004; Vargas et al., 2001). The host attacking behavior of *F. arisanus* parasitized 50% population of the fruit fly under orchards of mango (Purcell and Messing, 1996; Vargas et al., 2007). In Hawaii biological control of fruit flies with the help of *F. arisanus* reached up to 95% (Rousse et al., 2005) and these parasitoids are abundant in the horticultural ecosystem. The release of another parasitoid *Diachasmimorpha tryoni* at 20,000 per week was very effective in Hawaii (Wong et al., 1991) and *Psylattia fletcheri* significantly controlled the melon fruit fly (Purcell and Messing, 1996). One finding of Vargas et al. (2004) indicates the *P. fletcheri* suppress the 21-fold population of fruit flies and their own numbers increased 11 times.

In controlling of fruit flies, the presence of biological control agents, such as *Oecophylla longinoda* (red ants), *F. arisanus* (parasitoid wasps), and *Metarhizium* sp. (fungus) reduce infestation by predation of adult fruit flies, predation of third-stage larvae, destruction of pupa in the soil, and the repulsive effect of “pheromones” left by the ants on fruit so that flies are

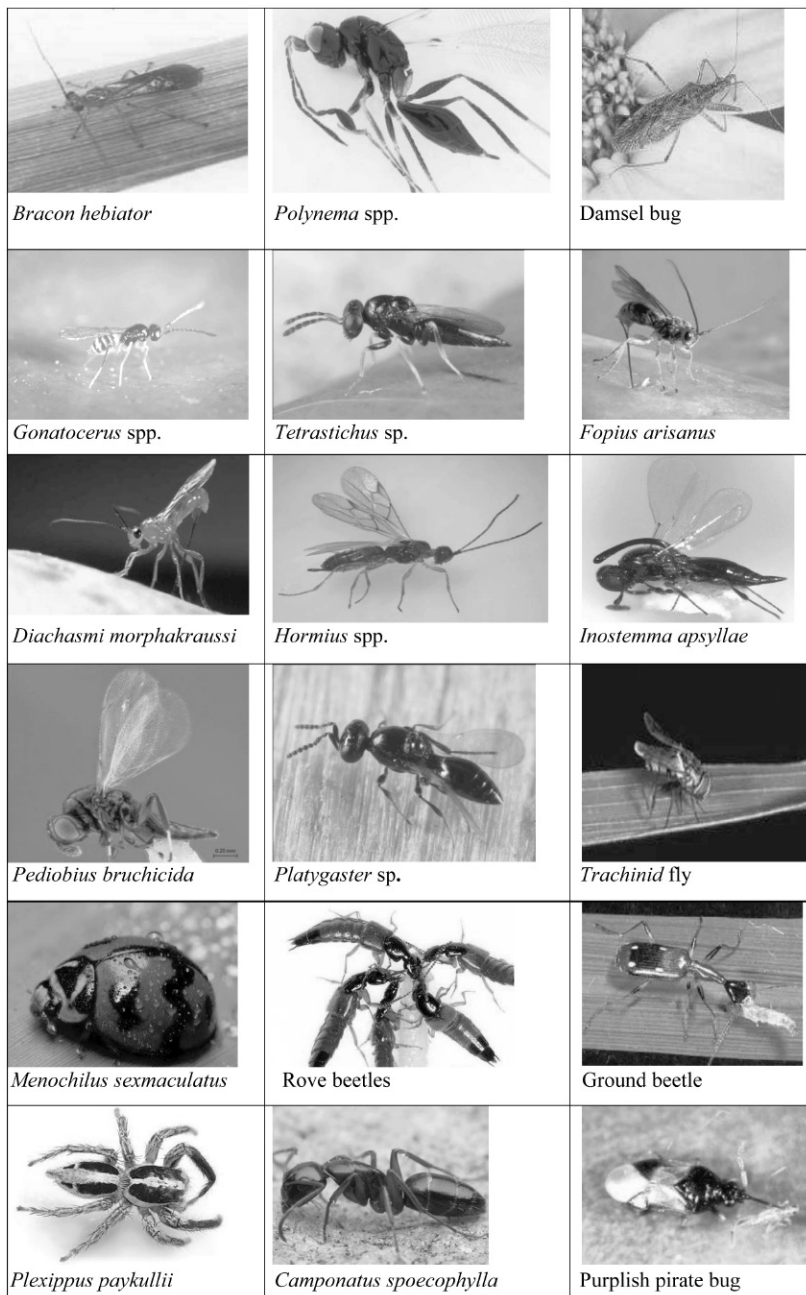


Figure 12.17 Commonly abundant biological control agent in fruit orchards.

Table 12.2 Natural enemies of some fruit pests

Name of insect	Natural enemies
Apple	
San Jose scale	<i>Encarsia perniciosi</i> ; <i>Aphytis diaspidis</i>
Plum scale	<i>Coccophagus ishii</i> ; <i>Blastothrix sericea</i>
Woolly aphid	<i>Aphelinus mali</i> ; <i>Coccinella septempunctata</i>
Aphid	<i>Episyrphus balteatus</i> ; <i>Sphaerophoria bengalensis</i>
Codling moth	<i>Trichogramma embryophagum</i> ; <i>Trichogramma cacaeciae</i>
Apple moth	<i>Myrmica</i> sp.
Citrus	
Leaf miner	<i>Cirrospilus quadristriatus</i> ; <i>Citrostichus phyllocnistoides</i> ; <i>Sympiesis purpureus</i> ; <i>Ageniaspis</i> sp.
Butterfly	<i>Melalophacharops</i> sp.; <i>Distatrix papilionis</i> ; <i>Trichogramma chilonis</i> ; <i>Holcojoppa coelopyga</i>
Psylla	<i>Tamarixia radiata</i>
Red scale	<i>Diaphorencyrtus aligharensis</i>
Whitefly	<i>Comperiella bifasciata</i> ; <i>Aphytis melinus</i> ; <i>Chilocorus nigrita</i> ; <i>Pharoscymnus horni</i>
Mealy bug	<i>Encarsia lahorensis</i> ; <i>Aschersonia aleyrodis</i>
Cottony cushion scale	<i>Coccidoxenoides peregrines</i> ; <i>Plesiochrysa lacciperdata</i> ; <i>Cryptolaemus montrouzieri</i> ; <i>Leptomastix dactylopii</i>
	<i>Rodolia cardinalis</i>
Mango	
Hopper	<i>Verticillium lecanii</i>
Scale insects	<i>Pteroptrix koebelei</i> ; <i>Encarsia citrine</i>
Mealy bug	<i>Coccidoxenoides peregrines</i> ; <i>Plesiochrysa lacciperdata</i> ; <i>Cryptolaemus montrouzieri</i> ; <i>Leptomastix dactylopii</i>
Fruit fly	<i>Terastichus</i> sp.; <i>Fopius longicaudatus</i> ; <i>Fopius vandendoschi</i> ; <i>Fopius ariasanus</i> ; <i>D. longicaudata</i>
Grape	
Mealy bug	<i>Anagyrus dactylopii</i> ; <i>Scymnus coccivora</i> ; <i>Allotropia japonica</i> ; <i>Mallada boninensis</i>
Guava	
Aphid	<i>Coccinella</i> sp.
Fruit fly	<i>Terastichus</i> sp.; <i>Fopius longicaudatus</i> ; <i>Fopius vandendoschi</i> ; <i>Fopius ariasanus</i> ; <i>D. longicaudata</i>
Mealy bug	<i>Cryptolaemus montrouzieri</i>
Ber	
Mealy bug	<i>C. montrouzieri</i>

Table 12.2 Natural enemies of some fruit pests (cont.)

Name of insect	Natural enemies
Pomegranate	
Butterfly	<i>Ooencyrtus papilionis</i> ; <i>Rahauri</i> sp.; <i>Telenomus cyrus</i> ; <i>Glyptapanteles vitripennis</i> ; <i>Trichogramma chilonis</i>
Whitefly	<i>Encarsia azimi</i>
Sapota	
Bud and fruit borer	<i>Xanthopimpla</i> sp.; <i>Cadurgia</i> sp.; <i>Calleida splendidula</i> ; <i>Parina nigrolineata</i>
Custard apple	
Mealy bug	<i>Spalgis epeus</i> ; <i>Cryptolaemus montrouzieri</i> ; <i>Scymnus coccivora</i>
Coconut	
Caterpillar	<i>Apanteles taragamae</i> ; <i>Bracon hebetor</i> ; <i>Goniozus nephantidis</i> ; <i>Elasmus nephantidis</i> ; <i>Brachymeria nosatoi</i> ; <i>Xanthopimpla punctata</i>
Rhinoceros beetle	<i>Santalus parallelus</i> ; <i>Scarites</i> sp.; <i>Harpalus</i> sp.; <i>Pheropsophus</i> sp.
Arecanut	
Mite	<i>Stethorus</i> sp.
Palm mite	<i>Stethorus keralicus</i> ; <i>Amblyseius channabasavanni</i>
Cashew nut	
Stem and shoot borer	<i>Metarrhizium anisopliae</i>
Mosquito bug	<i>Telenomus</i> sp.; <i>Erythmelus helopeltidis</i> ; <i>Leiophron helopeltidis</i> ; <i>Endochus inornatus</i> ; <i>Sycanus collaris</i>
Leaf miner	<i>Sympiesis</i> sp.
Leaf and blossom webber	<i>Apanteles</i> sp.
Coffee	
Stem borer	<i>Metapelma</i> sp.; <i>Allorhogas pallidiceps</i>
Berry borer	<i>Cephalonomia stephanoderis</i> ; <i>Prorops nasuta</i>
Shoot hole borer	<i>Tetrastichus xylebororum</i> ; <i>Pyemotes herfsii</i> ; <i>Callimerus</i> sp.
Green scale	<i>Coccophagus bogoriensis</i>
Mealy bug	<i>Coccidoxenoides peregrines</i> ; <i>Tetracnemoidea indica</i> ; <i>Blepyrus insularis</i>

discouraged from laying eggs in them. The soil treatment with *Metarrhizium anisopliae* mixed with sand or neem powder has been found highly effective to control the pupae of fruit flies in African countries (Ekesi and Billah, 2006; Ouna, 2010).

Soil inoculation of fungal pathogens creates a hostile environment for adult fruit flies or their larval and pupae developmental stages. However, it is nontoxic to beneficial parasitoids and because it can persist in the soil for more than a year, it is applied only once in a season (Ekesi and Billah, 2006).

The utilization of predators to manage fruit fly population has been reported from Benin. Reports revealed that *O. longinoda* significantly reduced the damage of the fruit fly. Although predation on adults of fruit flies took place, deterrence and disturbance by ants during oviposition seemed to be the most important causes of reducing fruit fly damage. The birds and rats also feed on caterpillars of fruit flies and the stem borer, resulting in limited infestation of fruit flies (Drew et al., 2005). The promotion of biointensive integrated programs to supply toxic-free grapes is needed in the current era because several times consignment was rejected due to the high residue of traditional insecticides. The application of *Bacillus thuringiensis* and mating disruption techniques are found exclusively effective for the management of insect pests of grapes and enhance the quality of grapes after harvesting (Ioriatti et al., 2005).

The microbial agent, for example, bacteria, protozoa, nematode, and fungi, also manages the insect population under horticultural ecosystems. The nematode *Neoplectana* sp. successfully controls larvae of *Anastrepha* sp. Bacteria *Bacillus thuringiensis* and protozoa *Nosema tephritidae* also control the fruit fly infestation in mango orchards in Hawaii (Badil et al., 2015).

18 QUARANTINE TREATMENT

Quarantine is a legislative (regulatory) attempt to exclude pests from invading into the areas where they do not exist by monitoring the import and export of plant, seed/planting material to prevent the spread of disease and pests. Insect pests may move along with the infected plants, seed, fruit, planting materials, or equipment.

Plant quarantine may, therefore, be defined as “Rules and regulations promulgated by government or governments to regulate the introduction of plants, planting materials, plant products, soil, living organisms, and so on, from one region to another with a view to prevent inadvertent introduction of exotic pests, weeds, and pathogens harmful to the agriculture, horticulture, or the environment of a country/region, and if introduced, to prevent their establishment and further spread” without adversely affecting the trade. Quarantine aims to prevent entry of dangerous pathogens, but not by completely blocking the movement of biological material.

Plant quarantine measures are of particular importance and relevant to countries like India, whose economy is largely based on agriculture. Quarantine not only helps to ward off the threats of exotic pests, but also aims to eliminate and prevent further spread of pests/pathogens (both indigenous and introduced) with restricted distribution within the country (domestic quarantine).

Government or governments through quarantine services offer beneficiaries, which are beyond the capabilities of individuals or that are difficult to obtain in some other way at a lesser cost. Thus, plant quarantine, in real sense, serves as a national service by monitoring and if required preventing the introduction of exotic pests/pathogens/weeds and their further spread. However, such endeavors could succeed only with the active support of all the administrators, general public, farmers, scientists, communication media, customs, and others.

Plant genetic stocks are a global resource meant for the welfare of humanity. Plant introductions could be in the form of new crops or new varieties for crop diversification, or germplasm in the form of primitive landraces or wild/weedy relatives of crop plants. Germplasm from the centers of origin and crop diversity may possess valuable genes for resistance against pests/pathogens, high yield, early maturity, cold, drought or salinity tolerance, and quality traits like increased oil, protein contents, etc.

Plant quarantine services are charged with the responsibility of preventing the entry of hazardous pests, pathogens, and weeds, but to deny entry to the valuable genetic resources would be against national interest. These activities are meant to help agricultural and horticultural development, and they are complementary to each other. Too much conservatism on the part of plant quarantine officials and too liberal an attitude on the part of plant introduction officials/breeders would be harmful. Plant quarantine officials must strive to provide adequate safeguards to allow the smooth flow of germplasm resources in a healthy state. They should also try to ensure that the germplasm, when received in quarantine station, is processed promptly and that the delays in release, if any, are purely due to biological considerations alone. At the same time, circumvention of plant quarantine must be avoided at all costs even if it means delaying the release or rejection of certain materials based on biological consideration. The two should work in unison as members of a single team. Together they should decide the type, quantity, and source of the material, and also the required quarantine safeguards. The plant quarantine officials should conduct research on developing sensitive and reliable methods of detection and salvaging treatments, or find alternatives to permit introduction of even high-risk genera, if the introduction of such materials is in the national interest.

18.1 International/Regional Cooperation

Plant quarantine, while being national in execution, is international in character. Therefore, international/regional cooperation is very necessary for achieving the objectives because plant genetic resources are a world

resource meant for the welfare of the human race as a whole. Cooperation on the following lines would greatly help in the safe exchange of germ-plasm materials and export or import of produce.

18.1.1 Biogeographical Regions

The eight biogeographical regions on the global basis are European and Mediterranean Plant Protection Organization (EPPO), Interafrican Phytosanitary Council (IAPSC) Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), Plant Protection Committee for the South-East Asia and Pacific Region (SEAPPC), Near East Plant Protection Commission (NEPPC), Comité Interamericano de Protección Agrícola (CIPA), Caribbean Plant Protection Commission (CPPC), North American Plant Protection Organization (NAPPO), Organismo Bolivariano de Sanidad Agropecuaria (OBSA), and ASEAN region grouping of Indonesia, Malaysia, Philippines, Thailand, and Singapore. These have been proposed for effective quarantine services within that geographic area and are separated by natural barriers like sea, high mountains, and deserts, making pest/pathogen dissemination extremely difficult so long as the exchange of genetic resources is judiciously regulated (Mathys, 1975). Accordingly, all countries in such a region must have common quarantine regulations because the larger the land mass covered by the same set of regulations, the greater is the protection afforded to the agriculture of the region. Based on this concept, countries, such as Indonesia, Malaysia, Philippines, Thailand, and Singapore of the ASEAN region have come to an official level understanding and have formulated a common set of quarantine regulations to protect the region against alien pests/pathogens. Understanding on the same lines among SAARC countries along with Afghanistan and Burma would be very useful in protecting the agriculture of the entire region. Such an arrangement will reduce duplication of efforts and operational costs.

18.1.2 Third-Country/Intermediate Quarantine

The concept of third-country quarantine is another example where international cooperation could play an important role in the safe transfer of plant genetic resources. This is particularly helpful for transferring high-risk tropical/subtropical plant genera from one country to another. The material could be grown, tested/indexed for hazardous plant pests and pathogens in a temperate country without much risk because either the possible hosts are not present or the environment is unfavorable for their establishment. Some centers that provide third-country quarantine facilities for transferring

genetic resources include the Sub-tropical Horticulture Research Station, Miami, USA for cocoa, coffee, rubber, and tea; University of Reading, UK for groundnut; and the Istituto Nazionale Per Piante de Legno, Torino, Italy for cassava.

Countries like Sri Lanka and Maldives or even some islands of India (Andaman group of islands) could even serve as third-country quarantines for temperate, high-risk genera. SAARC countries have already developed cooperative programs in many crops and quarantine could be incorporated in this agreement.

18.1.3 Consortium of Plant Quarantine Stations

This excellent concept, proposed by Kahn (1977), facilitates the exchange of genetic stocks and scientific information at international/regional level. Any material passing through a plant quarantine station will have very low pest/pathogen risk. The material so generated should be exchanged with other plant quarantine stations promptly, before it is distributed locally and gets contaminated with local pests/pathogens. Several quarantine stations working independently may be processing the same material (same crop or even same variety) at each station. Under the proposed consortium concept, different quarantine stations would undertake the processing of different materials (several accessions of the same crop or a group of crops at each station) and then share the material. This would avoid duplicate efforts, reduce costs of processing, and more material would be available with adequate quarantine safeguards. In the same spirit, scientific information (detection techniques, treatments, distribution of pests/pathogens) and antisera for serodiagnosis of viruses and bacteria could be shared by quarantine stations of different countries.

18.2 Pest Risk Analysis

Analysis of pest risk in plant introduction is essential to decide as to whether a particular planting material could be permitted entry or not. Such risk analysis provides sound biological basis to decide quarantine policies. The attitude toward “entry status” of a material may be liberal or conservative depending on the risks involved in its introduction. If risks are low, quarantine would be liberal in permitting the entry. However, if risks are very high, the material may be denied entry. Whether an introduced pest could establish, spread, and become serious, depends on three factors, namely, (1) availability of susceptible host in abundance; (2) ability of the introduced pest to multiply and spread rapidly; and (3) availability of favorable environmental

conditions. Agricultural practices and the pest management strategies in the country of introduction are also important. However, the host-pathogen-environment interactions are very complex and it is not always easy to understand them. As such, many times, our predictions about risks involved and quarantine importance of a pest may go wrong. Organisms of quarantine importance are the exotic pests/pathogens, which are considered to pose serious threats to agriculture and environment of a country or region, and include races and biotypes of indigenous pests and pathogens. Any *pest risk analysis* (PRA) should take into account the benefits that are likely to occur from the introduction of the planting material concerned and also the costs of quarantine inspection, treatment, including detention in the postentry quarantine facility and the cost of eradication, should an exotic pest get established. PRA addresses the injury or potential injury that a plant, animal, or pathogenic agent can cause in an area. Risk identification determines whether the organism in question qualifies as a quarantineable pest. Risk assessment establishes the probability of the pest being introduced to the country, for example, by wind, or via a vector species, and the chance that the pest will become established once introduced. Risk management aims to reduce the risk of introduction and establishment of pest species.

PRA should also consider factors, such as availability of trained personnel, efficacious detection techniques, treatments at the point of entry quarantine, knowledge about the life cycle of the pest, existence of races and strains, world distribution, modes of transmission, factors favoring establishment and spread of pests availability of safeguards (necessary manpower resources, chemicals, and equipment to contain and eradicate the pests), and adequacy of the survey and surveillance program. The action plan includes organizing PRA training, establishing working groups and holding a workshop attended by national and international experts to prioritize crops and commodities for PRA. Success or failure of plant quarantine measures would depend, to a great extent, on the ability of plant quarantine officials to detect pests and pathogens that may be associated with the introduced planting material. For quarantine purposes, techniques should be sensitive enough to detect even trace infestations or infections. This is particularly important in the case of pests/pathogens with very high multiplication rates like certain fungi, bacteria, viruses, and the insect.

A wide variety of pests and pathogens, such as insects, mites, nematodes, fungi, bacteria, viruses, viroids/ MLOs, spiroplasma, and weeds, are the objects for quarantine consideration. Similarly, planting material also may be introduced in a variety of forms, that is, true seed, corms, bulbs, rhizomes,

suckers, runners, budwood, scions, cuttings, and rooted plants. Therefore, detection techniques would vary depending on the type of material, the host species, and the type of pests/pathogens involved. Often, more than one technique would have to be used. Detection techniques may broadly be classified into two groups: (1) generalized tests that would reveal a wide range of pests/pathogens; and (2) specialized or specific tests that are used to detect specific pests/pathogens (Neergaard, 1977).

18.2.1 Generalized Tests

A widely used method is the inspection of dry seed with the naked eye or under the low power of a microscope. This method would reveal a wide range of free-moving insects, their eggs and larval stages, mites on or with the seed, weeds, soil, infected/infested plant debris, fungal fructifications like sclerotia, smut, and bunt balls, nematode galls, discolored or deformed seeds mixed with seed; oospore or bacterial crusts; acervuli, pycnidia, sclerotia, and even free spores of rusts, smuts, and many other fungi on the seed surface. Examination of dry seeds under UV or NUV light may reveal infections of certain fungi and bacteria through emission of fluorescence of different colors. Examination of seed washings may reveal surface contamination by rusts, smuts, downy mildews, and a large number of other fungi.

18.2.2 Specialized Tests

18.2.2.1 Insects

X-ray radiography has been used very successfully all over the world for the detection of hidden infestation (with no apparent sign of infestation on the seed surface) of insects, particularly seed infesting chalcids and bruchids. Seed transparency tests (boiling the seeds in lactophenol to make them transparent) may also be used for the detection of hidden infestation and the extraction of the insects for identification. X-ray radiography is also very effective in salvaging infested seed lots.

18.2.2.2 Nematodes

For the detection of seed-borne nematodes, seeds are soaked in water for about 24 h. This makes the nematodes active, which then come out of the seed into the water, or the seeds may be teased out with the help of forceps and a needle and examined for detection of nematodes under a stereo microscope. In rooted plants, the accompanying soil and plant debris may similarly be soaked in water and nematodes may be extracted for identification using nematological sieves or tissue paper.

18.2.2.3 Fungi, Bacteria, and Viruses

Serological tests are very effective for the detection and identification of viruses and bacterial pathogens and are being used in various plant quarantine stations with great success. Phage-plague technique is still more sensitive for bacterial pathogens as even strains of bacteria can be identified. Indicator test plants are also very helpful as they may reveal pathogenic races within a species of a fungus, bacterium, and specific strains within a virus.

18.2.3 Heat Treatment

Hot water treatment or hot air treatment are also used in quarantine for eradication of insects, mites, nematodes, fungi, bacteria, and viruses. The basic principle involved is that treatment temperature should be sufficiently high to kill the associated pest/pathogen but not the host. However, in most cases, the margin of safety is very narrow and, therefore, the temperature should be very accurately controlled. Some recommended hot water treatments (Kahn, 1977) are:

1. *Against nematodes*: Flower bulbs, 44°C for 240 min; chrysanthemum, 48°C for 25 min; potato tubers, 45°C for 5 min;
2. *Against insects and mites*: Narcissus bulbs, 44°C for 180 min; strawberry runners, 46°C for 10 min;
3. *Against viruses*: Grape vine, 45°C for 120–180 min; sugarcane sets, 50°C for 120 min.; potato tubers, 50°C for 17 min;
4. *Against fungi*: Celery seed, 50°C for 25 min; wheat seed, 52–54°C for 10 min.

18.2.4 International Quarantine

In 1951, the United Nations Food and Agriculture Organization (FAO) organized an International Plant Protection Convention (IPPC) with the aim of securing common and effective actions to prevent the introduction and spread of pests and diseases of plants and plant products, which is now regionally organized. Many countries established inspection stations at all ports. Today it is virtually impossible to ship nursery stock from one country to another or from one state (province) to another without the shipment being inspected and certified to be disease-free. A crucial process in quarantine regulation and the cooperation of countries in the IPPC is risk identification, assessment, and management.

18.2.5 Plant Quarantine Services in India

With a view to modernize, upgrade, standardize, and enhance the plant quarantine system, its capacities and the related legal and administrative framework, the government of India approved the notification of a new Plant Quarantine (Regulation of Import into India) Order, 2003 issued under DIP Act, 1914 incorporating the provisions of New Policy on Seed Development, 1988. Seed was not covered under the DIP Act until 1984, when the government of India brought forward a comprehensive Plants, Fruits and Seeds (Regulation of Import into India) Order, 1984. This new order is a step forward in harmonizing India's regulatory framework with the IPPC (1951) and internationally accepted standards and the tenets of the SPS Agreement of the World Trade Organization. Other supporting and managerial steps are also being taken to improve, to international standards, the entire gamut of the country's quarantine activity and phytosanitary border controls, including import and export inspections, on-field surveillance for pests and vectors, treatment standards and processes, and certification methodology.

India is making imports of plants and plant materials subject to PRA to protect its crops from risk of introduction of alien pests. Efforts are also under way to improve the export certification process and standards to ensure that such phytosanitary certification gives an assurance of freedom from quarantine and regulated pests and vectors, including alien species for importing countries to prevent the introduction and spread of exotic pests that are destructive to crops by regulating/restricting the import of plants/plant products and to facilitate safe global trade in agriculture by assisting the producers and exporters by providing a technically competent and reliable phytosanitary certificate system to meet the requirements of trading partners.

18.2.6 Major Activities

1. Inspection of imported agricultural commodities for preventing the introduction of exotic pests and diseases inimical to Indian fauna and flora
2. Inspection of agricultural commodities meant for export as per the requirements of importing countries under IPPC
3. Detection of exotic pests and diseases already introduced for containing/controlling them by adopting domestic quarantine regulations
4. Undertaking Postentry Quarantine Inspection in respect of identified planting materials
5. Conducting the PRA to finalize phytosanitary requirements for the import of plant/plant material

18.2.7 Available Plant Quarantine Facilities in India

1. 35 new plant quarantine stations are across the country at all major and minor ports.
2. Development of an integrated information management system.
3. An integrated PRA system and a national PRA unit for conducting integrated pest surveillance.
4. An integrated phytosanitary border control system.
5. A national phytosanitary database.
6. A national management center for phytosanitary certification to continuously review the national standards for export phytosanitary certification.
7. Establishment of advanced molecular diagnostic facilities at major plant quarantine stations for rapid pathogen detection.
8. Computerization and networking of all the plant quarantine stations.
9. Standardization of the export certification process so that uniform and credible certificates with a common format and seal are issued by all phytosanitary certification authorities, both in central and state governments, across the country.
10. Human resource development and skill upgrading or training programs for scientists, researchers, and others.
11. Obtaining ISO quality certification for major plant quarantine stations.
12. Production of guidelines for training of plant quarantine inspectors.
13. Production of guidelines for the development of new disinfection techniques and vapor heat treatment of fruit fly host commodities.
14. Development of fumigants as an alternative to the ozone-depleting methyl bromide.
15. Development of international standards for phytosanitary measures.
16. Planned production of guidelines for accreditation of postentry quarantine facilities and inspection.

The Directorate of Plant Protection, Quarantine, and Storage, headed by the Plant Protection Adviser to the government of India, is primarily responsible for enforcing the quarantine rules and regulations framed under the *DIP Act* in the country. For this purpose, plant quarantine and fumigation stations have been established at various international airports, seaports, and land custom stations where the incoming consignments are inspected, fumigated, or otherwise disinfested/disinfected before release to indenters. Consignments of plants/seeds for sowing/planting/propagation purposes, however, can only be imported through Amritsar, Bombay, Calcutta, Delhi, and Madras quarantine stations, where facilities in respect to well-equipped laboratories,

quarantine greenhouses and trained scientific and technical manpower are being strengthened to adequately meet the quarantine needs. Quarantine and fumigation stations under the Directorate of Plant Protection, Quarantine & Storage handle bulk imports for commerce and for planting.

18.3 Role of NBPGR

As the National Bureau of Plant Genetic Resources (NBPGR), New Delhi has been designated as the national nodal agency for exchange of germplasm material of agrihorticultural and agrisilvicultural crops for research purposes in the country; it has also been entrusted with the quarantine responsibilities in respect to germplasm of these crops. The Director of NBPGR has been empowered to issue permits for the import of seeds/planting materials for research purposes.

NBPGR has a separate Division of Plant Quarantine to meet the quarantine requirements in respect of the germplasm materials being exchanged through it. In certain crops, after laboratory examination at NBPGR, the exotic material is passed onto the specific crop-based institutes for postentry isolation growing, before it is released to the indenters. These institutes have established adequate postentry isolation growing facilities and required expertise is also available with them. These are the Central Potato Research Institute, Shimla; Central Tuber Crops Research Institutes, Trivandrum; Central Tobacco Research Institute, Rajahmundry; Sugarcane Breeding Institute, Coimbatore; and Central Plantation Crops Research Institute, Kasaragod.

NBPGR has established a regional Plant Quarantine Station at Hyderabad to fulfill the quarantine requirements of the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), National Institute of Rice Research, and other research organizations in the region. It is also proposed to establish quarantine facilities for temperate fruit crops at NBPGR's Regional Station, Bhowali, and an off-shore Quarantine Station at Port Blair in the Andaman Group of Islands for vegetatively propagated tropical crops. During the last years or so, a large number of exotic insects and mites, plant parasitic nematodes, plant pathogens, and weeds have been intercepted from the imported germplasm materials, many of which are of major quarantine significance and are not yet known to occur in the country. While processing the germplasm for quarantine clearance, all out efforts are made to salvage the infested/infected materials so that valuable exotic germplasm could be made available in a healthy state for exploitation in crop improvement programmes in the country.

The Enactment of New Policy on Seed Development (1989) by the government of India has made it obligatory for all plant breeders/researchers and users intending to import seed and planting material, to fulfill the following two mandatory requirements of Plants, Fruits and Seeds (Regulation of Import into India) Order 1989 (PFS Order 1989).

- Separate formats have been devised for applications for the issue of import permits and also for the permit letters issued for consumption purposes as opposed to those for propagative plant materials.
- Commercial imports of seeds of coarse cereals, pulses, oil seed, fodder crops, and planting materials of fruit plant species require prior clearance.
- Applications for seeds and planting materials must be accompanied by (1) a registration certificate issued by the National Seeds Corporation or the Director of Agriculture or Director of Horticulture of the state government, and (2) a certificate of approval of postentry quarantine facilities issued by the designated inspection authority.
- Permits are to be issued within a maximum period of 3 working days of submission of an application.
- PRA has been made a precondition for import of new agricultural commodities.
- Permits for import of soil or peat and for import of live insects, microbial cultures, or biocontrol agents are to be issued only by the Plant Protection Adviser, the technical head of the plant quarantine service.
- Permits for import of germplasm, genetically modified organisms, and transgenic material are to be issued by the director of the National Bureau of Plant Genetic Resources, New Delhi.
- Issued permits are valid for 6 months. This may be extended a further 6 months.
- Permits are not transferable and no permits are to issued for landed consignments.
- Relaxations from the conditions of the new order, necessitated by emergency or unforeseen circumstances, are to rest with the Ministry of Agriculture.

18.4 Phytosanitary Certificate

The second mandatory requirement is that of the Phytosanitary Certificate, to be met by the supplier of germplasm material. It should also be ensured that the seed material is not coated/ treated with chemicals/pesticides, and so on. This will facilitate proper laboratory inspection of imported material. It should also be ensured that the package of seed/planting material is

addressed only to the director, NBPGR, who is authorized to take delivery of the consignment and conduct required quarantine examination. The material, so introduced shall be made available to the indenter, after necessary quarantine clearance and national accessioning.

18.5 Some Important Points

The following points have been proposed to be considered before intending to exchange germplasm with other countries to ensure effective implementation of the said regulations laid down by the government of India.

- Always obtain an Import Permit from the Director, National Bureau of Plant Genetic Resources (Pusa Campus), New Delhi-110012 for importing germplasm of agrihorticultural crops. Apply on a proforma prescribed for the said purpose.
- Always obtain a Phytosanitary Certificate issued from NBPGR for plant materials to be exported to other countries from India.
- For importing transgenic material, send your request to Advisor, Department of Biotechnology (DBT), Ministry of Science and Technology, Block-2, C.G.O. Complex, Lodhi Road, New Delhi-110003 for seeking technical clearance for import. This is now mandatory as per Government of India Notification No.GSR 1067 (e) dated 5.12.89. After getting this technical clearance send your request to import of transgenic seed/planting material as per the regular procedure.
- For importing germplasm of the following crops route all requests through the directors of the respective ICAR crop based institutes to the Director, NBPGR.

18.6 National Coordination

Most of the plant material enters the country as air cargo or air mail parcels. Passengers going abroad also bring seed/planting material with them. The New Seed Policy now permits private enterprises to introduce more material in certain cases under an Open General Licence. Bulk consignments for consumption or sowing are brought by ships, and small research consignments through air freight or post. Therefore, the customs department, postal department, the International Airport Authority and Port Authority of India are also involved. Various research institutes under the Indian Council of Agricultural Research (ICAR) and the Council of Scientific & Industrial Research (CSIR) systems, agricultural universities, state departments of agriculture, and the private individuals/agencies are the ultimate users of the introduced germplasm material in crop improvement programmes.

Very effective linkages among all the government agencies are required so that while the introduced planting material is made available to the user clients without undue delay, all the required quarantine safeguards are observed to prevent introduction of foreign pests and diseases. The customs department, postal department, the International Airport Authority and Port Authority of India should ensure that the consignments/postparcels containing seeds/planting materials are cleared promptly and are sent compulsorily to the plant quarantine services. The consignments should never be released directly to the users. A Plant Quarantine Declaration Card, similar to the Customs Declaration Card should be introduced for passengers traveling to India. Any planting material declared or ceased by the customs department must be handed over to the plant quarantine officials for inspection and clearance. Officials of customs/postal departments should be made aware about the importance of plant quarantine through regular refresher courses. All international airports/seaports/international post offices should have plant quarantine counters along with the customs counters:

Various research institutes and agricultural universities can also contribute a great deal in this respect. They may develop some limited postentry quarantine facilities in the form of quarantine net houses/glass houses and the material, at least indented by them, could be grown in postentry quarantine under the supervision of plant protection scientists. The users of the introduced material, whether they are from the research institutes, universities, agriculture departments, or private individuals/agencies, could also contribute a lot in the smooth flow of planting material. They should always try to observe the plant quarantine regulations (requirement for import permit, phytosanitary certificates, etc.). Requests for planting materials from abroad should be channelized through appropriate authorities. For example, all requests for germplasm for research purposes should be made to the director, National Bureau of Plant Genetic Resources, New Delhi along with details of the material required, source country, name and address of the supplier, and so on, if available. Proper linkages, coordination of the efforts, and cooperation among the concerned agencies would go a long way in the smooth flow of material with required quarantine safeguards.

To prevent hidden infestation inside the fruit and check the spreading of insects from one place to another place quarantine treatment are important. However, preharvest approaches decrease the insect infestation of the fruit fly, but as per instruction of international trade we need quarantine treatment of harvested fruit. The following treatments are quite practicable before consignment exchange.

Mango should be passing through a quarantine treatment when shipped from one area to another area to inhibit fruit fly infestation. Recently, export quality of mango fumigated with ethylene dibromide (EDB) has been used for the quarantine purpose. The chemical treatment is a matter of concern worldwide, so heat and cold is used for quarantine purpose. Vapor heat treatment (VHT) is used commercially in Thailand (Unahawatii et al., 1986) and Philippines (Merino et al., 2000) to disinfest the fruit fly. In VHT, fruit are heated with water saturated air to a desirable temperature 46–47°C and heat for usually 10 min, then cooled under a water shower at ambient temperature and air dried, but sometimes VHT also damages fruit and causes fermented odor (Esguerra et al., 1990).

The use of modified atmosphere of 0.03%–0.26% oxygen with 72%–82% carbon dioxide for a period of 4 days at 20°C or controlled atmosphere storage with 2% oxygen and 50% carbon dioxide for up to 5 days at 2°C. Apparently these higher level of carbon dioxide can be used during short-term ambient temperature storage (Yahia and Hernandez-Moreno, 1993). The use of insecticidal carbon dioxide or oxygen levels to manage Caribbean fruit fly *Anastrepha suspensa* is now recommended in Mexico because of low cost and environmental safety; the treated fruit did not produce foul flavors or tissue injury and ripened normally (Yahia and Hernandez-Moreno, 1993).

For the export of papaya two-stage hot water dip treatment has been adopted as quarantine treatment. The standard double-dip treatment consists of immersing fruit in 42°C water for 30–40 min followed by a second immersion in 49°C water for 20 min (Couey and Hayes, 1986). This treatment only kills organisms near the surface of fruit and cannot be used on fruit that have ripened to the point where insects may have penetrated deeply; it also sometimes causes heat injury (Chan, 1986).

The combination of radiation and hot water double-dip method has been found very effective for the management of papaya fruit fly (Hayes et al., 1984). The atmosphere containing less than 1% oxygen and more than 60% carbon dioxide have been found very effective before storage of papaya (Brandle et al., 1983).

The hot water treatment and VHT are not applicable for guava due to thin peel and sensitivity (Yusof and Hashim, 1992). The coating of guava fruit with hydroxyl propyl cellulose, a nature seal and carnauba wax, are quite effective (Hallman et al., 1995).

The effective postharvest quarantine treatment that is not injurious to fruit and consumers should be applied to the export commodities. The alternative methods of toxic quarantine treatment, for example, hot water

treatment, cold water treatment, VHT, and radiation treatment should be promoted as per the nature of fruit in international trade (Robinson, 2005).

18.7 Insecticides

When all preharvest practices fail to manage the insect infestation then we should apply safe, recommended, economical, ecofriendly, easily degradable, less harmful to nontarget organisms and socially acceptable insecticides on fruit crops. The chemical insecticides are used to control insect infestation in orchards since human civilization. The poison bait along with traditional organophosphate insecticides has been used since the 20th century to control the fruit fly in several fruit production systems, but today, organophosphate insecticides are replaced with newer molecules of insecticides (Moreno and Mangan, 2000). Since the injudicious application of insecticides, the problem of insecticide residues has been raised and their permissible limit has been set by government agencies in India (Table 12.3). Insects of mango, guava, citrus, apple, papaya, grape, and other fruit may be managed by the spraying of chlorpyrifos methyl and ethyl, indoxacarb, spinosad, fludendiamide, imidacloprid, thiomethoxam, spinoturoam,

Table 12.3 Maximum residue limit of pesticides in fruit in India

Name of insecticides	Name of fruit	MRL (mg/kg)
Acephate	Mango	2.0
	Citrus	2.0
	Papaya	2.0
	Banana	1.0
	Litchi	1.0
	Apple	1.0
	Guava	2.0
Aldrin	All fruit	0.2
ANA	Pineapple	0.5
Benomil	Mango	2.0
	Banana	1.0
	Dry fruit	0.1
	Other fruit	2.0
Captan	All fruit	1.0
Carbaryl	Mango	1.0
	Guava	1.0
Carbendazim	Mango	2.0
	Banana	1.0
	Other fruits	5.0
	Dry fruits	0.1

Table 12.3 Maximum residue limit of pesticides in fruit in India (*cont.*)

Name of insecticides	Name of fruit	MRL (mg/kg)
Carbofuran	All fruit	0.1
Chlordane	All fruit	0.1
Chlorobenzilate	All fruit	1.0
	Dry fruit	1.0
	Walnuts	0.2
Chlorpyrifos	All fruit	0.5
Copper oxychloride	All fruit	20.0
2,4-D	All fruit	2.0
DDT	All fruit	3.5
Deltamethrin	All fruit	0.1
Dichlorvos	All fruit	0.1
Dicofol	All fruit	5.0
Dimethoate	All fruit	2.0
Dithiocarbamates	Cherries	1.0
Diuron	Citrus	1.0
	Grape	1.0
Dodine	Apple	5.0
Endosulfan	All fruit	2.0
Ethion	Peach	1.0
	Dry fruit	0.1
Fenitrothion	All fruit	0.3
Formothion	Citrus	0.2
	Other fruits	1.0
Inorganic bromide	All fruit	3.0
	Dry fruit	1.0
Lindane	All fruit	3.0
Malathion	All fruit	4.0
	Dry fruit	3.0
Monocrotophos	Citrus	0.2
	Coffee	0.1
Paraquate	All fruit	0.05
Parathion	All fruit	0.2
Phorate	All fruit	0.05
Phosalone	Pear	2.0
	Citrus	1.0
Phosphamidon	All fruit	0.2
Pyrethrins	All fruit	1.0
Thiometon	All fruit	0.5
Thiophanatemethyl	Apple	2.0
	Papaya	2.0
Trichlorofon	All fruit	0.5

sulfoxafloor, chlorantranileprole, fipronil flufenoxuron, Tebufenozide (Carlos et al., 2005; Ioriatti et al., 2005; Lucchi and Bagnoli, 2007).

18.8 Horticultural Ecosystem Analysis-Based Integrated Pest Management

Since the publication of a famous book “*Silent Spring*” by Rachel Carson in 1962, the people have come to know the adverse effects of traditional chemical pesticides on plants and their products, human beings, and the environment. Besides these, the indiscriminate use of chemical pesticides has also led to the development of pesticide resistance in pests and the problem of pest resurgence and replacement. In recent times, horticultural ecosystem analysis-based integrated pest management (HESAIPM) cannot only minimize or replace the usage of traditional chemical pesticides but also is found to be economical, effective, and efficient in insect-pest management programs.

The HESAIPM is based on environmental factors and biological factors in which orchards are located; both factors influence the existence of insects and their natural enemies. The process of observation, analysis, and decision making for appropriate pest management approaches is known as HESAIPM. In this approach fruit growers, horticultural experts, extension functionaries actively involved in analyzing orchard situations for insects, natural enemies, plant and soil conditions, and influence of environmental conditions to maintain healthy plants. If the insect and their natural enemies are available in a ratio of 2:1, then do not apply any chemical insecticides to control insects or an abundance of natural enemies is lower than insect population, we can suggest to fruit growers for release of predators, parasites, and parasitoids, application of biopesticides.

18.9 Methodology for HESAIPM

1. Visit the orchard at regular intervals
2. Randomly select 20–25 plants per acre and record the observations
3. Observations must include the stage of plants, number of healthy and affected branches, number and types of insects, number and types of natural enemies
4. Collection and identification of insects and their natural enemies
5. Analysis of situation
6. Decision making
7. Prediction of insect infestation
8. Formulation of appropriate pest management approaches

Always remember HESAIPM must be given more emphasis on natural enemies, environmental factors, and recapitulation ability of plants.

18.10 Ecological Engineering in HESAIPM

As we are aware of ecological balance of the nature, we must manipulate insect management practices to enhance natural enemies. The fruit growers must grow some flowering plants around the orchards to supply pollen and nectar for natural enemies at the adult stage. Through ecological engineering we can make provisions of shelter and alternate host plants for survival and development of natural enemies. The following activities should be taken for this purpose.

1. Grow flowering plants as borders of orchards or inside the orchard to attract natural enemies.
2. Some weeds, for example, *Ageratum conozides*, *Tridax procumbens*, and *Alternanthera* sp. act as a source of pollen and nectar of natural enemies so do not uproot these weeds.
3. Do not apply nonrecommended highly toxic insecticides during an abundance of natural enemies.
4. Intercropping with leguminous crops, which enhance soil fertility and natural enemies.
5. Apply organic manure and biofertilizer to maintain biodiversity.
6. Minimize tillage operation to allow hibernation of natural enemies.

19 CONCLUSIONS

The quality of fruit production is directly dependent on the preharvest management practices in terms of insect infestation. The preharvest approaches are especially critical for the determination of the shelf life of tropical, subtropical, and temperate fruit because typically long storage times to market are required and fruit are subjected to insect disinfection processes. The consideration of preharvest approaches properly will be helpful in growth and development, susceptibility to insect infestation and quality fruit production with insecticide residue-free fruit. The economic losses caused by several insects in fruit orchards by infesting stems, roots, and leaves we cited in this chapter only those insects that infestation responsible for deterioration of quality of fruit, as well as shelf life of the produce and their feasible and effective preharvest management practices. The most of farmers around the world applied traditional insecticides at regular intervals to control insect infestation but there are

several alternative methods, for example, orchard sanitation, physical and biological control, trapping and spraying of poison bait, sterile insect techniques, male annihilation techniques, and horticultural ecosystem analysis-based integrated pest management of insects along with newer and safest molecules of insecticides, which are economical, ecofriendly, and efficient in the control of insect populations, as well as optimize the yield by preharvest management.

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CHAPTER 13

Genetic Modification in Fruits and Vegetables for Improved Nutritional Quality and Extended Shelf Life

Khalid Z. Masoodi*, Saba Mir*, Shabir H. Wani**†, Farheena Shah*,
Minu B. Balkhi‡, Sajad M. Zargar*

*Division of Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

**Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, Wadura, Sopore, Jammu and Kashmir, India

†Michigan State University, East Lansing, MI, United States

‡GHHS Mirgund, J&K Govt., Srinagar, Jammu and Kashmir, India

1 INTRODUCTION

Fruits and vegetables are grown worldwide and make up a major portion of the human diet in many parts of the world. They play a significant role in human nutrition, especially as sources of vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber, and phytochemicals (Dias and Ryder, 2011). Fruits and vegetables in the daily diet have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases, such as diabetes, and some forms of cancer (Keatinge et al., 2010). Fruit and vegetable consumption worldwide is rising, reflecting the consumer's increased income, desire of diversity, and awareness of nutritional benefits. A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays, 2011; Kays and Dias, 1995). Leafy vegetables—of which the leaves or young leafy shoots are consumed—were the most often utilized (53% of the total), followed by vegetable fruits (15%), and vegetables with belowground edible organs comprised 17%. Many vegetable crops have more than one part used. Most of the vegetables are marketed fresh with only a small proportion processed because most vegetables are perishable. Consumption shortly after harvest guarantees optimal vegetable quality. About 3 billion people in the world are malnourished due to imbalanced diets (Keatinge et al., 2010; Pfeiffer and McClafferty, 2007). Underconsumption

of vegetables and fruits is among the top 10 risk factors leading to micronutrient malnutrition and is associated with the prevalence of chronic diseases (Dias, 2011; WHO, 2003). More than 70% of malnourished children live in Asia. At least half of the preschool children and pregnant women are affected by micronutrient deficiencies in Bangladesh, Cambodia, Nepal, and the Philippines (Talukder et al., 2010). Fruits and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases, such as cardiovascular disease (CVD) and certain cancers. According to *The World Health Report 2002*, low fruit and vegetable intake is estimated to cause about 31% of ischemic heart disease and 11% of strokes worldwide. Overall it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased. Recommendations in this direction tend to complement and reinforce other valid messages based on the long-known health benefits of consuming vegetables and fruit as dietary sources of fiber, proteins, and protective micronutrients. The recent Joint FAO/WHO Expert Consultation on diet, nutrition, and the prevention of chronic diseases, recommended the intake of a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases, such as heart disease, cancer, diabetes, and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less-developed countries. The recommendation thus adds to the already strong case for the health benefits to be gained from the consumption of fruits and vegetables

2 THE NEED FOR BIOTECHNOLOGY IN FRUITS AND VEGETABLE PRODUCTION

A number of challenges have called for the application of biotechnology in the production of fruits and vegetables. These are population increase, low nutritional quality, climate change, high perishability or postharvest decays, and short shelf life associated with fruits and vegetables. Genetic engineering has the potential to address some of these most challenging constraints faced by vegetables and fruit growers, which are not easily addressed through conventional plant breeding alone. Many vegetables exhibit a very short life span after harvesting and require very elaborate measures to expand their life. Reducing the rate of senescence in these crops is not an easy task either by conventional or biotechnological methods. The main obstacle to devising new technologies is the complexity of the problem

and lack of basic knowledge about the biochemical and cellular processes accompanying postharvest-induced senescence. This is accentuated by the extraordinary variety of tissue types that are commercialized. Early attempts to use genetic manipulation to alter senescence have been based on hormone physiology, either enhancing cytokinin production or blocking ethylene production or perception. In order to extend the postharvest life of leafy vegetables we first need to focus on the events that occur in regular leaves during senescence.

Most fruits ripen, deteriorate in appearance and eating quality, and succumb to postharvest diseases very rapidly after harvest. Poor postharvest characteristics, such as deficient flavor development, very short shelf life, quick softening, easy spoilage, sensitivity to low temperatures (chilling injury), and easy pathogen attack (fungi, etc.), are major constraints to profitability for the domestic market, and to the expansion of existing and new export markets. Among all fruits, tropical fruits are notorious for their poorer-than-average postharvest qualities. Two major obvious targets to improve the postharvest characteristics of fruits are (1) extension of shelf life and (2) resistance to pathogen attack. The ripening process involves a large number of biochemical pathways in the fruit that will result in marked changes in texture, taste, and color. At the molecular level there are a large number of genes involved and they are tightly regulated in order to induce the right changes at the right time in a highly coordinated process. In general, fruits are classified as climacteric or nonclimacteric depending on their patterns of respiration and ethylene synthesis during ripening. Climacteric fruits are characterized by an increased respiration rate at an early stage in the ripening process accompanied by autocatalytic ethylene production whereas nonclimacteric fruits show a different respiration pattern and display a lack of autocatalytic ethylene synthesis. Many of the economically important fruit crops are climacteric; therefore, a large amount of research has been devoted to studying the biochemical and molecular pathways operating during the climacteric ripening of fruits. Most of the genetic engineering approaches attempted in order to improve the shelf life and general appearance of fruits have centered on the set of genes controlling fruit firmness (membrane and cell wall properties) and the ripening rate (ethylene production or perception). These approaches have targeted endogenous genes with vital functions in the ripening process aiming to downregulate their activity by gene silencing. Postharvest decay of fruits and vegetables are a major challenge throughout the world. The degree of postharvest loss through

decay is well documented. In the industrialized countries, it is estimated that about 20%–25% of the harvested fruits and vegetables are decayed by pathogens during postharvest handling (Barkai-Golan, 2001; Droby, 2006; Sharma et al., 2009). The situation is far more exasperating in the developing countries, where postharvest decays are often more than 35%, due to inadequate storage, processing, and transportation facilities (Abano and Sam-Amoah, 2011). The use of synthetic fungicides, such as benomyl and iprodione to control postharvest diseases of fruits and vegetables is well known in scientific literature (Korsten, 2006; Singh and Sharma, 2007; Zhang et al., 2007). The health and environmental concerns associated with the continuous use of synthetic fungicides have alarmed legal enforcers and consumers to demand greener technology and quality products from the food industry as well as the scientific community. In the past 20 years, microbial antagonists like yeasts, fungi, and bacteria have been used with limited successes to reduce postharvest decays in fruits and vegetables (Barkai-Golan, 2001; Sharma et al., 2009; Singh and Sharma, 2007; Zhang et al., 2005, 2007). For instance, fungal diseases like gray mold, powdery mildew, and downy mildew in grapes do notably only cause losses in yield but also reduce wine quality (Compass, 2006). However, the advances in biotechnology can be employed to develop fruits and vegetables with improved quality and shelf life. The ability to maintain the quality of stored fruits and vegetables during postharvest storage is highly related to the physiological, biochemical, and molecular traits of the plant from which they derive (Lers, 2012). These traits are genetically determined and can be manipulated using genetic breeding and/or biotechnology. Published research results have revealed potential genes, which when manipulated can be used to improve shelf life and nutritional qualities of fruits and vegetables. Moreover, the nutritional value of fruits and vegetables depends on their composition, which shows a wide range of variation depending on the species, cultivar, and maturity stage. The composition of fruits and vegetables includes a great number of metabolites. It could be predicted that no single commodity might be rich in all these constituents, which might be one of the reasons that consumption fruit and vegetable is still below the dietary guideline goal. However, the biotechnological approaches have the potential to overcome these limitations, which is not possible by conventional breeding and the knowledge of these biotechnological approaches have not only led to major improvements in the extended shelf life of fruits and vegetables but improved nutritional quality as well.

3 TOMATO AS AN IMPORTANT MODEL SYSTEM FOR FLESHY FRUIT RIPENING

Tomato is the centerpiece system for genetic and molecular research in the family Solanaceae has emerged as a model for fleshy fruit ripening. It is due to its facilitating attributes including simple genetics, numerous characterized mutants, cross fertile wild germplasm to promote genetic studies and routine transformation technology. Recently it has been taken for genome sequencing by an international consortium currently funded and supported by 10 contributing countries. From the perspective of genetic and molecular research, tomato has advantages, such as ease of seed and clonal propagation, short generation time (approximately 45–100 days), efficient cross- and self-pollination ability, and year-round growth potential in the greenhouse has made tomato a plant of choice for fruit-ripening studies as well.

4 TOMATO RIPENING STAGES

Once the tomato fruit completes its development and attains final size then it is in mature green (MG) stage. The fruit then stops growing and starts ripening by sequential stage transition. Ripening process in tomato sequentially passes through six stages, based on the percentage of the external color: MG (no external red coloration), breaker (<10% red color at blossom end), turning (10%–30% of fruit surface having red color), pink (30%–60% of fruit surface having red shade), light red or orange (60%–90% of fruit surface having red color), and red (at least 90%–95% of fruit surface having red color). The key regulator for all the changes during ripening is the climacteric rise of ethylene observed in breaker stage.

Fruit ripening is a developmental process that is exclusive to plants whereby mature seed-bearing organs undergo physiological and metabolic changes that promote seed dispersal (Seymour, 1993). Anatomically, fruits are swollen ovaries that may also contain associated flower parts. Their development follows fertilization, and occurs simultaneously with seed maturation. Initially, fruits enlarge through cell division and then by increasing cell volume. The embryo matures and the seed accumulates storage products, acquires desiccation tolerance, and loses water. The fruit then ripens. Fruit ripening is a highly coordinated, genetically programmed, and an irreversible phenomena involving a series of physiological, biochemical, and organoleptic changes that finally lead to the development of a soft edible ripe fruit with desirable quality attributes (Seymour et al., 2002).

During maturation stage several structural and biochemical changes occur in fruit, which confers on them specific organoleptic qualities, such as modifications in the external aspect, texture, and flavor of the fruit. Although the specific biochemical programs resulting in ripening phenomena vary among species, changes typically include:

1. modification of color through the alteration of chlorophyll, carotenoid, and/or flavonoid accumulation;
2. textural modification via alteration of cell turgor and cell wall structure and/or metabolism;
3. modification of sugars, acids, and volatile profiles that affect nutritional quality, flavor, and aroma; and
4. generally enhanced susceptibility to opportunistic pathogens (likely associated with the loss of cell wall integrity).

The series of cell divisions followed by a phase of cell expansion stops after reaching maturity. The tomato maturation process is accompanied with alterations in the texture of the fruit, more specifically the loss of firmness, due to structural changes in the principle cell wall components (cellulose, hemicellulose, and pectin). The change in the color of tomato fruit results from transformation of chloroplasts into chromoplasts and from the degradation of chlorophyll, as well from the accumulation of pigments, such as carotenes and lycopenes, which are responsible for the orange and red color of the fruit (Gray et al., 1994). Finally, the accumulation of sugars, such as glucose and fructose and organic acids in vacuoles and the production of complex volatile compounds are responsible for the aroma and flavor of the fruit (Seymour, 1993).

5 BIOTECHNOLOGICAL APPROACHES FOR SHELF LIFE AND NUTRITIONAL QUALITY OF FRUITS AND VEGETABLES

Biotechnological approaches enable plant breeders to bring favorable genes, often previously inaccessible, into already elite cultivars, improving their value considerably and offering unique opportunities for extending the shelf life and improving nutritional quality of the produce (Dias et al., 2013). In this chapter we describe several advances of transgenic vegetables and fruits to nutritional quality and shelf life, very important for consumers. Many reviews have reported the wide range of determinants of desirable quality attributes in fresh fruits and vegetables, such as nutritional value, flavor, color, texture, processing qualities, and shelf life (Bapat et al., 2010; Vadivambal and Jayas, 2007). By regulating the activity of enzymes involved

in senescence of vegetables and fruit ripening, such as cell wall-degrading enzyme polygalacturonase, or ethylene biosynthesis, it is possible to control or delay the vegetable senescence and fruit softening allowing the vegetable and fruit to stay longer on the plant for greater flavor and texture development, and improving its shelf life. The shelf life of transgenic tomato fruits was reported to last for at least 60 days at room temperature without significant change in hardness and color. After 15–20 days of treatment of the transgenic fruits with ethylene, most of the tomatoes reached the ripe stage. RNAi technique has also been used to produce tomato fruit with delayed ripening using ACO gene.

Overexpression of Nr (wild-type) gene, in tomato using constitutive 35S promoter produced plants that were less sensitive to ethylene. As ethylene receptors belong to a multigene family, antisense reduction in expression of individual receptors did not show a major effect on ethylene sensitivity possibly due to redundancy except in case of LeETR4 (Ciardi et al., 2000). Antisense plants developed using LeETR4 under the control of CaMV35S promoter exhibited a constitutive ethylene response and were severely affected (Tiemann et al., 2000). The antisense plants that were developed using this receptor with fruit-specific promoter, fruits showed early ripening (Hackett et al., 2000; Kevany et al., 2008), developed transgenic Nr plants by inhibition of the mutant Nr gene. In these transgenic plants, normal ripening of Nr fruit was restored and fruit achieved wild-type levels of expression of ripening related (PSY1 and ACO1) and ethylene-responsive (E4) genes. Their study confirmed receptor inhibition as one of the modes of action of the NR (receptor) protein as in case of *Arabidopsis*. Fruit softening is one of the most prominent parameter in climacteric fruits. Softening of fruit occurs due to solubilization and depolymerization of cell wall hemicelluloses and pectin by various cell wall hydrolases (Brummell and Harpster, 2001; Rose et al., 2004). Due to accelerated fruit softening, excessive spoilage occurs, which needs to be checked. Transgenic rin plants, which accumulated reduced amounts of endogenous PG, provided clues to develop antisense PG transgenic under the control of E8 promoter. These transgenics produced fruit with PG enzyme activity that was 60% of wild-type and did not affect softening much. Downregulation of PG mRNA accumulation by constitutive expression of an antisense PG transgene driven by the cauliflower mosaic virus 35S promoter yielded transgenic fruits, retaining only 0.5%–1% of wild-type levels of PG enzyme activity though overall fruit ripening and softening was not affected (Rose et al., 2004; Saladié et al., 2007). Suppression of PME activity in tomato by introducing

antisense PME2/PEC2 transgenes under the control of the constitutive CaMV35S promoter modulated the degree of pectin methyl esterification. In transgenic antisense PME fruit esterification was higher than controls throughout ripening, but the fruit otherwise ripened normally (Nath et al., 2006). In another study, Phan et al. (2007) found antisense suppression of pectinesterase under CaMV35S promoter produced fruits with reduced PE activity and suppression in the rate of softening during ripening. In tomato, a large and divergent multigene family encodes EGases (cellulases), which consists of at least eight members. Rose et al. (2004) reported that mRNA accumulation of the highly divergent EGases LeCel1 and LeCel2 was suppressed individually by constitutive expression of antisense transgenes. In both cases, most suppressed lines showed decreased mRNA accumulation in fruit pericarp by 99% as compared to wild-type, without affecting the expression of the other EGase and fruit softening. Galactosidases in tomato are encoded by a multigene family having seven members (TBG1–7). These members show differential expression levels during fruit development (Smith and Gross, 2000). Transgenic plants have been developed using members of this family to reduce the softening process. Sense suppression by a short gene-specific region of TBG1 cDNA reduced TBG1 mRNA abundance to 10% of wild-type levels in ripe fruit, but did not reduce total exogalactanase activity and did not affect cell-wall galactose content or fruit softening (Carey et al., 2001). Antisense tomato beta-galactosidase 4 (TBG4) and 7 (TBG7) cDNAs driven by the CaMV35S promoter resulted in transgenic tomatoes with modulated fruit firmness in comparison to control fruit (Moctezuma et al., 2003). Overexpression of the Sl-ERF2 gene in transgenic tomato lines resulted in premature seed germination and enhanced hook formation of dark-grown seedlings, which is indicative of increased ethylene sensitivity (Pirrello et al., 2006). The expression of the mannanase 2 gene was upregulated in Sl-ERF2- overexpressing seeds, suggesting that Sl-ERF2 stimulated seed germination through the induction of the mannanase 2 gene. Fruits of this cultivar, called delayed fruit deterioration (DFD) undergo normal ripening but remain firm and show no loss of integrity for at least 6 months. Ripening DFD fruit interestingly showed minimal water loss by transpiration and elevated cellular turgor whereas expression of genes associated with wall disassembly were similar as in other cultivars (Saladié et al., 2007). Ethylene response factors (ERFs) play an important role in modulating ethylene-induced ripening in fruits. These ERFs belong to a multigene family and are transcriptional regulators. These mediate ethylene-dependent gene expression by binding

to the GCC motif found in the promoter region of ethylene-regulated genes. Modulation of expression of these individual ERFs in tomato has demonstrated their role in plant development and ripening. The sense and antisense LeERF1 transgenic tomato under the control of CaMV35 promoter were developed. Overexpression of LeERF1 in tomato caused the typical ethylene triple response on etiolated seedling. Antisense LeERF1 fruits showed longer shelf life as compared to wild-type tomato (Li et al., 2007). Based on biochemical and biomechanical analyses, this group has proposed a model in which softening of tomato fruit is affected by cuticle directly by providing physical support and by regulating fruit water status. Candidate gene/genes are not yet identified for this trait but once identified would be of much interest for biotechnological purposes. A new and important set of genes regulating different developmental processes involves micro-RNAs (miRNAs) (Jones-Rhoades et al., 2006). Though miRNAs and their targets have been identified in the number of plant species not much work has been carried out in relation to their involvement in fruit development and ripening. Recently (Yin et al., 2008; Zhang et al., 2008) identified a set of miRNA and their targets from tomato that were associated with the phase change from vegetative to generative growth. In addition, high throughput pyrosequencing has revealed micro-RNAs targeting genes that are involved in fruit ripening (Moxon et al., 2008). In apples, Dandekar et al. (2004) reported differential regulation of ethylene with respect to fruit quality components. A direct correlation was reported between ethylene and aroma production during apple ripening (Wang et al., 2007). Schaffer et al. (2007) identified 17 candidate genes that were likely to be the control points for ethylene with respect to aroma production. However, not all components of fruit quality are under the direct control of ethylene. Two MdERFs (ethylene response factors) were isolated from ripening apple fruit (Wang et al., 2007). MdERF2 expressed exclusively in ripening fruit whereas MdERF1 was expressed predominantly in ripening fruit with a small degree of expression in nonfruit tissues. The transcription of MdERFs was regulated positively by the ethylene signaling system. In a related study with two cultivars of apple, Zhu et al. (2008) characterized the expression patterns of AAT and ACS gene family members in order to examine the relationship with volatile ester production during on-tree and postharvest ripening. They found that differential expression of AAT genes contributed to phenotypic variation of volatile ester biosynthesis in the apple cultivars. The climacteric expression of MdACS1 that greatly enhanced the expression levels of MdAAT1 and

MdAAT2 genes was reported as the plausible reason for the emission of aromatic volatile esters. It was also suggested that the expression of MdACS3 might play a role on induction of AAT genes expression during early fruit development as it expresses prior to MdACS1. In a related research, [Nishiyama et al. \(2007\)](#) found that there was expressed suppression of the ACO gene of transgenic melon fruit when they examined the cell wall polysaccharide depolymerization and the expression of the wall metabolism-related genes. There was also a complete inhibition of softening in the transgenic melon fruits but were restored by exogenous ethylene treatment. Postharvest application of 1-MCP after the onset of ripening completely suppressed subsequent softening, suggesting that melon fruit softening is ethylene-dependent. There were, however, partial fragmentations (1038 bp cDNA) of melon invertase expressed in antisense orientation under the CaMV35S promoter observed by [Yu et al. \(2008\)](#). The transgenic melon fruits were 60% smaller in size and recorded increased sucrose and acidity invertase levels, with degraded chloroplast as a result of decreased photosynthetic rate than the control. In another study involving avocado fruits, [Tateishi et al. \(2007\)](#) found that three cloned members of β -galactosidases (PaGAL2, PaGAL3, and PaGAL4) played a significant role in the cell wall metabolism during fruits growth and ripening as well as AV-GAL1. The study of expression pattern of the isozymes by the same authors during avocado ripening found that the accumulation pattern of the gene transcripts and the response to ethylene gave a correlation between AV-GAL1 transcript and isozyme AV-GAL III. The authors therefore speculated that AV-GAL1, might have encoded the AV-GAL III and might be important for postharvest fruit softening while PaGAL2 was responsible for galactose metabolism both in expanding tissue and cell wall disassembly during ripening. In their research, they observed that PaGAL3 and PaGAL4 expression were strongly inhibited by ethylene and ripening signals suggesting that PaGAL2, PaGAL3, and PaGAL4 might have been involved in galactose metabolism of cells or cell walls during development and ripening. This could be the reason why postharvest biotechnology of avocado has been strongly limited in spite of the fact that it provided early clues to the ripening mechanism in fleshy fruit. [Symons et al. \(2006\)](#) have shown that brassinosteroids (BRs) (steroidal hormones) might be implicated in ripening of nonclimacteric fruits. The group isolated BR-6-oxidase gene homolog from grape and its function was checked by transgenic complementation of the tomato dwarf (dx/dx) mutant. The study showed that grape ripening was significantly promoted by exogenous application of BRs and ripening

could be delayed by brassinazole, an inhibitor of BR biosynthesis. Since exogenous BRs have also been shown to promote ripening in tomato it was speculated that common regulatory mechanisms might be operating early in the ripening processes of both climacteric and nonclimacteric species involving BRs. Recent advances in recombinant DNA technology and genetic engineering have opened up the possibility to manipulate ripening in fast perishable fruits like banana. Toward this, [Kesari et al. \(2007\)](#) and [Gupta et al. \(2006\)](#) reported many genes involved in ripening have been cloned and characterized. Ripening in banana is characterized by a biphasic ethylene production with a sharp early peak followed by a postclimacteric small peak ([Pathak et al., 2003](#)). During banana fruit ripening ethylene production triggers a developmental cascade that is accompanied by a huge conversion of starch to sugars, an associated burst of respiratory activity and an increase in protein synthesis. Other changes include fruit softening. Banana fruit softening is attributed to activities of various cell wall hydrolases. [Lohani et al. \(2004\)](#) found participation of various cell-wall hydrolases in banana softening during ripening. The enhancing and suppressive effects of ABA and IAA, respectively, on activities of different cell-wall hydrolases during ethylene-induced ripening in banana were also discussed. Decline in polyphenols, increase in activity of alcohol acetyl transferase, chlorophyll degradation, and so on, have been earlier reported during ripening in banana. [Liu et al. \(1999\)](#) have analyzed the expression of ACC synthase gene in association with ethylene biosynthesis and ripening in banana. [Huang et al. \(2006\)](#) have shown the presence of many isoforms of ACS other than MA-ACS1 (*Musa acuminata* ACC synthase 1) in banana. [Clendennen and May \(1997\)](#) reported a number of upregulated endochitinase, β -1,3-glucanase, and BanTLP (thaumatin like protein and metallothionein) as well as downregulated genes (class III chitinase and jacalin-related lectins) during ripening. Class III chitinase was postulated to fulfill a storage role in banana pulp. It is supposed to serve as an important source of amino acids for the synthesis of ripening associated proteins ([Peumans et al., 2002](#)). The role of expansin ([Sane et al., 2007](#); [Trivedi and Nath, 2004](#)) and polygalacturonase genes during banana fruit ripening has been investigated ([Asif and Nath, 2005](#)). In another study with apples, [Wang et al. \(2009\)](#) showed that null mutation in MdACS3 gene leads to longer shelf life. Out of the three genes in the MdACS3 family (a, b, and c) two of them (MdACS3b and MdACS3c) possessed 333-bp transposon-like insertion in their 5' flanking region, which was reported to have prevented transcription of these genes during ripening. A single nucleotide polymorphism in the coding region of

MdACS3a resulted in an amino acid substitution (glycine-289 → valine) in the active site that inactivated the enzyme. A review by [Bapat et al. \(2010\)](#) reported that two ripening-related genes (MaMads-rin and MaExp2) have been used for banana transformation to increase shelf life and fruit quality. Results indicated increment in shelf life both on plant and at postharvest. [Fraser et al. \(2002\)](#) investigated an increase in tomato fruit carotenoids phytoene, lycopene, β -carotene, and lutein in cultivar “Ailsa Craig.” Phytoene synthase from the bacterium *Erwinia uredovora* (crtB) has been overexpressed in tomato cultivar. Fruit-specific expression was achieved by using the tomato polygalacturonase promoter, and the CRTB protein was targeted to the chromoplast by the tomato phytoene synthase-1 transit sequence. Total fruit carotenoids of primary transformants [T(0)] were 2- to 4-fold higher than the controls, whereas phytoene, lycopene, β -carotene, and lutein levels were increased 2.4-, 1.8-, and 2.2-fold, respectively. The biosynthetically related isoprenoids, tocopherols, plastoquinone, and ubiquinone were unaffected by changes in carotenoid levels. The progeny T(1) and T(2) generations inherited both the transgene and phenotype. Ripe tomato fruits accumulate large amounts of lycopene and small amounts of β -carotene (provitamin A). Lycopene is transformed into β -carotene by the action of lycopene beta-cyclase (beta-Lcy). [Rosati et al. \(2000\)](#) introduced, via *Agrobacterium*-mediated transformation, DNA constructs aimed at up-regulating (OE construct) or downregulating (AS construct) the expression of the beta-Lcy gene in a fruit-specific fashion. Three tomato transformants containing the OE construct show a significant increase in tomato fruit β -carotene content. The tomato fruits from these plants display different color phenotypes, from orange to orange-red, depending on the lycopene/ β -carotene ratio. Fruits from AS transformants show up to 50% inhibition of beta-Lcy expression, accompanied by a slight increase in lycopene content. Leaf carotenoid composition is unaltered in all transformants. In most transformants, an increase in total carotenoid content is observed with respect to the parental line. This increase occurs in the absence of major variations in the expression of endogenous carotenoid genes. Current advances in genetic engineering of brassicas have enabled the production of plants with alterations in a range of vitamins or amino acids for improved human nutrition. In a study of ethylene-regulated and ethylene independent ripening pathways by [Silva et al. \(2004\)](#) in wild-type and AS3 transgenic melons, the AS3 transgenic melon fruits were reported to be firmer and higher in chlorophyll levels and acidity than their wild-type counterparts with no changes in carotenoid contents in both types. Vitamin E is a lipid-soluble

antioxidant, which includes tocopherols, have α , β , γ , and δ isoforms of tocopherol with relative vitamin E potencies of 100, 50, 10, and 3%, respectively. Conversion of γ -tocopherol to α -tocopherol in vegetable crops could increase their value and importance in human health because vitamin E reduces the risk of several serious disorders (e.g., cardiovascular diseases and cancer), slows aging, and enhances the function of the immune system. [Cho et al. \(2005\)](#) developed transgenic lettuce plants of the cultivar “Chungchima” expressing a cDNA encoding γ -tocopherol methyltransferase to improve tocopherol composition from *Arabidopsis thaliana*. Transgene inheritance and expression in transformed plants increased enzyme activity and conversion of γ -tocopherol to the more potent α form. [Wahlroos et al. \(2005\)](#) produced oilseed *Brassica rapa* with increased histidine content. Folate deficiency, which is regarded as a global health problem, causes neural tube defects and other human diseases. Foliates are synthesized from pteridine, *p*-aminobenzoate (PABA), and glutamate precursors. ([de La Garza et al., 2007](#); [Díaz et al., 2004](#)) developed transgenic tomatoes by engineering fruit-specific overexpression of GTP cyclohydrolase I that catalyzes the first step of pteridine synthesis and aminodeoxychorismate synthase that catalyzes the first step of PABA synthesis. Vine-ripened fruits contained on average 25-fold more folate than controls by combining PABA- and pteridine-overproduction traits through crossbreeding of transgenic tomato plants. The achieved folate level provides a complete adult daily requirement with less than one standard serving. [Grumet et al. \(2007\)](#) reported enhanced sugar and carotenoid accumulation whereas [Katzir et al. \(2008\)](#) reported a considerable reduction in aroma production for ACO1 antisense melons. Vegetables also offer consumers a diverse mixture of nutrients that promote human health more beneficially than dietary supplements. However, the ingestion of plant-based diets rather than diets that rely primarily on animal products could limit the intake of essential nutrients, such as calcium (Ca). Consequently, genetically engineering vegetables containing increased Ca levels may boost Ca uptake, thereby reducing the incidence of Ca deficiencies, such as osteoporosis. In this regard, [Park et al. \(2004\)](#) modified carrots to express increased levels of the plant Ca transporter sCAX1. These carrot lines were fertile and displayed no adverse phenotypes. Further, mice and human feeding trials demonstrated increased Ca absorption from sCAX1-expressing transgenic carrots vis-à-vis controls ([Morris et al., 2008](#)). This research supports alternative means of biofortifying vegetables with bioavailable Ca. Zinc, which is also an essential element in human nutrition, as its deficiency severely impairs organ function. In experiments to fortify

lettuce with this element, [Xiaofeng et al. \(2002\)](#) used *Agrobacterium*-mediated gene delivery of a mouse metallothionein mutant β -cDNA in the lettuce cultivar “Salinas 88.” The concentration of zinc in the lettuce transgenic plants increased to 400 $\mu\text{g/g}$ dry weight, which is considerably higher than in wild-type plants. Flavonoids are polyphenols whose dietary intake has the potential to prevent chronic diseases. [Schijlen et al. \(2006\)](#) introduced heterologous, flavonoid pathway genes—stilbene synthase, chalcone synthase, chalcone reductase, chalcone isomerase, and flavone synthase—to produce novel flavonoids in tomato fruit. These novel flavonoids—flavones and flavonols increased threefold, mostly in the Q12 peel, which had higher total antioxidant capacity. These findings add further support to the potential of engineering tomato fruit for accumulation of high levels of beneficial nutrients. Similarly, the polyphenol resveratrol, a stilbene, shows cancer chemopreventative activity and may prevent coronary heart disease and arteriosclerosis. [Liu et al. \(2006\)](#) in a quantitative analysis showed that resveratrol in transgenic lettuce plants was $56.0 \pm 5.52 \mu\text{g/g}$ leaf fresh weight, which is comparable to that in the skin of grape fruit (*Citrus* \times *paradisi* Macfad.). Flavonoids, such as anthocyanins are known as antioxidants in vitro and can reduce the risk of many diseases related to aging. However, some vegetable brassicas, such as cauliflower, are low in anthocyanins. In an attempt to manipulate pigment biosynthesis to increase the health benefits of brassica vegetables, the effect of a regulatory locus of flavonoid content was assessed. *Agrobacterium tumefaciens*—mediated transformation of a *Brassica oleracea* line—selected for high transformation ability by [Sparrow et al. \(2004\)](#), was used to produce plants transgenic for the maize *lc* (leaf color) locus. *Lc* is a regulatory gene in the anthocyanin pathway, and it is expected that its presence will increase the flavonoid content. Seedling explants were cocultivated with *A. tumefaciens* strain LBA4404 containing a binary vector Q27 with a neomycin phosphotransferase II (NPTII) gene. Under tissue culture conditions, *lc*-containing plants were green with no visible increase in anthocyanin production. However, after transfer to the greenhouse, the exposure to high-light intensity led to visible signs of pigmentation within 1 week. Increased pigmentation was apparent in stems, petioles, main leaf veins, and sepals. *Lc*-containing lines had 10–20 times higher levels of total anthocyanins than controls. In addition, antioxidant activity of *lc*-containing lines was 1.5 times higher than that of controls ([Braun et al., 2006](#)). The unique flavor and odor of alliums is derived from the hydrolysis of organo-sulfur compounds, which produces pyruvate, ammonia, and volatile sulfur compounds ([Randle and Lancaster, 2002](#)). This reaction is catalyzed by the

enzyme alliinase, which is contained in vacuoles within cells and released upon disruption of the tissue (Lancaster and Collin, 1981). Variations in the ratios of these volatile sulfur compounds are responsible for the difference in flavors and odors between *Allium* species (Randle and Lancaster, 2002). Along with health and nutritional benefits associated with these compounds, these thiosulfides are also major contributors to the bitter taste of some onions (Almeida, 2006; Randle and Lancaster, 2002). Three sets of transgenic onion plants containing antisense alliinase gene constructs (a CaMV 35S-driven antisense root alliinase gene, a CaMV 35S-driven antisense bulb alliinase, and a bulb alliinase promoter-driven antisense bulb alliinase) have been recently produced (Eady et al., 2003). Results from the antisense bulb alliinase lines have been much more encouraging, and three lines were produced with barely detectable bulb alliinase levels and activity. Progress has been confounded by the poor survival of transgenic plants. Crossing a nontransgenic open-pollinated parental line with a transgenic parental plant carrying a single transgene in the hemizygous state has conducted to a transgenic hybrid onion seed from these transgenic lines. Some resulting seeds produced by the nontransgenic parents will be hemizygous for the transgene and can be selected to give F1 heterozygous individuals containing the transgene. Self-fertilization of these individuals produces homozygous, hemizygous, and null F2 progeny for the transgene locus. These homozygous individuals can then be used to generate the bulk seed required for the production of commercial transgenic onion lines with less bitter taste. It was reported that tomato plants transformed with yeast SAM-DC gene under the control of E8 promoter showed improvement in tomato lycopene content, better fruit juice quality, and vine life (Bapat et al., 2010).

6 CHALLENGES ASSOCIATED WITH GENETICALLY MODIFIED FRUITS AND VEGETABLES

It is revealed that although biotechnological approaches are seen by the scientific community as a panacea to solve recent increased demands for fruits and vegetables, still the technology is more of a scientific jargon than a commercially viable entity. This is because of the dilemma and uncertainties that remain up to today regarding the consumption of biotechnological fruits and vegetables. Although the first biotech crop to be commercialized was a genetically modified tomato for processing as a consumer tomato paste, since then there have been comparatively few introductions of biotech fruits

and vegetables (Anthony and Ferroni, 2011). Reported cases with potential benefits for farmers in developing countries include virus-resistant papaya in China, now commercially grown, and, more recently, the high profile case of Bt eggplant, or brinjal, in India (Choudhary and Gaur, 2009). Due to the susceptibility of brinjal to the fruit and shoot borer insect, multiple insecticide applications are required to prevent uneconomic losses of yield in this crop. In India, the Indian Genetic Engineering Appraisal Committee recommended the commercial release of Bt brinjal (Event EE1) in 2010, but no authorization was given by the Ministry of Environment and Forestry (Jayaraman, 2010). A wide array of vegetables, such as tomato, broccoli, cabbage, and okra are also under development in India (James, 2010). In a study involving 77 fruits and vegetables and other specialty crops, Miller and Bradford (2010) attempted to understand the factors driving the lack of traits for commercialization. They reported that during 2003–08 more than 300 research papers were published describing more than 250 unique transgenic events for these kinds of crops of which some 20% of the papers were from China and India. The various researches addressed not just input traits, such as herbicide tolerance and insect resistance but also output traits, such as yield, postharvest quality, and modifications to compositions of oil, starch, protein, and nutrients. The primary conclusion was that the traits did not reach the market not because of poor performance or lack of grower interest but because of regulatory approval uncertainty and prohibitively high and uneconomic development and regulatory costs—a de facto barrier for technology deployment for smallholder farmers, even for high-value crops. It was established in surveys by private sector companies during 2008–12, that the cost of intervention, development, and registration of new traits for internationally traded crops, such as maize and soybean was as high as \$136 million for cultivation in two countries and for import approvals in at least five others. The breakdown cost analysis for regulatory scientific studies, registration, and regulatory affairs accounted for 25.8% of this total, \$35.1 million. Further McDougall (2011) reported that the time taken for registration has also increased, from a mean of 3.7 years for events sold before 2002 to a current estimate of 5.5 years. Recent reports in the EU member states indicate that while countries like Finland, Germany, and Greece have strongly opposed commercialization of GM crops including fruits and vegetables, Spain and UK do not fundamentally oppose cultivating GM crops but have used the precautionary principle. So the question remains, “Is biotechnology in fruit and vegetable plant production a commercial activity or simply research jargon?”

7 CONCLUSIONS

The biotechnological approaches to improve nutritional quality and shelf life of fruits and vegetables were reviewed. It was evident that developed biotechnological approaches have the potential to enhance the yield, quality, nutritional quality, and shelf life of fruits and vegetables to meet the demands of the 21st century and make important contributions to sustainable vegetable and fruit production by overcoming limiting factors, which are not easily addressed through conventional vegetable breeding alone. However, the biotechnological approaches for fruits and vegetables were more of academic jargon than a commercial reality. A barrier to the successful use of transgenic techniques might be the acceptance or lack thereof of transgenic fruit and vegetable crops by the public. To make sure that the current debates and complexities surrounding the registration and the commercialization of genetically modified fruits and vegetables are adequately addressed, various stakeholders in the industry, policy makers, private sectors, agriculturalists, biotechnologists, scientists, extension agents, farmers, and the general public must be engaged in policy formulations, seed embodiments, and products development. The full benefit of the knowledge can be reaped if there are total commitments by all stakeholders regarding increased and sustained funding, increased agricultural research and development, and less cost and time for registration and commercialization of new traits.

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CHAPTER 14

Preharvest Biofortification of Horticultural Crops

Arpita Das*, Samrat Laha*, Sanchita Mandal*, Sukanta Pal*,
Mohammed Wasim Siddiqui**

*Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

**Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

1 INTRODUCTION

Modern agriculture has been largely successful in meeting the nutritional demand of the poor populations in developing countries. In the past years agricultural research in developing countries mainly concentrated on production rather than nullifying the nutritional point. However, now is the high time when agriculture should concentrate on a new paradigm that will not only focus on food production, but deliver better quality food as well (Bechoff et al., 2009). A major challenge of our time is that one-sixth of the world's population suffers from hunger, a situation that is totally unacceptable. In addition, more than half of the global population, mostly pregnant women and children below the age of 5, are afflicted by a different form of food deficiency (IFPRI, 2014a). Micronutrient malnutrition, commonly known as “hidden hunger” has emerged as one of the major health problems worldwide. This “hidden hunger” is due to the quality, rather than the quantity, of the food available, and it is closely related to the fact that in many poor developing countries people rely only on mostly low-protein staple crops for food. Because foods that are high in micronutrients, such as vegetables, fruits, dairy, and meats are expensive, resource-poor people rely primarily on a few starchy staples that are rich in energy, but not in micronutrients. As a consequence, dietary diversity to achieve micronutrient intake adequacy becomes a luxury that the poor can often not afford. Though good progress has been made across the globe toward alleviating poverty, providing food and nutritional security, unfortunately many countries are not on track to achieve the goal of reducing the number of people suffering from hunger, malnutrition, and poverty (Gómez et al., 2013). However, the percentage of the poor has declined in all the regions. According to latest FAO estimates (Table 14.1,

Table 14.1 Population affected with malnutrition around the globe

Regions	Number of undernourished (millions) and prevalence (%) of undernourishment						
	1990–92		2000–02		2012–14		Change in number (%)
	Number	(%)	Number	(%)	Number	(%)	
Developed regions	20.4	<5	21.1	<5	14.6	<5	(–) 28.4
Developing regions	994.1	23.4	908.7	18.2	790.7	13.5	(–) 20.5
Africa	182.1	27.7	209.0	25.2	226.7	20.5	24.5
Sub-Saharan Africa	176.0	33.3	202.5	29.8	214.1	23.8	21.6
Asia	742.6	23.7	637.5	17.4	525.6	12.7	(–) 29.2
India	210.8	23.8	186.2	17.6	190.7	15.2	(–) 9.5
Latin America and the Caribbean	68.5	15.3	61.0	11.5	37.0	6.1	(–) 46.0
Oceania	1.0	15.7	1.3	16.5	1.4	14.0	40.0
World	1014.5	18.7	929.9	14.9	805.3	11.3	(–) 20.6

Source: From FAO, 2014. Food and Agriculture Organization of the United Nations. Available from: FAO, IFAD, WFP, 2014. The State of Food Insecurity in the World 2014: Strengthening the enabling environment for food security and nutrition. FAO, Rome.

Figs. 14.1 and 14.2), about 805 million people are estimated to be chronically undernourished in 2012–14, more than 100 million lower than the last decade, and 209 million lower than in 1990–92 (FAO, 2014). Many strategies, including supplementation, dietary diversification, and commercial fortification of foods have been deployed to overcome this problem. However, these remedies pose several problems due to the incapability of the target populations (especially in poor rural populations in developing countries) to consume balanced and supplementary diets, cost effectiveness, and nonsustainability over time. Therefore, developing micronutrient-enriched staple plant food is a powerful intervention tool that targets the most vulnerable people. These tools should be fully exploited by the nutrition and public health communities to combat micronutrient malnutrition (Graham et al., 2007).

Biofortification can be defined as a process of adding nutritional value to the crop. It refers to increase of bioavailable micronutrient content of food crops through genetic selection via plant breeding, biotechnological, and agronomic approaches. It is a crop-based technique, in which the

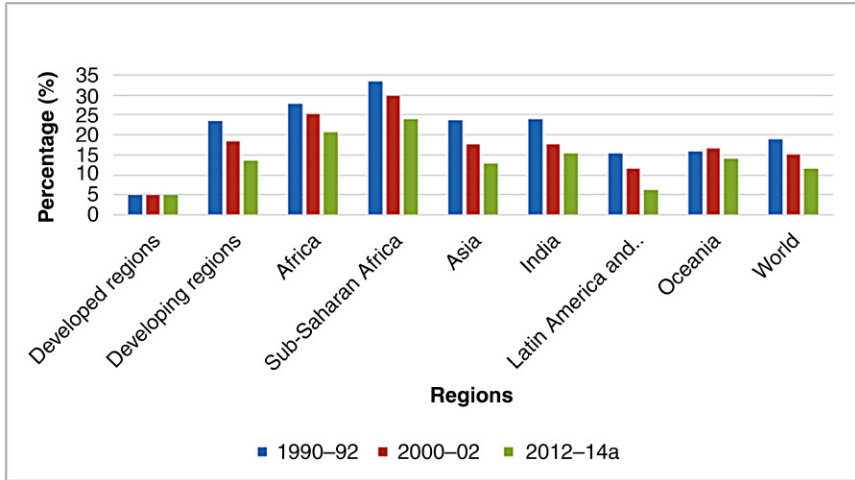


Figure 14.1 *Percentage (%) prevalence of undernourishment.*

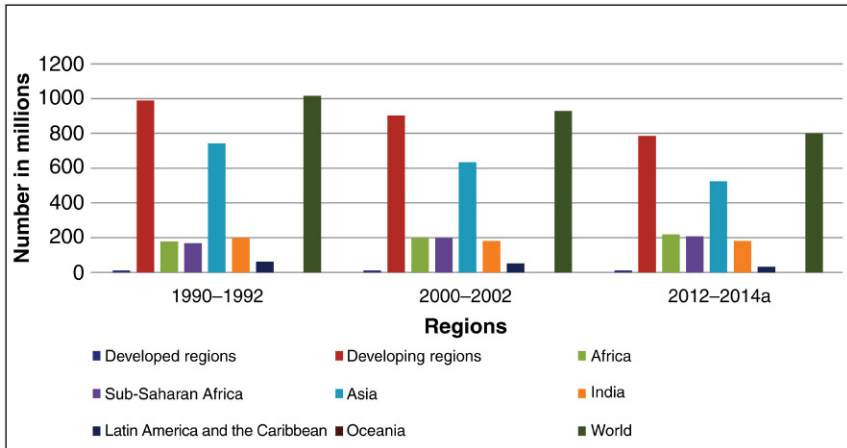


Figure 14.2 *Number of undernourished (millions).*

edible parts (grain, straw, root, and tubers) of food crops are enriched with micronutrients through appropriate breeding methods (Bouis, 2000, 2002; CIAT/IFPRI, 2004). In a broader effort fruits and vegetables have also been biofortified (Prasad et al., 2015). This branch of quality breeding is gaining prominence given the fact that it is a low-cost approach to enhance micronutrient status of edible food targeted toward the poor, who can hardly afford high-quality food. This approach therefore has immense potential to mitigate micronutrient malnutrition and ensure better health worldwide

(Waters and Sankaran, 2011). Introducing biofortified staple crops with increased nutrition content can therefore have a very big impact, as the strategy relies on improving an already existing food supply (Nestel et al., 2006). Biofortified staple foods may not deliver equally high levels of minerals and vitamins per day, compared to supplements or fortified food products, but they can increase micronutrient intake for the resource-poor people who consume them daily, and therefore complement existing approaches (Bouis et al., 2011). By enhancing the micronutrient content of these energy-rich staples, micronutrient intakes in general, and among the poor in particular, can be increased, thereby leading to a reduction in the prevalence of micronutrient under nutrition. The prime objective of biofortification is the nutritional enrichment of crops to address the negative economic and health consequences regarding vitamins and mineral deficiencies in human beings that can have a measurable impact on nutritional status.

The wide-scale adoption of cereal crops during the Green Revolution displaced much of the local production of fruits, vegetables, and legumes, which are the chief sources of micronutrients for most people (Welch and Graham, 2004). Fruits and vegetables are the source of carbohydrates, and are needed for providing energy to the body, including the nervous system and brain. These foods also provide significant amounts of dietary fiber that helps to improve digestive function and lower the risk for high cholesterol, heart disease, obesity, and diabetes. These are the store house of natural vitamins (vitamin A, folate, vitamin C, etc.), minerals, such as iron (Fe), zinc (Zn), selenium (Se), iodine (I), and potassium (K), which act as antioxidants that help to limit cell damage from free radicals. Numerous early studies revealed that there is a strong link between eating fruits and vegetables and protection against cancer. Therefore, the presence of fruits and vegetables in the diet make it complete and balanced. Fruits and vegetables have rich sources of genetic diversity for micronutrients, and hence they are highly amenable for biofortification through conventional and marker-assisted breeding programs of crops for combating malnutrition problem. This variability is the future repository of gene/quantitative trait loci (QTLs) governing important traits for disease resistance, abiotic stresses, and nutritional quality.

2 MICRONUTRIENT MALNUTRITION AND ITS IMPORTANCE

Humans require at least 49 nutrients to meet their metabolic needs (Table 14.2). Inadequate consumption of even one of these nutrients will result in adverse metabolic disturbances leading to sickness, poor health,

Table 14.2 List of essential nutrients for human life

Water and energy	Protein (amino acids)	Lipids-fat (fatty acids)	Macroelements	Microelements	Vitamins
Water	Histidine	Linoleic acid	Na	Fe	A
Carbohydrates	Isoleucine	Linolenic acid	K	Zn	D
	Leucine		Ca	Cu	E
	Lysine		Mg	Mn	K
	Methionine		S	I	C (ascorbic acid)
	Phenylalanine		P	F	B ₁ (thiamine)
	Threonine		Cl	B	B ₂ (riboflavin)
	Tryptophan			Se	Niacin
	Valine			Mo	B ₅ (pantothenic acid)
				Ni	B ₆ (pyridoxal)
				Cr	Folate
	V	Biotin			
	Si	B ₁₂ (cobalamin)			
	As				
	Sn				
	Co (cobalamin)				

Source: Data taken from Welch, R. M., Graham, R.D., 1999. A new paradigm for world agriculture: productive, sustainable and nutritious food systems to meet human needs. *Dev. Bull.* 49, 29–32.

impaired development in children, and ultimately large economic costs to society. There are 20 mineral elements that are essential for human health (Vander et al., 2001), including 7 major mineral elements (Ca, P, K, S, Na, Cl, and Mg) and 13 trace elements (Fe, I, Cu, Zn, Mn, Co, Cr, Se, Mo, F, Sn, Si, and V). These elements cannot be synthesized by the body and must be continuously supplied from foods. The recommended nutritional uptake of these essential elements, including vitamins, as well as their main physiological functions, is shown in Tables 14.3 and 14.4. Among the 20 essential elements, 5 are present in limited amounts in many foods, particularly in the diet of developing nations where people cannot accommodate a balanced diet. These are I, Fe, Zn, calcium (Ca), and Se. Importantly, the primary source of all nutrients for people comes from agricultural products. If the agricultural system fails to supply food containing adequate quantities of all nutrients, it results in micronutrient malnutrition and ultimately unhealthy

Table 14.3 Recommended nutrient intakes for males and females between ages of 25 and 50 years

Nutrient	Assessment	Male	Female
Energy (kcal)	AEA	2900	2200
Protein (g)	AEA	63	50
Proprovitamin A carotenoid (μg retinol equivalent)	RDA	1000	800
Vitamin D (μg)	RDA	5	5
Vitamin E (mg α - tocopherol equivalent)	RDA	10	8
Vitamin K (μg)	RDA	80	65
Riboflavin (mg)	RDA	1.7	1.3
Niacin (mg niacin equivalent)	RDA	19	15
Thiaming)	RDA	1.5	1.1
Pantothenic acid (mg/d)	ESADDI	4–7	4–7
Vitamin B ₆ (mg)	RDA	2	1.6
Vitamin B ₁₂ (μg)	RDA	2	2
Biotin ($\mu\text{g}/\text{d}$)	ESADDI	30–100	30–100
Folate (μg)	RDA	200	180
Vitamin C (mg)	RDA	90	60
Ca (mg)	RDA	800	800
P (mg)	RDA	800	800
Mg (mg)	RDA	350	280
Na (mg)	MR	500	500
K (mg)	MR	2000	2000
Cl (mg)	MR	750	750
Fe (mg)	RDA	10	15
Zn (mg)	RDA	15	12
Cu (mg)	ESADDI	1.5–3.0	1.5–3.0
Se (μg)	RDA	70	55
I (μg)	RDA	150	150
Mn (μg)	ESADDI	2–5	2–5
Mo (μg)	ESADDI	75–250	75–250
Cr (μg)	ESADDI	50–200	50–200
F (mg)	ESADDI	1.5–4.0	1.5–4.0

AEA, Average energy allowance; ESADDI, estimated safe and adequate daily dietary intakes; MR, minimum requirement; RDA, recommended dietary allowances.

Source: Data from FAO/WHO, 2000. The world health report 2000 health systems: improving performance. World Health Report, World Health Organization, Geneva.

Table 14.4 Main functions of important trace elements essential for humans

Elements	Functions
Proprovitamin A carotenoid	<ul style="list-style-type: none"> a. Powerful antioxidant that neutralizes free radicals—molecules that damage healthy cells—and increases the risk of accelerating the aging process and/or health conditions b. Promotes the growth of strong teeth and bones c. It is used to build new cells and is critical for normal brain development and nerve function d. Essential for the formation of visual purple, a pigment that allows you to see in dim light
Iron	<ul style="list-style-type: none"> a. Important part of hemoglobin b. Participates in the nitrogen body exchange and breathing process c. Catalyzes b, carotene into provitamin A carotenoid d. Induces antibodies synthesis and enhances immunity
Iodine	<ul style="list-style-type: none"> a. An essential constituent of the thyroid hormones thyroxine
Zinc	<ul style="list-style-type: none"> b. Promoting growth and development of humans a. Participates in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids b. Promotes children's intellectual development c. Accelerates teenagers' growth d. Affects the palate and appetite e. Affects male fertility
Selenium	<ul style="list-style-type: none"> a. Enhances immunity b. Antiaging c. Inhibit cancer d. Protects the heart e. Antagonist heavy metal
Copper	<ul style="list-style-type: none"> a. An important component of proteins and enzymes b. Closely related to human body hematopoiesis c. Affects antioxidant ability of body
Molybdenum	<ul style="list-style-type: none"> a. An important component of xanthine oxidase and aldehydes oxidase b. Takes part in the electronic transmission of cell c. Restrains the breeding of virus in cell
Chromium	<ul style="list-style-type: none"> a. Promotes protein metabolism and body growth b. Influences lipid metabolism c. An important part of glucose tolerance factor
Silicon	<ul style="list-style-type: none"> a. Plays an essential role in the development of bone b. Participates in the metabolism of the polysaccharide

(Continued)

Table 14.4 Main functions of important trace elements essential for humans (*cont.*)

Elements	Functions
Nickel	a. Is a component of hydrogenated enzyme b. Promotes the formation of insulin c. Lowers blood glucose
Cobalt	a. Is a component of vitamin B ₁₂ b. Participates in hemoglobin synthesis
Vanadium	a. Maintains normal metabolism of fat b. Is a constituent of nucleic acid c. Promotes the growth of bones and teeth
Fluoride	a. Plays an important part in the growth of bones and teeth
Tin	a. Has a function in the tertiary structure of proteins or other biosubstances b. Is used as catalyst for polymerization, transesterification, and olefin condensation reactions

Source: From Yin, X., Yuan, L., Liu, Y., Lin, Z., 2012. Phytoremediation and biofortification: two sides of one coin. In: Yin, X., Yuan, L. (Eds.), *Phytoremediation and Biofortification*. Springer, Dordrecht, Heidelberg, New York, London, pp. 1–6.

lives. Roughly more than one-third of the world's population is at risk of one or more micronutrient deficiencies in the world (Graham et al., 2007). The estimates indicate that about 7 billion people are suffering from either form of micronutrient deficiency; about 2 billion people worldwide are anemic, many due to Fe deficiency (WHO, 2016), 2 billion people suffer from I deficiency (de Benoist et al., 2008), and about 17.3% of the population is affected by Zn deficiency (Wessells and Brown, 2012). It is estimated that 250 million preschool children are provitamin A deficient, and a substantial proportion of pregnant women in at-risk areas are suffering from provitamin A deficiency (WHO, 2016). About one-third of preschool children in developing countries suffer from malnutrition, causing the deaths of 5–10 million of these children every year (Anderson, 2015; FAO, 2014). In addition, Se, Ca, magnesium (Mg), and copper (Cu) deficiencies are common in many developed and developing countries (Grusak and Cakmak, 2005; Rude and Gruber, 2004; Thacher et al., 2006; Welch and Graham, 2005). Therefore, these micronutrient deficiencies impose a considerable disease burden to the society through creating adverse functional outcomes, including stunting, increased susceptibility to infectious diseases, physical impairments, cognitive losses, blindness, and premature mortality. Marginal intakes of micronutrients have been shown to contribute to increased morbidity and mortality rates, diminished livelihoods, and adverse effects on learning

ability, development, and growth in infants and children. The extent of this disease burden is so high that the World Bank estimated the combined economic costs of micronutrient deficiency in developing countries could waste as much as 5% of gross domestic product (GDP). Globally, provitamin A, Fe, I, Zn, and Se deficiencies are reported to have the largest percentage disease burden among the micronutrients that are known to have a negative impact on public health (Black et al., 2008; Stein, 2010). Therefore, keeping these in mind, the discussion focuses on these important micronutrients.

3 FUNCTION, DEFICIENCY, AND ITS IMPLICATIONS AND BIOAVAILABILITY OF IMPORTANT MICRONUTRIENTS

3.1 Provitamin A Carotenoid

Provitamin A carotenoid denotes a group of carotenoid derivatives (retinal, retinol and its esters, and retinoic acid), which play an essential role in vision, immune response, epithelial cell growth, bone growth, reproduction, maintenance of the surface lining of the eyes, embryonic development, and regulation of adult genes (Tumuhimbise et al., 2013). Individuals lacking sufficient vitamin A in their bodies suffer from night blindness, which is the earliest symptom of vitamin A deficiency (VAD). VAD is the world's most common cause of childhood blindness in less-developed countries, particularly in sub-Saharan Africa and Southeast Asia where an estimated 250,000–500,000 children go blind each year (Bouis, 2003; WHO, 2003). Moreover, affected children have an increased risk of infection from other diseases, such as diarrhea and measles. Similarly, VAD causes night blindness in women during pregnancy and is responsible for nearly 600,000 maternal deaths due to problems related to childbirth each year. This deficiency has also been associated with elevated transmission of HIV from mother to child. It has been estimated that overcoming VAD could potentially decrease global child mortality by 23% (WHO, 2003).

The major sources of carotenoid in the diet are commonly found in animal products and from colored and leafy vegetables. Among the important precursors of provitamin A, the most prominent is the β -carotene. Bioavailability is defined as the efficiency with which ingested provitamin A carotenoid are absorbed and converted in to provitamin A carotenoid in the body. The preformed provitamin A carotenoid is easily absorbed by the intestinal tissue in the presence of fat. Excess carotenoid in our body is reserved in the liver. At the time of necessity, the reserved carotenoid is released from the liver through a highly regulated metabolic pathway.

However, the absorption of vitamin A is influenced by various factors. The chemical nature of physical binding with the food, presence of dietary fat, food products that inhibit the bioavailability, presence of bile salt, and pancreatic enzymes, food preparations are playing a pivotal role during absorption. The availability of preformed vitamin A is much greater than that of precursor carotenoids (Bates and Hesecker, 1994).

3.2 Iron

Iron (Fe) is an essential micronutrient for humans. As both an electron donor and acceptor, Fe plays a key role in many vital metabolic pathways, such as the electron transport chain of respiration (Gómez-Galera et al., 2010). Therefore, Fe is required for oxygen transport and energy metabolism in the body. If Fe intake is inadequate, the amount of hemoglobin in the red blood cells can fall leading to Fe-deficiency anemia, with symptoms of tiredness, weakness, and inability to concentrate. Moreover, Fe deficiency during childhood and puberty stage impairs physical growth, mental development, and learning capacity. In adults, it decreases the capacity to do physical labor. Severe anemia increases the risk of women dying during childbirth (Huang et al., 2012). In pregnant women, severe Fe-deficiency anemia is associated with intrauterine growth retardation, fetal and maternal morbidity and mortality, premature births, low birth weights, and increased risk of infection. Young children, whose mothers are anemic during pregnancy, suffer from impaired brain function, resulting in poor learning ability, decreased physical activity levels, and behavioral problems during adolescence (Black et al., 2008; WHO, 2003).

Consequently, Fe deficiency is the most common micronutrient deficiency in the world. It not only affects the health and development of people, but also hampers the social and economic development of countries due to physical decline of adults. The deficiency is more pronounced in developing countries. The World Health Organization (WHO) estimates that 39% of children younger than 5 years, 48% of children between 5 and 14 years, 42% of all women, and 52% of pregnant women in developing countries suffer from anaemia (WHO/UNICEF/UNU, 2001), with half having Fe-deficiency anemia (DeMaeyer and Adiels-Tegman, 1985).

Although Fe is the fourth most abundant element in the earth's crust, it is poorly bioavailable in soil because it binds rapidly to soil particles and forms insoluble complexes under aerobic conditions at neutral or alkaline pH. Dietary Fe exists as heme Fe or nonheme Fe. Heme Fe is found in animal foods and is primarily derived from the hemoglobin and myoglobin

found in meat while nonheme Fe is derived from plant and dairy products. Unlike heme Fe, which is well-absorbed nonheme Fe, consists mainly of Fe salts, which are bound to foods and therefore must be hydrolyzed or solubilized prior to absorption into the body (Baltussen et al., 2004). This depends largely on the presence of absorption enhancers or inhibitory substances in the meal. For example, reducing agents, such as ascorbic acid and cysteine, enhance Fe absorption while compounds, such as phytates, inhibit Fe absorption (Gurzaui et al., 2003). Similarly, carotenoid has been reported to enhance both Fe metabolism and absorption into the red blood cells (Cavalli-Sforza et al., 2005). Moreover, dietary Fe bioavailability is low in populations consuming monotonous plant-based diets with little meat (Zimmermann et al., 2005). Therefore, in plant-based diets, Fe absorption is often less than 10% (Hurrell, 2002; Zimmermann et al., 2005).

3.3 Iodine

Iodine (I) is an essential component of the thyroid hormones thyroxine and triiodothyronine, which regulate growth and development and maintain the basal metabolic rate (Gerber et al., 1999). Therefore, deficiency of I hinders the synthesis of thyroid hormones that regulate multiple functions, such as enhancing protein synthesis, regulating energy transfer, accelerating growth and development, and maintaining the structure of the central nervous system. The resulting physiological disorders and biological function abnormalities are called I-deficiency disease (IDD) (Upadhyay et al., 2002; Zimmermann, 2008). Another important IDD is goiter, which results from the lack of thyroxine, inducing the production of thyroid-stimulating hormone, which in turn causes the thyroid gland to swell. According to the recent statistics from WHO it was found that I deficiency is becoming a threat to the health of up to 1.6 billion people throughout the world (Bruno et al., 2008; Hetzel, 2005; WHO, 2003). India is one of the worst affected countries in the world, with more than 50 million cases of goiter and more than 2 million of cretinism.

Iodized salt and intake of seafood is the most common approach for dietary I supplementation (Andersson et al., 2005). However, because I supplementation may cause problems during food processing, it is difficult to control its loss during transport, storage, and food cooking (Winger et al., 2008).

However, in the case of I it is a well-known fact that I is characterized with bilateral threshold. Either overdose of I or deficiency of I can cause adverse effects (Wang and Zhang, 1985). Eighty percent of the I in

the human body and animals originally comes from edible vegetable foods under natural conditions (DeLong et al., 1997; Welch and Graham, 2005) and the bioavailability of I in food can achieve as much as 99%. Due to the fact that I in edible plant food originates from soil, the background concentration of I in soil as well as its bioavailability determines whether the consumed I can meet the need. However, results are largely affected by the I concentration and the chemical form supplied (Blasco et al., 2008) and by the growth substrate used (Weng et al., 2008).

3.4 Zinc

Zinc (Zn) is an essential trace element for plants (Broadley et al., 2007), animals (Prasad, 2008), as well as for microorganisms (Sugarman, 1983). It is one of the important constituents of most of the proteins and cofactors of the enzymes. This micronutrient is responsible for the normal expression of more than 20 physiological functions in organisms, including immune function, protein synthesis, wound healing, DNA synthesis, and cell division. A large number of proteins in biological systems need Zn to maintain their structural stability and transcription factors. Protection against infections and diseases is related to gene regulation and expression under stress conditions in which Zn is required (Prasad, 2010). Zinc is also a critical element required for detoxification of highly aggressive free radicals and for structural and functional integrity of biological membranes (Cakmak, 2000). Therefore, it is a necessary trace element that can support the normal growth and development during pregnancy, childhood, and puberty phase. Although Zn is an essential element for life, excess Zn can be harmful and can cause Zn toxicity to organisms.

Despite, of its immense role, Zn deficiency is among the major malnutrition in humans and has led to severe diseases, cellular disturbances and impairments, and even large mortality especially in infants and young children. Nearly 2 billion people are at risk of Zn deficiency, predominantly children and pregnant women (WHO, 2003). The mineral appears to be particularly important during periods of rapid growth, and insufficient intake during childhood and adolescence can delay growth, sexual development, and psychomotor development (WHO, 2003).

Like Fe the bioavailability of Zn from vegetarian diets is also likely to be less than that of nonvegetarian diets. Plant foods rich in Zn, namely, legumes, whole grains, nuts, and seeds contain phytic acid, an inhibitor of Zn bioavailability (Veenemans et al., 2011). Bioavailability of Zn is enhanced by dietary protein (Hashemipour et al., 2009), but plant sources of

protein are also generally high in phytic acid. Fe can have a negative effect on Zn absorption, if given together in a supplement, whereas no effect is observed when the same amounts are present in a meal as fortificants. Cadmium, which is increasing in the environment, also inhibits Zn absorption. The amount of protein in a meal has a positive effect on Zn absorption, but individual proteins may act differently; for example, casein has a modest inhibitory effect of Zn absorption compared with other protein sources. Amino acids, such as histidine and methionine, and other low-molecular-weight ions, such as EDTA and organic acids (e.g., citrate), are known to have a positive effect on Zn absorption and have been used for Zn supplements (Zhao et al., 2012).

3.5 Selenium

Selenium (Se) is a trace element that has a beneficial role in human beings in low concentration but at high levels it has a toxic effect. This element must be obtained through dietary sources. It is the main constituent of some particular proteins, called selenoproteins, which act to protect the body from free radicals as well as from oxidative damage. Without Se, the function of the Se-requiring proteins can be compromised, which results in the signs and symptoms of deficiency (Bleys et al., 2008). The deficiency of Se causes white muscle disease in human beings, which is characterized by muscular weakness and muscular dystrophy. The other deficiencies include reduced appetite, poor growth and reproductive function, and embryonic abnormalities (Lyons et al., 2005). Se is taken up from the soil and enters the food chain through plants. Therefore, the deficiency of Se has been noticed in parts of the world where there is low content of Se in soil (Rayman, 2004). Keshan disease is one of the endemic heart diseases caused by the deficiency of Se, which mainly affects children and women of child-bearing age. Therefore, Se acts as an antioxidant, which scavenges free radicals with health benefits, including the prevention of cancer and heart disease (WHO/FAO, 1998). At higher dosages Se may be toxic for all organisms. Toxic concentrations cause garlic breath, hair and nail loss, disorders of the nervous system and skin, poor dental health, and paralysis in humans (Li et al., 2012). There is also evidence for Se deficiency being involved in impaired immune function, and increased incidence of cancer, cardiovascular and other degenerative diseases, as well as overall mortality (Rayman, 2004).

Dietary sources of Se include green vegetables like spinach, nuts, as well as from the animal sources. The highest content are found in nuts

(0.53 mg/kg), followed by fish (0.3 mg/kg), eggs (0.19 mg/kg), and poultry (0.14 mg/kg). In general, the absorption of Se from the diet is thought to be high at around ~80% although high dietary sulfur can reduce absorption probably through competition between the chemically similar sulfur and Se species. Organically bound forms of Se have been shown to possess greater bioavailability than inorganic forms with selenite being less bioavailable than selenate. There is also good evidence to show that, at least with supplemental Se forms, organic Se species can prolong Se status better than inorganic Se species after supplementation has ended (Brown and Arthur, 2001). However, the bioavailability of other naturally occurring Se species is less well understood.

4 PHYSIOLOGY BEHIND MICRONUTRIENT UPTAKE, DISTRIBUTION, AND ACCUMULATION IN PLANTS, INCLUDING BIOSYNTHESIS OF PROVITAMIN A CAROTENOID

The physiological process controlling micronutrient efficiency and accumulation in plants and its bioavailability are the basic pillars before addressing biofortification program. Plants can only absorb mineral elements in their specific chemical forms, which are available forms for plants. Therefore, for a successful biofortification program, knowledge regarding the forms of mineral elements acquired by plant roots, and the restrictions to the supply and phytoavailability in the rhizosphere solution are essential. The rhizosphere process involves complex interactions between plant roots and rhizosphere microbes. Plant roots release a variety of organic compounds (such as organic acid, siderophores, and phenolics) that are the natural carbon resources for microbes (Bowen and Rovira, 1991) and accelerate micronutrient uptake through creating a favorable environment. Many strategies for the biofortification of crops with essential mineral elements rely on increasing the acquisition of these elements from the soil. However, it is obvious that if the soil contains insufficient amounts of these elements then they must be added to the agricultural system as fertilizers. If sufficient amounts of these elements are present in the soil, then the focus turns to increasing the supply and phytoavailability of these elements in the rhizosphere, and their uptake by plant roots and redistribution to edible portions, so that biofortification is effective.

Extensive studies have been carried out in plants and bacteria to understand the carotenoid biosynthetic pathway. In plants, it has been shown that four enzymes, namely, phytoene synthase (*Psy*), phytoene desaturase (*Pds*),

ζ -carotene desaturase (*Zds*), and lycopene cyclase (*Lcy*), are required to complete the biosynthesis of β -carotene from an intermediate compound geranyl geranyl diphosphate (GGPP) (Sandmann, 1994, 2001).

Generally, Zn can be absorbed via roots primarily as Zn^{2+} and/or $Zn(OH)_2$ at high pH level in the soil solution. Zn is transported either symplastically or apoplastically after being taken up through root cells (Broadley et al., 2007; White et al., 2002). There are various factors that control the absorption and desorption of Zn, including the total Zn content, chemical forms of Zn compounds, soil properties (organic matter content, carbonate, or phosphate content, granularity, pH), environmental conditions (temperature and humidity), concentrations of other trace elements, and relative biological activities (White and Broadley, 2009).

In most of the plant species Fe absorption takes place through the strategy where plant roots release protons into the rhizosphere, lower the pH of the soil solution, and increase the solubility of Fe^{3+} (Santi and Schmidt, 2008; Santi et al., 2005). After acidification, Fe^{3+} is reduced to Fe^{2+} by a membrane-bound ferric reductase oxidase (FRO) and transported into the root (Robinson, 1999). After entering the epidermis, Fe is required to be bound by chelating compounds, such as citrate and nicotianamine (NA) (Curie et al., 2009; Haydon and Cobbett, 2007). Depending on the Fe-chelate complex formed, different transport systems are involved in distributing Fe throughout the plant.

Se can be taken up by plant roots as selenate, selenite, or organoselenium compounds (Li et al., 2008; White et al., 2004, 2007) but cannot take up colloidal elemental Se or metal selenides (White et al., 2004, 2007). Selenate is transported across the plasma membrane of root cells by high-affinity sulfate transporters (Hawkesford and Zhao, 2007), while selenite is thought to be transported by phosphate transporters (Li et al., 2008). The former is rapidly converted to organoselenium compounds in the root, whereas the latter is delivered to the xylem and transported to the shoot, where it is assimilated into organoselenium compounds and redistributed within the plant in a manner analogous to S (Li et al., 2008).

I is present in the soil solution in trace amounts and most soil I is associated with organic matter, clays, and oxides of Fe and Al (Fuge and Johnson, 1986). The prevalent form of I in the soil solution is often iodide, but iodate may also occur depending upon pH and redox conditions (Fuge and Johnson, 1986; Kodama et al., 2006; Yuita, 1992).

The occurrence of these chemical forms in the rhizosphere solution is a function of the soil's physicochemical and biological properties, which

will ultimately determine the phytoavailability of these elements in the soil and high pH is often the major factor limiting the phytoavailability of these elements.

For proper accumulation of micronutrient (e.g., Fe and Zn, Se, and I) in edible tissue, several physiological barriers have to be eliminated. These barriers are the result of tightly controlled homeostatic mechanisms that regulate metal absorption, translocation, and redistribution in plants allowing adequate, but nontoxic levels of these nutrients to accumulate in plant tissues (Welch, 1993). There should be sufficient amounts of micronutrients in their available form in the rhizosphere for uptake. Sometimes changes or modification of root morphology, increasing surface area of root, stimulation of some root cell processes like the presence of organic matter, secretion of some biochemical by certain microorganism, changes of pH in the rhizosphere, presence of chelating agents and reductants can accelerate the micronutrient uptake process. During absorption, the transporters and ion channels involved in this process must be in active mode to facilitate accumulation of micronutrients from rhizosphere to apoplasm of root cell. Finally, after uptake of micronutrient via root cells these micronutrients should be translocated into sink- or target-edible tissue in bioavailable forms. Unfortunately, current knowledge of all of these processes is very limited and much more basic research is needed before food crops can be genetically modified efficiently in order to accumulate more bioavailable forms of micronutrients in seeds and grains through modern genetic engineering techniques (Fairweather-Tait and Hurrell, 1996).

5 CONVENTIONAL STRATEGIES FOR NUTRITIONAL ENHANCEMENT

The control of provitamin A carotenoid and mineral deficiencies is an essential part of the overall effort to fight hunger and malnutrition. Prior to focus on biofortification, there are various approaches that can be utilized for the nutritional improvement of the horticultural crops. These are discussed in the following sections.

5.1 Food Fortification

Food fortification and supplementation are one of the most cost-effective and long-term strategies to address global mineral and vitamin malnutrition (Horton, 2006). In the case of food fortification micronutrients are

added in the processed food. In many situations, this strategy can lead to relatively rapid improvements in the micronutrient status of a population, and at a very reasonable cost, especially if advantage can be taken of existing technology and local distribution networks (WHO and FAO, 2006). However, this strategy is difficult to implement specially in developing countries because of the high price of fortified foods, which are affordable by poor people. For successful implementation there should be proper coordination between processing and the supply chain to target a large number of populations for fulfilling their micronutrient demand. Since many parts of the world suffer from multiple deficiencies, strategies must also be developed to fortify foods simultaneously with several micronutrients without adverse interactions among them (Zimmermann et al., 2004). The successful examples are double fortification of salt with I and Fe to mitigate Fe and I deficiency, particularly for school children, adolescent girls, lactating and pregnant mothers, to mitigate the deficiency of these nutrients. Common approaches for Zn and Fe fortification have been implemented in the industrial world and some of the developing countries of Africa, Mexico, Bangladesh, and so on (IZINCG, 2007) as Zn and Fe deficiencies tend to go hand in hand. Fortification for provitamin A carotenoid content is also going on in some staple food crops like rice, in edible sugar, vegetable oil, skimmed milk, etc.

5.2 Supplementation

Supplementation is the term used to describe the provision of relatively large doses of micronutrients, usually in the form of pills, capsules, or syrups to improve nutritional health in a short-term way. It has the advantage of being capable of supplying an optimal amount of a specific nutrient or nutrients, in a highly absorbable form, and is often the fastest way to control deficiency in individuals or population groups that have been identified as being deficient (WHO, 2016). However, it is unsustainable for addressing large populations of developing countries, particularly very poor women and school children. Successful supplementation programs have been carried out for alleviating vitamin A, Fe, and folate deficiency. The distribution of vitamin A supplements has been one of the most cost-effective and successful acute intervention programs in the developing world (Shrimpton et al., 2002). Like fortification, successful supplementation strategies require a robust infrastructure and a government determined to improve the nutritional health of its population (Shrimpton et al., 2002).

5.3 Market-Driven Fortification

The term “market-driven fortification” is applied to situations where a food manufacturer takes a business-oriented initiative to add specific amounts of one or more micronutrients to processed foods (WHO and FAO, 2006). However, this type of fortification program must be regulated by the government. Generally this type of fortification program is well accepted in industrial areas. This can be helpful to mitigate the malnutrition problem, particularly for those micronutrients that are difficult to meet through normal diet. Examples include certain minerals (e.g., Fe, Ca) and sometimes selected vitamins (e.g., vitamin C, vitamin B₂). Public support for traditional fortification has recently been enhanced by new promotion and coordination efforts: Micronutrient Initiative (based in Canada), Flour Fortification Initiative (based in Emory University), Mid Day Meal Scheme (India), and the Global Alliance for Improved Nutrition (GAIN, based in Geneva), the Network for Sustained Elimination of Iodine Deficiency and the International Zinc Nutrition Consultative Group (Singh et al., 2014) are some of the initiatives.

5.4 Dietary Diversification

Linking cultivation of a variety of staple foods with a high vitamin and mineral content and awareness regarding balanced diet can produce better consumer behavior and overcome micronutrient malnutrition. The objective here is to diversify food cultivation and make a wider selection of foods with a high vitamins and mineral content so that consumers prepare more varied meals and have a more balanced diet. However, poverty and lack of awareness regarding nutrition are the major constraints of this effort. Promotion of kitchen gardens, inclusion of seasonal fruits and vegetables, and awareness generation regarding nutritional security through mass media are the various tools for approaching this. Alas, this type of effort is relatively difficult to sustain on any large scale.

6 BIOFORTIFICATION

In the introduction section, biofortification was already discussed that it is an agricultural process that increases the uptake and accumulation of mineral nutrients in agricultural products through plant breeding, genetic engineering, or manipulation of agricultural practices through agronomic approaches for improvement of nutritional content of the staple foods for eradicating hidden hunger. Conventional interventions have a limited impact, so biofortification has been proposed as an alternative long-term approach for

improving mineral nutrition (Zhu et al., 2007). In biofortification, strategies like conventional plant breeding or genetic engineering and agronomic interventions can increase the micronutrient status of the crops and their bioavailability. Unlike the continued financial outlays required for traditional supplementation and fortification programs, biofortification is more cost-effective and sustainable. Once the biofortified plants are developed and grown by the farmers, seeds can be multiplied, reproduced, and shared among the poor, with few additional costs occurring to maintain the high nutrient trait over time (Bouis and Welch, 2010). The biofortification strategy seeks to put the micronutrient dense trait in the most acceptable and high-yielding varieties and can target large numbers of undernourished population of rural areas. Economic studies have shown many potential health benefits of biofortification strategies, especially in combination with conventional strategies (Bouis et al., 2011; Stein et al., 2008). Therefore, for sustainable implications, conventional strategies can be adopted alone or in combination with different conventional strategies to alleviate micronutrient deficiency as shown in Fig. 14.3.

6.1 Genetic Biofortification

Genetic biofortification involves either classical breeding approaches or biotechnological approaches, including characterization and exploitation of genetic variation for mineral content, manipulation of gene makeup through

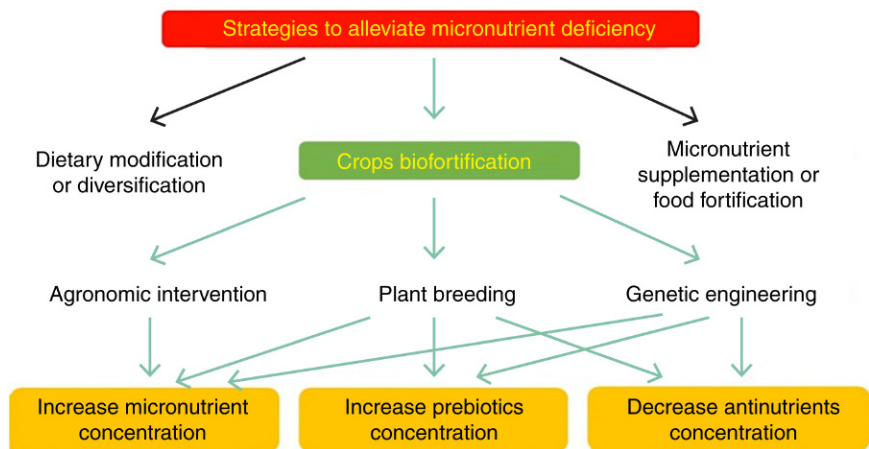


Figure 14.3 *Different strategies to alleviate mineral deficiency.* (From Huang, Y., Yuan, L., Yin, X., 2012. Biofortification to struggle against iron deficiency. In: Yin, X., Yuan, L. (Eds.), *Phytoremediation and Biofortification Two Sides of One Coin*. Springer Dordrecht Heidelberg, New York, London, pp. 59–74).

introgressing new traits controlling nutrient enhancement, reduction in the content of antinutritional factors, biotechnological approaches involving gene discovery and marker-assisted breeding. The cost of breeding is moderate and after the initial investment of developing fortified crops, no extra costs are met, making this strategy very sustainable to bring micronutrient sufficiency. Furthermore, the improved varieties can be shared internationally. Due to higher trace mineral content in biofortified seeds, better protection against biotic and abiotic stresses can be achieved, indirectly increasing yield. In addition, these micronutrient rich seeds and grains of crops increase crop productivity through longer root formation under micronutrient deficit conditions, allowing seedlings to scavenge more micronutrients and water, interestingly much of the developing world has significant areas of such problematic soils deficit in micronutrient.

6.1.1 Classical Plant Breeding

Traditional plant breeding primarily focused on enhancement of yield attributes and overcoming biotic and abiotic stresses and lack of priority on nutritional aspects leads to a decreased amount of nutrient status in the high-yielding varieties. However, recent progress in conventional plant breeding has given emphasis on fortification of important micronutrients and vitamins through a one-time investment. With the abundant natural genetic variations and centuries of conventional breeding experiences, plant-breeding strategy is widely accepted as a cost-effective and easily affordable solution among the staple food crops biofortification approaches. The genetic potential for increasing the concentration of bioavailable micronutrient (Fe, Zn, I, Se, and provitamin A carotenoid) in the edible tissue of several horticultural crops like I biofortification for tomato (Landini et al., 2011), spinach (Dai et al., 2006), cabbage, Chinese cabbage, Se biofortification for spinach (Zhu et al., 2003) and onion (Zhu et al., 2009), provitamin A sweet potato (HarvestPlus, 2014), cassava for Zn, Fe, provitamin A (HarvestPlus, 2014; Ngozi, 2013), Fe rich potato (Haynes et al., 2012; Paget et al., 2014), Zn- and Fe-fortified cow pea (Boukar et al., 2011), beans (Beebe et al., 2000; HarvestPlus, 2014), provitamin A-fortified banana (Prasad et al., 2015) have been discussed earlier. Before addressing the breeding program, certain criteria should be predetermined. These are: (1) there should be no compromise regarding yield potential of the fortified crops; (2) the micronutrient enrichment levels achieved must have significant impact on human health; (3) the micronutrient enrichment traits must be relatively stable across various edaphic environments and climatic zones; (4) ultimately, the bioavailability

of micronutrients in enriched lines must be tested in humans to ensure that they improve the micronutrient status of people preparing and eating them in traditional ways within normal household environments; and (5) consumer acceptance must be tested (taste and cooking quality must be acceptable to household members) to ensure maximum impact on nutritional health (Welch and Graham, 2004). For biofortification to succeed several factors have to be considered, starting with the identification of the targeted population and ending with the improvement of the nutritional status of this population. The “impact pathway for biofortified crops” as suggested by HarvestPlus is divided into three stages: (1) discovery, (2) development, and (3) dissemination of the newly developed plant variety (Fig. 14.4). In conventional plant breeding, the presence of genetic variations are essential for the development of new genotypes with high micronutrient and pro-vitamin A concentrations. However, since cultivated crops contain narrow genetic variations for micronutrient accumulation, species with promising genetic resources for higher micronutrient concentrations are needed. This includes the identification of the genetic variability of the targeted crop by screening varieties, which are able to accumulate high levels of the targeted

Discovery

- Identify target populations
- Set nutrient target level
- Screen germplasm and gene discovery

Development

Breed biofortified crops

- Test the performance of new crop varieties
- Measure nutrient retention in crop
- Evaluate nutrient absorption and impact

Dissemination

- Develop strategies to disseminate the seed

Promote marketing and consumption of biofortified crops

Improve nutritional status of target populations

Figure 14.4 Simplified diagram of the pathway for biofortified crops (HarvestPlus, 2009).

minerals (Ortiz-Monasterio et al., 2007). During the screening process, lines have to be identified, which accumulate and store a high proportion of the absorbed nutrients in their edible part and lines, which have an increased nutrient uptake while maintaining the high proportion of nutrients in the edible part (Calderini and Ortiz-Monasterio, 2003). In addition, germplasm with greater abilities to cope with adverse climate or soil conditions should be selected. In beans and pea regarding Fe and Zn levels up to a 6.6-fold variation have been reported (Gregorio et al., 2000). However, this genotypic variation is generally more reduced in tubers, like potato, sweet potato, and cassava (White and Broadley, 2003) and in fruits (e.g., Fe, Zn, Ca, and Mg concentrations) in strawberry differed less than twofold (Hakala et al., 2003). Breeders and molecular biologists also mining the targeted genes for micronutrient enrichment, once these are identified can be successfully transferred into the otherwise high-yielding but nutrient-poor genotypes through opting various breeding strategies. Once promising high-yielding, high-nutrient lines emerge, then these lines are tested in multilocation trials for confirming their stable performance for growing in megaenvironments and last, large-scale deployment of seeds of improved cultivars to farmers. Not only that, plant breeders could breed for genotypes that contain lower concentration of antinutrient or elevated concentration of promoters or can modify plant genetic architecture to silent or overexpress the genes encoding antinutrient or promoter, respectively. However, these should be done cautiously because some of the antinutrients, namely, phytate and polyphenols, play beneficial role by acting as anticarcinogenic also decreasing the risk of heart disease and diabetes. The pleiotropic nature of Fe and Zn association become a win-win opportunity to the plant breeder. Regarding bioavailability point of view it is important to determine the retention of micronutrients when screening and using genetic resources in breeding with food products in mind. Comparative analysis of Fe and Zn concentration of raw and cooked products indicated no significant differences due to “cooking” or “genotype \times cooking” interaction, which becomes a bonus to the plant breeder. From different research results it is confirmed that retention of micronutrient may also be genetically determined, which then adds retention heritability to the plant-breeding portfolio. Nutritional efficacy studies in human beings revealed that breeders can attain the nutrient targets already set because retention and bioavailability are higher than already assumed for determining target. Adoption of participatory selection can provide early feedback on farmer and consumer preferences and may speed delivery of biofortified varieties to targeted communities. Despite so many advantages

the success of conventional breeding is limited because it can only utilize the genetic variability that exists in nature. Furthermore, in some cases yield potential of the fortified crops has to compromise during their nutritional enhancement.

6.1.2 Special Breeding Approaches

To overcome the basic problem of classical plant breeding special breeding approaches are opted for nutritional enrichment of horticultural crops.

6.1.2.1 Mutation Breeding

Mutation breeding program is generally carried out for nutritional enhancement, where there is inadequate genetic variability in the population. In this breeding strategy genetic variability can be induced through physical and chemical mutagen. The FAO/International Atomic Energy Agency (IAEA) collaborative research focused on development of induced mutants containing higher concentration of micronutrient, or improving their bioavailability by reduction in the concentration of antinutritional factors like phytic acid and developed 776 mutants in this regard (www-mvd.iaea.org). This strategy can obviously reduce the recurring cost of food fortification or supplementation. However, keep in mind that the mutant must be acceptable by the farmers and consumers. Isolation of induced mutant in beans increases the bioavailability of Fe, Zn, and P in the edible tissue (Shetty, 2009). In cassava, three mutants have been isolated showing different sizes of starch grain. They have high-economic potential for industrial use of starch and influence on cooking quality. In banana, several mutants have been isolated for large fruit sizes having high provitamin A content (Jain, 2010).

6.1.2.2 Tissue Culture Technique

Plant tissue cultures can be defined as the culture of all types of plant cells, tissues, and organs under aseptic conditions. Nowadays, plant tissue culture is an integral part of molecular approaches for plant improvement and acts as an intermediary during gene isolation and genetic transformation. Tissue culture can be utilized to develop distant crosses between cultivated and wild species for transferring desirable traits related to nutritional enhancement. This technique becomes commercially successful in propagation of tuber crops like potato, sweet potato, and in fruit crops like banana. Desirable somaclones have been screened in these crops, which are either more nutritious, having good agronomic traits, or having reduced concentration of antinutritional factor.

6.1.2.3 Molecular Breeding and Marker-Assisted Selection

Nowadays, utilization of molecular markers associated with target alleles (MAS), determining variation for micronutrients, helps in identifying desirable segregants without direct selection at field condition and thereby considerably shortening the breeding cycle. The identification of certain QTL that control physiological activities, such as uptake, translocation, distribution, and sequestration of micronutrient in plants together with the confirmation of numerous enzymes involved in the micronutrient homeostasis in plants, contributes to better understanding of the mechanism for genetic breeding of crops with higher micronutrient content. Advances in nutritional genomics and efficient molecular marker techniques allow tracking complex traits and subsequent breeding strategy (DellaPenna, 1999). When the location of the gene for a desirable trait can be identified, a specific marker unique for that can be sequenced, which can be further use for MAS for monitoring the introgression or presence of that particular gene without any tedious morphological screening irrespective of growth stage. Using the successful deployment of novel marker system approaches, namely, SNP (single nucleotide polymorphism) genotyping, DArT (diversity arrays technology) marker analysis, genotyping by sequencing (GBS), and association study analysis in next-generation populations, such as backcross population, multiparent advanced generation intercross (MAGIC) population, natural population, targeting induced local lesions in genomes (TILLING) population, and recombinant inbred line (RIL), various QTL have been established for important agronomic traits in different crop species (Soren et al., 2016). This technique has also been used to find recessive traits in plants that cannot be located by conventional breeding or other techniques (Singh et al., 2016).

6.1.2.4 Transgenic Approach

In recent time genetic engineering attains considerable importance where conventional breeding fail to achieve the specific target particularly when the genetic variation is not enough and for that crops where sexual reproduction or conventional breeding methods are difficult to opt. In biofortification process also, genetic engineering can be utilized to fulfill one or more of the following goals (Zhu et al., 2007): (1) improve the efficiency with which minerals are mobilized in the soil, (2) redistribute micronutrients between tissues, (3) increase the efficiency of biochemical pathways in edible tissues, or even reconstruction of the selected path ways, (4) reduce the level of antinutritional compounds, and (5) increase the level of nutritional enhancer compounds.

In the case of Fe and Zn fortification, introduction of the gene for NA aminotransferase (Takahashi et al., 2001) has shown a better response to Fe and Zn accumulation in the transgenic plants. Amplification of the natural Fe store by expressing ferritin in bean, cassava, banana (HarvestPlus, 2014) is a further strategy for successful Fe accumulation in edible tissue. In case of cassava and sweet potato increased Fe content was achieved by the expression of the *FEA1* gene, from *Chlamydomonas reinhardtii*, in storage roots (Chávez et al., 2005). Another breakthrough in Fe biofortification is the development of plants expressing lactoferrin, a human Fe-binding protein present in milk that also has broad antimicrobial activity (Bethell and Huang, 2004). Recombinant human lactoferrin has been expressed in crops, like potato (Chong and Langridge, 2000). The amount of micronutrient absorption can also be increased by improving its bioavailability. This can be achieved by reducing antinutrients, such as phytic acid, poly phenolics, etc. In the case of Se biofortification the selenocysteine methyltransferase gene of *Astragalus bisulcatus* was introduced into *Arabidopsis thaliana* to overexpress Se-methylselenocysteine and γ -glutamylmethylselenocysteine in shoots (Ellis et al., 2004; Sors et al., 2005).

For provitamin A biofortification in banana, a similar transformation approach has been used from the knowledge generated from the Golden Rice project. The sequences for *Psy* from daffodil (*Narcissus pseudonarcissus*) (Ye et al., 2000) and *CrtI* from bacteria (*Erwinia uredovora*) (Misawa et al., 1990) have been isolated and used to isolate *Psy* homologues from crop plants, such as maize (Paine et al., 2005). Furthermore, using the available sequence information, the banana homologues of *Psy* have been isolated and sequenced from the high β -carotene bananas with the aim of transferring the sequences into cooking banana for the improvement of pVA content of the fruit (Mlalazi, 2010). Utilizing this approach bananas with up to 20 ppm provitamin A have been developed (HarvestPlus, 2014). In the same way, this approach is also being applied to other crops, including orange cauliflower, tomato, yellow potatoes, and golden canola (Susana et al., 2013). In the case of sweet potato and cassava β -carotene, enrichment of storage roots is conferred by two transgenes: the *Erwinia crtB* phytoene-synthase gene, and the *Arabidopsis* 1-deoxyxylulose-5-phosphate synthase (DXS) gene (HarvestPlus, 2014).

6.2 Agronomic Intervention

Agronomic biofortification of horticultural crops is a strategy to increase micronutrient concentration through applying mineral fertilizers in different

forms within a permissible limit. Like supplements and fortification, agronomic intervention is probably best applied in niche situations or in combination with other strategies (Cakmak, 2008). It is considered to be a flexible approach that can be used for all crop species and cultivars, and compared to genetic biofortification, it is considered a short-term solution without years of tedious crossing and backcrossing activities. However, the cost and environmental impact of the fertilizer application cannot be evaded. This strategy is lucrative when the micronutrient deficiency in the edible part reveals the absence of that mineral in the soil, minerals present in the fertilizer are mobilizable and proper distribution of micronutrient from the source to the sink in bioavailable form are feasible. Therefore, agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvement of the solubilization and mobilization of mineral elements in the soil (White and Broadley, 2009).

Generally, micronutrients are applied in root zone or in the form of foliar spray depending on their uptake efficiency, requirement, and phytoavailability. In case of Zn, the most widespread inorganic Zn fertilizer is Zn sulfate, along with Zn oxide and synthetic Zn chelates (White and Broadley, 2009). For improving agronomic effectiveness of Zn fertilizers it should be in the form of Zn-EDTA. In the case of soil application, certain agronomic strategies like reducing soil pH, adopting appropriate crop rotations, or introducing beneficial soil microorganisms contribute to high Zn phytoavailability (He and Nara, 2007; Rengel, 1999; White and Broadley, 2009). In the case of foliar spray, the use of soluble Zn compound can help for easy incorporation in the leaf apoplast. It has been found that foliar application seems to be more effective than soil application to reach the target concentration of 40 mg/kg in edible tissue (Cakmak et al., 2010; Wang et al., 2012).

Fe shows a low mobility in soil due to conversion of Fe^{+2} to Fe^{+3} when applied in the form of FeSO_4 in the root zone, makes it unavailable for plant absorption (Frossard et al., 2000). To overcome this, synthetic metal chelators and prebiotic, such as chelates and nitrogen, are often used along with soil Fe fertilizers, which can effectively increase mineral concentration in edible vegetable and fruit tissue (Shuman, 1998). For improving phytoavailability, soil acidification with elemental sulfur and increasing the nitrogen status of the plant can successfully alleviate the Fe accumulation up to threefold (Aciksoz et al., 2011). However, foliar sprays of FeSO_4 or chelates allow the direct uptake of Fe through leaves.

Se can be applied either in the rhizosphere or as a foliar spray in the form of sodium selenite and sodium selenate. In addition, the mixture of organic acids with Se-mineral fertilizers was used to chelate Se, which could obviously improve the acquisition of Se and elevate the utilization efficiency of Se fertilizers (Lynch, 2007; Morgan et al., 2005). For proper uptake of Se, root morphology and rhizospheric microorganisms also have an important role (Lynch, 2007). Therefore, the use of Se fertilizers both in soil and foliar application can reach the target concentration of 300 $\mu\text{g}/\text{kg}$, and it was performed on various fruits and vegetables (Liu et al., 2010; Lyons et al., 2005).

In the case of I, soil application in the form of iodide or iodate can positively increase accumulation of this nutrient in the edible part. The fertigation program in Northwestern China showed that I applied through fertigation resulted in very positive effects on food crops, including vegetables, human, and animal I status (Cao et al., 1994). Increasing I levels in the edible parts of vegetables through a soil fertilization strategy also seemed effective (Dai et al., 2004), although the target I concentration of 500 $\mu\text{g}/\text{kg}$ in edible tissue is hard to reach (Mackowiak and Grossl, 1999). Hydroponics is also more effective than soil application in promoting I absorption probably because the soil I can be retained by organic matter, bottlenecks of phytoavailability. Hydroponic culture, with I added to the nutrient solution, thus gives excellent possibility for biofortification of tomato, cabbage, and other. However, in the case of leafy vegetables foliar application of I is more effective than other methods of application (Mao et al., 2014).

6.3 Microbiological Intervention

6.3.1 *Plant Growth Promoting Rhizobacteria (PGPR) and Cyanobacteria*

These include a group of beneficial bacteria that helps in stimulating plant growth and improves crop yield, including micronutrition of crops. PGPR, the root colonizing bacteria, stimulate crop growth by different direct and indirect mechanisms. The mechanism involved in improving micronutrition includes sequestration and transformation by microorganisms in soil, such as production of acids, alkalis, etc; siderophore production influenced physiologically active Fe and production of antioxidants besides synergistic interaction between P and micronutrients (Balakrishnan and Subramanian, 2012). Some bacterial strains influence plant growth by synthesis of plant hormones and some strains increase minerals and nitrogen availability to the plants. Secretion of phytosiderophores by microorganisms and plants in restricted spatial and temporal windows represents an efficient strategy

for uptake of Fe and other micronutrients by plants from the rhizosphere. Analysis of the complex interactions among soils, plants, and microbes in relation to micronutrient dynamics represents a unique opportunity to enhance our knowledge of the rhizosphere ecology. Such progress can provide information and tools, enabling us to develop strategies to improve plant nutrition and health with a decrease in the application of chemical inputs. The use of PGPR is gradually increasing in agriculture, as it offers an attractive way to reduce the use of chemical fertilizers, pesticides, and related agrochemicals (Rana et al., 2012). Use of PGPR for the improvement of micronutrient deficiency is promising due to its ecological, economic, and ecofriendly nature (Altier et al., 2013). Microorganisms are significantly different than competing with higher plants for micronutrients (Stevenson and Luxmoore, 1991). Among bacteria, a lot of attention has been dedicated to the siderophore-mediated Fe uptake by *Pseudomonas fluorescense*. PGPR constitute a significant part of the important protective flora that benefit plants by enhancing root function, suppressing disease, and accelerating growth and development (Glick and Bashan, 1997). Cyanobacterial strains have the potential to inhibit pathogenic fungi that may be playing a role in bringing about a significant increase with respect to macro- (NPK) and micronutrient concentration (Rana et al., 2012). This emphasizes the importance of PGPRs as an easy, direct, and economically favorable strategy for biofortification. The PGPR may help the plants to develop deeper roots in mineral-deficient soils, produce ligands/siderophores or acids/alkali to mobilize macro-/micronutrients. Biofortification of crops through application of PGPR can be therefore considered as a possible supplementary measure, which along with breeding varieties can lead to increased micronutrient concentrations, besides improving yield and soil fertility.

6.3.2 Arbuscular Mycorrhiza Fungi

Most plants, including all major grain crops and almost all vegetables and fruits, are associated with mycorrhizal fungi that improves plant growth and productivity by influencing soil fertility, decomposition, cycling of minerals and organic matter, and the uptake of essential mineral elements from soils. The most widespread belowground associations of microorganisms with plants, mycorrhizal symbioses helps their plant hosts to the rich source nutrients required for their growth. This provides a continuous flow of energy-rich compounds that are required for nutrient mobilization and also a channel for the translocation of mobilized products back to their hosts. The arbuscular mycorrhizal (AM) and ectomycorrhizal mycelia are the most investigated

association, which improve the acquisition of mineral nutrients, already available in solution by means of extraradical mycelia (Singh et al., 2016). AM also provide an extensive surface area or network for nutrient uptake from soils with the help of these mycelia as physical extensions of the root system, which increase the surface area across to reach the nutrients available, besides providing a direct pathway for translocation of photosynthetically derived carbon to microsites having better accessibility. Depending on plant photosynthates as energy sources, the extensive AM mycelial systems (the vegetative parts of the fungus) effectively explore soil substrates and acquire soil inorganic nutrients, including major macronutrients N, P, and K and micronutrients Cu, Fe, and Zn (Caris et al., 1998), with some capacity for acquiring organic N and P. These symbiotic fungi, therefore, change, directly or indirectly, the mineral nutrition of plant products that are also essential for human. However, the role of mycorrhizas on mineral biofortification may be exploited through agricultural practices. Mycorrhizas can potentially offer a more effective and sustainable biofortification to limit global human malnutrition. AM inoculation has also been proved to increase growth and Zn nutrition in Zn-deficient soils, owing to better access of roots to native Zn and Zn added as fertilizer (Kothari et al., 1990). The mobility of Zn and Fe in soils is low. As a result, uptake of these nutrients by roots is limited (Barea et al., 2002). When no micronutrients were added to the soil, available Zn and Fe levels were low and a “depleted zone” of these nutrients formed around the roots. As a result, the uptake of these nutrients was limited in nonmycorrhizal plants. Mycorrhizal plants take up more metal nutrients via extraradical hyphae, which provide larger surface areas than the roots alone and reduce the distance for diffusion, thereby enhancing the absorption of immobile metal nutrients (Jakobsen et al., 1992). AMI (M+) fungi are widespread and agronomically important plant symbionts and often stimulate plant uptake of nutrients, such as Zn and Fe in deficient soils (Liu et al., 2002). Despite the availability of evidence for a significant role of AMF for Zn, Cu, Fe, and, to a limited extent, Mn in crop plant nutrition, the role of AMF in crop plant fortification is still in its infancy.

7 BIOAVAILABILITY IMPROVEMENT

Plant foods contain certain biochemical compounds that can either inhibit (inhibitor or antinutrient) or can promote (promoter and prebiotics) the absorption and bioavailability of essential microelements from the food sources (Table 14.5). The amount of these promoters and inhibitors

Table 14.5 Antinutrients and promoters in plant influencing micronutrient bioavailability

Antinutrients	Major dietary sources	Promoters	Nutrient	Major dietary sources
Phytic acid or phytin	Whole legume seed and cereal grain	Certain organic acid (ascorbic acid, fumarate, malate, citrate, etc.)	Fe and Zn	Fresh fruits and vegetables
Fibers (e.g., cellulose, hemicelluloses, lignin, cutin, suberin, etc.)	Whole cereal grain product	Hemoglobin	Fe	Animal meats
Tannins and other polyphenolics	Tea, coffee, bean, sorghum	Certain amino acid (e.g., methionine, cysteine, histidine, and lysine)	Fe and Zn	Animal meats
Oxalic acid	Spinach leaves, colocasia	Long-chain fatty acids (e.g., palmitate)	Zn	Human breast milk
Hemagglutinins (e.g., lectins, goitrogens)	Leguminous and wheat	Fats and lipids	Provitamin A carotenoid	Animal and vegetable fat
Goitrogens	<i>Brassicas</i> and <i>Alliums</i>	Fe and Zn	Provitamin A carotenoid	Animal meats
Heavy metals (e.g., Cd, Hg, Pb, etc.)	Contaminated leafy vegetables, root crops	β -carotene	Fe and Zn	Green and orange vegetables
		Selenium Inulin and other nondigestible carbohydrates (prebiotics)	I Ca, Fe (?), Zn (?)	Sea foods, tropical nuts Chicory, garlic, onion, wheat, Jerusalem artichoke

Source: From Graham, R.D., Welch, R.M., Bouis, H.E., 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. *Adv. Agron.* 70, 77–142.

are environment and genotype dependent. Current plant molecular and genetic modifications, combined with plant-breeding approaches, make it possible to reduce or eliminate antinutrients from staple plant foods, or to significantly increase the levels of promoter substances in the foods (Frossard et al., 2000). Most of the promoters are the plant metabolites and oligogenic. Thus, breeding for increased levels of these promoters should be relatively simple compared to breeding for higher concentrations of essential trace minerals, through regulating the absorption, translocation, accumulation in the edible tissue, which involve polygenes and gene \times environment interaction (Graham et al., 2001).

7.1 Promoters

Some organic compounds stimulate absorption of essential mineral elements by humans even in the presence of inhibitor in the food. These include ascorbic acid (vitamin C), β -carotene (provitamin A), protein cysteine, and various organic and amino acids like nicotinamine phytoferritin.

Enriching diet with β -carotene and ascorbic acid can increase the bioavailability of nonhem Fe to human (García-Casal et al., 2000). The effect of β -carotene on Fe availability is the result of increasing Fe solubility through formation of a vitamin A-Fe complex or may be the result of an indirect effect of β -carotene on Fe absorption and incorporation in the red blood cells. On the other hand, ascorbic acid can reduce the effect of phytate, an important antinutrient that reduces the nonhem Fe bioavailability. It is found that there is considerable intraspecific variation in both ascorbate and β -carotene concentrations in fruit and vegetables (Frossard et al., 2000).

There is also appreciable intraspecific variation in amino acid concentrations in edible tissues (Guzmán-Maldonado et al., 2000). Cysteine is the only free amino acid that enhances Fe absorption in human bodies (Glahn and Van Campen, 1997). A group of cysteine-rich, low-molecular weight polypeptides like metallothionein proteins (MTs) are used for this purpose (Cobbett and Goldsbrough, 2002). NA is a nonprotein amino acid biosynthesized by all higher plants, which can form stable complexes with ferrous Fe (Fe^{2+}). Several studies have shown that increasing NA levels in plants through molecular transformations results in greatly increased Fe concentrations in edible parts. Further, the Fe accumulated in the seed as the Fe(II)-NA complex appears to be highly bioavailable (Bouis and Welch, 2010).

Amplification of the natural Fe store like ferritin (Fe storage protein) is a further strategy to increase Fe accumulation in bioavailable forms in plants. Recombinant soybean ferritin has been expressed in several cereal

crops under the control of an endosperm-specific promoter (Drakakaki et al., 2005) and pea ferritin has been constitutively expressed in rice (Ye et al., 2008). Another breakthrough in Fe biofortification is the development of plants expressing lactoferrin, a human Fe-binding protein present in milk that also has broad antimicrobial activity (Bethell and Huang, 2004). Recombinant human lactoferrin has been expressed in crops, such as potato (Chong and Langridge, 2000).

Development of plants with higher phytate-degrading activity may result in more extensive phytate degradation in the human stomach, thereby increasing Fe bioavailability. For example, varieties that had greater mineral bioavailability due to improved breakdown of phytate was related to higher phytase levels (Lopez et al., 2001).

Staple food crops can contain prebiotics, that is, food substances that simulate the growth of beneficial microbiota (probiotics) in the human gut, among which the most studied are the nondigestible carbohydrates, such as inulin (a fructooligosaccharide) (HarvestPlus, 2014). Application of these prebiotics can positively enhance the bioavailability of some mineral nutrients (e.g., Fe, Zn, Ca, and Mg) in plant foods through overcoming the negative effect of inhibitor like polyphenols and phytate. Additionally, these prebiotics can improve the health and the intestine's ability to absorb and utilize numerous nutrients, regulate the immune system, and protect against invasion by pathogenic organisms. Thus, increasing the levels of prebiotics in staple food crops could be an extremely important strategy to enhance the nutrition and health of malnourished people worldwide (HarvestPlus, 2014).

7.2 Inhibitors

Beside promoters, plant food also contains some inhibitors in differing amounts depending on both genetic and environmental factors that reduce the efficacy and bioavailability of the micronutrient. Among these phytates, polyphenols, and tannins are the most important limiting factors in the absorption of Fe, Zn, and other micronutrient in human beings (Mendoza, 2002). Phytate occurs widely in plant tissues and is mainly concentrated in seeds. Phytate and polyphenols can bind to the cations along with other dietary components because of their large negative charge, making the cations (Fe^{3+} , Zn^{2+} , and Cu^{2+}) insoluble and unavailable for absorption by mucosal cells in the gut. Different strategies like the addition of *Aspergillus niger* phytase at ambient pH and temperature conditions can completely degrade phytate. Unfortunately *A. niger* phytase is heat-sensitive and denatured at

60°C, therefore, use of a thermotolerant phytase from *Aspergillus fumigatus* (Wyss et al., 1999) can solve the problem. In another strategy, several low phytic acid (lpa) mutants have been produced by nontransgenic techniques in crops (Banziger and Long, 2000). Sometimes there is negative correlation between phosphorus (P) with Zn and Fe bioavailability, nitrate with I bioavailability. Tannin concentration in edible tissues also varies greatly between varieties (Lin et al., 2005). Hence, breeding for reduced concentrations of these antinutrients appears feasible. Additionally, these antinutrients, may play important beneficial roles in human diets by acting as anticarcinogens or reducing the risk of heart attack and diabetes (Murgia et al., 2012; Zhou and Erdman, 1995).

The bioavailability of micronutrients in plant foods can be greatly affected by the composition of the diet (House, 1999; Van het Hof et al., 2000), various food processing techniques, meal components, and meal preparation techniques. Consumption of animal protein with plant foods high in antinutrients, such as phytic acid, can ameliorate the negative effects of the antinutrients on Fe and Zn bioavailability (Welch, 1993; Welch and House, 1995) due to presence of the sulfur containing amino acid, cysteine, which acts as a promoter (Fig. 14.5).

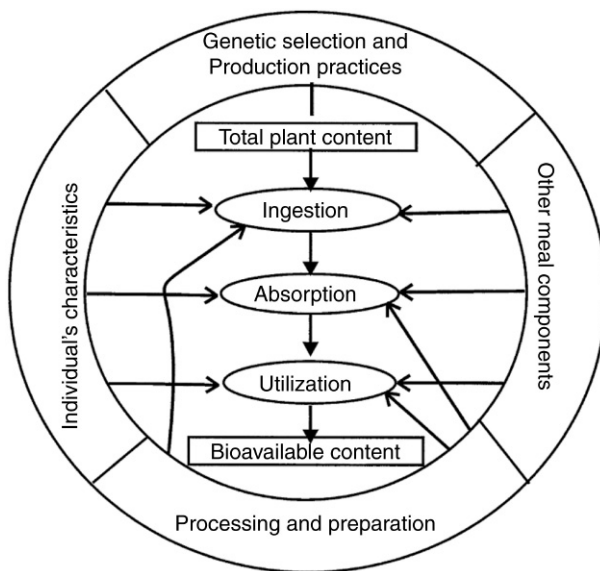


Figure 14.5 The complexities of bioavailability of nutrition in human guts. (from Welch, R.M. and Graham, R.D. 2003. Breeding for micronutrients in staple food crop from a nutrition perspective. *J. Exp. Bot.*, 55 (396), 353-364).

8 HORTICULTURAL CROPS TARGETED FOR BIOFORTIFICATION

Generally biofortification prioritizes the staple foods of the developing nation. The CGIAR (Consultative Group on International Agricultural Research) Micronutrients Project was established with an objective to assemble a package of tools that plant breeders could use to produce mineral and vitamin dense cultivars in wheat, rice, maize, beans, and cassava for Fe, Zn, and vitamin A (Bouis, 2003). However, among horticultural crops the main focus is given to the following fruits and vegetables: cassava, sweet potato, and banana for high provitamin A carotenoid content, potato, and common bean for high Fe and Zn content, cowpea for high Fe, spinach, onion, broccoli, cabbage, and lettuce for high Se content and tomato for I fortification. The Indian Agricultural Research Institute, New Delhi has also released several vegetable crops that are rich in vitamins and minerals, for example, vitamin A-enriched carrots, spinach, and pumpkin; vitamin C-enriched bitter melon, bathua, mustard, tomato, Fe, and Ca-enriched spinach and bathua and protein-enriched beans, lablab, French and garden peas. At the international level among the horticultural crops, the HarvestPlus Challenge Program (<http://www.harvestplus.org>), in collaboration with NARES (National Agricultural Research and Extension Services) and CGIAR centers carried out breeding activities for fortification of sweet potato, common bean, cassava, potato, banana, cowpea (Table 14.6).

8.1 Vitamin A Orange Sweet Potato (*Ipomea batatas*)

Sweet potato is widely consumed in sub-Saharan Africa (Woolfe, 1992). Most of the sweet potato varieties grown there are white-fleshed and thus lack β -carotene. International Potato Center (CIP) in South Africa and Uganda (HarvestPlus) and National Agriculture Research and Extension System (NARES) started a provitamin A biofortification project and the first biofortified crop with orange flesh was released in 2002. This variety has greater ability for provitamin A retention after boiling (80%–90%) or steaming (70%–75%) and also high-yielding and drought-tolerant (van Jaarsveld et al., 2006) breeders have produced several orange sweet potato (OSP) varieties with provitamin A content of 30–100 ppm, exceeding the target level of 32 ppm (HarvestPlus, 2014). Eight countries in Africa have released 46 improved sweet potato varieties since 2009, of which 31 are orange-fleshed. Biofortified varieties are now being introduced in many parts of Africa and South America, as well as China. In 2009, CIP launched its Sweet Potato for Profit and Health Initiative (SPHI), which seeks to deliver OSP to 10 million households in Africa by 2020.

Table 14.6 Biofortified crop varieties released globally

Crop	Target micronutrient	Micronutrient level (ppm)		Target countries	Leading institute	No. of varieties
		Base level	Target level			
Sweet potato	Provitamin A	2	32	Uganda South Africa Ghana Madagascar Malawi Mozambique Nigeria Rwanda Tanzania Zambia	CIP, NACCRI	77
Cassava	Provitamin A	0	15	DR of Congo Nigeria	IITA, CIAT, NRCRI	6
Banana	Provitamin A	10–18	17–106	DR of Congo Burundi Ghana Philippines Thailand New Guinea	Bioversity International	5
Bean	Fe Secondary: Zn	50	94	Rwanda DR of Congo Uganda	CIAT, RAB, INERA	19
Potato	Fe Secondary: Zn, vitamin C	19	48	Rwanda Ethiopia	CIP NARC	— ILL 7723
Cowpea	Fe Secondary: Zn	30	63	India	GBPUA and T, DBT	5

8.2 Vitamin A Cassava (*Manihot esculenta*)

Cassava is a perennial root crop native to tropical America and widely consumed in sub-Saharan Africa and parts of Asia. It is typically white-fleshed and classified as sweet or bitter, depending on the amount of cyanogenic compounds. Frequent consumption of cassava causes malnutrition of vitamin A, Fe, and Zn (Gegios et al., 2010). The strategy of HarvestPlus research

on cassava emphasizes developing genotypes with high concentrations of provitamin A carotenoids, mainly β -carotene, in the roots of agronomically superior varieties, as well as breeding for high Fe and Zn content. The International Center for Tropical Agriculture (CIAT), based in Cali, Colombia, coordinates the overall activities for cassava biofortification and has specific responsibility for research in Asia, Latin America, and the Caribbean; the International Institute for Tropical Agriculture (IITA), in Ibadan, Nigerian National Root Crops Research Institute (NRCRI) Nigeria, Institut National pour l'Etude et la Recherche Agronomiques (INERA) in the Democratic Republic of Congo (DRC) are responsible for cassava biofortification activities in Africa. The screening of genotypes revealed that genetic variation in Cassava roots for β -carotene is high (0–19 ppm) and orange-colored roots have 9–10 times more β -carotene as compared to white roots. First biofortified cassava varieties with 6–8 ppm β -carotene were released during 2011 (<http://www.harvestplus.org>). Research is being conducted to determine the retention of the β -carotene in yellow cassava roots after processing. Additionally, due to the presence of adequate variability in the Fe and Zn content of roots, work is going on to identify and select varieties that have both high provitamin A levels and good agronomic performance, with the highest Fe and/or Zn content (Chávez et al., 2000).

8.3 Vitamin A Banana/Plantain (*Musa paradisiaca*)

Breeding banana/plantain (*Musa*) is complex, as commercial varieties are sterile triploids (3X). Among the fertile groups, a high degree of cross incompatibility can exist. Further, the *Musa* crop cycle is long. Therefore, transgenic approach is suitable for banana biofortification. HarvestPlus initial screening for banana biofortification revealed high genotypic variation for provitamin A content (1–345 ppm) (<http://www.harvestplus.org>). Since 2006, Bioversity International has started work on vitamin A banana/plantain with the following objectives: germplasm screening from different regions; identification of proteins and enzymes responsible for the accumulation of provitamin A content; a genome-wide study of the main gene families involved in biogenesis carotenoid pathways; and, studies for nutritional profiling and bioaccessibility of provitamin A. Queensland University of Technology in Australia has developed transgenic bananas with β -carotene, vitamin E as well as Fe. The introduction of *psyB73* gene from maize and *crt1* from *Erwinia uredovora* led to the establishment of biosynthetic pathway for the production of β -carotene (<http://www.ogtr.gov.au>). In

India works initiated to transfer of specific traits for biofortification in two Indian banana varieties cv. Grand Nain and Rasthali (Prasad et al., 2015).

8.4 Iron Common Bean (*Phaseolus vulgaris*)

Common bean is the most common food legume in Latin America and eastern and southern Africa. It is a valuable candidate for biofortification because it is rich in protein, Fe, Zn, and certain other microelements that are generally found in low concentrations in most of the staple foods like cereal and tubers. The baseline Fe content of common bean is high at 55 ppm (Blair et al., 2010). HarvestPlus in collaboration with Center for Tropical Agriculture (CIAT) started biofortification program in Fe also considering Zn as a secondary trait. Initial screening revealed a satisfactory amount of genetic diversity for Fe (30–110 ppm) and Zn (25–60 ppm Zn) (Islam et al., 2002) and G14519 and G21242 are considered as high Fe genotypes in common bean. After screening and selection the promising high-Fe genotypes were utilized in crossing programs to combine the high-mineral trait in high yielding and acceptable agronomic background (Beebe et al., 2000). Finally, genotype \times environment (G \times E) interaction was tested and around 100 varieties were released within 2015 having nearly more than 100 ppm Fe content. With the help of advanced backcross population involving wild bean and cultivated bean, QTLs/genes for seed coat Fe and cotyledonary Fe and Zn were identified in two separate linkage groups (Blair et al., 2013). However, the most important QTL for Fe and Zn was identified and mapped on the same linkage group, which found to be overlapping for these traits (Blair et al., 2010). This indicates the pleiotropic nature of gene regulating high mineral trait and become a win–win opportunity for plant breeders for fortifying with these two micronutrients. The QTLs for Fe reductase activity in roots were also carried out (Blair et al., 2010). Interestingly, one of the QTLs identified for Fe content is localized with phaseolin locus, which encodes seed storage protein (Blair et al., 2009). On the other hand, ferritin is considered as a candidate for Fe accumulation, which is a storage protein for Fe and is colocalized with one of the QTLs (Blair et al., 2009, 2013).

8.5 Iron and Zinc Potato (*Solanum tuberosum* L.)

Potato is the staple food crop in many countries and contains high-quality protein, composed of all the essential amino acids. It is also a good source of energy, minerals (Cu, K, manganese, Mg, and phosphorus), vitamins (folate, ascorbic acid) (<http://nutritiondata.self.com/facts/>

[vegetables-and-vegetable-products/2770/](#)). Potatoes are also a good source of fiber, dry matter, and protein. Besides, this tuber also contains a variety of phytonutrients that have antioxidant activity. Among these, important health-promoting compounds are carotenoids, flavonoids, and caffeic acid, as well as unique tuber storage proteins, such as patatin, which exhibit activity against free radicals (Kawar and Kawar, 2016). Therefore, it is a valuable tuber crop, which can be utilized in fortification programs to combat micronutrient malnutrition. Micronutrient analyses of potatoes have focused primarily on Fe and Zn and the initial screening of germplasm accessions revealed 11–30 ppm Fe and 8–25 ppm Zn in existing potato varieties (<http://www.harvestplus.org>). Heritability of Fe and Zn concentrations in potato tubers is moderately high (Paget et al., 2014), and no negative correlation was found between micronutrient concentration and important resistance traits and tuber yield. Different promoters and inhibitors like ascorbic acid, phenols influenced the bioavailability of micronutrient absorption, were also considered. Fortunately, ascorbic acid is a promoter of Fe absorption present in potatoes. However, ascorbic acid is heat sensitive and a genotypic variation and cooking method significantly influenced the absorption and retention of micronutrient. HarvestPlus Challenge program in collaboration with CIP, Rwanda Agricultural Board (RAB), and the Ethiopian Institute for Agriculture Research (EIAR) conducted breeding as well as subsequent adaptive trials. Several promising clones have been screened out or evaluated and finally released conferring high Fe (35 ppm) supplemented with high Zn (29 ppm). Micropropagation of these promising clones is carried out for their distribution in the other targeted countries for nutritional enhancement (HarvestPlus, 2014).

8.6 Iron Cowpea (*Vigna unguiculata*)

Cowpea is generally cultivated and consumed by poor farming communities that derive their sustenance and livelihood from such crops (Carvalho et al., 2012). As a result, the cowpea is commonly referred to as poor man's meat, particularly among the inhabitants of rural areas and urban slums. HarvestPlus Challenge program started cowpea biofortification for Fe enrichment in collaboration with International Institute of Tropical Agriculture (IITA) responsible for cowpea biofortification in Nigeria followed by further collaboration with GB Pant University of Agriculture and Technology for the target country India (<http://www.harvestplus.org>). Initial screening in Nigeria identified considerable genetic diversity for the trait and identified genotypes having Fe content ranging 27–97 ppm and Zn content

23–62 ppm. Additionally, genotypes were also assessed for protein and total carotenoid contents. Studies revealed a positive correlation between protein, Fe, and Zn content. Biofortified couple lines were generated for high protein and minerals, including K, Ca, Fe, and Zn concentration (Santos and Boiteux, 2013). Within 2014, four biofortified cowpea varieties namely Pant Lobia-1, Pant Lobia-2, Pant Lobia-3, and Pant Lobia-4 enriched with Zn and Fe have been released in India, which are also photoinsensitive, heat tolerant, and early maturing. Some of the biofortified varieties are in the pipeline for release, which are resistant to important biotic stresses like yellow mosaic virus (HarvestPlus, 2014).

9 COST EFFECTIVENESS

Biofortified staple foods cannot deliver as high levels of minerals and vitamins per day as supplements or industrially fortified foods, but they can help to bring millions over the threshold from malnourishment to micronutrient sufficiency (Bouis, 2003). In principle, biofortification is an extremely attractive intervention, since a one-time investment in biofortified crop varieties yields a benefit stream year after year. The costs toward research are essentially the incremental costs for increasing or enhancing micronutrient density in food crops. These research costs are likely to be the single largest cost component of biofortification and are, of course, a one-time investment, which is incurred at the start of this program. There are virtually no recurring costs, except those involved in maintenance breeding, and in the case of fertilizer applications for agronomic intervention, in comparison, both fortification and supplementation involve recurring costs. It is also estimated that costs associated with plant breeding fall around an average of about US \$0.4 million/year/crop over a period of a decade globally (HarvestPlus, 2010). Moreover, where adequate and proper systems for dissemination of modern genotypes are in place (such as in South Asia), implementation costs are nil or negligible. However, in the case of developing countries, additional costs are incurred in establishing seed multiplication and delivery systems and creating both markets and consumer demand. For all crop–country combinations, biofortification can be rated as very cost effective, as costs are significantly below per capita income, which ranges from US \$365 in the Democratic Republic of Congo (DRC) to US \$3843 in India (Meenakshi et al., 2010). Therefore, biofortification interventions exhibit relatively high up-front costs in the first 6–10 years. These costs depend on many factors, such as the type of crop and micronutrient, size of

the target country, research infrastructure, and seed sector. As HarvestPlus and its partners make further progress in breeding and identifying cost-effective models for delivery, the costs to cover new regions and countries will decrease. The early phases of biofortification interventions generate public goods (e.g., knowledge about biofortified crops, breeding lines with high micronutrient content), which will need to be financed by governments and donors. In later phases, nutritional value becomes a standard target (i.e., mainstreamed) in public breeding programs, and biofortified germplasm is shared with the private sector, which can integrate it in its breeding programs so that in the long run, biofortification can be self-sustaining.

10 CHALLENGES

A penetrating stepwise analysis of challenges in biofortification reveals five sectors of goals that must be achieved to realize biofortification in horticultural crops, mostly vegetable, tuber, and fruit crops (Fig. 14.6). These are enlisted as follows:

- Setting appropriate target levels for the micronutrient content of biofortified staple foods to achieve adequate intakes.
- Retention of micronutrients in biofortified staple foods, that is, preserving the nutritional value.
- Bioavailability (Can plants provide a good source of bioavailable micronutrients?)
- Efficacy (determining the biological impact of biofortified crops in controlled feeding studies).
- Effectiveness (achieving population-level impact).

One of the major challenges in biofortifying vegetable and fruit crops is setting appropriate target levels for the micronutrient content in edible tissue to achieve adequate intakes. As a food-based strategy, the additional micronutrient intake resulting from biofortification would ideally be enough to fill the gap between current intakes and the amount that would result in the majority of the population having intakes above the theoretical mean dietary requirement level (the estimated average requirement, or EAR). However, with the use of this approach, there is not one unique answer that fits all possible target populations. Further, quantitative information on micronutrient intakes for most potential target populations does not exist or exists in very limited form. There are also differences in processing practices and inclusion of other foods that can result in large differences in the micronutrient content and bioavailability in the staple food. Again,

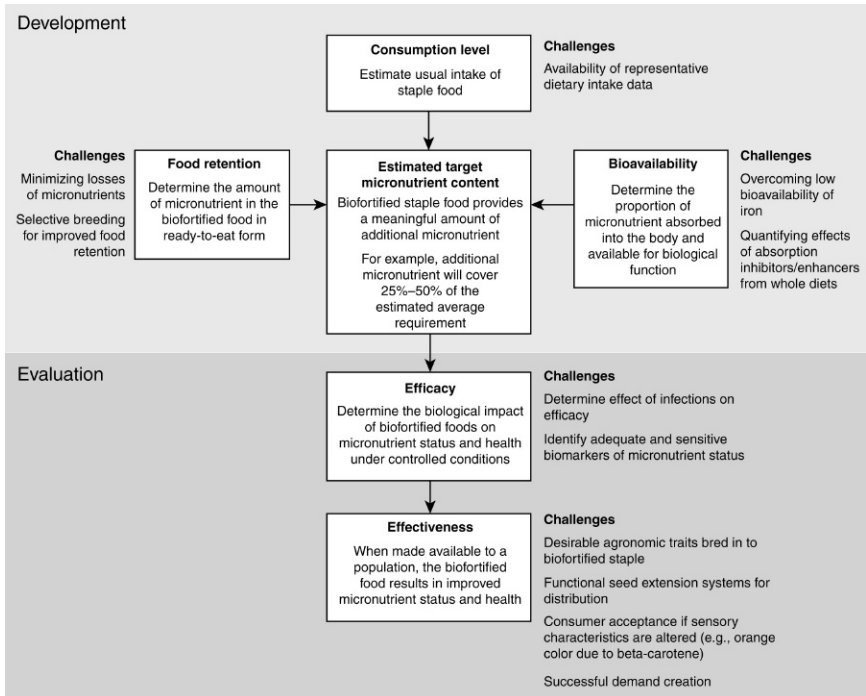


Figure 14.6 Summary of nutrition research and challenges for developing and evaluating biofortified foods as a viable public health intervention. (From Hotz, C., McClafferty, B., 2007. From harvest to health: Challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food Nutr. Bull.* 28 (2), 271–279).

as with universal fortification of staple foods, biofortification will lead to some degree of increased micronutrient intakes among individuals in all life stages. Progress in breeding to achieve target levels of nutrient contents is very promising for several crops. For example, in the coming years, meeting theoretical target levels for Zn and Fe will be achievable for beans, cowpea, and potatoes; and meeting target levels for provitamin A carotenoids may be achieved in bananas and plantains. Several crops simply do not contain enough natural genetic variation in provitamin A carotenoid content to expect reasonable increases in content through conventional breeding and therefore the use of transgenic approaches can be justified. Good potential, for example, exists for achieving important levels of provitamin A carotenoids in cassava. The target level for Fe will be difficult to achieve in most crops. This is largely because of the expected low bioavailability of Fe from most staple foods in unrefined form; very large increases in Fe intake would be needed to result in biologically important increases in the amount of

absorbed Fe. This justifies the importance of identifying and testing various plant compounds that can substantially improve the bioavailability of Fe, and of determining the impact and agronomic feasibility of reducing the content of known inhibitors of Fe bioavailability, mainly phytate (Hotz and McClafferty, 2007).

The second most important factor is to understand the retention of micronutrients in biofortified staple foods, that is, preserving the nutritional value. In humans, retinol is the main biologically active form of vitamin A. Plant foods do not contain vitamin A in the form of retinol, but some are rich sources of provitamin A carotenoids that can be converted to vitamin A in the body. Losses and degradation of provitamin A carotenoids with storage, processing, and cooking are potentially important and need to be quantified in biofortified crops. In horticultural crops, orange-fleshed sweet potato is a very rich source of the major provitamin carotenoid, β -carotene. The majority of β -carotene is maintained after sweet potatoes are boiled. For example, the true food retention of β -carotene of the orange-fleshed sweet potato ranged from 83% to 92%, depending on the exact boiling procedure. In contrast, processing of sweet potatoes to make flour can result approximately 60% to less than 15% food retention of β -carotene, depending on the variety (Hotz and McClafferty, 2007). For cassava, preliminary data suggest that the mean food retention of carotenoids after boiling is about 50%, but it ranges widely from 27% to 83%, depending on the specific genotype (Chávez et al., 2007). Once they are verified, factors responsible for higher food retention of β -carotene may become other traits for which cassava is selectively bred. The highly variable and potentially low food retention of provitamin A carotenoids in different staple foods highlights the need to accumulate further data on food retention and to take them into account when setting appropriate target levels for provitamin A carotenoid content in biofortified foods. Losses of Fe and Zn with typical household cooking procedures are not typically very high in regard to vegetables. For example, in cooked beans, 75%–90% of Fe and 85%–90% of Zn is retained, depending on the boiling time and whether water is drained.

The third challenge would be to understand bioavailability (Can plants provide a good source of bioavailable micronutrients?). Provitamin A carotenoids must be released from the plant matrix and incorporated into micelles, absorbed, and then cleaved to form retinol. The conversion of provitamin A carotenoids is not 100% efficient, thus making plant-based diets poorer sources of vitamin A activity. The efficiency with which provitamin

A carotenoids are converted to retinol in humans is often expressed as the retinol activity or equivalency. It has generally been assumed that 12 μg of all-*trans*- β -carotene is required to produce 1 μg of retinol (West et al., 2002) although this equivalency rate depends on factors, such as the food matrix and the presence of dietary fat, which improves carotenoid bioavailability. The retinol equivalency of provitamin A carotenoids from specific foods has largely been determined from the change in serum retinol concentration after consumption for a specified period of time, although this is not the most reliable way of determining retinol equivalency. Stable isotope tracer techniques have been developed and, when combined with long-term consumption, can be used to quantify changes in retinol stores (Haskell et al., 2005). The role of promoters and nutrients regarding the interaction with Fe and Zn during digestion to alter their bioavailability has already been discussed.

The next succeeding challenge is determining efficacy (the biological impact of biofortified crops in controlled feeding studies). In order to be accepted as a viable, cost-effective strategy to improve the health and development of micronutrient-deficient populations, the biological impact of biofortification needs to be demonstrated. Studies to determine the efficacy of biofortified foods measure the effect of consumption on biological and/or functional indicators of micronutrient status under controlled study conditions in comparison to a similar but nonbiofortified genotype of the same staple food (Hotz and McClafferty, 2007).

The final challenge ascertains effectiveness (achieving population-level impact). Even when they have been shown to be efficacious, the large-scale impact of biofortified crops on the micronutrient status of populations will depend on many factors unrelated to nutrition. To be accepted by farmers and consumers, the new biofortified crops will have to have an equivalent yield, good resistance to biotic and abiotic stresses, and acceptable sensory and cooking qualities. The existence of an effective seed and rural extension system for multiplication and dissemination of new varieties will also be important for an effective biofortification program. Although increased Fe and Zn contents of crops are very unlikely to cause changes in color or taste, increased content of provitamin A-rich biofortified foods will result in a noticeable color change and could potentially affect taste. Studies to assess consumer preferences and perceptions are needed. If the problem is not insurmountable, a strong demand creation component will be needed, including effective nutrition education and social marketing, to promote consumption of such products. Good success was achieved with

the introduction of orange-fleshed sweet potato in Mozambique by using intensive demand creation (Hotz and McClafferty, 2007)

11 FUTURE NEEDS

Biofortification for the future needs to amalgamate several disciplines of study, and not limited to plant-breeding, plant physiology, biotechnology, and agronomy toward producing micronutrient rich crops. At the same time screening, identification, and selection of local landraces and wild types having higher potential to assimilate and accumulate micronutrient and their utilization in breeding can be of utmost importance for future biofortification programs. Elucidation of the mechanisms of mineral translocation from soil to seed, fruit, or other consumable edible parts in vegetable and food crops has to be conducted to realize this objective. Hence, advanced knowledge in the basic understanding of the rate-limiting steps of micronutrient acquisition and translocation in soil-plant system should be generated. Biofortified crops have to be analyzed in detail for safety issues before making them available to the consumers. Important information lapses exist in bioavailability of micronutrients in agronomic and horticultural crops and mineral distribution pattern in plant system. The loss of micronutrients during processing has not been properly documented for most of the crops and needs to be explored. Some of the important strategies would be transferring genes for higher Fe and Zn content through molecular cytogenetics, minimizing the loss of micronutrients during postharvest processing by uniform allocation of minerals in the edible tissue, manipulation of phytic acid level to enhance the bioavailability, etc. Recently, fertilizer products of nanoscale levels are viewed as a potential agro-input for precise micronutrient management even at very low application rates. Consequently, strategic utilization of these nanobased micronutrient fertilizers may help in biofortification process. To date, the biofortification technique is confined to some major crops and some crops with local relevance. In the same line, there is a need to explore all the crops that are directly or indirectly associated with micronutrient deficiencies. However, before we use this tool effectively for mitigating micronutrient malnutrition, some questions always remain about its scientific feasibility, adoption probability at farmers and consumer level, economic viability, and production stability (Nestel et al., 2006). In this background, the success of a biofortification program is directly associated with improved policies, including nutrition education, marketing, agricultural policy, and finally public acceptance (Singh et al., 2016).

12 CONCLUSIONS

In the future, mineral and vitamin deficiencies are expected to be more threatening, and biofortification strategy is a potential tool for addressing the problem. Given a low-cost, easy, and crop-based approach, biofortification techniques hold a great promise for mitigating the micronutrient malnutrition problem in the developing world. Significant progress has been made in this line, and future strategic research and appropriate policy could lead to biofortification as a great success in the coming years to change billions of people from nutrient deficiency to nutrient sufficiency.

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CHAPTER 15

Biofortified Vegetables for Improved Postharvest Quality: Special Reference to High-Pigment Tomatoes

Riadh Ilahy*, Mohammed Wasim Siddiqui**, Imen Tlili*,
Chafik Hdidder*, Nouri Khamassy*, Marcello Salvatore Lenucci†

*National Agricultural Research Institute of Tunisia, Tunis, Tunisia

**Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

†University of Salento, Lecce, Italy

1 INTRODUCTION

Biofortification is the development of micronutrient-/phytonutrient-rich staple crops using efficient traditional breeding techniques or modern biotechnology tools (Nestel et al., 2006). However, even if in some cases, the use of transgenic approach is crucial, consumer concerns about genetically modified organisms is increasing, which shifts most of the efforts toward the use of naturally biofortified crops. The valorization of naturally biofortified crops is of great importance since the differences with currently and ordinary grown cultivars (cvs) is clearly visible [high-pigment (*hp*) and purple tomatoes, and colored potatoes].

Tomatoes contain a plethora of nutritional and bioactive nonnutritional compounds contributing to the functional and postharvest quality of tomato fruit. Breeding objectives have changed over time with the cvs released and modifications of growing systems. The breeding history has passed through four phases: breeding for yield in the 1970s, for shelf life in the 1980s, for taste in the 1990s, and since then for nutritional value (Bergougnoux, 2014). Although the *hp* and purple tomatoes exemplify the later alternative, they are recently considered also as a starting point for multiple-trait breeding since those mutants exhibit, besides higher functional quality, extended shelf life as compared to ordinary tomato genotypes (Butelli et al., 2008; Siddiqui et al., 2015).

The potato is the fourth most important food crop in the world after rice, wheat, and maize, and is the only major food crop that is a tuber.

Besides being a rich source of starch, potatoes contain also good quantity of secondary metabolites that play a key role in a number of processes (Friedman, 1997; Ilahy et al., 2013). Many of the compounds present in potato are important because of their positive effects on human health, which make them highly desirable in the human diet (Katan and De Roos, 2004). Postharvest practices widely change the nutritional composition in white-fleshed potato tubers. However, a lower impact was detected on colored potato cvs. In this context, similarly to *hp* and purple tomato cvs, colored potato cvs can be used for multitrail breeding as well.

Although these aspects are increasingly supported by recent findings, limited data links directly the postharvest quality in various crops, particularly *hp* and purple tomato, as well as colored potato cvs to their initial antioxidant content.

In this review we will attempt to review the importance of the beneficial phytochemicals in some biofortified crops (*hp* and purple tomatoes, and colored potatoes) and to highlight the possible impact of such phytochemicals in postharvest quality of such crops with a special reference to *hp* tomato cvs.

1.1 High-Pigment Tomato

Besides the economic importance and the nutritional value of its fruits, tomato (*Solanum lycopersicum* L.), one of the most important agricultural crops worldwide, is rich in a plethora of natural antioxidants and bioactive compounds.

Tomatoes contain different classes of substances with antioxidant properties contributing to the functional quality of the fruit. Breeding objectives have changed over time with the cultivars released and modifications of growing systems. The breeding history has passed through four phases: breeding for yield in the 1970, for shelf-life in the 1980, for taste in the 1990 and since then for nutritional value (Bergougnoux, 2014). Although, the *hp* mutations exemplify the later alternative, such genetic resources are being considerably being used for multi targeted breeding (both shelf-life and functional quality).

Several *hp* tomato mutants have been reported in the literature. Among these are the *hp1* (*hp1*, *hp1w*), *hp2* (*hp2*, *hp2j*, *hp2dg*) (Levin et al., 2006), *hp3* (Galpaz et al., 2008), and Intense pigment (Ip) (Lavi et al., 2009). The spontaneous mutant *hp-1* was first identified in 1917 at the Campbell Soup Company farms (Riverton, NJ) (Reynard, 1956). However, the *hp-2* mutant was reported in the Italian tomato cv San Marzano in 1975 (Soressi, 1975).

The *hp-1* and *hp-2* mutations map to different chromosomes (Van Tuinen et al., 1997; Yen et al., 1997). The locus of the *hp-1* mutation has been mapped to chromosome 2 adjacent to the 45 s rDNA locus, and it codes for DNA Damage-Binding Protein1 (DDB1) (Lieberman et al., 2004; Liu et al., 2004; Yen et al., 1997). However, the *hp-2* gene maps to tomato chromosome 1 and encodes the Arabidopsis (*Arabidopsis thaliana*) homolog of Deetiolated1 (DET) (Mustilli et al., 1999; Van Tuinen et al., 1997). The *hp-3* shares some *hp* mutant characteristics. However, unlike *hp-1* and *hp-2*, *hp-3* is not a photomorphogenic mutant, but rather it harbors a mutation in the zeaxanthin epoxidase (ZEP) gene, which reduces the abscisic acid (ABA) levels in this mutant (Galpaz et al., 2008). The tomato intense pigment (*IP*) genotype was originated from a cross between the wild species (*Solanum chmielewskii*) and the cultivated tomato (*Solanum lycopersicum*). It has been characterized as having both increased content of soluble solids (Azanza et al., 1994; Rick, 1974; Robinson et al., 1988) and visual pigmentation of its immature, as well as mature fruits (Kerckhoffs et al., 1997). Similar observations were reported also for *hp* tomato cvs (Ilahy et al., 2011a,b), which suggests that besides their high lycopene content, those cvs exhibit also various agronomic desirable traits, such as high soluble solids and extended shelf life with respect to ordinary tomato cvs.

High-pigment tomato cvs have been used in various breeding programs aiming, first, primarily to produce fresh market tomatoes with desirable postharvest quality. Then, as consumers have become increasingly concerned about physicochemical, organoleptic, and health-giving qualities of tomato fruits, the aim shifted to producing high functional quality fruits.

1.2 Purple Tomato

Fruits and vegetables are essential components of the human diet, and their concentrations of bioactive compounds beneficial to human health is becoming an important quality trait (Ilahy et al., 2011a,b, 2016). Recently, considerable attention is being given to the presence of anthocyanins in tomato fruits (Butelli et al., 2008; Gonzali et al., 2009; Lila, 2004). Regarding their beneficial health effects, anthocyanins and flavonoids are increasingly becoming the most important targets for improving the nutritional value of foodstuffs. Anthocyanins are natural bioactive pigments that have beneficial health effects but, unfortunately, are not present or present at very low levels in tomato fruits.

Regarding the beneficial effects of anthocyanins supported by recent investigations, Butelli et al. (2008) highlighted the interest in the production

of tomato cvs enriched with these valuable bioactive compounds. In this context, a purple tomato cv highly enriched with anthocyanins was developed. The line with the strongest visible pigmentation accumulates 3 mg/g fresh weight (fw) anthocyanins (Butelli et al., 2008), which is the highest value obtained so far in this species. This concentration is comparable to the levels found in blackberries and blueberries. In addition to being enriched with anthocyanin, these fruits also prolonged the life of cancer-susceptible mice, suggesting that they have additional health-promoting effects. In fact, these particular compounds boost nutritional value, and various health-promoting activities are increasingly ascribed to them (Butelli et al., 2008; Gonzali et al., 2009).

Nevertheless, it is widely recognized that tomato antioxidant act as a whole “portofolio” of compounds in balanced concentrations. Those compounds synergize in various levels to exert their positive effects (antioxidant, antiradical, radical scavengers, chain breaking...). The production of purple tomatoes where the biosynthetic pathway was switched for the production of anthocyanins at the expense of other basic tomato antioxidants, such as lycopene will probably lead to unbalanced dietary effects in vivo, even if anthocyanin in vitro effect is confirmed.

2 BENEFICIAL PHYTOCHEMICALS IN TOMATO FRUITS

Tomatoes have gained attention as a source of carotenoids, mainly lycopene and β -carotene, vitamins E and C (Abushita et al., 2000; Ilahy et al., 2016; Lenucci et al., 2006). Both ascorbic acid (AsA) and its oxidized form, DHA, contribute to vitamin C content. Vitamin E is essential for normal growth and development of the human body, and its deficiency often leads to clinical abnormalities. It has also been proposed that vitamin E enhances some biological properties of lycopene, such as inhibition of cell proliferation (Pastori et al., 1998) and LDL oxidation, and reduction of aortic valve lesion (Balestrieri et al., 2008). Yellow, tangerine, apricot, orange, orange-red, *hp*, and purple tomato cvs differ not only by their flesh color but also by their carotenoid contents (Frecknall and Pattenden, 1984).

2.1 Vitamin E

R,R,R- α -tocopherol is the most biologically active form of vitamin E. Lenucci et al. (2006) reported that α -tocopherol content was very high in *hp* tomato hybrids attaining 22 mg/kg fw in HLY13 and 16 mg/kg fw in HLY18 (Table 15.1). In mature tomato fruits, naturally occurring mutations

Table 15.1 α -Tocopherol ascorbic acid and dehydroascorbic acid of high-lycopene tomato cvs

Attribute(s)	Country	Range	Reference(s)
α -Tocopherol (mg/kg fw)	Italy	6–22	Lenucci et al. (2006)
AsA (mg/kg fw)	Tunisia	125.3–186	Ilahy et al. (2016)
	Italy	121–157	Lenucci et al. (2006)
	India	280.6–400	Siddiqui et al. (2014)
DHA (mg/kg fw)	Tunisia	86.9–150.6	Ilahy et al. (2016)
	Italy	0–110	Lenucci et al. (2006)
	India	n.d.	

that increase carotenoid content, including lycopene, are also characterized by a dramatic increase in plastid biogenesis and in the production of other compounds, such as vitamin C and flavonoids (Mochizuki and Kamimura, 1984). Regarding purple tomatoes enriched with anthocyanin, information on their content of α -tocopherol is not available. However, it seems that engineering metabolic pathways toward the overproduction of anthocyanin phenolic will be at the expense of other metabolic pathways among other metabolic pathways in tomato fruits.

2.2 Ascorbic Acid

AsA is taken as an index of quality for fresh product. It has been reported that tomato fruit has a moderate amount of vitamin C (20 mg/100 g of fw). Ilahy et al. (2011b) reported that total vitamin C content attained 352.8 mg/kg fw in the high-lycopene tomato cv HLY13. Generally, higher total vitamin C content was detected in high-lycopene tomato cvs compared to ordinary cvs. The pattern of vitamin C during ripening was also genotype dependent. Since higher AsA content was observed in red-fruited cvs compared to yellow-fruited cvs, high-lycopene tomato cvs seems to accumulate higher AsA content than traditional cvs. In a recent trial, Ilahy et al. (2016) reported that among the three fractions (peel, pulp, and seeds), skin fraction contained the highest levels of ascorbic acid, which ranged from 261.5 to 344.6 mg/kg fw in high-lycopene tomato cvs compared to 170.5 mg/kg fw in the ordinary cv Rio Grande (Table 15.1).

2.3 Carotenoids

Tomatoes are a good source of carotenoids, lipophilic pigments synthesized and stored within chromoplasts (Ilahy et al., 2015). Lycopene is the major

Table 15.2 Carotenoid content of high-lycopene tomato cvs

Attribute(s)	Country	Range	Reference(s)
Total carotenoids (mg/kg fw)	Tunisia	200–277.71; 167–280	Ilahy et al. (2009, 2016)
	Italy	252.3	Ilahy et al. (2011b)
	India	n.d.	
Lycopene (mg/kg fw)	Tunisia	118.2–210.15	Ilahy and Hdidier (2007)
	Italy	175–253	Lenucci et al. (2006)
	India	30.5–41.8	Siddiqui et al. (2014)
β -Carotene (mg/kg fw)	Tunisia	8–19.4	Ilahy et al. (2011a); Hdidier et al. (2013)
	Italy	11–20	Lenucci et al. (2006)
	India	n.d.	
	Israel	11.2	Levin et al. (2003)

carotenoid present in red-ripe tomato fruits, with β -carotene is present in lower amounts. Lycopene is a powerful natural antioxidant. It exhibits the highest physical quenching rate constant with singlet oxygen among dietary carotenoids (Agarwal and Rao, 2000; Di Mascio et al., 1989; Stahl and Sies, 1996), thus reducing the risk of several important pathologies, such as cardiovascular diseases and some cancer typologies (Clinton, 1998; Rao, 2006). Ordinary tomato cvs accumulate lycopene levels not higher than 150 mg/kg fw (Ilahy et al., 2009, 2010, 2011a,b, 2016). However, *hp* mutations (*hp-1*, *hp-2*, *hp-2j*, *hp-2dg*, and *ip*) are best known for their positive effect on carotenoid content, mainly lycopene in red-ripe fruits (Ilahy et al., 2016; Lenucci et al., 2009; Levin et al., 2004; Mochizuki and Kamimura, 1984). Many authors highlight significant variations in carotenoid content among high-lycopene tomato varieties. Lenucci et al. (2006) reported that lycopene values ranged from 175 to 253 mg/kg fw in cvs HLY13 and Kalvert respectively (Table 15.2). Ilahy and Hdidier (2007) and Ilahy et al. (2009, 2010) reported that lycopene content was 52%–170% higher in high-lycopene cvs compared to traditional tomato cvs.

High-lycopene tomato berries also contain moderate amounts of α - and β -carotenes and lutein (Ilahy et al., 2011b; Lenucci et al., 2006). In field trials conducted in Southern Italy, Ilahy et al. (2011a) and Hdidier et al. (2013) reported β -carotene levels ranging from 8 to 19.4 mg/kg fw in *hp* cvs HLY02 and HLY18 respectively compared to 5.8 mg/kg fw in the ordinary cv Donald. Similarly, Bino et al. (2004) reported 200% higher β -carotene content in *hp* tomato cv Manapal with respect to its isogenic counterpart.

Tomato color mutants contain different carotenoid compositions. In fact, yellow tomato mutants contain phytofluene, α -carotene, substantial levels of neurosporene, and high content of β -carotene. Tangerine tomato mutants accumulate *cis*-phytofluene, α -carotene, β -carotene, and high levels of phytoene, *cis*-neurosporene, and lycopene. Apricot tomato mutants accumulate phytofluene, neurosporene, *cis*-lycopene, α -carotene, and substantial levels of β -carotene. Orange tomato mutants contain phytoene, α -carotene, neurosporene, and high levels of β -carotene. High-pigment tomato cvs accumulate very high levels of various carotenoids (phytoene, phytofluene, lycopene, and β -carotene). Other carotenoids are also present at lower concentration, such as neurosporene, *cis*-neurosporene, and α -carotene (Frecknall and Pattenden, 1984).

2.4 Phenolics and Flavonoids

Phenolic compounds are important secondary metabolites in plants. The antioxidant activities of total phenolics in several plant extracts have been largely reported, suggesting their preventive role in reducing the risk of cardiovascular diseases in humans. Tomato berries are also a good source of flavonoids that accumulate especially in the peel (Ilahy et al., 2016). Flavonoids are health-protecting components in the human diet because of their capacity to activate endogenous antioxidant defense systems and signaling pathways (Meiers et al., 2001; Williams et al., 2004). It has been reported that high-lycopene tomato cvs accumulate considerable levels of phenolics and flavonoids. The advanced breeding line HLT-F51 showed the highest total phenolics and flavonoid contents (259.31 mg gallic acid equivalents (GAE)/kg fw and 473.74 mg rutin equivalents (RE)/kg fw respectively) compared to the cv Rio Grande (171.0 mg GAE/kg fw and 132.59 mg RE/kg fw respectively) (Ilahy et al., 2009). Ilahy et al. (2011a,b), studying different high-lycopene tomato cvs, reported total phenolics levels ranging from 105.8 mg GAE/kg fw in cv Lyco 1 to 394.5 mg GAE/kg fw in cv HLY 02 and flavonoids content ranging from 105.6 mg RE/kg fw in cv Lyco 2 to 511.9 mg RE/kg fw in cv HLY 13 (Table 15.3).

Regarding purple tomato cvs, recently Espin et al. (2016) compared the phenolic profile in tomato cvs consisting of two yellow cvs, Caltura and Pelileo, and two purple cvs, namely, Giant Purple and New Zealand purple. The authors found that the main anthocyanin compounds in purple tomato cvs were delphinidin 3-O-rutinoside, cyanidin 3-O-rutinoside, and pelargonidin 3-O-rutinoside. The total anthocyanin concentration ranged from 102.35 to 168.88 (mg/100 g dw).

Table 15.3 Total phenolics and total flavonoids of high-lycopene tomato cvs

Attribute(s)	Country	Range	Reference(s)
Total phenolics (mg GAE/kg fw)	Tunisia	216.1–256.2	Ilahy et al. (2016)
	Italy	1200–1330	Lenucci et al. (2006)
	India	179.1–301.4	Siddiqui et al. (2014)
Total flavonoid (mg RE/kg fw)	Tunisia	222.0–552.1	Ilahy et al. (2016)
	Italy	168–470	Lenucci et al. (2006)
	India	120–200	Siddiqui et al. (2014)

3 ANTIOXIDANT PROPERTIES

The evaluation of antioxidant potential is becoming increasingly pertinent in the food industry as it provides useful information with regard to the health-promoting and functional quality of raw material without the analysis of each single compound (Siddiqui et al., 2014). Ilahy et al. (2011b) assessed hydrophilic (HAA) and lipophilic antioxidant activity (LAA) of different high-lycopene and ordinary tomato cvs. HAA values in tomato pulp ranged from 155 to 266 μM Trolox/100 g fw compared to 114.4 μM Trolox/100 g fw in Rio Grande. The LAA in high-lycopene tomato pulp was higher and ranged from 237.3 to 340.4 μM Trolox/100 g fw compared to 139.9 in Rio Grande (Table 15.4).

Regarding purple tomato cvs, Espin et al. (2016) reported that the antioxidant activity in giant purple and New Zealand purple tomato cvs was very high using various antioxidant activity assays (FRAP, TEAC, and ORAC) (Table 15.5). The obtained values were similar or higher than those

Table 15.4 Hydrophilic and lipophilic antioxidant activity of high-lycopene tomato cvs

Attribute(s)	Country	Range	Reference(s)
HAA	Tunisia	185–266 μM Trolox/100 g fw	Ilahy et al. (2016)
	Italy	2.67–4.07 mM FRAP/g fw	Lenucci et al. (2006)
LAA	India	n.d.	
	Tunisia	237.3–340.4 μM Trolox/100 g fw	Ilahy et al. (2016)
	Italy	1.75–2.67 mM FRAP/g fw	Lenucci et al. (2006)
	India	n.d.	

Table 15.5 Antioxidant activity of different purple tomato cvs

	Antioxidant capacity (TEAC values)*		
	FRAP	ABTS	ORAC
Giant purple	15 ± 0.2a	70 ± 0.8a	202 ± 1.4a
New Zealand purple	50 ± 0.8b	89 ± 1.2b	325 ± 3.8b

Each value represents the mean ± SD ($n = 6$). Values for the same antioxidant capacity assay followed by different letters are significantly different by ANOVA test ($P < 0.05$).

* TEAC values: μmol Trolox showing the same antioxidant capacity as a gram of dry pulp.

Source: Adopted from Espin, S., Gonzalez-Manzano, S., Taco, V., Poveda, C., Ayuda-Durán, B., Gonzalez-Paramas, A.M., Santos-Buelga, C., 2016. Phenolic composition and antioxidant capacity of yellow and purple-red Ecuadorian cultivars of tree tomato (*Solanum betaceum* Cav.). Food Chem. 194, 1073–1080.

of *hp* tomato cvs. Although the reported increased antioxidant activity in those purple cvs, their marketability and consumption will have a major hurdle to overcome related to consumer concerns about GMOs and metabolic engineering in food crops as well.

4 TOMATO HEALTH BENEFITS

Increased consumption of fresh or processed tomato products (canned tomatoes, sauce, juice, ketchup, soup, etc.) is directly associated with a reduced risk of contracting several widespread human pathologies, including cardiovascular diseases; prostate, lung, and stomach cancers; osteoporosis; and UV radiation-associated skin disorders (Erdman et al., 2009; Fernández-García, 2014a,b; Qu et al., 2013; Soares et al., 2014). Flavonoids, phenols, ascorbic acid (vitamin C), tocochromanols (vitamin E), and carotenoids, mainly lycopene, are important bioactive molecules of ripe tomato fruits (Ilahy et al., 2015, 2016; Lenucci et al., 2006). These compounds synergize to exert positive effects on human health through oxidative and still not fully understood nonoxidative mechanisms (Erdman et al., 2009; Fernández-García, 2014a,b; Soares et al., 2014). Consequently tomato fruits are increasingly considered as “functional food” (Ilahy et al., 2011a,b, 2015, 2016; Siddiqui et al., 2014).

Regarding purple tomato cvs rich in anthocyanin particularly, various health-promoting activities were ascribed to these compounds (Clifford, 2000). Many studies have linked those compounds with antioxidant, anti-inflammatory, and anticarcinogenic properties; protection against heart and cancer diseases; and the reduction in the risk of diabetes and cognitive function disorders (Pojer et al., 2013; Tsuda, 2012). However, it should be underlined that most of the clinical studies were conducted

using ordinary and/or naturally biofortified tomato fruits/products such high-lycopene tomatoes. Engineered tomato crops, such as purple tomato cvs should be submitted to clinical trials (supplementations/intervention studies) to confirm effective *in vivo* antioxidant activity and beneficial effects since *in vitro* antioxidant activity will not lead to the same effect *in vivo* (Erdman et al., 2009).

5 COLORED POTATOES

Besides starch, potatoes is a source of secondary metabolites, which play a key role in a number of processes (Friedman, 1997; Ilahy et al., 2013). Many secondary metabolites in potatoes are important because of their positive effects on human health, which make them highly desirable in the human diet (Katan and De Roos, 2004). Although the consumption of white-fleshed potato tubers is the highest with respect to other colored potato cvs, recently attention has increasingly shifted to colored potato tubers due to their attraction pigments with supposed beneficial effects for human health (Ilahy et al., 2013).

White-, yellow-, and dark yellow-fleshed potatoes contain carotenoids, phenolics, and flavonoids, with dark yellow-fleshed potato cvs containing the highest total carotenoid content (up to 1000 $\mu\text{g}/10\text{ g fw}$). However, purple- and red-fleshed potato cvs contain anthocyanins, with purple-fleshed potatoes containing the highest anthocyanin levels (368 mg/10 g fw) (Ezekiel et al., 2013; Lewis et al., 1998).

5.1 Phenolic and Flavonoids

Polyphenols include more than 8000 identified substances divided into groups according to their chemical structure (phenolic acids, stilbenes, coumarins, lignins, and flavonoids) (Ross and Kasum, 2002). Polyphenols are the most abundant antioxidants in our diet (Manach et al., 2005), and potato tubers represent a major source of these compounds. Phenolic compounds represent a large group of minor chemical constituents in potatoes, which play an important role in determining their organoleptic properties. Additionally, phenolics have a wide array of health-providing characteristics (Bravo, 1998) and therefore have potential for use as functional food for improving human health.

Purple- and red-skinned tubers contained twice the concentration of phenolic acids as white-skinned tubers. Tuber flesh contained lower concentrations ranging from 100 to 600 μg of phenolic acids

and 0 to 30 μg of flavonoids. It was also reported that purple- or red-fleshed cvs had three to four times the concentration of phenolic acids of white-fleshed cvs. All of these data suggest that integrating the new colored potato tubers in the diet in various forms will be beneficial for human health by increasing the intake of valuable compounds with high antioxidant activity.

Flavonoid content in potatoes ranged from 200 to 300 $\mu\text{g}/\text{g}$ fw (Lewis et al., 1998). The flavonoids, in order of abundance in potato tubers, were reported to be catechin, epicatechin, erodictyol, kaempferol, and naringenin (Brown, 2005). Flavonols, such as rutin are also present in potato. The flavonol content of potato is not significantly high, but these could be considered as a valuable source of these compounds because of their high consumption (Tudela et al., 2002).

Anthocyanins, a subgroup within the flavonoids, unlike tomato fruits, are present in substantial amounts in pigmented flesh potatoes. Anthocyanin levels ranged from 5.5 to 35 mg/100 g fw in potatoes (Brown, 2008). Lewis et al. (1998) reported that purple- or red-fleshed cvs accumulate twice the flavonoid content of white-fleshed cvs, with concentrations being considerably higher in skin, approaching 900 mg in purple-fleshed and 500 mg in red-fleshed types per 100 g fw.

Red- and purple-fleshed potatoes had acylated glucosides of pelargonidin, while purple potatoes had, in addition, acylated glucosides of malvidin, petunidin, peonidin, and delphinidin (Brown, 2005; Lachman and Hamouz, 2005). Fossen and Andersen (2000) reported that the anthocyanins of the purple-fleshed (cv Congo) potatoes consisted of ferulyl gluco- and ghamno-pyranosides of malvidin and petunidin, novel anthocyanins. Besides, potato peel contained quercetin, a flavonol with antioxidant activity; such activity in flavonols has been attributed to their action as free radical acceptors. Red purple potato tubers accumulate higher levels of neochlorogenic acid, caffeoyl putrescine, chlorogenic acid, cryptochlorogenic acid, caffeic acid, and kaempferol-3-rutinoside as compared to yellow-, white-, and white/purple-fleshed potato cvs.

6 POTATO HEALTH BENEFITS

Consumers are becoming increasingly interested in foods carrying health benefits beyond basic required nutrients. Various health-promoting activities, such as antibacterial, antiinflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic, and vasodilatory were ascribed to

the antioxidant and free radical scavenging properties of phenolics, reported several years ago (Alan and Miller, 1996; Amakura et al., 2000; Manach et al., 2005; Pojer et al., 2013).

Potatoes are a major dietary source of phenolics and a number of antioxidant compounds. These antioxidants obtained from potatoes have free radical scavenging effects, and decrease the risk of coronary heart diseases by reducing blood serum cholesterol accumulation and by enhancing the resistance of vascular walls. From a dietary point of view, potatoes are second only to tomatoes in the total intake of polyphenols by humans. Potato peel, which is discarded as a by-product from potato industries, is reported to possess strong antioxidant properties. In this regard, they reported that potato peel contains phenolic acids, the largest portion of which consists of chlorogenic acid (50.31%). Other phenolic compounds, such as gallic (41.67%), protocatechuic (7.81%), and caffeic (0.21%) acids were also present in potato peel. Additionally potato peel extract showed similar antioxidant capacity to famous commercial antioxidants, such as BHA and BHT. In this context, there is a need to consume potato tubers along with their skin.

It has been reported that chlorogenic acid is the main phenolic constituent in potatoes (Mattila and Hellstrom, 2007). Interestingly, chlorogenic acid is also well known for its large health-giving properties, including (1) protecting against main degenerative diseases, cancer, heart disease (Nogueira and do Lago, 2007), hypertension (Yamaguchi et al., 2007), and viral and bacterial diseases; (2) inhibiting the matrix metalloproteinase (MMP)-9, a key enzyme responsible for tumor invasion and metastasis (Jin et al., 2005); (3) slowing down the release of glucose into the bloodstream (Bassoli et al., 2008); and (4) blocking nitrosamine formation through competitive reaction with nitrite and binding the carcinogen benzo(a)pyrene in a cellulose model system (Friedman, 1997).

Colored purple potatoes naturally accumulate high levels of anthocyanins, which function as antioxidants. These compounds are best known to prevent diseases, such as cardiovascular diseases, cancer, and diabetes (Konczak and Zhang, 2004; Reddivari et al., 2007). Colored potato extracts and an anthocyanin-enriched fraction have been reported to suppress lymph node carcinoma of the prostate and prostate cancer-3 cell proliferation (Reddivari et al., 2010). Polyphenol- and anthocyanin-rich purple potato flakes were found to play an important role in the protection against oxidative damage in rats fed a high-cholesterol diet (Han et al., 2007), and red potato flakes improved the antioxidant system by enhancing hepatic superoxide dismutase mRNA in rats (Han et al., 2007). Because of their high

carotenoid content, potatoes are also particularly beneficial for eye health (Tan et al., 2008; Wang et al., 1999).

Colorful fleshed potatoes (red or purple) owe their characteristic coloring to polyphenolic compounds, anthocyanins, occurring both in flesh and in peel (Andre et al., 2007). The average content of polyphenolic compounds in red- and purple-fleshed potatoes range from 2–3 times higher with respect to potatoes of yellow or white color of flesh. Anthocyanins are mainly responsible for the antioxidant activity and possess a wide array of biological functions. Positive effects of potato anthocyanins on scavenging free radicals, antimutagenicity, and anticarcinogen and antihypertensive activity have been reported.

7 POSTHARVEST QUALITY OF BIOFORTIFIED VEGETABLES

Among the important challenges for cultivation of vegetable crops are postharvest losses and reduced quality due to fruit senescence and pathogen infection. It has been hypothesized that crop biofortification will contribute to minimize those losses by extending the shelf life while enhancing the functional quality of fruits and vegetables. In this context, several attempts have been made to increase the functional quality of various fruits and vegetables using traditional breeding techniques (*hp* tomato cvs, colored potato cvs) or genetic engineering (purple tomato cvs). Nevertheless, consumer concerns about GMOs shift most of the efforts toward naturally biofortified crops.

Shelf life is defined as the period where a fresh produce keeps its sensory, functional, and nutritional attributes in optimal conditions perceived by the consumer as acceptable for consumption. It is widely considered that within a group of fresh produce, there is a genotypic difference in phytochemicals, shelf life potential, as well as potential quality. Postharvest fresh produce quality decreases during storage after harvest. Various methods are adopted to maintain this quality under postharvest conditions, but cannot be improved. Therefore, preharvest strategies, such as breeding biofortified new cvs and high-keeping-quality crops must be used to enhance fresh produce quality (Siddiqui et al., 2015).

Recently, Siddiqui et al. (2015) reported that several pre- and postharvest efforts have been suggested to improve the shelf life of commercially grown tomatoes. However, finding cvs with increased shelf life has not yet been achieved. In this context the authors focused on the characterization of two color mutants [*dg* (BCT-115) and *ogc* (BCT-119)], one ripening

mutant [*rin* (BCT- 111)], with seven traditional ordinary tomato genotypes [Berika (high lycopene) Punjab, Chhuhara, FEB-2, BCT-53, Patharkutchi, CLN-B, and CLN-R] using different physicochemical and subjective variables to acknowledge the storage potential at 25°C and RH 80%–82%. The authors found significant variations in storage life among all tomato genotypes. Considering the all storability assessment data, it can be summarized that mutants carrying *rin* and *dg* genes, respectively, showed the highest shelf life (>18 days). Thus, the present study clearly indicates selecting the cv having a long shelf life could improve marketability of tomato fruits for relatively longer period.

In addition, Zhang et al. (2013) reported that the enrichment of anthocyanin, a natural pigment, in tomatoes can significantly extend shelf life, delay the overripening process, and reduce susceptibility to gray mold in anthocyanin-rich tomatoes, commonly referred as purple tomatoes. For purple tomato fruit, 49 days of storage at 18°C were required to observe 50% of the fruit softened, equivalent to the level of softening observed in ordinary red ripe tomato fruit at 21 days. Furthermore, complete collapse was observed in purple fruit after 10 weeks' storage, compared to only 5 weeks for red fruit. Accumulation of anthocyanin results in high hydrophilic antioxidant capacity, reducing the increase in ROS levels that occurs late during fruit maturation and offers anthocyanin-rich purple tomatoes twofold longer shelf life as compared to ordinary red-fruited tomato cvs.

Regarding potato cvs, although data on the postharvest quality of colored potato cvs is rather scarce, it is widely recognized that for ordinary (white- and yellow-fleshed) potato cvs, postharvest storage generally increases total phenol content in the tubers. The trend (increase or decrease) is rather dependent on the storage period, temperature, genotype, and the considered potato fraction. However, in pigmented potato cvs rich in anthocyanin, the determination of anthocyanin levels in 14 potato clones freshly harvested and after 135 days of storage at 4°C and 86% relative humidity revealed no significant change in anthocyanin content of the tubers. This trait is very important with respect to ordinary potato cvs since colored potato cvs can retain their fresh status for a longer period under storage conditions (Ezekiel et al., 2013; Jansen and Flamme, 2006).

Boiling and microwaving did not cause any changes in the phenolic acid content but rather caused 16%–29% decrease in anthocyanin content (Mulinacci et al., 2008). Thermal processing causes anthocyanin degradation (Patras et al., 2010) and anthocyanins are enzymatically degraded in the presence of polyphenol oxidase. Therefore, potato cv biofortifications

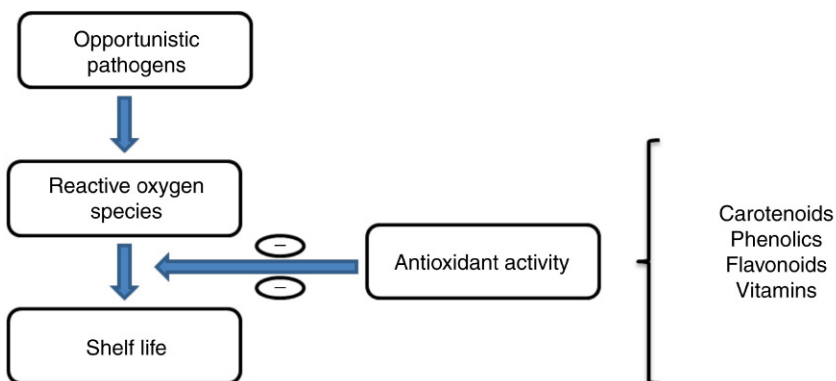


Figure 15.1 Proposed roles of different types of antioxidants in extending shelf life of different crops following postharvest storage pathogen infection.

attempts are meaningless if the targeted compounds do not survive different processing treatments in reasonable amounts (Ezekiel et al., 2013).

A proposed correlation between higher functional quality and increased shelf life suggests that different antioxidants exert an antioxidant activity that counters ROS generated from opportunistic storage pathogens and therefore increases shelf life and retains fresh-like status of fruits and vegetables (Fig. 15.1).

8 CONCLUSIONS AND FUTURE TRENDS

High-lycopene tomato cvs might be beneficial not only for consumers but also for growers, breeders, and processors looking not only for functional foods but also for increased shelf life as a result of their functional quality. Using such cvs, processors can guarantee the deliverance of products with high levels of beneficial ingredients.

Although the general trend is a decrease of antioxidant levels in organically grown tomatoes as compared to conventionally grown ones, the use of high-lycopene tomatoes will overcome this decrease by obtaining organic fruit with high-lycopene contents and extended shelf life.

Processed fruit and vegetables are expected to have a lower health-protecting capacity than fresh ones. The treatments and preservation methods applied during the processing steps are generally believed to be responsible for this depletion of valuable naturally occurring antioxidants in the final product. Various attempts have been made to reduce processing damage, which showed diverse weaknesses. However, recently the development of

tomato crops with an increased content of bioactive compounds represents different and rapidly developing tools for improving the health properties not only of raw materials but also of the processed final products.

Natural colorants and antioxidants present in purple- and red-fleshed potato cvs can be used for developing functional foods. Pigmented potatoes may also serve as potential source of natural anthocyanins for use in the food industry since the cost of potato production is relatively low with respect to the other horticultural crops.

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CHAPTER 16

Preharvest Fruit Bagging for Better Protection and Postharvest Quality of Horticultural Produce

Ram R. Sharma*, Vijay R. Sanikommu**

*Indian Agricultural Research Institute, New Delhi, Delhi, India

**Central Institute for Arid Horticulture, Bikaner, Rajasthan, India

1 INTRODUCTION

Fruit undergoes several physical, physiological, and chemical changes during growth and development. During this period, the fruits are highly susceptible to insect-pest infestations, birds, diseases, and mechanical damage, which in turn reduce their commercial value and thereby cause significant economic losses. To prevent these losses caused by biotic and abiotic factors, several good agricultural practices (GAPs) are increasingly becoming popular throughout the world (Sharma, 2009). Further, the development of alternative techniques to improve the appearance and quality of fruit and reduce pest and disease infestation is becoming increasingly important as global chemophobia and environmental social awareness is increasing day by day. Thus, more emphasis is being placed on reducing the use of pesticides to ensure workers' safety, consumer health, and environment protection (Sharma et al., 2009). Of several such alternatives, preharvest fruit bagging has emerged as one of the best approaches in various parts of the world (Sharma et al., 2014a). In this technique, individual fruit or a fruit bunch is bagged on the tree for a specific period to get the desired results. It is a physical protection technique generally applied to many fruits, which improves the visual quality of fruits not only through the promotion of coloration but also through the reduction of the incidence of cracking and russeting (Sharma et al., 2014a). However, it can also change the microenvironment of fruit development, which has multiple effects on internal fruit quality. Of late, this technique has been extensively used for several fruit crops to improve peel color and to reduce disease, insect-pest incidence, mechanical damage, sunburn, and bird damage, and also to reduce agrochemical residues on the fruit surface (Amarante et al., 2002a; Bentley and

Viveros, 1992; Hofman et al., 1997; Joyce et al., 1997; Xu et al., 2010). Preharvest fruit bagging has been the conventional practice in Japan, Australia, and China for commercial crops, namely, peaches, apples, pears, grapes, and loquats, in order to optimize fruit quality through reduced physiological and pathological disorders and to improve fruit appearance (Amarante et al., 2002b), leading to increased market value (Joyce et al., 1997). Interestingly, some countries, such as Mexico, Chile, and Argentina, won't import apples if they are not bagged. Several authors have reported contradictory results on the effects of preharvest bagging on fruit size, maturity, peel color, flesh mineral content, and fruit quality, which might be due to differences in the type of bag used, the stage of fruit development when bagged, the duration of fruit exposure to natural light after bag removal (before harvesting), and/or fruit- and cultivar-specific responses (Fan and Mattheis, 1998; Hofman et al., 1997). In this review, an attempt has been made to compile the diverse information on this approach to better understand the researchers and extension personnel working in this and related fields.

2 EFFECTS OF PREHARVEST BAGGING ON FRUITS

2.1 Fruit Size and Weight

Fruit size and weight increases gradually after fruit set till maturity. When bags are applied to fruit at some developmental stages, they may influence fruit physiology, which directly or indirectly contributes to the growth and size of the respective fruits. The voluminous literature has been contradictory with respect to the effects of fruit bagging on fruit size and weight, which may reflect differences in the type of bag used, fruit age at bagging, fruit and cultivar response, prevailing climatic conditions, and the conditions under which fruit is held after harvest (Chen et al., 2012; Estrada, 2004a,b; He et al., 2003; Huang et al., 2007; Sun et al., 1995; Wang et al., 2002; Zhen et al., 2000; Zhou et al., 2012). Many researchers have reported the positive influence of fruit bagging on fruit size and weight, while some others have reported a negative influence and a few others have observed no effect of bagging on fruit size or weight.

2.1.1 Increase in Fruit Size and Weight

The positive influence of fruit bagging on fruit growth, size, and weight has been reported by Asrey et al. (2011); Chonhenchob et al. (2011); Harhash and Al-Obeed (2010); Watanawan et al. (2008); Xu et al. (2008); Yang et al. (2009); and Zhou et al. (2012). For example, Xu et al. (2008) reported that

bagging with plastic bags increased the average fruit weight of carambolas, and that it was the highest when bagging was carried out 10 days after full bloom. [Watanawan et al. \(2008\)](#) reported that bagging “Nam Dok Mai #4” mango fruits with two-layer paper (black inside with brown, brown and waxed and white outside), newspaper, and golden paper bags for 52 days increased fruit weight. [Yang et al. \(2009\)](#) reported that the bagging of longans promoted fruit development, resulting in larger-sized fruit. Similarly, [Jaroenkit et al. \(2010\)](#) reported that the bagging of longan “Daw” fruit in season caused fruits to be much bigger and longer than the control fruits. [Harhash and Al-Obeed \(2010\)](#) reported that bunch bagging of the date palm cultivars “Succary” and “Khalas” with blue bags increased bunch weight significantly. [Chonhenchob et al. \(2011\)](#) studied the effect of preharvest bagging using different wavelength-selective bags on “Nam Dok Mai #4” mangoes in Taiwan and reported that bagging increased fruit weight, size, and sphericity over unbagged fruit. The individual fruit size of the “Xiangtian” olive was improved by using Shengda bags in China ([Zhou et al., 2012](#)).

2.1.2 Reduction in Fruit Size and Weight

Some researchers have reported that preharvest fruit bagging reduces fruit size and weight. For instance, [Witney et al. \(1991\)](#) found that bagging reduced the weight of the apple “Sundale Spur Golden Delicious” at harvest. [Estrada \(2004a\)](#) found that the kind of bag used had a significant effect on fruit weight, and the highest weight was obtained in fruit without bags for all six mango cultivars. [Murray et al. \(2005\)](#) reported that the fruit of “Laetitia” and “Songold” plums, when covered with shade netting, were smaller in size than uncovered ones. [Xu et al. \(2010\)](#) tried bagging for the loquats “Baiyu” and “Ninghaibai” and reported that bagging decreased the weight of fruit. [Hudina and Stampar \(2011b\)](#) reported that the bagging of “Conference” pears reduced fruit weight to such an extent that fruits were under 110 g, which therefore had a negative effect on consumer appeal and marketability. Bagging has also been reported to reduce fruit size and weight in pomegranates ([Hussein et al., 1994](#)), apples ([Arakawa, 1988](#)), and bananas ([Hasan et al., 2001](#)).

2.2 Fruit Maturity

Fruit bagging has a significant influence on fruit maturity, along with growth and development. However, scientists have reported contradictory results as well for the effects of preharvest bagging on fruit maturity ([Costa et al., 2002](#); [Debnath and Mitra, 2008](#); [Kim et al., 2008a, 2010](#); [Rodrigues](#)

et al., 2001; Wang et al., 2010b). For instance, Johns and Scott (1989) reported that bagging reduced the time to maturity in bananas significantly. Meanwhile, Costa et al. (2002) reported that the bagging of “Nanicão” banana bunches with polyethylene sacks decreased the interval between the emergence of the inflorescence and harvest. Similarly, Rodrigues et al. (2001) evaluated the effect of polyethylene banana bunch covers and reported that time to harvest was reduced by 12 days in the second crop. Debnath and Mitra (2008) reported that it was possible to stagger the harvesting period of litchis over 30 days, because cellophane paper bags advanced commercial maturity by 12 days while brown paper or newspaper (biodegradable) bags delayed maturity by 10 days. Kim et al. (2008a) reported that the time to maturity of “Janghowon Hwangdo” peach fruits bagged with white paper was faster by 6 days compared to fruits bagged with newspaper bags or unbagged fruits. Wang et al. (2010b) reported accelerated fruit maturity (10 days) in “Wanmi”, a late-ripening peach cultivar, using preharvest fruit bagging. In contrast, Ju (1998) reported that bagging did not affect time to fruit maturity of “Delicious” apples. From this discussion, it appears that fruit bagging can be used to stagger the time to fruit maturity in peaches, litchis, and bananas, which may indirectly help to make fruits available in the market and fetching good prices for a longer time.

2.3 Fruit Appearance

Fruits are liable to acquire several physical defects, namely, blemishes, abrasions, and injuries, during cultivation, harvesting, packing, and transportation, resulting in reduced appeal to consumers and loss of market value. Studies have indicated that preharvest fruit bagging has been widely used to improve the commercial value of fruit (Wu et al., 2009; Asrey et al., 2013). For example, as early as 1956, Berill (1956) reported covering banana bunches with plastic or burlap to protect the fruits from blemishes. Amarante et al. (2002b) have reported that preharvest bagging of the pear “Doyenne du Comice” increased the percentage of fruit accepted for export, primarily by reducing bird damage and skin blemishes. Katagiri et al. (2003) studied the effect of bagging on the “Fuyu” persimmon to increase fruit marketability by improving the appearance of the fruit, and reported that fruit blemishing had been reduced by the earlier bagging treatment. The major effect of bagging on increased marketability was due to the drastic reduction in blemishes (caused by exogenous factors such as wind) and the prevention of rainwater and pesticides from penetrating the cracks. Similarly, Faoro and Marcia (2004) reported that fruit bagging of the “Housui” pear resulted in a

better fruit appearance, as the fruits were uniform in size, with smooth and shiny peels. Jia et al. (2005) reported that “Hakuho” peaches covered with orange bags appeared bright red and had high L values, both of which accounted for their good visual quality.

In the Philippines the mango industry suffers from various problems, such as scab; misshapen, distinct veins; undersized fruits; wind scar; mottling; sooty mold; and sap burn. All these problems were reported to be reduced significantly with fruit bagging and resulted in mango fruits acceptable for export (Bayogan et al., 2006). Huang et al. (2007) reported that bagging could increase the proportion of “Cuiguan” pear fruits without, or with only a few, russets, and that bagging made the skin brighter. Lin et al. (2008) reported that the bagged fruits of “Cuiguan” and “Hosui” pears were brighter and more attractive, with fewer russets and visible dots than non-bagged fruits, which increased their market value. Furthermore, due to bagging, there was an increase in the percentage of extra class and class I fruits and a reduction in the number of defective (blemished, sunburned, and cracked) fruits. Similarly, the bagged litchi fruits were free from blemishes due to the minimal incidence of sunburn and fruit cracking, and had added luster on the peel, and hence they were more appealing to consumers (Debnath and Mitra, 2008). Furthermore, Sarker et al. (2009) reported that the physical fruit quality (the percentage of black spots) of bagged mango fruits was better than unbagged healthy fruits, which helped their market appeal. Wu et al. (2009) also reported that bagging was effective in improving the appearance of “Zill” mango fruits. Xu et al. (2010) tried bagging “Baiyu” and “Ninghaibai” loquats with different materials and reported that bagging promoted a better fruit appearance. In bananas, Muchui et al. (2010) have reported that banana fruits grown under covers had no blemishes at all and were attractive to consumers at a glance, while unbagged fruits had black spots and blemishes caused by thrips and freckle fungi attacks. After completing a fruit-bagging experiment on the “Apple” mango, Mathooko et al. (2011) reported that the bagged fruits had a smooth texture and a spotless light green color, and hence bagged fruits were rated superior in terms of general appearance and overall acceptance, which led to improved exports and better prices for mango farmers.

2.4 Fruit Color Development

Fruit color is the basic point of attraction for consumers. Attractive color improves the physical appearance of the fruit, which helps to get a better price in the domestic or export market. Several studies have indicated that

preharvest fruit bagging can have significant influence on fruit color promotion or inhibition.

2.4.1 Color Promotion

The majority of studies have reported that fruit bagging improves the color of fruits by accumulating anthocyanin content. For example, in litchi, semi-transparent CP bags developed excellent peel coloration in fruit (Chen and Li, 1999; Hu et al., 2001). In apples, the red color is due to anthocyanins, for which UV light is essential (Saure, 1990). In earlier studies, fruit bagging was reported to inhibit color development in apples (Kikuchi, 1964; Proctor and Lougheed, 1976); however, it has now been established that fruit bagging is an effective way to promote anthocyanin synthesis and improve fruit coloration in apples (Sharma and Pal, 2012c). It is believed that bagging increases the light sensitivity of fruit and stimulates anthocyanin synthesis when fruits are reexposed to light after bag removal. However, the direct effect of fruit bagging is not to promote, but to inhibit, anthocyanin synthesis (Ju et al., 1995a,b; Kim et al., 2010). Bagged fruits are capable of synthesizing anthocyanin when they are exposed to light for few days before the actual date of harvest (Ju, 1998; Sharma et al., 2013).

The peel color of unbagged pear fruits is a darker green compared to the brighter green of bagged pears. Amarante et al. (2002a) reported that preharvest bagging of pears (from 30 days after full bloom till harvest) improved the skin color of fruits, giving them a more attractive light green color without reducing blush on the exposed side of the skin. Similarly, red Chinese sand pears (*Pyrus pyrifolia* “Nakai”) that were bagged until harvest had a yellow tinge, with a higher lightness value and a higher hue angle than control fruit (Huang et al., 2009). “Hakuho” peaches bagged with orange paper bags had a significantly higher skin L^* and h° and smaller C^* values than those subjected to other treatments (Jia et al., 2005). In grapefruit, Hwang et al. (2004) found that bagging only slightly enhanced pulp color during maturation, while skin color was affected significantly. Mazarro et al. (2005) reported that bagging with a polyethylene or a butter-paper bag improved the color of figs significantly over unbagged fruit or those covered with brown kraft paper bags. Singh et al. (2007) reported that simple newspaper bagging in “Allahabad Safeda” guavas produced fruits of a higher yellow index than unbagged fruits. Signes et al. (2007) reported that preharvest bagging with cellulose bags increased uniformity in color development, even in the black grape variety “Perla”. Watanawan et al. (2008) reported that bagging mango fruits with two-layer paper bags for 52 days increased

peel color development from green to yellow. Bagging with black paper could help in the degreening of “Harumanis” mangos, and it was found that that one layer of black paper was sufficient to degreen the fruits (Ding and Syakirah, 2010). However, in “Keitt” mangos, white paper bagging increased the percentage of yellow skin area as compared to the control fruit (Hofman et al., 1997). Jaroenkit et al. (2010) reported that bagging “Daw” longans during preseason, on-season, and off-season periods indicated that bagging caused the skin to be more yellowish-green or golden yellow than control and nonbagged fruits. Zhou et al. (2012) investigated the effect of bagging with different bags on “Xiangtian” olives and reported that the attractive golden yellow color of fruits was obtained with Shengda double-layer bags in particular. The color intensity of the pitaya was found to be significantly increasing with white paper bags and black polyethylene bags (Tran et al., 2015)

2.4.2 Color Inhibition

As stated above, the basic effect of fruit bagging is to inhibit color development rather to promote it (Proctor and Loughheed, 1976); this, however, largely depends on the stage of development at which the fruit is bagged, the bagging date, the kind of bag used, the date of bag removal, and the climatic conditions of the area (Amarante et al., 2002a; Ju et al., 1995a,b). Studies conducted by scientists have reported that fruit bagging inhibited color development in some fruits and therefore support this statement. For example, Ju (1998) reported that bagging inhibited anthocyanin accumulation in the fruit peel of “Delicious” apples. However, when fruits treated with three-layered bags were exposed to light, they started to accumulate anthocyanin rapidly, and anthocyanin accumulation reached a maximum after 3 days of light exposure (Sharma et al., 2014a). Kwan et al. (2000) reported that bagging “Yuzu” citrus fruits (*Citrus junos* L.) before early September with recycled Japanese phone book paper (PBP) resulted in worse coloration, whereas bagging on September 20 and thereafter showed similar coloration as the nonbagging treatment. However, when fruits were bagged in PET and BP on September 25, fruit coloration occurred earlier than in fruits bagged with PBP or in nonbagged fruits. Jia et al. (2005) found that bagging increased hue angle and decreased chroma in peaches, yet the appearance of bagged fruit was less attractive. Similarly, the color development in apples was reported to be inhibited by preharvest bagging (Wei et al., 2005). Murray et al. (2005) reported that bagged plum fruits had a greener ground color and a poorer red color than unbagged fruit. Wei

et al. (2006) reported that the content of anthocyanin was notably lower in bagged “Red Fuji” apples than in the control fruits; however, after 5 days of bag removal, its content surpassed that of the control group and was 1 time more than the control fruits at harvest. Takata et al. (2006) found that there was no significant difference in the rate of reddish-pulp development in “Takei Hakuho” peaches between unbagged fruit and those in white and in orange bags. However, fruits covered with orange and with black bags showed a lower rate of reddish-pulp development at harvest. Kim et al. (2008b) reported the more positive influence of white-coated bags on the appearance of “Janghowon Hwangdo” peaches over white paper, yellow-coated paper, yellow paper, and newspaper bags. Lin et al. (2008) reported that the bagged fruit of the “Cuiguan” and the “Hosui” pear were brighter than nonbagged fruit. Xia et al. (2009) reported that the anthocyanin content of bagged “Jiang Su Red Fuji” apples was notably lower than that of control fruits during fruit development, but that a very rapid increase was observed after bag removal, even surpassing the control fruits. Hudina and Stampar (2011a) reported that the appearance of bagged “Concorde” pear fruits was less attractive; their color was a muddy yellow and the extent of russetting was much higher compared to unbagged fruit. In contrast, Hofman et al. (1997) reported that bagging did not affect the pulp color of “Keitt” mangoes.

2.5 Incidence of Insect pests

Preharvest fruit bagging is a good technique to keep a physical separation between environment and the produce. One of the most significant influences of fruit bagging has been its effect on protecting fruit from the damage caused by insect pests (Table 16.1). For instance, bagging of individual guava fruit with paper bags has been shown to reduce the incidence of fruit fly (Pereira, 1990). Hofman et al. (1997) reported that the bagging of mango fruits with brown paper bags was effective for the control of the mango fruit fly. Similarly, Singh et al. (2004) reported a reduction in fruit fly incidence with fruit bagging, and Sarker et al. (2009) conducted studies with different bagging materials (black polybag, transparent polybag, brown paper bag) to control mango fruit fly attacks on “Langra” and “Khirshapat” varieties, reporting that although all bagging materials gave 100% protection against the fruit fly infestation of mango fruits, the bagging of fruits with brown paper bags was found to be the best for protecting mango fruits (Table 16.1). Further, Morera-Montoya et al. (2010) observed that the use of nylon bags offered the highest protection for guavas against the fruit fly.

Table 16.1 Effect of preharvest fruit bagging on the incidence of insect pests

Fruit crop	Bagging date/time	Bagging material	Insect pest controlled	References
Apple “Imperial Gala”	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	Fruit fly (<i>Anastrepha fraterculus</i>) Oriental fruit moth (<i>Grapholita molesta</i>) Apple leaf roller (<i>Bonagotasa lubricola</i>) Woolly apple aphid (<i>Eriosoma lanigerum</i>)	Rosângela et al. (2011a)
Apple “Fuji Suprema”	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	Fruit fly (<i>A. fraterculus</i>) Oriental fruit moth (<i>G. molesta</i>) Apple leaf roller (<i>B. lubricola</i>)	Rosângela et al. (2011b)
Apple “Royal Gala”, “Suprema”	15 and 7 days before harvesting	White paraffinic paper bags or transparent polypropylene microperforated bags	Most harmful insects	Santos and Wamser (2006)
Apple “Granny Smith”	At golf-ball fruit size	Brown paper bags	Reduction in fruit infested by codling moth	Bentley and Viveros (1992)
Apple “Royal Delicious”	30 days before harvesting	Spunbonded light-yellow colored bags	Reduction in San Jose scale incidence	Sharma and Pal (2012a,b)
Guava	6–9 weeks before harvesting	Biodegradable films (BF30, BF50, BF70) from cassava starch and poly(butylene adipate–coterephthalate) (PBAT) by extrusion	Fruit fly (<i>Anastrepha</i> spp.), guava weevil (<i>Conotrachelus psidii</i>)	Bilck et al. (2011)
	175 days of fruit set	Waxed paper, nylon fabric, Taiwan bag, and PBP	Fruit fly	Morera–Montoya et al. (2010)

(Continued)

Table 16.1 Effect of preharvest fruit bagging on the incidence of insect pests (*cont.*)

Fruit crop	Bagging date/time	Bagging material	Insect pest controlled	References
Caimito	At marble stage	Single-layer nonwoven spunbonded polypropylene fabric bags White TNT	Fruit fly Larvae of fruit fly (83.3% controlled)	Sharma and Nagaraja, 2016 Nascimento et al. (2011)
Pomegranate “Mridula”, “Ganesh”, “Jyoti”, “Ruby”, “Jalore Seedless”	60–70 days before harvesting	Parchment bags	Nearly 90% reduction in the incidence of anar butterfly (<i>Deudorix (Virachola) isocrates</i>)	Bagle (2011)
Mango “Langra”, “Khirshapat”	30 days before harvesting	Bagging materials (black polybag, transparent polybag, brown paper bag)	100% control of fruit fly	Sarker et al. (2009)
Mango “Carabao”	60 days before harvest	Brown paper bags	Complete reduction in fruit-fly incidence	Buganic et al. (1997)
Litchi	Bagging after 1 week of fruit set	Cellophane paper bags, brown bags, and newspaper bags (biodegradable)	Reduced incidence of stalk-end borer (<i>Conopomorpha cramerella</i>)	Debnath and Mitra (2008)
	Bagging after 1 week of fruit set	Cellophane paper bags, brown bags, and newspaper bags (biodegradable)	Reduced incidence of stone borer (<i>Platyplepa</i> sp., <i>Conogethes</i> sp.)	Debnath and Mitra (2008)

Likewise, [Bilck et al. \(2011\)](#) reported the beneficial effects of fruit bagging with biodegradable films for reducing the incidence of the guava weevil. However, this pest-management technique was found to have a significant impact on the genetic diversity and genetic makeup of the targeted insect populations ([Zheng et al., 2015](#)).

The incidence of the codling moth was reported to be reduced by bagging “Granny Smith” apples ([Bentley and Viveros, 1992](#)). [Rosângela et al. \(2011a,b\)](#) reported that regardless of the material used, bagging reduced the damage caused by the fruit fly, oriental fruit moth, apple leaf roller, and woolly apple aphid ([Table 16.1](#)). San Jose scale is a serious insect pest of apples in India, and causes severe losses. It has been reported that bagging with light-yellow spunbonded bags reduced its incidence significantly in “Royal Delicious” apples ([Sharma and Pal, 2012a,b](#); [Sharma et al., 2014b](#)). Further, the pomegranate suffers a lot from the fruit borer, and its incidence was significantly reduced with white TNT bagging ([Bagle, 2011](#)). [Buganic et al. \(1997\)](#); [Debnath and Mitra \(2008\)](#), and [Santos and Wamser \(2006\)](#) have also reported a reduction in harmful insects in mangoes, litchis, and apples, respectively, by using preharvest fruit bagging ([Table 16.1](#)). From the foregoing discussion, it can be appreciated that preharvest fruit bagging can be a useful approach to produce fruits with the least incidence of insect pests, for which several insecticides are used.

2.6 Incidence of Diseases

As specified earlier, fruit bagging maintains a physical separation between the environment and produce, resulting in the creation of a hurdle for the successful entry of pathogens onto the fruit surface. A review of literature on this subject has revealed that fruit bagging can protect from the invasion of pathogens in some fruit; yet in others, it may not have any effect ([Amarante et al., 2002a](#); [Chonhenchob et al., 2011](#); [Gao et al., 2007](#); [Guo et al., 2005](#); [Senghor et al., 2007](#); [Sui et al., 2005](#)) ([Table 16.2](#)). In mangoes, several studies have been conducted on the effect of bagging on disease incidence, which have revealed that bagging mango fruits with different bagging materials reduced the incidence of the most dreaded diseases of mangoes, such as anthracnose and stem-end rot ([Chonhenchob et al., 2011](#); [Hofman et al., 1997](#); [Senghor et al., 2007](#)). Similarly, postharvest fungal attack on litchi fruit was also reduced by bagging ([Kooariyakul and Sard-sud, 1997](#)). In loquats and guavas, fruit bagging reduced the incidence of fruit rot and anthracnose, respectively ([Ko et al., 2010](#); [Martins et al., 2007](#)) ([Table 16.2](#)). [Sharma and Pal \(2012a,b\)](#) observed that bagging reduced the

Table 16.2 Effect of preharvest bagging on the incidence of diseases in fruits

Fruit crop	Bagging date/time	Bagging material	Disease incidence	Reference(s)
Apple	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	No control of apple scab, bitter rot, and moldy core	Rosângela et al. (2011a)
Apple “Fuji Suprema”	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	No effect on apple scab, bitter rot, and moldy core	Rosângela et al. (2011b)
Apple “Royal Delicious”	30 days before harvesting	Spunbonded light-yellow colored bags	Reduction in fly speck and sooty blotch	Sharma and Pal (2012a,b)
Apple	Different stages of fruit development	Kobayashi, Tongle, and Qianwei bags	Slight increase in black-dot and red-dot diseases of bagged fruits	Qin et al. (2012)
Apple	During fruit development	Plastic bags	Increased incidence of superficial scald	Fan and Mattheis (1998)
Mango “Nam Dok Mai #4”	45 days after full bloom	Plastic bags with wavelength-selective characteristics	Reduction in the incidence of most postharvest diseases	Chonhenchob et al. (2011)
Mango “Keitt”	100 days before harvest	White paper bags	Incidence and severity of anthracnose and stem-end rot was reduced	Hofman et al. (1997)
Mango “Carabao”	60 days before harvest	Brown paper bags	Significant reduction in anthracnose and stem-end rot	Buganic et al. (1997)
Mango “Dashehari”	During fruit development	White or brown paper bags	Significant reduction in anthracnose and stem-end rot	Pandey and Tandon (2004)
Mango	During fruit development	White bags	Control of <i>C. gloeosporioides</i>	Senghor et al. (2007)
Guava	One month before harvesting	Paper bags	Reduction in black spot (<i>Guignardia psidii</i>) and anthracnose (<i>Colletotrichum</i> spp.)	Martins et al. (2007)
Loquat	During fruit development	Plastic bags	Reduction in fruit rot (<i>Alternaria</i> sp.)	Ko et al. (2010).
Pear “Huangguan”	During fruit development	Three-layered bags	Increase in the incidence of browning spot	Wang et al. (2011)

incidence of the sooty blotch and fly-speck diseases in apples. At the same time, Rosângela et al. (2011a,b) reported no effect on apple scab (*Venturia inaequalis*), bitter rot (*Colletotrichum gloeosporioides*), and moldy core (*Alternaria* sp.; *Fusarium* sp.) from bagging. However, in contrast, Fan and Mattheis (1998) reported that bagging during fruit development increased superficial scald and eliminated stain during cold storage. Similarly, bagging has been reported to increase the incidence of black-dot and browning-spot diseases in apples (Guo et al., 2005; Hao et al., 2004; Hao et al., 2011; Wang et al., 2011) (Table 16.2). These reports on the effects of preharvest fruit bagging suggest that this practice can be a favorable practice for producing quality fruits, even without, or using minimal, chemicals that are used for controlling postharvest diseases in fruits.

2.7 Physiological Disorders

Physiological disorders are inanimate anomalies in fruits, which are not caused by insect damage or pathogen invasion, but are the result of the deficiency or excess of a nutrient, low or high temperature, or high or low ethylene or respiration rates (Sharma, 2009). Several disorders have been reported in fruits that affect yield and quality, and several approaches have been adopted for their management. Studies have shown that fruit bagging can be used to reduce the incidence of some disorders in fruits (Table 16.3), and hence this approach is being used extensively in some countries to reduce the problem of sunburn and fruit cracking. Fumuro and Gamo (2001) observed that black stain (BS) is a serious problem for the skin of the “Shinsyu” persimmon, which can be reduced significantly by bagging all the fruit on the tree with paper bags 50–35 days before harvest. Fruit bagging has also been reported to reduce the incidence of sunburn in apples (Bentley and Viveros, 1992; Sharma et al., 2014b) and pears (Amarante et al., 2002b), fruit drop in longans (Yang et al., 2009) and carambolas (Xu et al., 2008), stone cells in pears (Lin et al., 2008), fruit splitting in nectarines (Ding et al., 2003), fruit spot in citrus (Kwan et al., 2000), russetting in apples (Rosângela et al., 2011b) and pears (Amarante et al. (2002a) and bitter pit, cork pit, and brown core in apples (Sharma and Pal, 2012a,b) (Table 16.3). Similarly, bagging reduced fruit cracking in pomegranates (Abdel-Rhman, 2010) and reduced the percentage of tip-cracked fruit in dates (Kassem et al., 2011) and lenticel discoloration in mangoes (Mathooko et al., 2011; Rymbai et al., 2012). However, in contrast, Han et al. (1999) reported an increased incidence of water core in the Japanese pear “Hosui” when using polyvinyl chloride bagging from 90 days after full bloom to

Table 16.3 Effect of preharvest fruit bagging on physiological disorders in fruits

Fruit crop	Bagging date/time	Bagging material	Disorder	References
Apple	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	Increase in russetting	Rosângela et al. (2011a)
Apple “Fuji Suprema”	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	Reduction in bitter-pit incidence	Rosângela et al. (2011a)
Apple “Fuji Suprema”	40 days after flowering	Transparent microperforated plastic or nontextured fabric bags	Reduction in russetting and bitter pit	Rosângela et al. (2011b)
Apple “Gamhong”	4–5 weeks after full bloom	Ca-coated paper bags	Reduction in bitter pit	Kim et al. (2008a)
Apple “Royal Delicious”	1 month before harvesting	Spunbonded light-yellow colored bags	Reduction in the incidence of bitter pit, cork pit, and brown core	Sharma and Pal (2012a,b)
Apple “Granny Smith”	At golfball fruit size	Brown paper bags	Reduction in sunburn	Bentley and Viveros (1992).
Carambola	10–17 days after full bloom	Plastic bags	Reduction in fruit drop	Xu et al. (2008)
Date palm “Zaghoul”	At pollination time	Transparent and blue polyethylene bags	Reduction in tip-cracked fruit	Kassem et al. (2011)
Lonagn “Chuliang”	34 days after anthesis	White adhesive-bonded fabric bags (WAFB) and black adhesive-bonded fabric bags (BAFB)	Reduction in fruit drop	Yang et al. (2009)

Mango “Apple”	40–50 days before harvesting	White bags	Reduction in lenticel discoloration	Mathooko et al. (2011)
Nectarine	During fruit development	Plastic bags	Reduction in fruit splitting	Ding et al. (2003)
Pear “Doyenne du Comice”	30 days after full bloom	Microperforated polyethylene bags	Reduction in russeting	Amarante et al. (2002a)
Pear “Conference”	During fruit development	Plastic bags	Reduction in sunburn	Amarante et al. (2002b)
Pear “Cuiguan”	35 days after full bloom or 20 days after full bloom	Paper bags once or twice	Reduction in stone cells	Lin et al. (2012)
Pear “Cuiguan”, “Housi”	28 days after full bloom	Haoguo bags (a two-layer paper bag), Jiatian bags (a one-layer paper bag), common bags (a two-layer paper bag)	Reduction in stone cells	Lin et al. (2008)
Persimmon “Shinsyu”	50–35 days before harvest	Paper bags	No BS	Fumuro and Gamo (2001)
Yuzu (<i>C. junos</i> L.)	Bagging before early September	Recycled Japanese PBP, BP, or black PET bags	Significant reduction in fruit spot injury	Kwan et al. (2000)

harvest. Similarly, Rosângela et al. (2011a) have reported an increased incidence of russetting in apples after fruit bagging with transparent microperforated plastic or nontextured fabric bags.

2.8 Postharvest Quality of Fruits

The eating quality of fruit includes attributes, such as total soluble solids, acidity, and so on. The ultimate aim of any producer is to produce fruits of better quality that are acceptable to most consumers. These eating-quality attributes are also invariably influenced by fruit bagging (Table 16.4). For example, Bentley and Viveros (1992) reported that the sweetness of “Grunny Smith” apples was improved by brown paper bags when the fruit was bagged at the golfball-size stage of fruit development. Similarly, improvement in soluble solids has also been reported in loquats (Xu et al., 2010), “Red Globe” grapes (Zhou and Guo, 2005), peaches (Kim et al., 2008b), guavas (Singh et al., 2007), pears (Lin et al., 2012), mangoes (Watanawan et al., 2008), litchis (Debnath and Mitra, 2008; Purbey et al., 2016) and apples (Sharma and Pal, 2012a,b). Further, a reduction in soluble solids and/or acidity after fruit bagging has been reported in apples (Chen et al., 2012), plums (Murray et al., 2005), pears (Hudina and Stampar, 2011b; Lin et al., 2008), and mandarins (Hiratsuka et al., 2012) (Table 16.4). Interestingly, no effect from fruit bagging was observed on soluble solids and/or acidity in “Yali” pears (Xin and Zhang, 2003), Nashi pears (Faoro and Marcia, 2004), peaches (Jia et al., 2005), apples (Xia et al., 2009), longans (Yang et al., 2009), and bananas (Muchui et al., 2010) (Table 16.4). Some authors have reported that bagging can influence the vitamin content of fruits. For instance, (Wang et al., 2006) reported an increase in flesh lycopene and the β -carotene content of oranges, while (Sharma and Pal, 2012a,b) reported on andan increase in the ascorbic acid content of apples. In contrast, Jia et al. (2011) reported a reduction in the β -carotene content in the peel and flesh of “Fuji Yanfu-3” apples (Table 16.4).

2.9 Activities of Enzymes

During fruit development several biochemical changes occur, and all these reactions are catalyzed by various enzymes. Detailed perusal of the available literature has revealed that preharvest bagging plays a pivotal role in influencing the activities of certain enzymes. For instance, Ju et al. (1995b) found that fruit bagging significantly inhibited both phenylalanine ammonia-lyase (PAL) activity and anthocyanin synthesis, while bag removal enhanced both of them. They further reported that although PAL catalyzes a reaction to produce precursors of anthocyanin synthesis, under conditions of sufficient

Table 16.4 Effect of preharvest fruit bagging on quality of different fruits

Fruit	Quality attribute(s) affected	References
Apple	Improvement in fruit sweetness Slight reduction in total soluble sugar content Reduction in β -carotene content in peel and flesh Improvement in soluble solids and ascorbic acid content	Bentley and Viveros (1992) Chen et al. (2012) Jia et al. (2011) Sharma and Pal (2012a,b); Sharma et al. (2013, 2014a,b)
Banana	No effect on the content of soluble sugars, reducing sugar, and TTA No adverse effect on total soluble solids, TTA, and firmness	Xia et al. (2009) Muchui et al. (2010)
Guava	Increase in soluble solids concentration (SSC)	Singh et al. (2007); Sharma and Nagaraja (2016)
Grapes	Increase in soluble sugars	Zhou and Guo (2005)
Litchi	Significant improvement in TSS/acid ratio	Debnath and Mitra (2008)
Longan	No significant effect on sugar content	Yang et al. (2009)
Loquat	Increase in soluble solids and decline in acidity Content of sucrose, glucose, and soluble solids increased, while the content of fructose, sorbitol, and TTA decreased Improvement in total sugar content	Liu et al. (2004) Ni et al. (2010) Xu et al. (2010)
Mandarin	Reduction in sugar and organic acid content	Hiratsuka et al. (2012)
Mango	Significant effects on SSC, TTA, SS/TTA ratio, vitamin C	Watanawan et al. (2008)
Nashi pears	No effect on total soluble solids	Faoro and Marcia (2004)
Orange	Increase in flesh lycopene and β -carotene content	Wang et al. (2006)
Peach	No effect on total soluble solids and TTA Improvement in SSC	Jia et al. (2005) Kim et al. (2008b)
Pear	Reduction in soluble solids and increase in TTA Negative impact on sucrose and sorbitol content Double increase soluble solids	Lin et al. (2008) Hudina and Stampar (2011b) Lin et al. (2012)
Plum	Reduction in the content of sugar and acid Reduction in SSC	Xin and Zhang, 2003 Murray et al. (2005)

precursors, changes in anthocyanin accumulation can occur independently of PAL activity. Wang et al. (2000a) investigated the influence of maturity and bagging on the relationship between anthocyanin accumulation and PAL activity in “Jonathan” apples and reported that maximal PAL activity is not the only regulating factor for anthocyanin accumulation in bagged mature and ripe apples. In nonbagged and shaded apples, anthocyanin accumulation and maximal PAL activity increased as the apples developed from the immature to the initial-ripe stage and then decreased at full-ripe stage. Hu et al. (2001) reported that bagging of “Feizixiao” litchis enhanced their color development, which was associated with the metabolism of phenols and flavonoids, and the activities of PAL and polyphenol oxidase (PPO). Zhou and Guo (2005) reported that fruit bagging in “Red Globe” grapes increased acid invertase (AI) activity, which was responsible for the accumulation of higher sugar in the bagged fruit. Ni et al. (2010) found that the activities of AI and neutral invertase (NI) in the bagged loquat fruits were lower than those in the nonbagged fruit, and the activities of sucrose synthase (SS) and sucrose-phosphate synthase (SPS) in the bagged fruit were higher than those in the nonbagged fruit. The activities of SDH (sorbitol dehydrogenase) and SOX (sorbitol oxidase) in the bagged fruit were lower than those in the nonbagged fruit, suggesting that bagging mainly increased the products of photosynthesis by enhancing the activities of SS and SPS. Wang et al. (2010a) reported that the activities of SOD, POD, CAT, and APX in the bagged apple fruits were higher than those in unbagged ones. Chen et al. (2012) reported that PAL activity in different cultivars of bagged apples tended to rise, and then declined after bag removal, while that of the control fruits decreased slowly. On the second day after removal, the activity of PAL in treated fruit exceeded that in the control fruit and reached a maximum on the 6th day after bag removal. Hiratsuka et al. (2012) reported that the PEPC (phosphoenolpyruvate carboxylase) activity peak in mandarins was 90% of the control fruits after fruit bagging. Thus, just before their color development, mandarin fruit assimilated CO₂ actively through photosynthesis and PEPC. However, these activities are inhibited by bagging, likely resulting in lower sugar content at harvest. Thus, from this discussion, it can be concluded that fruit bagging may enhance or inhibit the activities of enzymes involved in color synthesis and quality improvement.

2.10 Phenolic Compounds and Antioxidant Activity

Phenolic compounds are secondary metabolites, which act as antioxidants and protect us from several diseases. The literature reveals that fruit bagging

can also influence the level of phenolic compounds and antioxidant activity in fruits. The contradictory effects of bagging on phenolic-compound accumulation in fruits might be due to differences in cultivars, material of bagging, bagging date, bagging period, date of bag removal, and climatic conditions. For example, [Ju et al. \(1995b\)](#) reported that simple phenol concentration increased up to 60 days and then declined in “Delicious” apples when bagged. [Xu et al. \(2010\)](#) reported that the total phenolic and flavonoid content in loquats was decreased by bagging treatments, with the lowest occurrence in the fruits bagged with TGDPB. Bagging also decreased the total antioxidant capacity (AOX) of the fruit pulp, which again was lower in TGDPB-treated fruits than in those bagged using OWPB. [Wang et al. \(2010b\)](#) reported that bagging did not affect the chlorogenic acid and catechol content of either fruit peel or flesh in “Wanmi” peaches. [Hudina and Stampar \(2011a\)](#) reported that bagged fruit of “Concorde” pear fruits had a significantly lower concentration of phenolics in the peel, while there was no significant difference in the pulp. Similarly, [Hudina and Stampar \(2011b\)](#) reported that bagging of “Conference” pears increased some phenolic compounds, such as epicatechin and caffeic acid, in skin; these compounds were highest in bagged fruit. The removal of bags seven days before harvest decreases the arbutin content of the pulp and also total sugar in the whole fruit, and thus the fruit may have a poorer eating quality. Further, quercetin 3-O-rhamnoside content in skin decreased in both years among fruit bagged until harvest. Similarly, [Hudina et al. \(2012\)](#) reported that bagging “Concorde” European pear fruits decreased the content of phenolic compounds such as catechin, chlorogenic acid, epicatechin, p-coumaric acid, quercetin 3-O-galactoside, quercetin 3-O-glucoside, and quercetin 3-O-rhamnoside in skin. [Chen et al. \(2012\)](#) reported that fruit bagging decreased most of the phenolic compound concentrations in both peel and flesh of three apple cultivars, namely, “Golden Delicious”, “Red Delicious”, and “Royal Gala”.

2.11 Aroma Volatiles

Most fruits are liked by consumers because of their pleasing aromas and flavors. The characteristic flavor and aroma of different fruits is contributed by various sets of volatile compounds. Since bagging intercepts light, it may indirectly influence the synthesis of various flavor-contributing volatiles. For instance, [Jia et al. \(2005\)](#) reported that bagging of “Hakuho” peaches with orange paper bags did not affect total aroma volatile production by whole fruit, but significant differences were observed in the aroma volatile

content between skin and flesh, which increased the fruit flavor significantly. Zhang and Jia (2005) found a significant increase in the synthesis of volatile compounds in “Hujingmilu” peaches, which was later supported by Li et al. (2006), who concluded that high-quality “Hujingmilu” peaches can be produced when bagged with a single layer of orange paper that allows 27% light transmittance. Such single-layer bagged fruit showed the highest level of γ -decalactone, the main characteristic aroma of peaches, and total lactones at the firm-ripe and full-ripe stages. In contrast, Wang et al. (2010b) reported that bagging did not affect the content of keracyanin or quercetin-3-rutinoside in fruit flesh during fruit development. However, they further reported that keracyanin and quercetin-3-rutinoside levels were significantly reduced in the peel of fruit bagged before ripening compared to non-bagged fruit peels and recommended that, considering the large changes in volatiles, “Wanmi” peaches should be harvested 126–147 DAFB, about 1 month ahead of maturity. In “Hanfu” apples, the total content of aroma in fruits with double-layer paper and reflective film bags was 40.03% and 20.33%, respectively, lower than that of control fruits (Li et al., 2011). While bagging increased the total content of esters, it decreased the total content of alcohols and aldehydes.

2.12 Fruit Firmness

Fruit firmness is one of the important indicators for harvest maturity, which ultimately determines the postharvest life of a particular fruit. A few studies conducted on this subject have shown that preharvest fruit bagging can influence fruit firmness at harvest. For example, Bentley and Viveros (1992) reported that the fruit firmness of “Granny Smith” apples was improved by brown paper bags when bagged at the golfball-size stage of fruit development. Hofman et al. (1997) reported that the fruit firmness of mangoes was not affected by white paper bags. Faoro and Marcia (2004) studied the effect of bagging on fruit firmness and reported that bagging did not affect the fruit firmness in “Nashi” pears. Murray et al. (2005) subjected the Japanese plum cultivars “Laetitia” and “Songold” to different levels of shading by bagging entire scaffold branches with shade netting and reported that bagged plums were firmer than unbagged plums. Singh et al. (2007) reported that bagging of “Allahabad Safeda” guavas produced fruits with a soft texture. Rosângela et al. (2001a) reported a reduction in the fruit firmness of apples after bagging, while Sharma and Pal (2012a,b) reported retention of firmness with bagging at harvest and during subsequent cold storage in “Royal Delicious” apples.

2.13 Pesticide Residues

To combat different biotic stress-causing agents, several chemicals (insecticides, fungicides) have been used of late. The residues of these chemicals have been proved to be hazardous for consumers, and hence nonchemical approaches in the form of GAPs are gaining popularity throughout the world. Fruit bagging, which provides a physical barrier between the applied chemical and the produce, can be a useful approach in reducing pesticide residues on fruit (Chen et al., 2006; Han et al., 2015; Kim et al., 1988; Lin et al., 2008, 2009; Liu et al., 2003; Shaomin et al., 2002). However, only a few studies have been conducted in this area. To cite the reported work, it is important to mention the work conducted by Wang et al. (2003), who found that bagging had no effect on the taste of *Litchi chinensis* fruit, but reduced the residue of fenpropathrin and trichlorphon on the fruit. Liu et al. (2003) reported a reduction in the pesticide residue in “Red Fuji” apples. Similarly, Chen et al. (2006) reported that nonbagged apples have higher heavy-metal (Pb, Cd and Cr) and pesticide-residue content than bagged apples, and that single-layer bagged apples have a higher content than double-layer bagged ones; it was concluded that apple bagging is an effective measure in ensuring apple sanitation and safety. Debnath and Mitra (2008) reported that litchi fruit developed inside the bags were free from the residues of agrochemicals. Further, Lin et al. (2008) reported that the residues of chlorpyrifos, carbendazim, and cyhalothrin in fruits were also greatly reduced by bagging in “Cuiguan” and “Hosui” pears. Similarly, Lin et al. (2009) reported that residues of cyhalothrin and carbendazim were much lower in the bagged fruits of “Cuiguan” pears and recommended that double bagging was more effective in producing high-quality pear fruits.

2.14 Bird Damage

Birds act as major enemies for certain fruit crops, namely, bananas, mangoes, apples, dates, and more, especially at the ripening stage, and they cause considerable damage. Several conventional approaches, such as the beating of drums, stretching of reflecting ribbons in the fields, and so on were adopted traditionally, but the birds are smart enough to get acclimatized to these practices very soon. In our opinion, fruit bagging can be the best practice to produce sound and bird damage-free fruits, if employed at the right stage of fruit development. Some studies have reported that fruit bagging helped to reduce the damage caused by birds in different fruits (Amarante et al., 2002a; Harhash and Al-Obeed, 2010; Hofman et al., 1997; Joyce et al., 1997).

3 BAGGING DATE

In fruit bagging, individual fruits (e.g., apples, mangoes, guavas), panicles (e.g., litchis), or fruit bunches (e.g., grapes, bananas) are covered by a polyethylene, newspaper, or other type of bag during the developmental stage. One major question that arises is when to bag the fruit/bunch/panicle. To answer this complicated question, several experiments have been conducted on different fruits by scientists across the world to judge the effect of bagging at different times on appearance, color, insect pests, diseases, and disorders, and the results have shown a difference of opinion among researchers (Li et al., 2005). The date of bagging has a pronounced influence on the fruit color of “Yuzu” citrus fruit (Kwan et al., 2000), as bagging before early September with recycled Japanese PBP resulted in a worse coloration than that of control fruits, whereas bagging on September 20 and thereafter resulted in a similar coloration to nonbagged fruit. Sharma et al. (2016) studied the influence of staggered fruit bagging in “Royal Delicious” apples and found that fruits bagged 60 days after full bloom developed excellent color with a high anthocyanin content. Hu et al. (2001) reported that bagging in “Feizixiao” litchi fruits should be done 15 days after full bloom until harvest for better coloration.

BS is a major problem in the “Shinsyu” persimmon (*Diospyros kaki* L.), and in order to reduce it Fumuro and Gamo (2001) covered all fruits of each tree with paper bags on September 17 (the beginning stage of coloration); they reported that bagging significantly decreased the occurrence of BS and increased the ratios of BS-free fruit by 4–7 times over the nonbagged ones, and suggested that “Shinsyu” persimmons should be bagged 50–35 days before harvest for the prevention of BS. Costa et al. (2002) reported that bagging bunches of “Nanicao” bananas with polyethylene sacks on three dates (May 21, 1996; December 17, 1996; and February 26, 1997) produced no significant difference in the emergence of inflorescence and harvest.

Katagiri et al. (2003) bagged “Fuyu” persimmon fruits with white paper bags at different dates and found that bagging from early August or mid-September until early December markedly reduced blemishing and improved appearance compared to the control group. Early removal of the bags in late October, however, caused an increase in blemishing by harvest time. Faoro and Marcia (2004) studied the effect of bagging with different paper bags on two bagging dates, 34 and 83 days after full bloom, and reported that the use of small transparent paraffin paper bags or large brown kraft paper bags 34 days after full bloom, and also the use of small transparent paraffin paper bags at 34 days followed by the use of a double large bags

at 83 days after full bloom, resulted in a better fruit appearance (uniform, with a shiny and smooth skin color and small lenticels). [Xu et al. \(2008\)](#) studied the effects of bagging dates on carambolas and reported that the best fruit quality could be obtained if bagging was carried out 10 days after full bloom, and that the effects of fruit bagging on fruit width and length varied with bagging date. [Wang et al. \(2010b\)](#) reported that bagging in “Wanmi” peaches was done at the early stage of endocarp hardening to study its effect on volatiles and polyphenols; they reported that the total volatile compounds and esters of bagged fruits was significantly lower than that of nonbagged fruits. [Mathooko et al. \(2011\)](#) reported that bagging in mangoes 70 days after bloom (DAB) promoted color and reduced blemishes and produced high-quality fruits, leading to improved exports and better prices for mango farmers. [Lin et al. \(2012\)](#) reported that bagging “Cuiguan” pears once (covering fruit with large paper bags 35 DAB) or twice (covering fruit with small paper bags 20 DAB followed by large paper bags 45 DAB) was more helpful in producing top-grade fruit than bagging once. In contrast, [Beasley et al. \(1999\)](#) reported that neither bagging date nor bagging material (paper/plastic) led to significant differences in either skin or flesh calcium concentration of “Kensington” mango fruits. Similarly, the marble stage of fruit development was reported to be best in “Álphonso” mangoes ([Haldankar et al., 2015](#)). [Sharma and Nagaraja \(2016\)](#) reported that the bagging of guava fruits at the marble stage of fruit development is best for getting cent percent of fruit free from fruit-fly incidence.

4 KIND OF BAG

The type and material of the bag may have a significant influence on a fruit, and a bag recommended for one fruit may not work well for another fruit ([Hong et al., 1999](#)). Several studies have been conducted on this subject and research findings have demonstrated contradictory results ([Table 16.5](#)). For example, [Kwan et al. \(2000\)](#) reported that the type of bag has great influence on “Yuzu” fruit and recommended black polyester (PET) bags and BP (gray-colored paper bags) for better coloration. Further, [Coelho et al. \(2008\)](#) experimented with 14 different types of bags in “Aurora 2” peaches and reported that bagging with transparent polypropylene microperforated bags can be recommended because these bags allow the producers to see the color of the fruits at harvest time, making the harvesting operation easier. Though bagging with nylon bags was found to reduce fruit-fly infestation

Table 16.5 Effect of different kinds of bags on color, insect pests, disorders, and quality of different fruits

Fruit	Bagging material used	Best recommendation	Positive influence(s)	References
Apple	Blue, light-yellow, red, and green spunbonded single-layer bags	Light-yellow bags	Improvement in color, firmness and reduction in storage disorders	Sharma and Pal (2012a,b); Sharma et al. (2013, 2014a,b) Dong et al. (2007)
	Different types of bags	Paper bags	Better absorption of calcium in fruits	
Carambola	Plastic bags, self-made newspaper bags, and nonwoven cloth bags	Plastic bags	Increase in fruit size, higher SSC	Xu et al. (2008)
Date palm	Black or blue polyethylene bags, white “agrisafe” (polypropylene fleece), and paper bags	Black or blue bags	Increased rate of ripening	Awad (2007)
	Black, white, blue, or yellow plastic bags	Blue plastic bags	Accelerated fruit-ripening process	Harhash and Al-Obeed (2010)
Fig	Polyethylene bags, butter-paper bags, brown kraft paper bags	Polyethylene bags	Improvement in color and quality	Mazaro et al. (2005)
Guava	Waxed paper, nylon fabric, Taiwan bags, and PBP	Nylon bags	Highest protection against fruit fly	Morera-Montoya et al. (2010)
	PP nonwoven bags, nettings, brown paper bags	PP nonwoven bags	Best protection from fruit fly	Sharma and Nagaraja (2016)
Litchi	Cellophane paper, adhesive-bonded fabric, newspaper, and kraft paper	Cellophane or fabric bags	Better coloration in fruits	Hu et al. (2001)

Loquat	One-layer white paper bags (OWPB) and two-layer paper bags with a black inner layer and a gray outer layer (TGDPB). Eleven kinds of paper bags	OWPB	Promotion in appearance	Xu et al. (2010)
		Shengda paper bags	Improvement in the appearance of fruits by reducing rate of strain, fruit rust, splitting fruit, and anthracnose	Liu et al. (2004)
Mango	Black polybags, transparent polybags, brown paper bags	Brown paper bags	Reduction in the incidence of fruit fly, maintains higher TSS, and better physical quality of fruits	Sarker et al. (2009); Haldankar et al. (2015)
	Bags made of old newspaper, brown and black paper Wavelength-selective bags (no pigment, yellow, red, blue/violet, and blue) and kraft paper bags with black paper liner	Brown and/or black paper VM and V plastic bags	Improvement in peel color Improvement in fruit weight and peel glossiness	Ding and Syakirah (2010) Chonhenchob et al. (2011)
	Two-layer paper (black inside with brown, and waxed and white outside), newspaper and golden paper bags	2-layer paper (brown outside and black inside)	Improvement in fruit weight and peel appearance	Watanawan et al. (2008)
Nashi pear	Small transparent paraffin paper bags, large brown kraft paper bags	Small transparent paraffin paper bags	Development of uniform color, shine and smooth skin color with small lenticels in fruit	Faoro and Marcia (2004)

(Continued)

Table 16.5 Effect of different kinds of bags on color, insect pests, disorders, and quality of different fruits (*cont.*)

Fruit	Bagging material used	Best recommendation	Positive influence(s)	References
Peach	White, orange and black bags	White or orange bags	Better coloration of pulp	Takata et al. (2006)
	14 different types of bags	Transparent polypropylene microperforated bags	Better color of fruits	Coelho et al. (2008)
	Coated white paper, white paper, coated yellow paper, yellow paper, and newspaper	White-coated bags	Improvement in appearance and accumulation of higher amount of anthocyanin	Kim et al. (2008b)
Pear	Haoguo bags, Jiatian bags, and common bags	Haoguo bags	More attractive surface, higher soluble solids, and lower acid content in fruits	Lin et al. (2008)

in guavas (Morera-Montoya et al., 2010), the incidence of black-dot and red-dot diseases in apples was found to be higher in the fruits bagged with different kinds of specially designed bags, namely, Kobayashi, Tongle, and Qianwei (Qin et al., 2012; Wang et al., 2000b).

5 DATE OF BAG REMOVAL

The date of bagging and type of bag used, which influence fruit color, size, and/or quality, not only are important, but the date of bag removal also plays an important role. For instance, initial bagging studies in apples have reported strong color inhibition, primarily because bagging intercepts light, which is absolutely needed for anthocyanin synthesis. However, later experiments have revealed that the date of bag removal has a greater influence on color development in apples. In general, if bags are removed from fruits on the day of harvest, it is likely that fruits will have poor color development; however, if bags are removed 3–7 days before the actual day of harvest, the apples are more likely to develop better and more attractive color than unbagged fruits (Ju, 1998). Thus, it appears that reexposure of fruits to sunlight after bag removal is a must for anthocyanin synthesis to bounce back. To cite a few examples, Fan and Mattheis (1998) reported that enclosing “Fuji” apple fruits in paper bags 2 months after full bloom delayed and reduced red color development, but external surface color changed significantly within the first 4 days after bag removal. Ju (1998) reported that reexposing bagged apples for 3 days to sunlight helps in the accumulation of maximum anthocyanins. Hu et al. (2001) reported that retaining cellophane or fabric bags on “Feizixiao” litchis until harvest is essential for better coloration. Further, Huang et al. (2009) reported that the bags should be removed at least 10 days before harvest in order to obtain red Chinese pears and sand pears with an attractive appearance and good inner qualities. Qu et al. (2012) reported that anthocyanin content in “Fuji” apples was noticeably lower than that of control fruit at bag removal, but rapidly rose following removal. The anthocyanin content exceeded the control value on the 6th day following removal and was approximately double the control group on the 8th day after bag removal.

6 CONCLUSIONS

From the above-mentioned discussion it can be concluded that preharvest fruit bagging is a simple, farmer-friendly technology, which is safe to use and has multifarious effects on the physical appearance and quality of

produce. Further, it is the safest approach for the protection of fruits from several insects, diseases, and disorders. This approach is an integral part of fruit production in several developed countries in the world, yet it needs popularization in developing countries as well. By and large it is a laborious process, and development of biodegradable bags that can decompose after use is needed. Moreover, we need to standardize specifications in terms of kind of bag, date of bagging, removal of bags, and so on, to harvest maximum benefits from this ecofriendly technology.

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Preharvest Modulation of Postharvest Fruit and Vegetable Quality

Edited by **Mohammed Wasim Siddiqui, PhD**

Assistant Professor and Scientist, Department of Food Science and Post-Harvest Technology, Bihar Agricultural University, Sabour, India

Preharvest Modulation of Postharvest Fruit and Vegetable Quality focuses on the potential yield quality, quantity, and safety benefits of intervention during growth. Among the several factors responsible for overall quality of produce, about 70% comes from preharvest conditions while only 30% postharvest factors affect the quality. In fact, with the very best of postharvest knowledge and technologies available, the best that can be achieved is a reduction in the rate at which products deteriorate as they progress through their normal developmental pattern of maturation, ripening, and senescence. Therefore, it is very important to understand what preharvest factors influence the many important harvest quality attributes affecting postharvest deterioration and subsequently, the consumers' decision to purchase the product in the market. *Preharvest Modulation of Postharvest Fruit and Vegetable Quality* is a unique addition to maintain and modify the postharvest quality of fresh produce in terms of safety and nutrition. The information provided within the text can be used to extend the shelf life of fruits and vegetables by retaining nutritional and cosmetic appeals.

Key Features

- Presents the preharvest factors that influence important harvest quality
- Includes up-to-date information on preharvest factors that modulate postharvest biology
- Identifies potential methodologies and technologies to enhance preharvest interventions

About the Editor

Dr. Mohammed Wasim Siddiqui is an established postharvest researcher, academic, and editor and presently affiliated to Department of Food Science and Post-Harvest Technology, Bihar Agricultural University, Sabour, India as an Assistant Professor and Scientist. He is an author or co-author of 36 peer reviewed research articles, more than 30 book chapters, and several conference papers. He has 18 books to his credit published by Elsevier, USA, CRC Press, USA, Springer, USA, & Apple Academic Press, USA. He is the founding editor of two book series namely *Postharvest Biology and Technology* and *Innovations in Horticultural Science* being published from Apple Academic Press, New Jersey, USA. He is an editorial board member and active reviewer of several international journals. Dr Siddiqui has received more than 15 awards and fellowships in recognition of research and teaching achievements from national and international organizations.



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