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Fruit Industry

Edited by İbrahim Kahramanoğlu and Chunpeng Wan

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Meet the editors

İbrahim Kahramanoğlu is an associate professor in the Faculty of Agriculture, European University of Lefke, Northern Cyprus. He is an expert in horticultural production, postharvest biology and technology, and good agricultural practices. His main areas of studies include postharvest physiology and handling of fruits, natural and novel technologies for handling and storage, digital and precision farming for sustainability, and value adding to horticultural crops. He has authored various books, book chapters, conference papers, and scientific publications.

Dr. Wan is a professor in the College of Agronomy, Jiangxi Agricultural University (JXAU), Nanchang, China. He received his MS in Traditional Chinese Medicine (TCM) from Jiangxi University of Traditional Chinese Medicine, and his Ph.D. from Nanchang University, China. Dr. Wan joined the Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, USA, as a visiting scholar and postdoctoral fellow in 2011 and 2014. His research interests include phytochemistry and human health benefits, postharvest biology, and the technology of citrus fruits. His research continues to be funded by federal, state, and other agencies. Dr. Wan has edited various books and authored more than 150 publications.

Contents

Preface

Fruits are an excellent source of important vitamins, minerals, phytochemical compounds (e.g., anthocyanin, flavonoids, lycopene, carotenoids, phenolic, etc.), and dietary fiber. These phytochemicals protect plants against macro- and micro-animals and improve their adaptability to different environments, including stress conditions (salinity stress, drought stress, high-temperature stress, etc.). The fruit phytochemicals also provide support to human health. Eating a diet high in fruits is believed (and scientifically confirmed for specific cases) to reduce the risk of or help cure numerous diseases, including certain cancers, cardiovascular diseases, diabetes, and hypertension. No single fruit can provide all essential nutrients to human beings, in whom a diversified diet with fruits and vegetables is recommended.

Dietary diversity, both within and across food groups, is a key strategy for a healthy life. The World Health Organization (WHO) recommends adults eat about 300–400 g of fruits and vegetables daily. According to data provided by the Food and Agriculture Organization (FAO), fruits account for the fourth highest amount (76.79 kg/capita/year) and share (10.71%) of annual food supply after cereals, vegetables, and milk groups. It is estimated that if the global population continues to increase, fruit production will need to increase about 50% by 2050 to feed the world's population.

The world has been challenged by food safety and food insecurity issues for several decades and this is expected to continue in the future. Food insecurity is mostly driven by incorrect horticultural practices (e.g., misuse and excessive use of agrochemicals, fertilizers, and irrigation), the increase in the human population, and shifts in people's dietary characteristics. From the 1950s to today, the global population has increased more than threefold, and thus it is necessary to increase the food supply. However, at the same time, it is very important to protect natural resources and ensure the sustainability of horticultural production to be able to guarantee food security in the future.

Agricultural activities have dramatically changed in the last ten decades. The shifts in agricultural systems and new technologies in farming helped to increase crop yield for a short time, but the negative impacts of such actions (i.e., agrochemicals, high use of fertilizers, monoculture, etc.) appeared later. Some examples of these negative impacts are food safety issues (related to agrochemicals), water pollution, air pollution, loss of biodiversity, salinity, the resistance of pests to chemical control, and so on.

Fruits are botanically diverse, seasonal, and perishable. They play an important role in the global economy. The fruit industry, which includes the selection, reproduction, production, harvesting, handling, preservation, and marketing of fruits, is very important for the human diet and the sustainability of Earth. Each and every step taken in the fruit industry must aim to achieve fruit safety and security.

Our planet provides diverse climates and natural resources (soil, water, air, heat, light, biodiversity, climate, etc.) for horticultural production, providing ample opportunities for the development of the fruit industry. In the present era of globalization, it has become very important to produce more at less cost to be able to survive in the market. However, it is also very important to protect natural resources and ecosystems to ensure sustainability in horticultural production and in human life. As such, this book presents and discusses current information on the fruit industry, highlighting new topics for further research and industrial development.

This book is a useful resource for farmers, students, teachers, professors, scientists, food packers and sellers, and entrepreneurs in the fresh fruit industry. I would like to thank my co-editor, chapter authors, author service manager, and all who have contributed directly or indirectly to bring the publication of this book.

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Section 1

Postharvest Management

Chapter 1

Perspective Chapter: Traditional, Innovative and Eco-Friendly Methods for Postharvest Storage of Fruits

İbrahim Kahramanoğlu, Serhat Usanmaz and Chunpeng Wan

Abstract

Fruits are among the most important elements of human diet. It is also well known and scientifically confirmed that fruit-based diet helps to protect human health and prevent many human diseases, mainly because of the high contents of vitamins, minerals, and phytochemicals. Since the human population on the earth is increasing, the need for fruits is also increasing. However, at the same time, the main factors of fruit production, that is, soil, water, and climate, are being damaged by human activities. Therefore, the production of the fruits and vegetables is becoming difficult. Furthermore, nearly 30% of fruits do not reach the consumers because of the postharvest losses along the fruit value chain. Therefore, prevention of the postharvest losses is highly important for ensuring the sustainability of life through consumption of wholesome fruits. In this chapter, we aim to list and discuss the traditional, innovative, and eco-friendly methods for postharvest storage of fruits. We also aim to provide most current information about these methods and provide practical information for students, scientists, farmers, food packers & sellers, and entrepreneurs engaged in fruit storage.

Keywords: agrochemicals, edible film packaging, innovative packaging, modified atmosphere packaging, traditional storage

1. Introduction

Food insecurity is reported to be the world's most crucial problem in the near future. Shifts in human diet, non-diversification in production and consumption (selection of nearly 200 crops for horticultural production), monoculture, excessive and mis-use of pesticides and fertilizers, reduction in soil fertility, reduction in water quality and quantity and increase in human population are the most important causes of food insecurity [1–3]. For example, only four crops (sugar cane, maize, wheat and rice) accounts half of global primary crop production and consumption in 2019. However, fruits are a major part of human diet and of horticultural production. They are very important for healthy life [3]. The most commonly produced fruits globally include bananas, apples, grapes and citrus [4]. Agricultural activities have significantly changed over the last 10 decades. Most of these changes, such as use of high yielding varieties and agrochemicals, cause an increase in the crop yield and quality in the beginning. However, excessive and mis-use of these activities resulted into negative impacts on soil, water and biodiversity, and adversely affected agricultural sustainability [5]. Aside the problems of production and storage, the uneven distribution among population also drive food insecurity [6].

The total agricultural primary products was 9.2 billion tons in 2018 [4], which is 50% more than the total agricultural primary products recorded in year 2000. Fruits and vegetables accounted for 10% and 12% of the global primary products for year 2018, respectively. Although there is an increase in the amount of production, the hunger is on the rise, which is estimated to be 690 million people undernourished in 2019 [4]. It is noteworthy that as hunger is increasing, food losses (from postharvest o distribution, excluding retail level and homes) is also increasing. The Food Loss Index (FLI) of Food and Agriculture Organization (FAO), based on the models developed by official records, noted 13.8% and 13.3% food loss globally in year 2016 and 2020, respectively. Among the crop groups, fruits and vegetables accounted for about 22%, which is highly related to their perishable nature [4]. The addition of the losses at retail level and homes, the postharvest losses may reach up to 50%, which means about half of the quantity of fruits and vegetables produced were not consumed and lost due to several factors such as poor postharvest handling practices, high physiological and respiration activities, high moisture content, microbial infection etc. This loss is highly crop dependent and handling practices are very important for the prevention of these losses [7, 8].

The main causes of the postharvest losses (senescence and deterioration) are the respiration, transpiration and diseases and these are highly dependent on prevailing temperature, relative humidity, light, atmospheric composition and ethylene concentration of the surrounding environment [8, 9]. Since the beginning of agriculture, human beings have developed several practices for reducing postharvest losses of fruits and vegetables. Additionally, agrochemicals have important role in controlling postharvest pathogens. In today's world, where there is an increasing negative awareness on agrochemicals, the eco-friendly methods are having more attention by the consumers. The developments in technology also made it possible to better understand fruit physiology and develop some innovative techniques for fruit storage. However, there is not a clear distinction among the traditional, innovative and eco-friendly methods for storage of fruits and vegetables (**Figure 1**). This chapter therefore aimed to discuss and summarize up-to-date information about these traditional, innovative and eco-friendly methods for fruit storage for practical information for readers.

2. Traditional methods

Since the beginning of horticulture and production of horticultural crops, human beings are trying to find suitable ways for different products to improve their storability. Most of these traditional techniques are mainly simple and in line with ecocentric philosophies of the adopted societies. These techniques mostly aim to convert these perishable products to more stable products for improving storage life and to eliminate toxicity. Some of these traditional methods are listed below in separate headings and discussed briefly.

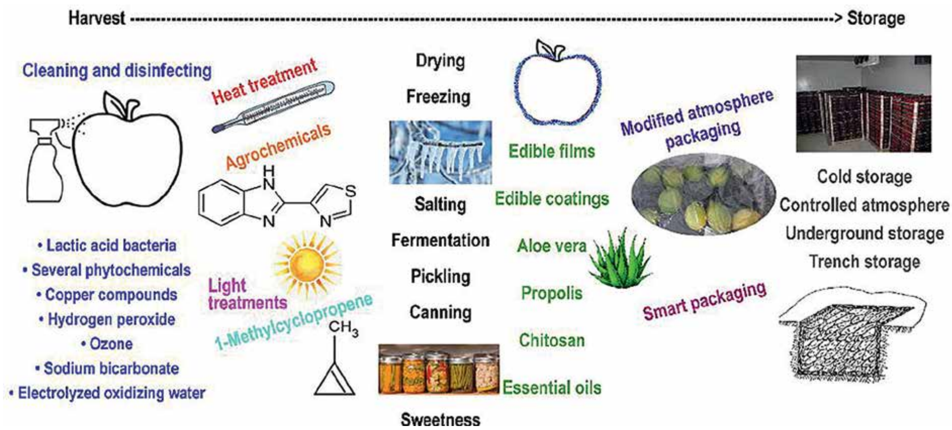

Figure 1.
List of different traditional, innovative and eco-friendly methods for postharvest storage.

2.1 Underground storage

Temperature is one of the most crucial environmental factor affecting the postharvest storability of products, by stimulating/reducing pathogen development and fruit ripening/senescence. Increase in temperature increases the pathogen growth rate and stimulates fruit ripening. Thus, reduction of the temperature is so crucial during postharvest storage. However, lowering temperature too much (below 7 C) for a longer duration may cause chilling injury on many subtropical fruits [8].

People have historically learned how to preserve/store foods (including fruits and vegetables) and developed some techniques for food preservation. This was necessary, because the climate was/is not suitable for growing same fruits and vegetables throughout the year on the same place. People have understood that harvested fruits and vegetables are alive and they can be stored longer if the respiration and transpiration can be stopped or slowed which reduce the spoilage. During the colonial era, the use of cold storage, ice boxes or ice houses were not common. The ice houses and temporary food storage emerged during the beginning of nineteenth century. However, ice was difficult and expensive to obtain at that time. Hence, the underground rooms were more common to keep fruits cool [10].

2.2 Cold storage

Storage is the act of storing fruits and vegetables in a safety place being ready for consumption but not being used at that time. It aims to prevent fruits and vegetables from deterioration for a specific time period [11]. Moreover, cold storage is so crucial and important way of fruit storage, which helps to reduce respiration and transpiration and so delays senescence and prevent deterioration [8]. Therefore, the idea of cold storage was reported to date back to ancient times. The first forms of cold rooms were formed from the ice blocks which lead to the developments in ice industry in 1800s. During 1830s, ice became among the most important marketing items. After the works of Benjamin Franklin and John Hadley in 1758 (about cooling an object with the help of evaporation on volatile liquids), in 1820 Michael Faraday liquefied ammonia by applying high and low pressures, and then in 1834 Jacob Perkins invented the first vapor-compression cooling system. Then the refrigeration equipment became popular in meat industry [12]. The mass production and use of the refrigerators were reported to begin around 1918 in USA [13].

2.3 Drying

Drying is among the most used traditional methods for food preservation. In this method, the fruits and vegetables were dried to reduce the water content (dehydration). Removal of water helps to inhibit the growth of food pathogens. According to Nummer [14], drying of fruits dates back to ancient times in Middle East and Asia around 12.000 BC. Mostly, sun drying, air drying or wind drying had been used for evaporation purposes for dehydration. Nowadays, with the help of technology, food dehydrators can be used for same purpose, which provides quick and consistent results than traditional methods [15]. Drying the fruits results with a reduced water and increased sugar concentration, which ensure longer storage duration and sweeter taste. However, drying the fruits significantly changes the structure of fruits and makes them different than the fresh ones. For example, grapes become raisin, while plums transform into prune. Besides to freeze-drying, some forms of light (ultraviolet light, X-rays or ionizing radiations) can also be used for sterilization and dehydration [16].

2.4 Freezing

Freezing fruits and vegetables for ensuring preservation dates back to prehistoric times. People used ice and snow for preserving their hunts and then used for fruits and vegetables. It slows the movement of molecules and enzyme activity in the fruits and pathogens, and delays spoilage by causing pathogens to enter into dormant stage. The frozen water in the fruits and vegetables become unavailable for the pathogens. However, it is known that most of the pathogens remain dormant during frozen and can be problem after thawing. Frozen fruits became popular after 1930s [14]. Depending on the physiology of the products, the fruits and vegetables can be kept safely for 3–12 months. The critical temperature is −18°C and products should be kept at or below this level. Packaging is also recommended in suitable (i.e. plastic) bags to prevent freezer burn. It is not recommended to freeze hot fruits and the refrozen the thawed products [17]. Freezing alone does not change the nutrient contents of the fruits. On the other hand, freezing slows the activity of several enzymes (which may increase deterioration, if active) but not halt their activity. Enzyme activity can be neutralized in frozen fruits by the acids but not in the vegetables which are low acidic. Therefore, partial cooking in boiling water is recommended for vegetables to slow/prevent deterioration. This process is known as blanching. Lack of oxygen or freezer burn, especially under longer storage may cause change in color. Slow freezing may be problematic for several fruits, especially for pomegranate arils. It creates large ice crystals in the fruits, which may damage the cells during thawing and dissolve emulsions. Therefore, fast freezing is recommended in such products. Thawing is as important as with freezing. The most safety way of thawing was reported to be under cool conditions, such as refrigerator or cold water. After thawing, the products must be cared carefully as they are perishable and consumed in a short time as possible [18].

2.5 Salting

Salting is a type of drying, mainly used before refrigeration. It is used to draw water out of the fruits and vegetables which provides similar advantages with drying and prevents food deterioration [19]. Sodium plays an important role in reducing pathogen growth and improves texture. A number of other sodium-containing compounds are also used for increasing the safety and shelf life of foods or creating physical properties [20]. There are two ways of salting. In the dry method, the fruits or vegetables are surrounded in salt and left till dry (water is drawn out into the salt). The second method is wet curing. In this method, salt is dissolved in water and is called as brine. Then the fruits or vegetables are placed in brine and left in cool dry place [21]. Salt reduces the activity of water in foods. This is because the sodium and chloride ions tents to associate with water molecules [22]. Salt also cause osmotic shock on microbial cells, loss of water from microbe cells and finally death of the pathogens [23].

2.6 Fermentation

It is the process of converting carbohydrates to alcohol or other organic acids by using microorganisms (mostly yeasts) or oxygen-free conditions. This process is used to produce alcoholic drinks including wine and beer. This is not well used in fresh fruit industry but most commonly used for bread, cheese, yoghurt, wine, beer, vinegar and olives [14]. Salt has an important role in food fermentation. Products like pickles and cheese obtain most of their characteristics from the lactic acid bacteria. Salt inhibits the growth of many spoilage bacteria while favoring the growth of salt-tolerant lactic acid bacteria [24].

2.7 Pickling

Pickling is another method of fruit or vegetable preservation which takes place in acids, especially vinegar. Vinegar is an end product of fermentation. Firstly, the carbohydrates are fermented into alcohol and next the alcohol is oxidized into acetic acid by some bacteria. Wines and beers can be used to produce vinegars [14]. Pickling is most commonly performed by placing the fruits or vegetables in water, salt, some herbs and vinegar or lemon. Pickling may require boiling the fruits or vegetables in the salt mixture. After the food is infused by the pickling solution, it must be placed in an airtight container [10].

2.8 Canning

Another important method for food preservation is the application of canning technology. In this method, the fruits or vegetables are placed in cans or similar materials and heated to destroy pathogens and inactivate enzymes. The cans are being cooled after heating and this creates a vacuum seal. Thus, the entrance of external pathogens is also being prevented [14]. This method was firstly developed in France, between 1795 and 1809, with the aid of French government to feed their citizens during the wars [10].

2.9 Sweetness (jam)

Boiling the fruits (whole, pulp or parts) in sugar mixture or sealing those in honey are among the other methods of food preservation. The use of honey is not new and has been used for thousands of years for fruit preservation [10]. For this purpose, fruits should be ripe and firm. After harvest, fruits should be washed, generally peeled, pulped (removing seeds), and sugar (generally in the proportion of 1:1 or 1:0.5 w/w) is added. Then the mixture is being boiled with continuous stirring. Sometimes citric acid can be added. The fruit-sugar mixture is cooked till the soluble solids concentration reached 65–70%. Hereafter, the jam is filled hot into sterilized bottles, capped and stored (even at ambient temperatures). High sugar content of jams makes the moisture unavailable for the growth of postharvest pathogens and other microorganisms.

2.10 Trench storage

Trench is an excavation in the ground, commonly deeper than is wide and narrow than its length. Trench storage is a traditional method for food preservation. It is suitable for preserving late-maturing varieties of different fruits [25]. In these systems, the trenches are filled with wet sand (3–7 cm), then the fruits are placed in (30–60 cm) and finally covered with maize straw or reed mat to control temperature. This method is beneficial for especially apples [25]. In this method, the trench (together with wet sand) provides moisture for the fruits and keep the fruits cold. It is the traditional way of modern cold storage rooms.

2.11 Heat treatment

Postharvest heat treatments (mostly as air or water) have a long history in fruit preservation. Although it is a traditional method, there are numerous recent studies about innovative applications of heat. Heat treatments have several advantages on fruit storage, (1) regulating products' response to cold, (2) direct control of pathogens, (3) improving products' tolerance against pathogens, (4) cleaning products and (5) maintaining products' quality during storage [26–28]. Empirical studies are required for determination of the best temperature and duration for different types of heat application for different varieties of crops. Mostly temperatures from 30 to 40°C for hot air treatments (HAT) for a duration of range from hours to days were reported to be effective in conditioning treatments for different product varieties [26]. Moreover, hot water treatments (HWD), are generally applied at temperatures from 45 to 55°C for a few minutes (3–5 min) for controlling postharvest pathogens [29]. Biochemical reactions in living organisms are highly affected by temperature. Change in the temperature around the plants and/or fruits signals the metabolism to make regulations in cell function and metabolism for preventing heat-related harms [30]. Heat stress cause metabolic imbalance in crops, affects cell membranes and proteins and alters several enzymatic reactions, including reactive oxygen species (ROS) [31, 32]. These changes in the metabolism improves products' tolerance against pests and helps to maintain products' quality. The heat can also directly damage the existing pathogens' cells and control the growth and development of diseases. Peroxidase (POD) and superoxide dismutase (SOD) enzymes can alleviate lipid peroxidation, thus the chilling injury [33, 34] and polyphenol oxidase (PPO) enzymes enhance fruits' resistance against pathogens [35, 36]. Heat treatments also prevent or delay the occurrence of chilling injury [26]. The heat-shock proteins (HSPs) increase after heat stress and protects products against chilling injury. However, these HSPs disappear quickly when the fruits are placed in ambient air conditions [37]. Not only in traditional application, but also for commercial applications today, the heat treatment is commonly used and known as safe, effective and physical [29].

2.12 Agrochemicals

The first use of fungicides dates back to seventeenth century. Firstly salty water treatment on grain and then copper sulphate (1760) took place for controlling grain bunt. Sulfur (1824) and lime sulfur (1833) were then used for pathogen controlling of food [38]. The developments in the fungicide history was continued with bordeaux mixture (1885) and mercury chloride (1891). This was continued with farmer prepared inorganic preparations till 1940s and the industrial & commercial fungicides were developed in 1940 with chloranil and dichlone active ingredients [38]. Since then, fungicides are widely used in controlling funguses during production and after harvest, mainly because of their easy to use and ability to bring about quick results in the food products. It is important to note that most of the pathogens which damage the products after harvest require a field application is also necessary to reduce/eliminate infections [39]. Fungicides applications can be done for controlling the infections on the fruits and/or vegetables, and also can be done in cold rooms or storage rooms to reduce/eliminate the sources of infections. Application of fungicides to the products can be done in different ways, mostly as dipping the products into the solutions, spraying onto the products, as volatiles/gas into the environment (fumigants), treating as wraps or embedded in coatings/films. Dipping and spraying are the two most common applications. The active ingredients of thiabendazole (from benzimidazoles group) and imazalil (from triazoles group) are among the most common fungicides which have been widely used against several important pathogens including *Penicillium* and *Colletotrichum*. Both fungicides are approved by European Union and are in use as of February 2022 [40]. Moreover, sulfur dioxide as fumigant is widely used against gray mold (*Botrytis cinerea*). For each and every application of the fungicides, it is a must for checking the suitability of the active ingredients and the chemical against the target pathogens and product. This is very important for the elimination of the chemical residues on the products. The doses of the chemicals must be used correctly according to the recommendations and fungicide rotation must be done in store houses to prevent fungicide resistance in the pathogens. Similar with the other agrochemicals, the fungicides have permitted limits on crops as the maximum residual limit (MRL) [41].

2.13 Cleaning and disinfecting

Cleaning and disinfecting are among the most important measures for better storage of fruits and vegetables. It is highly recommended to be carried before other handling practices for fruits and vegetables. This is a traditional measure but is being innovated continuously and several eco-friendly methods are also being used for cleaning. Hygiene is very important for the products health and quality both during production and storage. It is very critical to reduce (if possible eliminate) the sources of pathogens before storage. For this reason, a good knowledge is required about the type of the pathogen, its characteristics and life cycle. Therefore, cleaning of the pathogen sources prior to storage is strongly recommended for each product [39]. Chlorine was among the most widely used chemicals for product disinfection. The chlorine dioxide has ability to penetrate into the cell membrane of the target microorganisms and inhibit the metabolic functions [42]. However, it is now prohibited in many European countries because of its potential harms on human health and increased public concern about its use [43]. It was reported that there can be some by-products of chlorine, such as chloroform and chloramines, which are carcinogenic [44]. There are some biological or chemical alternatives of chlorine in which some of them are more effective and safer than chlorine. Some of them are listed below and explained briefly. Some of these techniques are not traditional, but are listed below:

- Lactic acid bacteria (LAB) are classified as Generally Recognized as Safe (GRAS) and produce some antimicrobial compounds, which help them to be used as disinfecting agent [45]. An example of these compounds is *Bacteriocin nisin*. This may form pores on the cell membrane and cause cell death [46]. In a study, Bari et al. [47] used nisin at 50 ppm for 1 min on broccoli and reported high efficiency against *Listeria monocytogenes*.

- Several phytochemicals. In their natural structures, a lot of plants are capable of producing phytochemicals (secondary metabolites) which have several roles in plants body, including control of pathogens. Phytochemicals are mainly divided into three groups as terpenes, phenolics and nitrogen/sulfur containing compounds [48]. The mod of action of these phytochemicals vary widely, but main impact takes place by increasing membrane permeability of the cells and leading leakage of cell compounds. Essential oils cover the most important part of these phytochemicals, such as carvacrol from thyme, citral from vitrus and etc. [48, 49].

- Copper compounds. Copper is essential for many microorganisms at very low concentrations. However, its higher concentrations may damage membrane integrity and stimulate the development of free radicals, which results with the cell death [50]. On the other hand, its high concentrations can be toxic and this limits its use in food industry. Because of this, the combination of copper with other compounds, i.e. lactic acid or hydrogen peroxide is suggested [51].

- Hydrogen peroxide (H_2O_2) is a reactive oxygen species which has antimicrobial characteristics due to its ability to form cytotoxic species [52]. The most important disadvantage of this compound is its fast decomposition, which is being not effective in cross-contamination [53]. Moreover, it may cause browning onto the vegetables, i.e. lettuce [54].

- Ozone (O_3) is a gas which can be dissolved in water. Ozone fumigation is among the important sanitizing agents having high potential against a wide range of pathogens [55]. Very small concentrations (1–5 ppm) are enough when dissolved in water for controlling most of the pathogens, but higher concentrations are necessary for gas applications [56]. It has several important disadvantages and higher attention is required during application. First of all, it can be toxic because of its respiratory tract and irritation ability [57], it decomposes rapidly and unstable [58] and corrosive for equipment [59]. However, it was approved as a disinfectant for water and decontaminant for products [60].

- Sodium bicarbonate. It is another important GRAS food additive. Palou et al. [61] suggested that it can be used for controlling molds at citrus fruits caused by

Penicillium italicum and *Penicillium digitatum*. In a recent study, Vilaplana [62] reported that the postharvest application of 298 mM (2.5%) sodium bicarbonate is effective in improving the storability and shelf life of pitaya fruits, by reducing weight loss, retaining color and firmness, slowing down the changes in solible solids concentration, titretable acidity and pH and most important controlling the black rot caused by *Alternaria alternata*. It was also suggested that the combination of sodium bicarbonate with hot water or *Bacillus amyloliquefaciens* improves the efficacty against pathogenic decay at mandarin fruits [63].

- Electrolyzed oxidizing water (EOW) is a new, innovative technique for food storage industry. It is an activated water. It is formed by electrolysis of sodium chloride by passing it from chamber containing an anode and a cathode [64]. EOW is low-cost, eco-friendly and safe method [65, 66]. Its ability to be produced on-site is important for the elimination of exposure risks [67]. It is deactivated when contacts and reacts with organic matter and tap water can easily dilute it from the environment [66].

3. Innovative methods

The above listed traditional methods have been still using in handling practices and still have important role in product preservation. As described above, some of these techniques have been developed and some innovations were applied to the techniques. Besides to that, there are some other newly developed innovative techniques in postharvest handling. Some of these methods are listed and described below.

3.1 Controlled atmosphere (CA) storage

Respiration rate of the products is strongly related with the product senescence [68]. The increase in the respiration increases the fruit senescence. Thus, the reduction of the respiration rate is known to improve products storability by delaying the fruit ripening and product senescence. This can be achieved by modifying the atmosphere surrounding the product. The modification must achieve a reduced oxygen level and increased carbon dioxide level for being able to reduce respiration rate. However, it is also strongly important not to eliminate oxygen level in the surrounding atmosphere, which can cause anaerobic respiration and alcohol production in the products, which could result into bad smell and decreased marketability [68].

Since the beginning of technological developments, people have tried to modify surrounding atmosphere of the products for increasing storability [69]. Controlled atmosphere (CA) storage is among these technical, which simply aims to monitor and adjust of the O_2 and CO_2 levels within gas-tight cold rooms [70]. The combination of CA rooms (so control of temperature and relative humidity) with ethylene control lowers the metabolic activity of products and improves storability. In these systems/rooms, the atmospheric composition inside the air-tight room must be controlled regularly and maintained during storage. In the CA storage rooms, there are two common ways of regulation of the room atmospheric composition, these are: static and dynamic. Products generates the atmosphere by respiration in cold rooms and it is being regulated by ventilation and scrubbing in the static method, whereas a supply of gas concentration is required in the dynamic system. There are different systems used for controlling/regulating oxygen and carbon dioxide concentrations. Simply the oxygen level inside the room is reduced to pre-defined (well-studied) desired level by nitrogen flushing, and then carbon dioxide is being injected with a gas generator [71]. CA storage is reported to be an advantageous method for the preservation of climacteric fruits (having increased respiration rate after harvest), i.e. apples, mangoes, bananas, papayas, etc. The effectiveness of CA storage is not very high on the non-climacteric fruits and some others with slow respiration rate [68].

3.2 Modified atmosphere packaging (MAP)

Respiration is the basic process in freshly harvested fruits and vegetables causing deterioration and it is mainly dependent on the atmospheric composition (mostly the level of O_2 and so CO_2) as well as on the temperature, ethylene and water vapor. Therefore, regulating the gas concentrations in the surrounding atmosphere of the fruits (or other food products) is highly important for reducing respiration and increasing storability of the products. Reduction of O_2 and elevation of CO_2 can delay deterioration of fresh horticultural crops [72]. However, it is highly dependent on the type of commodity, cultivar, maturity and temperature. At this time, some important methods are coming to forefront to regulate atmospheric composition around the fresh products. When the generation and stabilization of favorable atmosphere are obtained by packaging refrigerated produce in closed polymeric films of reduced dimensions (bags, boxes, pallets), the technique is called MAP. Modified atmosphere packaging (MAP) is a dynamic process of altering gaseous composition (mainly oxygen and carbon dioxide) inside a package. If the volume of gas with a different atmospheric concentration was introduced at the time of sealing the package (active MAP) or simply the bag was sealed with atmospheric air (passive MAP). The passive MAP is an old technique and is not used well, while the active MAP constitutes the most important share in the MAP use. The interaction between the respiration rate (RR) of the crop and the permeability of packaging material are the two important elements of modified atmosphere packaging, with no further control exerted over the initial gas composition [73]. The permeability coefficients of the materials for O_2, CO_2, N_2, and H_2O is very important. Also the type of the crop, growing conditions, physiological stage and storage time are important for the selection of appropriate packaging material. The most used materials for MAP are low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), ethylene-vinyl alcohol (EVOH), ethylene vinyl acetate (EVA), and etc. Each of these polymeric materials offers different mechanical characteristics and fundamentally different permeability to O_2, CO_2 and water vapor. Such as: polyamide has very low permeability to O_2 and CO_2, polypropylene and EVA has moderate permeability and LDPE has high permeability [74]. It is also reported to be successful for the prolongation of storage duration of several fruits by slowing down respiration, reducing weight loss, reducing sensitivity to ethylene, reducing development of some physiological disorders (i.e. chilling injury), maintaining product quality and preventing decay [75, 76]. This techniques (MAP bags) is an important way for extending the storability of fresh products.

3.3 1-Methylcyclopropene (1-MCP)

1-Methylcyclopropene (1-MCP) is an ethylene inhibitor. Ethylene is a colorless gas (plant hormone) which plays an important role in the postharvest life of horticultural crops, especially the climacteric ones. It is also known as the ripening hormone. It is produced by the climacteric plants during ripening and it speed up the ripening and respiration of climacteric fruits (apples, banana, mangoes, apricot, tomato, etc.), while it damages the postharvest quality of non-climacteric fruits (grape, lemon, eggplant, cherry, watermelon, etc.) at the same time. It can also be produced by petrol combustion engines [77]. However, the higher concentration or continuous production/supply of ethylene damage the quality of the climacteric fruits too. Therefore, the control of ethylene is important to improve the storability of fresh horticultural crops. Thus, 1-MCP interact irreversibly with ethylene receptors and avoids its physiological stimulus [78]. The active ingredient of 1-MCP is currently (as of February 2022) approved by European Union and can be used in postharvest storage of fruits and vegetables [40]. Therefore, it is used in postharvest storage to down regulate the ripening of fruits. Its postharvest and preharvest application was reported to delay ripening of many fruits [79].

3.4 Light treatments

Light has fundamental roles in many biochemical reactions, including well-known photosynthesis. It also has important impacts on physical and chemical process and its range and dose are the most important factor. The higher energy of the light might be toxic, while the lower doses can be ineffective [80]. The electromagnetic spectrum of light ranges from long radio waves to gamma (γ) rays [81]. The different ranges of the spectrum has been tested for different purposes in horticulture, including production and postharvest storage [82]. With the help of the LED technology, which has ability to produce light from a narrow bandwidth of wavelengths, including UV LEDs, IR LEDs, and LED blue lights [82]. The non-ionizing irradiations (UV-C [100–280 nm], UV-B [280–315 nm], UV-A [315–400 nm] and blue light [400–500 cn]) of the light and ionizing irradiations (gamma (γ) and X-rays) may have different impact on the products' quality. This impact can be direct, by inhibiting the growth of postharvest pathogens, or indirect by inducing the resistance of products to the several pathogens and/or storage stress conditions [80, 83]. UV-C is the most tested light treatment in postharvest studies, especially against postharvest pathogens and was reported to be very effective [84, 85]. The duration and intensity of the light is so crucial and there are numerous different studies with different fruits and vegetables. Besides to UV light, blue light is also reported to have high efficacy in postharvest studies. It is mostly absorbed by plant tissues and have job in several metabolic reactions [86]. It has both role in growth promotion in plants and damages in fungal cells. It was reported to induce ferroptosis (nonapoptotic type of iron-dependent cell death) in fungal cell by activating ROS production in the cell wall and damaging the DNA [87]. Therefore, the light irradiation, especially the UV-C and blue light have significant advantages in postharvest studies and are powerful tools in postharvest handling with the help of technology [80].

3.5 Smart packaging

In these systems, the packaging is embedded intelligent (agri 4.0) sensor technology is embedded in the packaging material to monitor the status of the products during storage. The data generated about the quality is used to regulate storage conditions for improving product and customer safety [88]. Several different principles (sensors, indicators or radio frequency identification-RFID) are being used in these systems to monitor the temperature, atmospheric composition, microbial growth, integrity indicators and freshness indicators of the products [89]. Monitoring of these factors and quality parameters enables the active and quick response to the changing factors. For example, adjusting of the atmospheric composition is so crucial for prevention of the respiration rate and so the postharvest losses. Similarly, control and management of temperature and relative humidity are crucial both for fruit quality and for the control of pathogens. Thus, the smart and innovative technology makes this possible without the need for mental control, but with the artificial intelligence. Therefore, smart packaging may help to actively prevent fruit spoilage, improve/maintain the fruits' characteristics (i.e. taste, aroma, appearance, etc.), hence extending the storability of products. However, the integrity of artificial intelligence into these systems is new, where some doubts exist about its performance and it is more expensive than traditional methods [90]. Sooner or later, smart packaging techniques will be highly developed and introduced into the market; which is hoped to reduce postharvest losses.

4. Eco-friendly methods

Because of the negative impacts of agrochemicals on the environment and human health and the damages on the ecosystem caused by the petroleum-based packaging materials, there is a trend in postharvest studies to develop and introduce the use of eco-friendly alternatives in postharvest handling of fruits and vegetables. Some eco-friendly alternatives which are promising alternatives to agrochemicals and petroleum-based packaging materials have been listed and discussed. It is believed that these methods will be upgraded, modified appropriately in postharvest handling of fruits and vegetables globally in future.

4.1 Edible film packaging and edible coatings

Edible film packaging is a thin layer (with less than 0.3 mm thickness) [91] formed from the combination of biopolymers and various additives which is prepared before application and then adhered to the product surface and can be consumed as an integral part of the food product [92]. Edible coating is similar with the edible films, but formed as thin layer directly on the product surface [93]. Their application onto the products is differs from the edible films, and are applied in liquid form by dipping the products into the solutions. Both the edible films and edible coatings have high attraction by the consumers and environmentalist, because of their biodegradable characteristics as compared with the plastic packaging materials. These materials, similar with the plastic packaging materials, creates a modified atmosphere around the products and helps to regulate respiration and transpiration [94, 95]. Several other benefits of the biomaterials used in the edible film industry are prevention of microbial decay (both by inducing product resistance or direct control of pathogens), reducing weight loss, improving product appearance, maintaining the composition and concentration of phytochemicals and etc. [96]. Proteins, polysaccharides, lipids and the secondary metabolites of plants are being used for the production and use of edible films [95, 96].

Wheat gluten, corn zein and soy protein are important protein sources for edible films and coatings, where oils and waxes are the most used lipid-based materials. Plant- (cellulose, gums, starch, pectin, etc.) or animal- (chitosan and chitin) based polysaccharides are on the other hand are being highly used for production of edible films and coatings [95]. The mechanical characteristics (elasticity, elongation and tensile strength) are among the most important characteristics of edible films and coatings [97]. Starch, with its gelatinization characteristics has been used in bio-packaging universally. Another important bio-polymer is the alginate, which has ability to form hydrogels and encapsulation barrier [98]. Chitosan (discussed below separately) has recently been involved in food packaging industry for edible films and coatings. The ability of the materials to provide a barrier for the transfer of gaseous and water vapor, ability to improve food storability and processing techniques are the most important points to consider for the selection of the right material. The polysaccharide-based materials and chitosan have are nonpolar to the aroma compounds and produce a good barrier [99]. Alginate and cellulose, on the other hand, are hydrocolloid-based materials which have higher ability to retain moisture [100]. To sum up, edible films and coatings helps to reduce transpiration and respiration, retard ethylene biosynthesis, stimulate the biosynthesis of several enzymes (i.e. PPO), stimulate antifungal activity of products, enhance the activity of secondary metabolites and so protect the products' storability.

4.2 *Aloe vera* application

The perennial *A. vera* plant, belonging to the family of Xanthorrhoeaceae, has been widely used in medicine and traditional medicine for curing several human diseases. Besides to that, the gels of *A. vera* have an important role in food industry, mostly as a source of edible film or coating [101]. It has ability to reduce respiration and transpiration and delay food deterioration. Besides to this general advantage of edible films and coatings, the *A. vera* plant extracts or gels, have antimicrobial ability to control several microorganisms, including postharvest fungi. In a research by Nabigol and Asghari [102], it was found that the A. vera gel have high ability to stop the mycelium growth of *Penicillium digitatum* and *Aspergillus niger*. Similarly, Kator et al. [103], suggested that *A. vera* gel application prevents decay at tomato fruits. The number of examples can be extended to several other fruits, including pineapples, nectarine, grape, plum, strawberry and etc. [104–108].

4.3 Propolis application

Propolis (bee-glue) is a natural resinous produced by honeybees from plant exudates. Previous studies revealed that the propolis includes wide variety of phytochemical compounds including phenolic which have been linked with its beneficial characteristics [109]. The sources of plants and the season are highly affecting the chemical composition of the propolis. It has been using in traditional and scientific medicine for several decades and noted to have wound healing ability since 300 BC [109]. Moreover, it was reported to have high benefits in postharvest handling of fruits and vegetables. Studies suggested that the high concentrations of cinnamic acid, ferulic acid and caffeic acids provides anti-microbial ability for the propolis extract [110]. Similar with the light and edible materials, the positive impacts of propolis on the control of pathogens can be in two modes of action as direct control or improving resistance of the products [109]. Extracts of propolis had been suggested to reduce gray mold (*Botrytis cinera*) at pomegranate fruits [76], anthracnose at mango fruits [111] and *Penicillium digitatum* at orange fruits [112]. Besides to the control of postharvest pathogens, several studies suggested that the propolis extracts reduce weight loss and chilling injury, maintains soluble solids concentration, titratable acidity, ascorbic acid, total phenolic content, antioxidant activity, textural quality and overall acceptability of several fruits, including pomegranates, mango, papaya, banana and orange [76, 111, 113–115].

4.4 Chitosan application to stored fruits

Chitosan is a linear polysaccharide which is obtained from the exoskeleton of insects and the shells of crustaceans (i.e. crab, shrimp, lobster, etc.). It has a wide area of use in different sectors including agriculture, medicine, cosmetics, textile, food and biotechnology. The use of chitosan as a supplement to edible films and coating had been reported to have high potential for maintain product quality and reducing pathogen growth. It is a biocompatible polysaccharide with intrinsic antimicrobial characteristic [116]. The chitosan was approved in the European Union (Reg. EU 662014/563) for plant protection purposes. It was suggested that chitosan have three separate characteristics, which makes it an important alternative in postharvest. It has an ability to produce biofilms on the applied surface [117], it has high antimicrobial ability [118] and it has ability to stimulate the defense mechanism of the products [119, 120]. The exact mechanism behind the antimicrobial activity of chitosan has not been completely understood yet. The polycationic structure of the chitosan has been suggested as one of the reasons of the mechanism. The chitosan is positively charged which causes it react with cell membranes which are negatively charged [121]. This connection then damages the membrane permeability, inhibits DNA replication and finally cause cell death [122]. As mentioned above, chitosan stimulate the defense mechanism of products against pathogens. Several different defense related mechanisms have been reported to be stimulated by the application of chitosan, including pathogenesis-related (PR) proteins [123], several secondary metabolites (i.e. chitinase, lignin, phytoalexins, etc.) [124, 125] and reactive oxygen species (ROS) [126].

4.5 Essential oils application to stored fruits

Essential oils (EO) are complex and concentrated hydrophobic liquid containing volatile aromatic substances derived from plants. These oils are mostly composed of terpenes, phenols, esters, alcohols, nitrogen and sulfur compounds [127]. EOs are commonly extracted by steam distillation and can also be derived by cold pressing, solvent extraction and wax embedding. EOs can be used in different industries including cosmetics, perfumes, air fresheners and as food additive. Several studies have reported that the different EOs have higher efficacy in controlling postharvest diseases [128]. The application of the EOs can be as direct application (as vapor) or incorporation into films, coatings or washing materials [41]. Some recent research noted that the essential oils of myrtle (*Myrtus communis* L.) leaves [129], black cumin oil [130], and lemongrass oil [131] have high potential of promoting storability of loquat, apricot and strawberry fruits, respectively.

4.6 Plant-derived methods

Besides to the essential oils, several different plant extracts or plant-derived materials have been reported to have significant positive influence on the prevention of product quality and preventing the growth and development of several postharvest pathogens [132]. For example, ethanolic extracts of garlic have been reported to control *Penicillium* sp. in citrus fruits [133]. In a different study, the extracts of guava leaves and lemon have been suggested to improve the storability of banana fruits [134]. In another study, the aqueous extracts (10% and 20%) of neem, chinaberry, and marigold were noted to improve the storability of guava fruits [135]. On the other hand, cinnamon, pimento, and laurel extracts were tested against postharvest gray mold at apple fruits. The in vitro studies were found to be effective, while the in vivo studies with fruits were found to be ineffective [136]. The success of the plant extracts might be because of the essential oils (explained above) or other forms of secondary metabolites. It is clear from the above examples that the plant-derived biomaterials have significant positive impact on the storage quality of fruits and vegetables. Therefore, the development and use of plant-derived materials is a promising alternative for synthetic chemicals which can improve the storage quality of the products.

5. Conclusions and recommendations

Reduction of the postharvest losses is so crucial for ensuring food security on the earth and eco-friendly management of postharvest losses is so important for its sustainability and ensuring food safety. Thus, the use of eco-friendly postharvest technologies, i.e. edible films, is valuable for reducing postharvest losses throughout the value chain. Aside from the selection and use of traditional and innovative techniques in postharvest handling is so crucial, where a proper postharvest handling includes at least cleaning, selection, grading, packing and storage. Where applicable, curing and/or heat treatment before storage; pre-cooling; packaging with plastic or edible materials; and regulation of the surrounding atmospheric composition are essential and highly recommended for successful postharvest handling. They have vital role in keeping products' quality and storability during supply chain. It is highly important to focus on eco-friendly alternatives in all kind of application for every step and to develop eco-friendly & innovative technologies to overcome postharvest losses.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 2

Postharvest Management and Marketing of Apples in Mexico

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Abstract

México produced 745,820 tons of apple fruit in 2020 and does not supply the national demand, so imports must be made. Apple production is from July to October in the year, the highest production is in September and is destined for national consumption. In the fresh market, the highest sale price is in July and August, but as of November the price drops. For that reason, the business producers use refrigeration. The refrigerators can be 1500 cubic meters or higher, and cooled with harmless gases to avoid damage to the atmosphere, the refrigerator must have a temperature of 3.3 to 3.9° C, a CO_2 concentration of less than 10% and a concentration of oxygen from 2.0 to 2.5%. In the region the best refrigerators use liquid nitrogen and can take out their apple slowly each month to supply the market at a better price. To keep the fruit in CA until January of the following year, to have prices higher than $50 Mexican pesos per kilo (2.45 US dollars).

Keywords: refrigerants, control atmosphere, marketing

1. Introduction

Mexico has an area of 57,186 ha of apple tree, of which 43,200 ha are irrigated (75.5%), the production in 2020, was 745,820 tons, if the average apple consumption per capita is 8.8 kg [1], for that reason only 84,753 inhabitants had access to the national apple, which represents less than 1% of the population in Mexico [2], therefore there are production deficit of apple in Mexico, and the rest of the population would not have access to the apple in their annual diet, to supply this demand with the apple production of Mexico, the producers who select the apple sell it in the fresh market as rural development producers and those who select and refrigerate it to have a better price are business producers who are in the irrigated areas.

The biggest amount of apple in Mexico is imported from January to August of each year from the United States and Chile (**Figure 1**), the national production is only from August to December, very few producers make refrigeration storage to have apple until March. This causes greater apple imports, with capital flight and representing an increase in imports each year [3].

The apple is a climacteric fruit, after cutting the fruit rapid increase the respiration rate, in appearance it changes as softening, de-greening, wax accumulation and aroma

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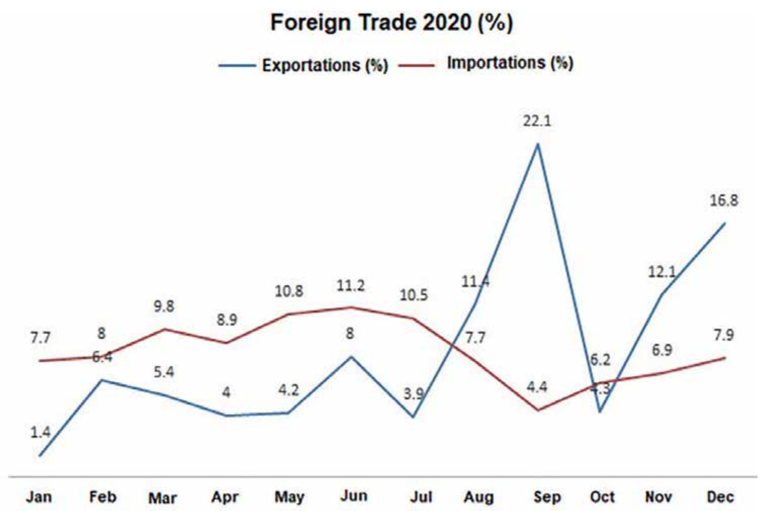

Figure 1.
Importation and exportation of apple in México.

production, inside the fruit increase the activity of organic acids, lipid, starch, sugar with reduced respiration with cuticle covers and cooling is the best way to known fruit storage potential [4].

Imports start from January, where most domestic production has already been sold and consumed. The varieties that enter by import are Red Delicious, Golden Delicious and Gala, are those that have had acceptance in the national market [5].

The apple harvest in Mexico begins in July until October and the highest production is in September (**Figure 2**), in this month if you want to store to have apple every month until March, you must have infrastructure of refrigeration rooms and only business producers have it and can get a higher sale price.

The state with the highest production, largest area and highest yield in Mexico, is the region of Cuauhtémoc and Guerrero in Chihuahua, a northern state of the country bordering the United States and the apple zone is on the mountain range of the Western Sierra Madre (**Table 1**), in this region of greater production is where

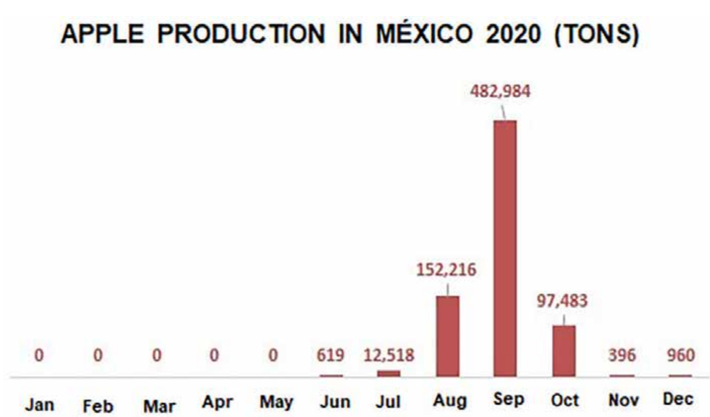

Figure 2.
Apple production in Mexico in moths.

Entity	Superficies (ha)			Production (tons)	Yield (t/ha)
	Planted	Harvested	Sinister		
Chihuahua	33,936.25	32,429.75	0	627,603.13	19.35
Puebla	7499.00	6487.90	0	34,557.23	5.33
Durango	6577.04	6473.09	0	7085.20	1.09
Coahuila	5802.00	5725.00	0	44,748.22	7.82
Nuevo León	1290.00	1233.00	28	3110.20	2.52
Chiapas	1196.25	984.25	0	3460.51	3.52
Veracruz	833.00	822.00	0	9204.20	11.2
Hidalgo	804.30	744.50	0	3048.68	4.09
Oaxaca	768.01	721.16	0	2468.69	3.42
Zacatecas	701.5	558.5	0	4036.77	7.23

Table 1.
Apple production by the first 10 states in México.

refrigeration is mostly used to have apple until March, its use is for fresh consumption, the varieties that are produced are Golden, Red Delicious, Starkrimson and Rome Beauty.

The second region in importance is the state of Puebla, with two regions, Zacatlán which is located in the Eastern Sierra Madre, where Golden apple, Red and regional creole are produced, and Huejotzingo which is located on the slopes of the Iztaccíhuatl volcano whose production is for cider with creole trees that, over the years, they have improved them for the production of this drink. In Zacatlán very little apple enters refrigeration, the surplus of national consumption, they market it to Belize, a neighboring country with a subtropical climate with a border with Mexico.

The third region in importance is the Sierra de Arteaga, which is located in the Eastern Sierra Madre and includes the states of Coahuila and Nuevo León, where you have Gala apples, Red type, Golden type mainly, business producers market it as a fresh market and rural producers use it for preserves, jams and wines that they sell on Sundays in the public square in the views that tourists make to their communities. Business producers refrigerate it and market it in supermarkets.

The last region of apple importance in Mexico is Canatlán in the state of Durango, which is located in the western Sierra Madre and is mainly produced apple of red type, and the Golden type of regional varieties, this state for being south of the state of Chihuahua, mainly its market is fresh and few producers refrigerate the apple to market it.

These four regions represent 96.1% of the national production and 91.5% of the area planted with apple in Mexico, therefore, apple production is governed by these regions, and only less than 15% use refrigeration systems to market the apple when the imported apple enters the country [6].

2. Postharvest management

The apple is harvested when it begins to change color, the seeds become dark, the fruit is easily cut from the tree and the water content in the fruit is reduced, which allows us to estimate a good storage of the apple [7]. These specimens are indicators of the firmness of the fruit, the change of color and the breathing of the fruit, which is inversely proportional to the firmness. Most apples in their ripening increase ethylene production which if it is not controlled cuase senesce in apple storage [8]. There are genotypes with minimal ethylene production to reduce storage problems [9].

The intensity of respiration of a fruit, depends on its degree of development, it is measured as the amount of CO_2 (mg) released from each kilogram of fruit per hour [10]. Respiration depends on the water content, because if there is a low water content at the time of harvest, the apple fruit when breathing dehydrates during storage, ages and lowers its value in the market.

There are products to reduce the respiration of the apple, bioplastics and conservation in controlled atmosphere with refrigeration that will increase the shelf life of the apple fruit, so the sale price at harvest that is $ 15 / kg (0.73 USD), will increase at least $ 35 / kg, (1.71 USD) being able to reach up to $ 50 / kg (2.45 USD) in April which is the time of greatest import of apple from abroad.

The new varieties do not allow the fall of the fruit, they stay in the tree to be cut, this was a problem in the harvest because a lot of fruit was fallen and at the passage of the tractor with the boxes to place the harvest, it destroyed all fallen fruit. Its harvest is carried out in cloth bags (**Figure 3**), with a lower opening so as not to damage the apple and to be able to deposit it in the harvest box; the dimensions of the box are 1 × 1 × 1 m, one cubic meter [11].

The boxes with the fruit are taken to packing plant to enter a washing and waxing process. There are several methods to prolong the post-harvest life of the apple, which are: storage at low temperatures, the use of plastic packaging to create modified atmospheres, the application of hydrothermal treatments, irradiation and formulations containing biological agents, among others.

The controlled atmosphere (CA) and the use of bioplastics turn out to be the best alternative to prolong the shelf life of the fruit. So, to start you must have a controlled and automated atmosphere cooler for when there are failures in the electrical power [12].

The apple fruit is a climacteric fruit because its epidermis can be consumed, different from orange and avocado, whose bioplastic covers are not for consumption with epidermis [13].

Bioplastics are high molecular weight long chain polymers. They are based on waxes or other products (such as polysaccharides), and their use is to maintain the organoleptic quality of fruits during their shelf, commercialization and export processes [14].

Bioplastics reduce the rate of respiration and dehydration of coated products, in addition, these coatings allow the incorporation of food additives (antimicrobial agents, antioxidants, mineral salts, etc.) that slows down enzymatic browning by oxidation, the appearance of physiological disorders such as surface scalding, microbial growth, loss of texture, weight loss and total acidity due to the fermentation of sugars in the fruit; it allows to control wrinkles, increases the marketing period and improves their appearance by providing them with shine [15].

The fruit once harvested should be washed with detergents and fungicides such as Imazalil (maximum concentration allowed of 2 mg/kg) [16], Thiabendazole (allowed interval of 0.02 to 10.0 ppm) [17], Orthophenylphenol (0.4 mg/kg bw/day) [18], and then dried with air. The drying temperature can be up to 30°C, the higher the drying temperature, the greater the impact on the organoleptic and physiological characteristics of the fruit, since the higher the drying temperature the greater the dehydration of the fruits, reducing their weight.

Figure 3.
Bag to deposit apple when harvesting from apple tree.

Edible coatings have good results in the control of weight loss, have fewer metabolic problems (fermentations) and greater reduction in senescence, but provide little shine. The bioplastics that will be used are: beeswax, carnauba wax, shellac, candelilla wax, chitosan plus oleic oil and lemon oil and imported edible coatings such as sodium alginate and Hydroprofil-Methyl cellulose [19].

For disease control during cold storage, the agriculture sustainability in horticultural production has options like wheat gluten, corn zein, soy protein, oils, waxes, starch, pectin, A. vera polysaccharides, cellulose, plant gums and secondary metabolites which are citral, eugenol, thymol [20].

The use of bioplastics is made at harvest when the apple has greater firmness and greater hydration in its tissues. Once it goes through the waxing, it is selected by size and the packaging is made for marketing in the fresh market. The packers select by size (**Table 2**) and color, the size is given by the NMX – FF – 061 – SCFI – 2003

Grade	Commercial size	Equatorial diameter (cm)		Fruit weight (grams)	kg per box (grams)
	Number of apples per box	Class	Average		
Extra	48	Greater than 9.2	Greater than 9.2	375	18.00
Extra	56	8.4 a 9.2	8.80	324	18.14
Extra	64	8.1 a 8.9	8.50	284	18.18
Extra	72	7.8 a 8.6	8.20	253	18.22
Extra	80	7.5 a 8.3	7.90	227	18.16
Extra	88	7.2 a 8.0	7.60	207	18.22
First	100	6.9 a 7.7	7.30	182	18.20
First	113	6.6 a 7.4	7.00	162	18.31
Second	125	6.4 a 7.2	6.80	145	18.13
Second	138	6.3 a 7.1	6.70	131	18.08
Second	150	6.2 a 7.0	6.60	122	18.30
Third	163	6.0 a 6.8	6.40	111	18.09
Third	175	5.6 a 6.4	6.00	105	18.38
Fourth	198	5.2 a 6.0	5.60	91	18.02
Fourth	216	4.8 a 5.6	5.20	85	18.36
Marble	234	4.0 a 4.8	4.40		
Mean					18.19

Table 2.
Mexican classification norm for packing apple fruit.

Box Size	Diameter		Weight	
	Inches	mm	grams	ounces
234	2 1/4	57.2	79	—
216	2 5/16	58.7	86	3.1
198	2 3/8	60.3	94	3.4
175	2.46	62.5	—	3.8
163	2 9/16	65.1	114	4.1
150	2 5/8	66.7	124	4.5
138	2 11/16	68.3	135	4.8
125	2 3/4	69.9	149	5.4
113	2 13/16	71.4	165	5.9
100	2 15/16	74.6	186	6.7
88	3	76.2	211	7.6
80	3 3/16	81	232	8.4
72	3 5/16	84.1	258	9.3
64	3 3/8	85.7	291	10.5
56	3 1/2	88.9	332	12
48	3 5/8	92.1	387	14

Table 3.
USDA Washington standards for apple fruit.

standard, which is a Mexican classification system that considers size as extra, first, second, third, fourth and marble [21]. There is the USDA Washington Standards (**Table 3**) rating system, which uses the size of the box as a reference, if the box is 175 has apple with a diameter of 2.46 inches, a weight of 3.8 ounces, the commercial box will have 175 apples [22].

The commercial box is made of cardboard and the harvest box is made of wood, both types of box can be stored in refrigeration, because in supermarkets the two presentations are placed, the wooden box only has a single variety of apple (**Figure 4**), and

Figure 4.
Wood box presentation in supermarket to commercialization.

Figure 5.
Carton box presentation in supermarket to commercialization.

they are considered in bulk at a price of $ 40 / kg (1.94 USD) in March; in cardboard boxes you can present several varieties for the taste of the consumer (**Figure 5**) to be taken by customers and carry the amount they need, but the price rises to $ 50/kg (2.45 USD) in March, when the import of apple is greater in Mexico.

3. Refrigeration

A CA is an environment that is artificially produced, in which the oxygen, nitrogen and carbon dioxide concentrations as well as the temperature and humidity are regulated [23]. Firmness, soluble solids and acidity are the most important quality indices relating with best sales. Reduction in temperature and O_2 or increse in CO_2 in CA storage reduce the rate of loss in acid [24].

Figure 6.
Liquid nitrogen tank and controller.

The cooling process is to circulate refrigerant gases to reduce or maintain the temperature below the ambient temperature. The refrigerant gas begins in an initial state (liquid or gaseous), to go through a series of processes and return to its initial condition. This series of processes are known as the cooling cycle. This cycle will be repeated as many times as necessary to absorb heat through the refrigerant [25].

The use of refrigerant gases is in full evolution and modernization due to the application of the European F-Gas regulation, whose main objective is the reduction of the use of hydrofluorinated greenhouse gases (GHG) by 70% by 2030. This regulation is associated with the application of the Tax on fluorinated gases entered into force since January 1, 2014, because hydrofluorinated carbon gases (HFCs) have a high Global Warming Potential (GWP), which has caused professionals and manufacturers of HFC gases to look for alternatives and substitute gases that are compatible with

Figure 7.
Automatic nitrogen compressor.

the refrigeration equipment already installed and that are equally efficient in their application [26].

Low-GWP refrigerant gases such as R-407c have been generated, is a mixture of hydrofluorocarbons used as a refrigerant. It is an azeotropic blend of R 32, R 125, and R 134a, his applications include residential and commercial air conditioning systems, and some commercial refrigeration systems. The R134a refrigerant is mainly used as a refrigerant in automobile air-conditioning and commercial refrigerant applications and the R410A refrigerant is used in new residential and commercial air conditioning systems.

A cooling room of 13 × 16 × 6 meters by 2021, has a cost of $3048,893 (147,717.7 USD), but operates with 404 Y refrigerant gas, which is a hydrofluorocarbon (HFC) refrigerant. However, these low-WGP gases will not be able to be used in 2022 for new equipment,

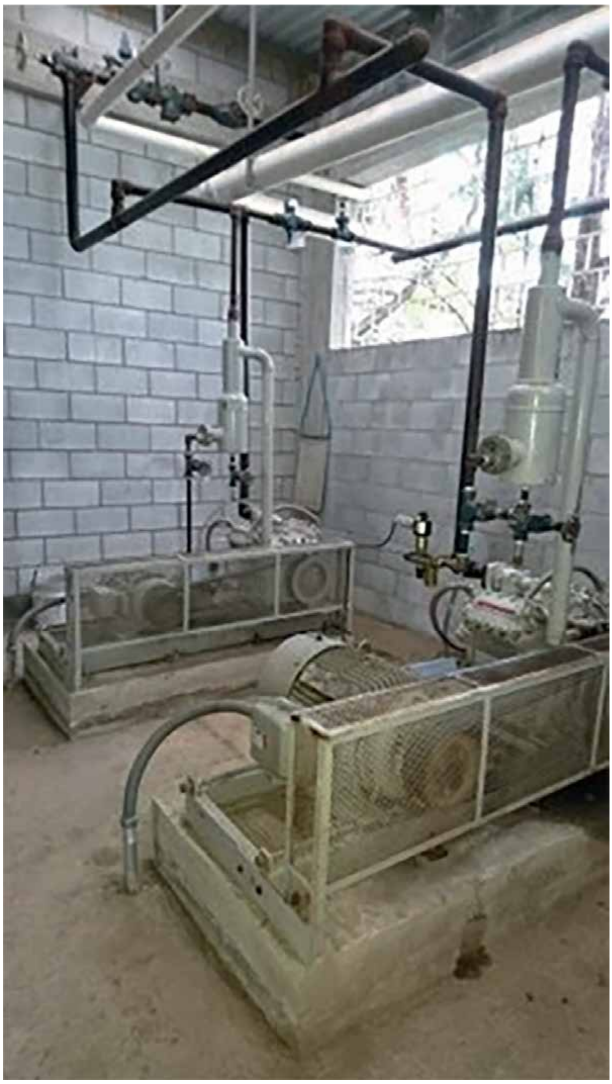

Figure 8.
Nitrogen refrigerant pumps.

Figure 9.
Expansion device that distributes the cold.

Figure 10.
Nitrogen recycling as refrigerant and automation panel.

although they do not yet have a deadline for service and maintenance, to conserve the quality of apples fruit [4].

The conditions of refrigeration gases to reduce the effect of greenhouse gases have caused ammonia (NH_3) to be considered again, it is a gas whose use dates back to the nineteenth century and its application in commercial refrigeration equipment is widespread even today, in medicine, livestock, agriculture, industry, hospitals, hotels or airports and other areas where refrigeration is needed [27]. NH_3 as a refrigerant has the ability to achieve cooling at temperatures down to −70°C, but great care must be taken in its handling because it is a colorless, odorless, tasteless and deadly gas.

The use of liquid nitrogen in the refrigeration and freezing of food is booming because it is a gas that does not affect the ozone layer, although its implementation is expensive. Liquid nitrogen reaches extremely cold temperatures (−70°C), making it the fastest method of individual food freezing [28].

Figure 11.
Wood box with apple in stacked order into the refrigerator.

The operation of ammonia cooling consists of a liquid nitrogen flow reservoir and controller (**Figure 6**) from there it passes to a compressor (**Figure 7**) that compresses to condensation temperature the dry gas that comes from the separator at evaporation temperature and pumps the gas for cooling to the condenser (**Figure 8**). The coolant arrives from the condenser to the expansion device that distributes the cold in the room where it is located at the top of the room where the apple boxes are (**Figure 9**) and then recycles it to continue with the cooling cycle (**Figure 10**).

A CA cooler with nitrogen cooling of 4 × 6 × 4 m has a cost of $ 3,781,646 (183,219.3 USD), if we compare it with the cooling with HFC, it is higher but with less volume, which indicates that cooling with nitrogen has more cost.

The refrigeration system must keep the apple at a temperature of 3.3 to 3.9° C, a CO_2 concentration of less than 10% and a concentration of oxygen from 2.0 to 2.5%, with a relative humidity of 90%, for a storage period of 6 months [29], this means that if harvested in August, the apple would be coming out in its entirety by March. The wooden drawers with the apple are stacked in the cellar with refrigeration (**Figure 11**), so that they are taking out boxes every month according to the needs of the market.

4. Merchandising

The apple of the business producers would leave in March at the price of $ 50 per kilogram (2.45 USD) in cardboard boxes and every month in box from September to March in wooden box at a price of $ 40 per kilogram (1.94 USD), the price is to recover the costs of investment in energy, which now in Mexico with the energy reform, it is unknown what the cost of energy will be, this is a formal trade with supermarkets y only use brand names when they are associated with the supermarket, the name of apple orchard is only identification.

Figure 12.
Apple with tamarind at $12 per piece.

Figure 13.
100% apple artisan liquor at $ 300 per liter.

The apple of rural production that does not have refrigeration systems, in Mexico it is known as informal trade for that reason do not use brand names, the sold as an added value in sweets (**Figure 12**), alcoholic beverages (**Figure 13**), jams, juices, cider and very little as a fresh market, so the apple is a profitable crop and that one apple per day is recommended in the work or school week as a snack [30].

5. Conclusions

The CA is the best form to maintain and commercialization of the refrigerated apple to give it added value, the business producer uses refrigerators to store the apple for 6 months from September to March in Mexico.

Liquid nitrogen is the best option for cooling, but its cost is high as those cooled with GHG.

In the selection of the crop to be stored, the apple is washed, dried, covered with bioplastic or organic products, and separated by size, to be stored.

Mexico has four apple-producing regions, even so, it does not supply the national demand, so imports must be made.

The commercialization of the apple of the rural producer is carried out after harvest and is sold as a snack, entremes or appetizer.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Indigenous and Improved Postharvest Handling Methods and Processing of Fruits

Oluyinka Adewoyin, Adebayo Ibidapo, Lydia Babatola, Folasayo Fayose, Anthony Ekeocha and Temidayo Apata

Abstract

After harvesting, fresh fruit's quality cannot be improved but it can be maintained. Fruits should be harvested at the appropriate maturity stage and size. Harvesting of fruits at improper maturity stage reduces shelf-life. Time of harvest, method of harvest, tools used in harvesting also contribute to the wholesomeness of harvested fruits. Fruits are living organisms that continue their living processes after harvest; therefore, their handling directly affects freshness as well as optimum flavor. Maintaining cool temperatures, appropriate air combination to maintain the quality of fruits, producers, handlers, and retailers are to ensure that fruits going for processing, marketing, or into storage are at the best quality state. Indigenous handling refers to the native, age-long, cultural system of postharvest handling of horticultural crops. Postharvest handling comprises interconnected activities from harvest to sorting, grading, preservation, transportation, packaging, processing, marketing, and decision by the consumer to accept or reject the food. Improvement is the enhancement made on the traditional postharvest handling methods to reduce losses of agricultural produce by at least 5%. Various means have been developed over time to handle and preserve food and particularly fruits over ages of technology advancement from the Stone Age.

Keywords: fruits, postharvest handling methods, processing, indigenous, improved postharvest methods

1. Introduction

Foods are substances consumed by living organisms to satisfy the appetite, meet physiological and chemical processes of growth, supply energy and facilitate adaptation to climate change [1]. Agriculture evolved from the gathering of fruits and vegetables from the wild, before the domestication of animals and cultivation of crops. Man has devised various methods by which these fruits are kept and handled. The principles adopted over time are to control agents of deterioration to maintain fruit quality [2–4]. Agents of deterioration are microbial activities; effects of temperature resulting in early senescence and death of tissue due to interruption of metabolic rate as a result of high or extremely low temperature; Loss of moisture through evaporation and transpiration which causes shriveling; low shelf life due to ethylene biosynthesis; low relative humidity; inappropriate proportion composition of air; Inappropriate use of herbicides; hormones; pesticides and insecticides [5–10]. The nutritional quality of harvested fruits is also affected by other factors such as light, water activity and oxygen. Davey further affirmed that temperature and relative humidity were important factors in maintaining the quality of fruits after harvest [11]. Wilson also asserted that deterioration of fresh commodities can result from physiological breakdown due to ripening, water loss, physical damage, and invasion by micro-organisms and their interactions with temperature and relative humidity of the storage conditions [12]. Fruits for export require more attention and appropriate postharvest handling methods because the producers aim at getting the best return from the produce. John stated that maturity at harvest is one of the most important factors that determine the shelf-life and final fruit quality in mature fruits [5]. Fruits harvested at immature stage became insipid with bad flavor soon after harvest. Fruits require very scientific postharvest handling methods with cold storage at an exact temperature, suitable air movement and appropriate humidity [13]. The objective of studying post-harvest handling is to create an understanding of all operations from harvesting to distribution to facilitate proper technology in each step and in such a way as to minimize losses and maintain quality as high as possible during the distribution chain. This chapter takes an overview of the indigenous and improved postharvest handling methods and processing of fruits considering the total postharvest chain from harvesting methods, harvesting tools and implements, transportation, storage and processing. It identifies aspects of critical postharvest losses and finds solutions that would lead to a remarkable reduction in postharvest losses in quality and quantity of harvested fruits, thereby increasing the quality and quantity of marketable products.

2. Indigenous postharvest handling practices of fruits

Indigenous postharvest handling practices of fruits go along with a lot of inappropriate handling methods resulting in huge postharvest losses as a result of knowledge gap for all stakeholders in the postharvest food chain of fruits. Wounding, bruising, and physical injury imparted on the produce from rough and abusive harvest practices and postharvest handling methods will result in significant produce quality loss and an increase in postharvest decay. In **Figures 1**–**4** careless handling of fruits were observed which will result in internal bruising, abnormal physiological damage, splitting and skin breaks. Skin breaks provide sites for infection by disease organisms causing decay. Enzymes contained in the cells of fruit tissues may be released as a result of mechanical damage during postharvest handling. These enzymes break down cellular material. Chemical reactions catalyzed by the enzymes result in the degradation of quality leading to off-flavors, deterioration of texture, and the loss of nutrients [14]. Chemical reaction occurs when fruits are damaged by falling, breaking, crushing, cutting, insect punctures and peeling. These damages release enzymes that trigger chemical reactions such as rancidity in fruits, deterioration of chlorophyll pigments and flavor changes [15]. Other major chemical changes which occur are lipid oxidation and non-enzymatic browning. This leads to deterioration in sensory quality, changes in the color and flavor of foods. The lipid oxidation rate is influenced by light, water activity, local oxygen concentration, high temperature, and the presence of

Figure 1.
Harvested lemon packed in worn-out sharp locally made basket.

Figure 2.
Fruits carelessly handled at retail point.

catalysts such as iron and copper [16]. In **Figure 1**, it was observed that the indigenous bamboo basket is already weak and fruits can easily be bruised by the sharp edges of the basket due to weakness in the basket the fruit can fall off during transportation, loading and unloading from one destination to the other. **Figures 2** and **3** showed the

Figure 3.
Inappropriate and careless handling of fruits.

Figure 4.
Mishandling of fruits during transportation.

exposure of fruits to direct sun which will increase the internal temperature and speed up chemical processes in the fruit. Food spoilage may be defined as any change that renders food unfit for human consumption. Every change in food that causes the food to lose its desired quality and eventually become inedible is called food spoilage or rotting [17]. Damage restricts the use of produce, whereas loss makes its use impossible. Quality attributes describe the traits that make fruits acceptable to consumers such as nutritional composition, freedom from defects such as cuts, over-ripeness, spots, and disease infections. Quantitative and qualitative losses occur at all stages in the post-harvest handling system and distribution chain of fruits from harvesting, through handling, packing, processing, storage and transportation to final delivery of the fresh produce to the consumer [18]. Factors affecting post-harvest losses vary from place to place depending on the season, the genetic constitution of the crop, postharvest management practice, temperature and relative humidity (**Table 1**). Various authorities have estimated that 25–70% of fresh fruit and vegetables produced are lost after harvest [19]. Further studies revealed 20–40 percentage loss in developing countries [20]. Kereth et al. [12, 21, 22] estimated that from 5 to 25% of fruit from the farm gate never reaches the consumer. Post-harvest losses of banana, citrus, grapes, apples,

Postharvest value chain	Activities	Causes of losses	Percentage loss	Solution
Farm gate	Harvesting, sorting, grading and sizing.	Mechanical, Physiological, pathological	5%	Harvest timely properly, and with pedicel, removal of infected fruit
Packing house operations	Packaging, sorting, grading, sizing, trimming, washing, degreening	Mechanical damage, pathological losses due to contamination	5–10	Avoid exposure to direct sun-ray, careful handling
Transport	Handling, sorting, grading	Mechanical losses due to mishandling, physiological changes, pathological damages due to action of micro-organism	5–10	Appropriate packaging method to prevent moisture loss, appropriate container, avoid hard packages
Wholesale	Packing, sorting, grading, at wholesales point	Mechanical damages due to leveling, pathological damages due to the micro-organism, physiological damage due to moisture losses	10–25	Avoid exposure, store under shade, appropriate packaging materials, use of refrigerator or evaporative coolant structure, avoid delay, separated infected produce, avoid heaping especially fruit and vegetable
Retail	Buying and selling, packing sorting, and grading.	Pathological losses, slight mechanical damage, moisture loss.	5–10	Appropriate packaging, adequate storage facilities, avoid exposure, store under shade.
Consumers	Buying and selling, palatability test, choices.	Physiological losses, pathological losses.	5–10	Well ventilation, appropriate package, use of refrigerator, use of evaporative coolant, washing

Table 1.
Identification of critical causes of postharvest losses and the solutions.

avocado, and papaya were reported to be 20–80, 20–95, 53, 14, 43 and 40–100% respectively in developing countries [23, 24]. Food loss assessment provides the basis for programs aimed at reducing postharvest losses [7, 25–28].

2.1 Harvest indices, tools, containers, storage temperature and relative humidity

In most developing world, orchards were established for a long period up to 50–100 years old. Fruits are plucked from the tree with hand by hired skilled laborer's with the use of an indigenous bamboo ladder and the fruits are placed in a bamboo basket. Fruits are conveyed by the farmer with the use of a basket or jute bag to the collection sites. The fruits are thrown to the ground from a height of about one meter. In Nigeria, a harvesting knife (usually referred to as 'go-to-hell'), consisting of a sickle-like metal head attached to a long wooden handle is employed in harvesting these fruits like orange, pear, African star apple, cashew from the trees (**Table 2**). The impact on the ground due to fall from the tree is reduced by gathering straw on the floor around the tree or heavy mulch is place on the ground with a thick depth of leaves. Sometimes a long cloth is attached to the tree branches from one end to the other and the fruits fall on the cloth without touching the ground. Sometimes the branches of the tree are shaken with hand and fruits will fall from the tree to the ground. The fruits would then be conveyed using the basket to the primary assembly point which is usually unprotected from environmental hazards such as heavy rain or sunshine until they produce are transported to wholesale markets. The delicate nature of the fruits and internal flesh should always be kept in mind while harvesting and handling produce. Physical damage is pronounced in the indigenous harvesting system due to the lack of knowledge and training.

2.2 Distant market

Primary Collection Centre (Farm gate).

In the indigenous settings, open ground is used where fruits are heaped on bare ground for transportation to distant markets (**Figure 5**). At this point, there is no sorting, grading, sizing, precooling or washing. The fruits are packaged in baskets or used rice bags or jute bags and then loaded in Lorries or commuter vehicles and then transported to wholesale point or distant market which is usually on market days. The traditional method utilizes a local basket for packing and transportation of fruits. A sizable quantity of the fruits gets damaged in transit. The loading of the vehicles also exceeds the vehicle's carrying capacity on very rough roads that promotes vibration and jostling of the produce on vehicles that are poorly maintained where the shock absorber may not be well fixed with poor ventilation. The loading and unloading of the vehicle are not done with careful handling; the produce is thrown over high elevations and over wide distances which promotes cuts and bruises. The environmental conditions in these center's may promote excess heat, low humidity, mechanical damage, improper postharvest handling methods, poor sanitation, and poor environmental control. The field containers should be put under shade to minimize produce heating in the interval between harvest and transport to the packinghouse. In the improved postharvest handling system efforts to control these factors are often very successful. In the improved system, air-conditioned structure is usually erected where packing house operations takes place such as sorting, grading, washing, disinfestation, degreening, waxing packaging. The plastic fruit crates 'area' are a more suitable option in this system [29–31]. The modern fruit plastic crates take care of problems such as

S/N	Fruit	Botanical name	Origin	Harvest indices	Storage T°C RH%	Ethylene Sensitivity	Harvest Containers	Harvest tool
1.	African star apple	*Chrysophyllum albidum*	West Africa	Colour change green to orange, ease of abscission	28 ± 2°C 90–95%	Insensitive	Harvesting basket.	Long harvesting knife
2.	Apple	*Malus domestic*	North America	Colour changes from leaf green to yellowish-green, firmness	0°C 90–95%	Sensitive	Apple basket	Apple picker Ladder
3.	Apricot	*Prunus armeniaca*	China Central Asia	Harvest when fruits are fully colored	0°C 90–95%	Sensitive	Gently place in a basket	Hand picking
4.	Avocado	*Persea Americana*	Africa	Colour change, Ease of abscission	6–7°C 85–95%	Sensitive	Cloth bag	Pruner
5.	Banana	*Musa domestica*	South East Asia	Colour change dark green to light green, peel/pulp ratio	18°C 80–85%	Sensitive	Gentle handling of bunches	Cutlass, Sharp knife
6.	Chestnut	*Castanea sativa*	Asia Minor	Fullness of size, Browning of pods	0°C 90–95%	Insensitive	Basket or wooden boxes	Long stick or hand plucking
7.	Cherimoya	*Annona cherimola*	Tropical America	changing in color from a darker to a light green or greenish tan	15–30°C 40–90%	Sensitive	Plastic box or Paper boxes	Hand picking
8.	Cashew	*Anacardium occidntale L*	South eastern Brazil	ease of abscission, Acidity level, firmness	30°C 67%	Insensitive	Basket, plastic boxes	Hand picking. Vibrator
9.	Custard Apple	*Anonnona squamosal linn*	South America	Colour change slightly yellow. Firmness	25–30°C :60–70%	Sensitive	Paper boxes, plastic boxes	Handpicking
10.	Clementine	*Citrus clementine*	Misserghin, Algeria	Fully yellow	10°C 90–95%	Insensitive	Plastic boxes	Hand picking
11.	Dates	*Phoenix dactyifera*	Westen Parkistan, Egypt	Fruit turns brown	0°C 85–95%	Insensitive	Jute bag	Hand picking

S/N	Fruit	Botanical name	Origin	Harvest indices	Storage T°C RH%	Ethylene Sensitivity	Harvest Containers	Harvest tool
12.	Elephant Apple	*Dillenia indica*	Indonesia	Colour change from dark green to olive green	0°C 90–95%	Sensitive	Harvesting boxes	Hand plucking
13.	Fig	*Ficus carica*	Northern Asia Minor	Ease of abscission, aroma, colour	0°C 50%	Sensitive	Harvesting boxes	Hand plucking
14.	Grape	*Citrus paradise*	North America	Ease of abscission, aroma, colour Firmness	0°C 90–95%	Insensitive	Harvesting boxes	Hand plucking
15.	Guava	*Psidium guajava*	Mexico, Central America, Northern South America	TSS acid ratio, specific gravity and colour	5°C 75–85%	Sensitive	Harvesting boxes	Hand plucking
16.	Jaboticaba	*Pliniacauli flora*	Brazil	Change in colour. Fragrance	13–15°C 90–95%	Insensitive	Harvesting boxes	Hand plucking
17.	Jackfruit	*Artocarpus heterophylluus*	Western Ghats of southern India	Firmness, ease of abscission	13°C 85–90%	Sensitive	Harvesting box	Long harvesting knife
18.	Kiwi	*Actinidiade liciosa*	Southern China	Firmness, ease of abscission. Increase in sugar content	−0.5°C 90–95%	Sensitive	Harvesting box	Hand plucking
19.	Lime	*Citrus aurantifolia*	Southern Iraq and Persia	Fragrance, colour change	9–10°C 85–90%	Insensitive	Harvesting boxes	Hand plucking
20.	Lemon	*Citrus limon*	North-western India	Ease of abscission, colour change from dark green to light green	10–13°C 85–95%	Insensitive	Harvesting boxes	Hand plucking
21.	Mammee Apple	*Mammee Americana*	South America Hawaii AfricaAsia	Colour change, firmness, ease of abscission	November, December January 0°C 90–95%	Sensitive	Harvesting boxes	Hand plucking

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S/N	Fruit	Botanical name		Origin	Harvest indices	Storage T°C RH%		Ethylene Sensitivity	Harvest Containers	Harvest tool
22.	Mango	*Mangifera indica*		South East Asia	Olive green colour, smooth and shiny, TSS 10–14% Starch content, flesh colour.	April–June	12°C 90–95%	Sensitive	Harvesting boxes	Hand plucking
23.	Noni	*Morinda citrifolia*		South Asia	Colour change from light green to yellowish green. Firmness, Ease of abscission	November, December January	10–13°C 85–95%	Sensitive	Harvesting boxes	Hand plucking
24.	Sweet Orange	*Citrus sinensis*		Southern China, Northern and East India and South East Asia.	Ease of abscission, colour change from dark green to light green	November, December January	0–9°C 85–90%	Insensitive	Basket. Harvesting box	Hand plucking
25.	Olive	*Olea europaea*		Mediterranean basin	Ease of abscission, Firmness	August— November	5–10°C 85–90%	Insensitive	Basket. Harvesting box	Hand plucking
26.	Peach	*Prunus persica*		China	Ease of abscission, golden colour, firmness	May— September	−0.5–0°C 90–95%	Sensitivity	Basket. Harvesting box	Hand plucking
27.	Passion fruit	*Passiflora eludes*	Southern Brazil	Ease of abscission, dark green to light green depending on the variety	December – January in the tropics	5–10°C 85–90%		Sensitive	Basket. Harvesting box	Hand plucking
28.	Pomegranate	*Punica granatum*	Iraq and Himalayas in northern India	Sugar percentage should be 12–16% and acid percentage 1.5–2.5%,	September–November	5°C 90–95%		Insensitive	Basket or cloth bag	Hand plucking
29.	Pawpaw	*Carica papaya*	Panama and Columbia	Patches of yellow, Jolliness of seed	August – September	7–13°C 85–90%		Sensitivity	Harvesting boxes	Long stick or hand plucking

S/N	Fruit	Botanical name		Origin	Harvest indices	Storage T°C RH%	Ethylene Sensitivity	Harvest Containers	Harvest tool
30.	Pear	*Pyrus communis*	China	Ease of abscission	May – August	−1.5 – 0.5°C 90–95%	Sensitivity	Harvesting box	Long harvesting knife
31.	Plum	*Prunus domestica*	Eastern Europe and China	Fragrance, firmness, Ease of abscission soluble solids 17 percent	July – September	-0.5°C 90–95%	Sensitivity	Harvesting box	Pluck with hand
32.	Pineapple	*Ananas comosus*	South America	Ease of abscission, colour change, taste.	May – August	7–13°C 85–90%	Insensitive	Harvesting crates	Sharp knife or cutlass
33.	Persimmon	*Diospyros kaki*	China	Ease of abscission, firmness	November–January	−1°C 90%	Sensitivity	Paper Boxes	Pluck with hand
34.	Raspberry	*Rubus spp.*	North America	Ease of abscission, colour change, taste.	April–August	−0.5-0°C 90–95%	Insensitive	Paper Boxes	Pluck with hand
35.	Strawberry	*Fragaria spp.*	North America	Ease of abscission, colour change, taste	April–August	0–0.5 °C 90–95%	Sensitive	Paper Boxes	Pluck with hand
36.	Tangerine	*Citrus reticulate*	South Asia	Ease of abscission, colour change, taste.	April–November	9–10°C Humidity: 85–90%	Insensitive	Basket. Cloth bag. Boxes	Pluck with hand

S/N	Fruit	Botanical name		Origin	Harvest indices	Storage T°C RH%	Ethylene Sensitivity	Harvest Containers	Harvest tool
37.	African Walnut	*Plukenetia conophora*	Tropical West African nations	Ease of abscission	September–December	10–20°C 85–95%	Insensitive	Jute bag or boxes	Pluck with hand
38.	Watermelon	*Citrullus lanatus*	Egypt	Colour change from dark green to light green, sound, browning tendrils	April – September	10–15°C 90%	Insensitive	Jute bag or boxes	Pluck with hand
39.	Plantain	*Musa paradise*	South Asia	Colour change from dark green to light green, blackness of tip, peel/pulp ratio, blackness of tip, peel/pulp ratio,	January – March	18°C 80–85%	Sensitive	Gentle handling of bunches	Cutlass, Sharp knife

Table 2.
Harvest indices, tools, containers, storage temperature and relative humidity.

Figure 5.
Harvested fruits kept under a tree to prevent exposure to direct sunlight to reduce internal heat.

bruises, cuts and other likely mechanical damage. For example, reducing mechanical damage during grading and packing greatly decreases the likelihood of postharvest disease because many disease-causing organisms would enter through wounds [32–36].

2.3 Indigenous temperature management practices of fruits

Farmers usually employ hired labour or collaborate among themselves by mobilising each other for harvest as early as 6 am when the temperature is low before 12 pm. The harvested produce is moved to the market for early sales to consumers or retailers. Sometimes, the produce harvested in the evening will be spread on flat surfaces overnight not allowing the produce to overlap one another; this will be sold in the market in the next morning. Oranges, Grapes, pear, mango, guava, African star apple, Avocado pear, African star apple are harvested when the color changes from deep green to slight yellow. At this stage, the produce can be transported to distant markets (**Figure 6**).

2.4 Improved temperature management practices

The improvement in temperature management requires rapid removal of the field heat through: hydro-cooling, packaging in iced containers, top icing, evaporative cooling, room cooling, forced air cooling, serpentine forced air cooling, vacuum cooling, and hydro-vacuum cooling. The cold chain system is required in the value chain of fruits from the farm gate to the consumer.

Figure 6.
Primary assembly point.

2.5 Indigenous pre-harvest quality management

Farmers have the age-long practice of keeping the best of their harvest as seed for the next season with the concept of maintaining the appropriate genetic resources from generation to generation to maintain produce qualities. The land preparation is done thoroughly to minimize weed infestation which reduces the quality of harvested produce. Farmers depend on accumulated hand-on experience or indigenous knowledge on when to harvest and how to harvest. Leguminous food crops are planted like cocoyam, melon, wrapping leaves, and vegetables are intercropped with the fruit trees to supply staple food for the household and regular income. It also reduces maintenance cost.

2.6 Improvement on pre-harvest quality management

Pre-harvest factors affect the rate of deterioration in the following ways:

Genetic factors: The rind thickness, skin layer affects rate deterioration. Some fruits outer layer has been improved to prolong shelf -life.

Maturity: Fruits should be harvested at the proper maturity stage to give the best quality.

Seed Selection: The right seed that is disease free and give the best quality output should be used.

Site Selection: The soil fertility status must support growth and development and insect pest status should be low.

Tools and Implements: These must be free from disease and the most appropriate implement for harvesting of that particular fruit.

Climatic conditions: Erratic changes in temperature will reduce the quality of fruits in terms of water stress, which will reduce the liquid content of fruits while excess water will result in rot, absence of cold period will affect the formation of the orange color in citrus.

Cultural practice: All cultural practices must be done appropriately and timely. Planting must not be delayed in other to avoid pest peak period.

Use of chemicals: Use of various chemicals in excess should be avoided.

Water stress: Irrigation should be planned along with field establishment.

Disease, insect, rodents: Activities of field pest reduce quality of produce through boring, feeding and laying of egg. Often disease and pest are transferred from field to storage and along the postharvest chain.

Injury: This can be caused by insect pest or farm implements, sometimes lack of moisture in the atmosphere may result in cracking of fruits.

Irregular weeding: Weeds serve as alternative host for diseases and pests; therefore, it must be controlled.

Management practices: This can also affect postharvest quality. Produce that has been stressed by too much or too little water, high rates of nitrogen, or mechanical injury (scrapes, bruises, abrasions) are susceptible to postharvest diseases. They are also susceptible to mold and decay caused by fungus *Rhizoctonia*, as a result fruits lying on the ground, and it can be alleviated by using mulch (**Figures 7** and **8**) [37, 38].

Figure 7.
Fruit display by retailer with partial provision of shade.

Figure 8.
Fruits in display at retail market with minimum shade.

2.7 Indigenous postharvest quality management

Transportation to the market is done by trucks, lorries, wheelbarrows, and motorcycles. During loading, fruits are carried in basket on head and thrown over straw or fruits already present inside the truck from a meter height. These practices result in bruising of the fruits and as a result become unmarketable. Fruits have to face the same fate of rough handling during unloading. Storage is a much-neglected aspect in the whole process and there is no permanent structure for storage in any point of time during the whole process of harvesting to marketing. Rough handling practices are practiced during loading of the field containers in transit. Throwing of the field containers and excessive drop at high heights is practiced by the handlers. The consequences of these undesirable practices included noticeable physical injury and bruise damage to the product. The likelihood of postharvest decay of the injured items is high. The process of loading the field containers onto the transit vehicle and unloading at the packinghouse needs closer supervision [2]. Indigenous method of temperature management includes exposing harvested fruit to frozen air at night, or putting fruits in water immediately after.

2.8 Improved postharvest handling methods of fruits

Harvesting and handling;

1. Harvesting should be done with extreme care due to soft tissue of the fruits

2. Produce should be harvested at the best quality for storage to prolong shelf life.
3. Harvesting should be done under cool condition
4. Harvest in batches to give highest quality available to customers.
5. Plant at different times using varying varieties to extend harvest season.
6. Hold produces in a shaded area before transportation
7. Poor quality produce should be removed to avoid contamination and distractions.
8. Avoid increase in temperature and moisture loss during transportation and storage.
9. Commodities for export must be handled carefully.
10. Use appropriate tool, container and implements during handling

2.9 Evaporative cooling system as improvement on the use of clay pot to preserve fruits

Different types of Evaporative Cooling Systems have been developed for the small- and large-scale storage systems.

i. *Pot-in-Pot:* This comprises of two clay pots in which a smaller pot with the smaller mouth is placed inside a bigger one and the interspace filled with riverbed sand. Fruits for storage are kept inside the smaller pot and covered with an insulating material. The riverbed sand is kept moist by wetting it once daily. Fruits kept in it can remain fresh for up to 1 month.

ii. *Metal-in-Pot:* This is similar to pot-in-pot except that the smaller inner pot is replaced with a metal pot – an old kerosene tin. The working principles are the same.

iii. *Wall-in-Wall:* This is the commercial size of (i) above. It involves building a block inside another block with a small door made of wooden materials. The inner part of the small block is provided with shelves. Tap water pipe could be connected to the interspace for the case of water supply to wet the riverbed sand. It could keep fruits for 1–3 months without a problem. This structure of size measuring 2.5 m × 2.5 m × 2.5 m could be used to store 15–20 baskets of fruits at a time.

iv. *Metal-in-Wall:* This is similar to (iii) above but for the fact that the inner block wall is replaced with a metal tank that has shelves inside. The working principles are the same.

v. *Bamboo Coolant Structure:* The base of the cooler is made by a large diameter tray that contains water. Bricks are placed within this tray and an open ware cylinder of bamboo or similar materials is placed on top of the bricks. Hessian cloth is wrapped around the bamboo frame, ensuring that the cloth is dipped in water to allow it to be drawn up the cylinder's wall and food kept in the cylinder with a lid placed on the top.

vi. *Charcoal Cooler:* The charcoal cooler is made from an open timber frame of approximately 50 mm × 25 mm in section. The door is made by simply hinging one side of the frame. The wooden frame is covered in mesh, inside and out leaving a 925 mm (1″) cavity; this is filled with pieces of charcoal. The charcoal is sprayed with water and when wet provides an evaporative cooling effect. The framework is mounted outside the house on a pole with metal to deter rats and a good coating of grease to prevent ants from getting to the food stored.

vii. *Almirah Cooler:* The Almirah cooler is a more sophisticated cooler that has a wooden frame covered with cloth. There is a water tray at the base and on top of the frame into which the cloth is dipped, thus keeping it wet. A hinged door and internal shelves allow easy access to the stored produce.

Various researches were carried out to investigate the effectiveness of the evaporative coolant structure in prolonging the shelf life of fruits [39]. Babatola and Adewoyin [40] observed that Cucumis sativus stored best for 3 weeks under the refrigerator followed by evaporative coolant structure and then open shelf. The evaporative coolant stored Cucumber fruit effectively for 2 weeks. Babatola [41] also investigated the effect of storage conditions on nutrient composition and quality of *Capsicum frutescens* under three storage conditions. Observations were made on colour, firmness, weight loss, disease incidence and pungency level of pepper fruit, it was observed that pepper fruits kept well for 21 days in the evaporative coolant structure at a temperature of 20–22°C. Babatola [42] further investigated the effect of NPK fertilizer levels on the growth, yield and storage of pepper on *Capsicum annum*. The result showed that fruits stored in the refrigerator stored best for 3 weeks, followed by evaporative coolant structure which stored for 2 weeks while fruits under the ambient deteriorate rapidly after 4 days. Another research was conducted on the postharvest quality of okra fruit under three storage conditions. Evaporative coolant structure was found to store okra effectively for 2 weeks [43]. The physicochemical changes and shelf life of guava as influenced by postharvest condition were observed, refrigeration was observed to prolong shelf life for 16–28 days followed by evaporative coolant structure [44]. Further research on the influence of storage conditions, such as deep freezing, refrigeration and evaporative coolant structure on the quality of varieties of carrots showed that carrots stored best in the deep freezer at the temperature of 0–4°C in terms of color, firmness and disease infection [45].

Postharvest technology is crucial in agricultural production and utilization system. It plays a key role in loss reduction, value addition, food security, employment and income generation [38, 39]. A postharvest technology revolution is essential with strong linkages of storage, marketing and distribution. Inappropriate postharvest management system resulted in large quantity of fruits gets damaged during the process of handling, transportation and marketing **Figure 3** [36, 37]. Due to the absence of proper storage and marketing facilities, farmers are forced to sell their produces at throw-away prices. Sometimes farmers do not even get the two-way transportation cost, so they would rather dumb their produce near the market area than bear the transportation cost required for taking the product back. It is of utmost importance to identify all aspects of critical postharvest losses and find solutions that would lead to a remarkable reduction in postharvest losses.

Processing is a postharvest activity carried out to maintain or raise the quality of produce or change the form or characteristics of fresh produce, spoilage agent must be destroyed without ruining the nutritive value or palatability of the farm produce. Processing easily destroys Vitamin C in fruits, especially where heat is used. Produce that has been processed can also be stored to prevent spoilage and extend storage life; hence we have the term preservation. Processing and preservation aim at achieving the following goals:

i. Increase the shelf life of the produce.

ii. Increase variety in the diet by providing a range of attractive flavours, colours, aromas and textures.

iii. Increase the economic value of the product by raising its quality.

iv. To create a new product.

v. To remove inedible parts of produce.

vi. Facilitate efficient and easy transportation

vii. Reduce bulkiness

viii. Produce easy to display

ix. Increase sales

x. It makes fruits available where it is not produced

xi. It makes fruits available throughout the year e.g., dried mango, pineapple.

xii. Stabilizes price

xiii. Reduce postharvest losses

xiv. Value addition is possible

xv. The waste from processing can be used to generate income.

3. Conclusion

Fruits are to be harvested at the appropriate maturity stage and size to prolong shelf life. Major losses in the postharvest chain of fruits are due to mechanical damages, physical bruises, a physiological disorder due to high temperature and unhygienic conditions. Time of harvest, method of harvest, tools used in harvesting, transportation affects wholesomeness or increased rate of deterioration of harvested fruits. The inappropriate postharvest management system in the postharvest chain of fruits results in huge losses during the process of harvesting, grading, packaging, handling, transportation and marketing. These losses are due to inappropriate harvesting methods and tools, unavailability of cold chain systems, absence of appropriate storage facilities and poor marketing strategies. To maintain and effectively preserve fruit quality. Postharvest handling must be efficient, rapid and coordinated by ensuring immediate removal of field heat, reducing damages by protecting from sun and unhygienic conditions. Knowledge on specific produce handling method, market requirement, appropriate container and simplified packing line is essential to achieve uniformity and ensure produce are properly placed and strapped for delivery to consumers. Workers involved in critical postharvest handling steps are to be well trained, remunerated and equipped with appropriate tools, risk-free and conducive working condition.

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Section 2

High-Density Planting

Chapter 4

Dwarfing Rootstocks for High-Density Citrus Orchards

Mateus Pereira Gonzatto, Sabrina Raquel Griebeler and Sergio Francisco Schwarz

Abstract

There is a worldwide trend regarding high density of fruit planting. In the last four decades, the Brazilian citriculture had increased the average planting density by more than 80%. The main reasons for this increase are the fast return on invested capital, the easiest management of cultural practices, and the control of strategies epidemics-associated (e.g., *Huanglongbing*). In that regard, the use and development of dwarf and semi-dwarf rootstocks are essential. The main dwarf rootstock known in citriculture is the Flying Dragon trifoliate orange [*Poncirus trifoliata* (L.) Raf. var. *monstrosa* (T. Itô) Swing.] which greatly reduces the canopies volume allowing the design of dense and ultra-dense orchards. Currently, several citrus breeding programs are producing new cultivars of dwarf and semi-dwarf rootstocks. In this chapter, citrus rootstocks with dwarfing potential were approached including physiological aspects, horticultural performance, and behavior to phytosanitary problems. In addition to Flying Dragon, there are other dwarfing rootstocks which are hybrids of trifoliate oranges, like citrandarins, citrangedarins, citrumelandarins, and citrimonianandarins. Dwarfing rootstocks are one of the leading alternatives for citrus orchards in high-density planting systems.

Keywords: *Poncirus trifoliata* Raf., *Citrus* spp., Flying Dragon trifoliate orange, citrandarin, citrangedarin, citrumelandarin

1. Introduction

The high-density planting allows an increase in fruit yield per area in the initial years of production and a reduction in the payback period despite the higher cost of implementing the orchards. Therefore, it has been a trend to enhance the production system [1, 2]. Moreover, high-density planting improves orchard harvesting since there is no need for ladders [3, 4], and also reduces loss by diseases such as *Huanglongbing* (HLB) [5]. However, this technology requires effective control of the canopy growth of citrus plants. A suitable planting density with restricted space available for the growth of these plants and avoidance of excessive intercrossing of scions must be employed [3].

In Southeast Brazil, citrus orchards have shown an increase in planting densities, from an average of 337 trees per hectare in the 1980s to over 600 trees per hectare

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since 2013. Since 2014, a stabilization in planting densities of around 650 trees per hectare is noted [6].

Two main classifications of citrus plants size are proposed. To Castle and Phillips [7], a classification was recommended according to the height or volume of scions into four categories regarding a standard plant: dwarf, semi-dwarf, semi-standard, and standard. Dwarf and semi-dwarf occur when the plant is 40% and 40–60% of the standard size, respectively. Semi-standard arises to plant with a size of 60–80% of the standard. Standard, on the other hand, is used for plants having 80–100% of the standard size. In this classification, the standard used was plants grafted onto a rough lemon (*Citrus jambhiri* Lush.). Another classification was proposed by Bitters et al. [8] in which a tree with a height of 6.0 m or more was used as the standard. Sub-standard plants, semi-dwarf plants, and dwarf plants were the ones which have a reduction of 25%, 50%, and 75%, respectively regarding the standard.

Many factors affect citrus plants' size such as rootstock, variety, soil and climate conditions, cultural treatments, and phytosanitary conditions [3]. The effective control of the citrus growth plants can be achieved by means of several strategies: (a) use of scions with restricted growth potential; (b) pruning; (c) biological agents, usually viroids and viruses; (d) restriction of the root system growth; (e) use of dwarfing, semi-dwarfing and intergrafted rootstocks; (f) sloping planting of seedlings, and (g) use of plant growth regulators [3, 9]. Among them, probably the most effective is the use of dwarfing rootstocks [7] because few scion varieties have significantly reduced growth by themselves. In mandarin trees, some cultivars of the Satsuma group (*Citrus unshiu* Marcovitch) such as 'Clausellina' and 'Hashimoto' are highlighted due to their small growth [10].

This chapter presents the most important rootstocks for citrus high-density orchards in addition to the most recent alternatives. The main characteristics and horticultural performance of rootstocks were approached.

2. Dwarfing rootstocks and high-density citrus orchards

A dwarfing rootstock is the one that in combination with any canopy genotype, generates a mature tree with a height of no more than 2.5 m, independent of environmental and/or viral influences [11]. Currently, several materials from directed hybridization are classified as dwarfing. Nevertheless, few of them seem to provide an increment in the trees yield efficiency. Diversely, Flying Dragon [*P. trifoliata* (L.) Raf. var. *monstrosa* (T. Itô) Swing.] had a high yield efficiency [12–16] probably due to distinct dwarfing mechanisms [17].

In Brazil, densified citrus orchards have densities between 600 and 1250 plants ha^{-1}, with distances of 4–6 m between rows and 2–3 m between plants [2, 5]. Differently, ultra-dense orchards are those where planting densities vary from 1500 to 2000 plants ha^{-1}. Theoretically, these ultra-dense-orchards reach up to tens of thousands of trees per hectare, within the concept of "meadow orchard" [9]. In recent work in India with Nagpur mandarin onto Rangpur lime, a high-density was considered as the one bearing between 555 and 625 plants ha^{-1} and an ultra-high density planting the one varying from 1250 and 2500 plants ha^{-1} [18]. In Japan, long-term experiments were accomplished in 'Wase' satsuma mandarin (*C. unshiu* Marc. var. *praecox* Tan.) to evaluate orchards with densities of up to 10,000 plants ha^{-1} [19, 20].

Densification is a common practice in mandarin orchards in Southern Brazil. There is a spacing recommendation for *P. trifoliata* varying from 6.5 m × 3.0 m to 5.0 m × 2.0 m (512–1000 plants ha^{-1}), depending on soil fertility. For mandarin plants grafted onto Flying Dragon, a spacing of 4.5 m × 1.5 m and 4.0 m × 1.0 m (1480 and 2500 plants ha^{-1}) is recommended in high and low fertility soils, respectively [21]. In Southeast Brazil, row spacing of 4–5 m and plant spacing of 1.5–2.5 m is recommended for the Flying Dragon rootstock [22]. In Florida, despite its limited commercial use, a spacing of 5–7 ft (1.52–2.13 m) is recommended between plants grafted onto Flying Dragon. Instead, for common trifoliate orange, there is a spacing recommendation of 6–8 ft (1.83–2.44 m) [23].

In Iran, three planting spacings/densities were evaluated on 'Unshiu', 'Clementina', 'Page' and 'Ponkan' mandarin grafted onto Flying Dragon (4 m × 4 m, 625 plants ha^{-1}; 4 m × 3 m, 833 plants ha^{-1}; and 4 m × 2 m, 1250 plants ha^{-1}). There was no effect of planting density on yield per plant. Nevertheless, yield per area increased significantly at the highest planting density. 'Ponkan' mandarins showed a better yield performance, while 'Page' mandarins had the worst yield performance and the lowest growth in the first five productive years of the orchard [22]. In later studies, the mandarin tree 'Span Americana', an early variety from the same group as 'Ponkan', performed well over Flying Dragon. Then, it has shown potential for densely planted orchards [23]. Furthermore, reports indicate a better performance of densified systems in citrus plants having columnar canopies.

Biological agents are also explored in citrus high-density planting toward the re-engineering of citrus seedlings. As recently shown, the use of Citrus dwarfing viroid (CDVd) into trifoliate orange rootstock 'Rich 16-6' (*P. trifoliata* (L.) Raf.) reduced canopy volume by up to 50% [24]. Beyond size reduction, the canopy of CDVd-infected trees had a long-lasting phenotype regarding Flying Dragon rootstock. Further, a report aimed to discover the mechanism of CDVd dwarfism. The understanding of this mechanism would allow the development of commercial products absent infectious agents [25].

2.1 Flying Dragon trifoliate orange

The most important and well-established citrus dwarfing rootstock is the Flying Dragon trifoliate orange, also known as Hiryu or Japanese Hiryo [26–28]. The Flying Dragon [*P. trifoliata* var. *monstrosa* (T. Itô) Swing.] originated as a mutant of a non-dwarfing trifoliate orange [*P. trifoliata* (L.) Raf.]. Besides, it has not undergone sexual recombination suggesting a great degree of kinship between *P. trifoliata* and Flying Dragon genotypes [29, 30].

A large number of studies over Flying Dragon rootstock in several environments and with many citrus scions are reported [4, 12–16, 22, 23, 26, 28, 31–37]. Due to the several advantages, it is an interesting alternative for densification of citrus orchards [28], mainly for 'Tahiti' lime [35] and mandarins [22].

The trees grafted onto Flying Dragon rootstock are small or dwarf sized, with a maximum height between 2.5 and 3.0 m [26, 28]. Flying Dragon features curved thorns and tortuous trunk, unlike common trifoliate orange (**Figure 1**). These two characteristics are morphological markers of the dwarfing effect, due to gene linkage or pleiotropy [29]. The tortuosity inheritance seems to be linked to three nuclear genes (Cr1, Cr2 and Cr3), in which the Flying Dragon genotype is entirely heterozygous (Cr1cr1Cr2cr2Cr3cr3), with seedlings of tortuous phenotypes showing

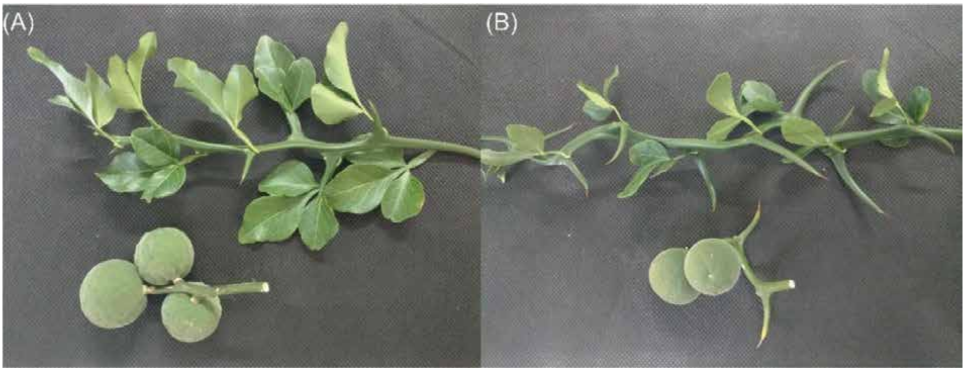

Figure 1.
*Fruit and branches of trifoliate oranges. (A) common trifoliate orange (*P. trifoliata*); (B) Flying Dragon trifoliate orange (*P. trifoliata *var.* monstrosa*).*

low canopy growth, while the phenotypes with straight structures showed a great variability [27].

As there is a great genetic proximity between Flying Dragon and the trifoliate orange, they have many similar characteristics [10]; excellent fruit quality of the scion variety [38], late maturation, tolerance to cachexia, and sudden death. They are also resistant to the nematode *Tylenchulus semipenetrans* as well as to *Citrus tristeza* virus and to citrus gummosis (*Phytophthora* spp.). Beyond, both rootstocks are susceptible to citrus decline, exocortis, and burrowing nematode (*Radopholus similis*) as well as a high tolerance to cold and waterlogging and low tolerance to drought [39–41]. Further, HLB incidence on Flying Dragon is lower than on Rangpur lime and the other three semi-standard rootstocks. The reduction in canopy volume seems to influence host–vector relationships [42].

Furthermore, incompatibilities between *P. trifoliata* and several canopies such as: 'Pêra', 'Rio Seleta' and 'Crescent' orange; 'Murcott' tangor; 'Sicilian' and 'Eureka' lemon are described [40, 41, 43]. There are also incompatibilities in Flying Dragon under 'Lima-da-Persia' [*Citrus limettioides* (Christm.) Swingle] and kumquat (*Fortunella* sp.) in South Brazil [44]. In Spain, there is a record of incompatibility when the scion is 'Eureka' [10].

In Brazil, there are five cultivars registered as *P. trifoliata* var. *monstrosa* (T. Itô) Swing. in the Ministry of Agriculture, Livestock and Supply (MAPA). Cultivars were also registered by other agricultural Brazilian institutes. Two were registered by the Agronomic Institute of Campinas (IAC, 'IAC 848 Davis A' and 'IAC 718 Flying Dragon'). One cultivar was registered by the Agriculture Research and Extension Agency of Santa Catarina State (EPAGRI, 'Flying Dragon') and by the Rural Development Institute of Paraná (IAPAR-EMATER, 'IPR 150'). Additionally, the 'Citrolima Flying Dragon' is also registered [45].

Flying Dragon has some disadvantages, like the low nucellar polyembryony, requiring a very strict selection of seedlings to be grafted [46–48]. Besides that, this rootstock has seed germination and seedling uniformity between 80 and 90% [39]. It requires a longer period for commercial seedling formation than other rootstocks, mainly in low-temperature climates [49]. It is also a drought-sensitive dwarf rootstock [13], requesting a regular rainfall distribution. If the region has a well-defined dry period, irrigation is needed [50]. Nevertheless, under non-irrigated conditions and in soil without chemical restrictions, Tahiti lime grafted onto Flying Dragon

(1157 plants ha^{-1}) and grown in a no-till system intercropped with *Urochloa ruziziensis* developed higher fruit production in the first 5 years. Apart from that, Tahiti limes grafted on Flying Dragon also showed reduced water stress, a better soil chemical and physical characteristics regarding tilled orchards [51].

The use of trifoliate oranges as rootstock demands specific soil fertility conditions, because of its high demand for nutrients [52]. These rootstocks have restrictions on the presence of toxic aluminum in the soil solution, and consequently, a restriction to acidic soils not corrected with liming [53]. With regard to Flying Dragon, there is poor performance in basic pH soils [39] due to the high requirement for iron [52]. Unlike plants of Citrus genus which have few or no root hairs under field conditions, trifoliate oranges can develop root hairs when grown in sand culture [54].

Flying Dragon is employed to Satsumas mandarins culture (*C. unshiu*) in protected structures of Japanese regions and in the southern United States [55]. In Southeast Brazil, it was used in 3% of total citrus seedlings produced in 2020, mostly to produce seedlings of 'Tahiti' lime (*C. latifolia)* [52], allowing planting densities of up to 2500 plants ha^{-1} [35]. Hence, in the first 3 productive years of the orchard, an increase in scions volumes and of the yield at the highest densities was observed [35]. For 'Tahiti' lime under irrigated conditions, there was a reduction in the yield efficiency regarding non-irrigated conditions. This lower yield efficiency is associated with an increase of more than 70% in scion volume due to irrigation [37].

In New Caledonia [28], the economic performance of 'Tahiti' orchards over 13 years under two installation conditions were compared: a high-density planting where trees were grafted onto Flying Dragon and a conventional orchard, where trees were grafted onto 'Volkamer' lemon. For high-density planting, the density was set at a rate of 1000 trees ha^{-1} while for the conventional orchard the density was 208 plants ha^{-1}. The installation cost of the densified orchard was 2.6-folds higher than the conventional orchard because of the higher seedling cost and the planting labor. However, the recovery of invested capital occurred in 4 years for the densified orchard and in seven years for the conventional orchard. Furthermore, the cost of production was US$ 0.30 and US$ 0.57 per kg of fruit produced in the high-density plantation and conventional plantation, respectively. Therefore, the high-density orchard generated over 13 years a gross revenue 3.3-folds higher than a conventional orchard. In contrast, densified conditions with mechanical pruning seem to be inappropriate for 'Valencia' orange, 'Hamlin' orange, and 'Murcott' tangor (2020 plants ha^{-1}) due to the small yields obtained [31].

Another option for Flying Dragon use is as an interlock to modulate vegetative growth. Nevertheless, there is a strong interaction with the rootstock and scion varieties employed. When intergrafted between rootstocks such as 'Swingle' citrumelo or sour orange, or scions as 'Star Ruby' grapefruit (*C. paradisi*) or 'Michal' mandarin (*C. clementina* × *C. tangerina*), Flying Dragon seems to reduce the vegetative growth [56]. On the other hand, when intergrafted under 'Tahiti' lime, the effect depends on the rootstock. A reduction in scion was observed when Flying Dragon was grafted onto 'Catania 2' Volkamer lemon (*Citrus volkameriana* Ten. & Pasq.). Oppositely, an increase in vegetative growth was seen when it was grafted onto *P. trifoliata* 'Davis A'. No effect on scion size was noticed when the Flying Dragon was grafted onto 'Morton' citrange and onto the 'Swingle' citrumelo [37].

Orange "Navelina" and lemon 'Kütdiken' plants intergrafted on Flying Dragon using sour orange as rootstock had 41.1% and 22.5% of growth reduction compared to non-intergrafted plants, respectively. In parallel, during winter and spring, the presence of the intergraft increased net CO_2 assimilation for both canopies. To Navelina orange trees, an increase in transpiration was also observed [57]. In 'Mexican' lime trees onto Alemow (*Citrus macrophylla*) rootstock, the use of Flying Dragon interstocks reduced the canopy volume by more than 60%. Then, there is the maintenance of fruit yields at commercially acceptable volumes (80 kg ha^{-1} ano^{-1}). Also, this would be a viable approach for regions with HLB endemic occurrence [58].

As regards to dwarfing mechanism, the canopies grafted on the Flying Dragon trifoliate tree had a reduced sap flux compared to plants grafted on common trifoliate orange [38]. In comparison to common trifoliate trees, the Flying Dragon reduced the hydraulic conductivity of rootstock and of graft union regions. Further, a restriction of carbohydrate flow to the roots through the grafting region was described [59]. Besides, Flying Dragon had stem and roots xylem vessels larger as well as a lower phloem percentage than the vigorous rootstock Rough lemon (*C. jambhiri)* [60]. The lower carbohydrate flow through the grafting region to the roots supports the reduced root system and the increased production efficiency, expressed in mass of fruit per unit of canopy volume. Additionally, there was a reduction in net CO_2 assimilation, of stomatal conductance. A reduction of transpiration between 12:00 and 3:00 p.m in plants grafted onto Flying Dragon compared to those onto common trifoliate orange was noted [59].

Phytohormones have different behaviors on dwarfing and vigorous rootstocks. The highest indolacetic acid (IAA) level was found in 'Eureka' lemon new shoots on 'Swingle' citrumelo and the lowest was found on Flying Dragon. Opposite effects were seen to abscisic acid (ABA). Higher content of ABA was seen in 'Eureka' lemon new shoots on Flying Dragon while lower ABA content was observed on 'Swingle' citrumelo. An assumption is that higher ABA levels account for plant growth reduction [61]. Likewise, the exogenous use of gibberellic acid and inhibitors of its synthesis are involved in the control of vegetative growth in citrus [62, 63].

Recently, *P. trifoliata* as a rootstock displays a potentiality to increase DNA demethylation and the amount of *24-nt small RNAs* on the orange scion compared to *P. trifoliata* grafted onto itself [64]. The evidence of possible epigenetic modifications imparted by grafting may also be associated with the mechanism of rootstock dwarfing.

2.2 Others dwarfing rootstocks

Several rootstocks are classified as dwarfing: 'Cunningham' and 'Yuma' citranges, 'Cuban' pummelo, *Citrus ichangensis*. Besides, other species belonging to the Rutaceae family, such as the genus *Hesperetusa, Citropsis, Clymenia, Eremocitrus e Microcitrus* have also a dwarfing effect [8, 26]. Nonetheless, in many cases, the dwarfing effect is not fully established. It is not clarified whether this effect is due to the rootstock effect itself, to the environment interaction with viral agents, or even to difficulties linked to rootstock/scion incompatibility [26].

In Spain, two dwarfing rootstocks developed by IVIA (The Valencian Institute of Agrarian Research) highlighted: Forner-Alcaide (FA) 418 and FA 517. The rootstock FA 418 is a citrangedarin, a hybrid of Troyer citrange (*C. sinensis* × *P. trifoliata*) with common mandarin (*Citrus deliciosa* Ten.). FA 517, is a citrandarin (*Citrus nobilis* Lour × *P. trifoliata*). Both rootstocks provided a large reduction in canopy volume and a significantly increase in yield efficiency of Navel orange compared to 'Carrizo' oranges. Along with that, both rootstocks induced the production of good quality fruit, with FA 517 inducing early entry into production. On pioneers roots (diameter 2–4 mm), FA 517 had a higher frequency of xylem vessels with diameters greater than 30 μm, while FA 418 showed higher densities of xylem vessels. These rootstocks reduced the hydraulic conductance, the net CO_2 assimilation, the stomatal conductance, and the transpiration. A greater reduction was noticed in Navel orange over FA 418 [65]. Yet, the rootstocks were tested under ultra-high-density conditions, between 1250 and 3333 plants ha^{-1}, and with mechanical harvesting (over-row continuous canopy shaking harvester) [66, 67]. Additionally, FA 517 exhibited resistance to Citrus tristeza virus, to *Phytophthora* sp. gummosis, and to nematode *Tylenchulus semipenetrans.* More, a good performance in saline and clayey soils resulting in high fruit yields beheld. On the other hand, FA 418, induced a greater reduction of tree size. Although larger fruits were obtained, they were more susceptible to the nematode *Tylenchulus semipenetrans* and to *Phytophthora* gummosis. Advantageously, FA 418 is tolerant to *Citrus tristeza* virus [68].

In USA, rootstock US-897 citrandarin was released by the US Department of Agriculture with the goal of reducing the size of plants in high-density orchards. His rootstock is used on 7% of the citrus orchards in Florida to high-density plantings in field and in protected structures. US-897 rootstock is a cross of Cleopatra mandarin (*C. reshni* Hort. ex Tan.) × Flying Dragon trifoliate orange (*P. trifoliata*). The yield of trees grafted onto US-897 is low. Nonetheless, due to the small size of the trees, calculations of the potential yield per hectare at the predicted optimum spacing can be very high. Despite the fruit good internal quality of US-897, its size can often be below average. It has tolerance to *Citrus tristeza* virus, citrus nematodes, the *Phytophthora–Diaprepes* complex, and high pH [69]. In-row spacings of 8 and 10 ft (2.42 and 3.05 m) are recommended for this rootstock [39].

Farther, new rootstocks have been produced by the University of Florida breeding program. Many of them are tetraploids, somatic hybrids, or sexual hybrids of two somatic hybrids. Among them, citrandarin UFR-6 (*C. reticulata* 'Changsha' + *P. trifoliata* '50-7') is a candidate for high-density plants, producing small plants, cold-hardy plants, and fruits with high soluble solids content [69, 70]. Beyond that, it is tolerant to *Phythophtora* gummosis and to *Citrus tristeza* virus. Plant spacing between 6 and 8 ft (1.83–2.44 m) is recommended [39].

Table 1 summarizes the characteristics of rootstocks discussed so far: Trifoliate orange, Flying Dragon, FA 148, FA 517, US 897, and UFR 06. In this table, aspects related to horticultural performance and biotic and abiotic stresses are compiled.

In Brazil, the citrus breeding program of EMBRAPA (Brazilian Agricultural Research Corporation) has developed rootstocks by hybridization. Among the genotypes tested for the 'Valencia' orange, four are important with respect to dwarfing effect along with relatively high fruit yield, high yield efficiency, and good fruit quality. These rootstocks are three citrandarins (TSKC × TRFD-003, TSKC × TRFD-006, TSKC × TRFD-007) and one citrumelandarin (TSKC × CTSW-058), hybrids of Common Sunki mandarin [*Citrus sunki* (Hayata) Hort. ex Tan.] with Flying Dragon trifoliate orange [*P. trifoliata* var. *monstrosa* (T. Itô) Swing.] or with Swingle citrumelo (*C. paradisi* Macfad. × *P. trifoliata*). Apart from that, TSKC × TRFD-003 citrandarin had a similar drought tolerance to Santa Cruz Rangpur lime (*Citrus limonia* Osbeck) [71]. Also, in the 'Valencia' orange several other materials seem to have dwarfing potential. Among them, the TSKC × (LCR × TR)-059 citrimoniandarin had a high yield efficiency, a dwarfing effect, an induction of earlier fruit-bearing with higher quality, and good drought tolerance [72, 73].

In other experiments with 'Valencia' orange in southeast Brazil, tetraploid Swingle citrumelo had a dwarfing performance which reduced canopy volume by 77% compared to the vigorous standard. In this same experiment, citrandarins offspring of Flying Dragon had no dwarfing behavior [74]. In 'Tahiti' lime, TS × PT 14 citrandarin had a dwarfing behavior and potential tolerance to HLB [75].

Features	Trifoliate orange	Flying Dragon	FA 148	FA 517	US 897	UFR 06
Horticultural performance						
Yield per tree	L-I	L-I	I	H	L	H
Yield efficiency	I-H	H	H	H	H	H
Earl bearing	I	I	I	H		
Fruit size	Sm-I/L*	Sm-I/L*	Lg	I-Lg	Sm-I	Sm-I
Fruit quality	H	H	H	H	H	H
Maturity	I	I				
Abiotic stress						
Drought stress	P	P				
Salinity	P	P	G	I-G		[I]
Flooding	I	I				
Freeze	G	G			[I]	G
Soil high-pH	P	P	G	G	[I]	
Soil low-pH	P	[P]				
Biotic stress						
Tristeza	T	T	T	R	T	T
Phytophthora	T	T	S	T	T	T
Nematode	T	T	S	R		

[a]*Sm–small; L–low; I–intermediate; Lg–large; H–high; P–poor; G–good; R–resistant; S–susceptible; T–tolerant. [ ] - Any symbol in brackets indicates a probable or expected behavior.*
**Conflicting data in the literature.*

Table 1.
Main features of dwarfing citrus rootstocks and common trifoliate orange (a semi-dwarfing rootstock) [39, 52, 68][a].

Other dwarfing rootstocks are also approached. In hot arid climate in India, Fremont mandarin had dwarfing behavior when grafted on *Citrus pectinifera*, probably a *Citrus depressa* Hayata, compared to vigorous rootstocks (Karna khatta and Rough lemon) [76]. In China, hybrids of Ziyang Xiangcheng (*Citrus junos* Sieb. ex Tan.) and trifoliate orange (*P. trifoliata*) were researched and ZZ6, ZZ31, and ZZ948 rootstocks showed strong alkaline tolerance (pH 8.2). Among these hybrids, ZZ6 and ZZ948 were rootstock dwarfing type [77].

There are several potentially dwarfing rootstocks that have emerged in recent decades from worldwide breeding programs. Nevertheless, the confirmation of these potentials needs further investigations. In those investigations, it is required the evaluation of these materials under different scions and at different environments for a minimum period of time, to generalize this information [78].

3. Conclusions

Citrus dwarfing rootstocks are the main options to enable the development of designed orchards in high and ultra-high density planting systems allowing easy mechanical harvesting and cultivation under protected structures. Further, it is paramount to perform a regional studies of different dwarf rootstocks genotypes and their interaction with the different scions as well as economic feasibility. With a well-conducted and designed studies, it is possible to establish recommendations and guide the correct management of dense citrus orchards in different environments. Therefore, the reduction of plant growth provided by rootstocks can be a management strategy to improve the orchard horticultural performance.

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Section 3

Pest Management

Chapter 5

Insect Pest Management in Fruit Production

Murat Helvacı

Abstract

Several pests cause destructive damages in fruit production. All of the pests cause yield loss but some of these cause transmission of virus diseases. For example, Brown citrus aphid transmits Citrus Tristeza virus in citrus production. Historically, broad-spectrum pesticides were used to prevent the yield loss and transmission of bacteria and virus diseases in the world. These pesticides cause several problems including environmental, human health and also cause negative effects on soil health. At the same time, pesticides have other potential negative effects including food safety. For all these reasons, alternative management methods such as biological, biotechnical, sterile insect techniques are used in fruit growing all over the world.

Keywords: biological control, biotechnical method, pest, sterile insect technique

1. Introduction

The word "pest" describes an organism that harms crops, harms or irritates animals or humans. Agricultural pests include insects, weeds, bacteria, viruses, fungi and animals that reduce crop yield relative to the potential yield that would be possible in a pest-free world [1]. Some of the epidemic diseases have been carried by insects. In the 14th century, the Bubonic Plague epidemic disease influenced the population of Europe negatively and this disease was transmitted by fleas. Each insect species has different periods in its life cycle. The most important biological forms are "complete metamorphosis" and "incomplete metamorphosis" forms. In complete metamorphosis, the adult insect lays its egg in plant tissue or soil. The larvae that emerge from the eggs do not resemble the adult insect. As they feed and develop, they molt and become pupae. Pupation takes place on the plant or mostly underground. After a certain time, adults emerge from the pupa and the life cycle continues in this way. In incomplete metamorphosis, the nymphs that emerge from the eggs that the adult female gives birth are very similar to the adults. They look like a miniature of the adult. However, the wings are not developed. These nymphs molt as they feed, and their resemblance to the mother increases after each molting period [2]. The attacking of several harmful insect's damages plant leaves, buds, stems, fruits, flowers and seeds, causing significant crop losses and decrease the market value of crops. For this reason, applying of management methods against pests is significant in the agricultural production [1].

Pest control aims to safely maintain economic, effective and long-term pest control. Generally, it contains suppressing pest populations to economic injury levels rather than eradicating the pest completely. Many pests negatively affect agricultural production in the world. Many methods are used by the producers to minimize the quality and quantity losses of these pests in agricultural production. The main of these methods, which are considered for Plant Protection or Agricultural Control, are cultural measures, quarantine measures, mechanical and physical methods, biological method, biotechnical method, chemical method and integrated pest management, which expresses the combination of the necessary ones [3]. Today, chemical applications are made for producers in terms of ease of application and results [4]. Depending on pesticides for plant protection is related to undesirable effects on the environment, health, and the sustainable effectiveness of their use. The emergence of synthetic pesticides has made it possible to simplify crop systems and abandon more complex crop protection strategies [5]. Pesticides are chemical matters used to decrease the devastating effects of living forms such as rodents, insects, animals, weeds, fungi, which live on or around plants, human and animal bodies, and reduce or damage the nutritional value of food sources during production, storage and consumption. Pesticide term includes all of the chemicals classified as an insecticide (use for harmful insects), herbicide (use for weeds), fungicide (use for fungal diseases), rodenticide (use for rodents), molluscide (use for slugs), avicide (use for birds), acaricide (use for acars), ovicide (use to kill eggs of harmful insects), bactericide (use for bacterial diseases), nematicide (use for nematodes), etc. [6]. However, using of pesticides raises several environmental concerns, including human and animal health hazards. Food contaminated with toxic pesticides is associated with serious effects on human health, as it is the basic necessity of life. More than 98% of applied insecticides and 95% of herbicides end up somewhere other than their target species, including non-target species, air, water and soil. However, pesticides can contaminate soil, water and vegetation. In addition to killing insects or weeds, pesticides can be toxic to several other organisms, including birds, fish, beneficial insects and non-target plants [7]. Many harmful insects cause economic losses in fruit growing. For example, Stem borer (*Zeuzera pyrina* L., Lepidoptera: Torticidae) is an important pest that causes tree death by attacking stems. On the other hand, aphids, especially *Aphis pomi* De Geer (Homoptera: Aphididae), are serious pests as young pomegranate leaves are highly susceptible to aphid attacks. Although, these harmful negatively affect pomegranate production, the market value of pomegranate fruits is mostly affected by Citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), Medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) and Pomegranate butterfly, *Deudorix livia* (Virachola) (Klug) (Lepidoptera: Lycaenidae) [8]. In addition to this, Olive fruit fly (*Bactrocera oleae* Gmel.) (Diptera; Tephritidae) is a major harmful insect of olive and If management methods are not adequately implemented, large product losses can reach up to 80% in olive oil-producing areas and up to 100% in table olive growing areas [9] and Medfly (*C. capitata* Wiedemann) (Diptera; Tephritidae) can cause 20–25% losses in citrus fruits, 91% in peaches, 55% in apricots and 15% in plums [10]. Considering the damage done by insects in fruit growing; insect pests such as Mediterranean fruit fly and Olive fruit fly lay their eggs in the fruit (oviposition damage) and cause fruit drop; pests such as aphid, thrips and whitefly act as vector insect and cause virus diseases to spread. For instance; in most cases, there is a very close relationship between the parasite and the vector, and often the vector is the only means of transmission.

The simplest form of spread is known as mechanical transmission. Typically, the insect picks up the parasite on its body surface while feeding on the host organism and may release the parasite into a new host body or contaminate the food that will later be eaten by the host. However, many insects pests feed on plant sap and blood in vertebrates and can mechanically transmit pathogens and parasites through contamination of the proboscis [11]. It also has many undesirable effects such as resistance to diseases, insects and weeds. For this reason, since issues such as human health and the protection of biodiversity are kept at the forefront, the issue of chemical control has begun to be questioned [4]. In this study, the subject of chemical control and other control methods which are used as a management method against insect pests that cause economically significant losses in agricultural production is included.

2. Management methods of insect pests

Today, there are pests such as insects, diseases, weeds and animal pests (birds, rodents, etc.) that cause economic losses in agricultural production. Insects and other species that damage crops and also infect humans or animals are therefore pests that should be controlled as much as possible [1]. Some of the harmful insects play a role as vector insect in the spread of important diseases such as virus diseases. In addition, weeds host many disease agents and harmful insect species. Vector insects include aphids, whiteflies and thrips. To give an example, the Brown citrus aphid is the most significant vector insect of citrus tristeza virus (CTV) due to its superior vector productivity, especially for vigorous strains [12]. In the management against these insect pests, Integrated pest management (IPM) is an oncoming based largely on the information of pest biology and ecology to allow farmers to make tactical decisions to optimize ecologically and economically sound control of harmful organisms (pathogens, weeds, insects, vertebrates) [13]. Among these different methods of management; the method of suppressing the pest population with beneficial insects “biological control”; the method of management with using traps, “biotechnical control”; “cultural method”, a method of management with using agricultural methods such as plowing, crop rotation; “chemical control” method with using pesticides such as insecticide, fungicide, acaricide; Methods such as “physical control”, which includes methods such as manually collecting individual pests, pruning damaged plant tissues and removing excessively damaged plants, are applied. In this study, information is given about these control methods which are applied against pests that cause significant damage and economic losses in fruit growing.

2.1 Chemical method

Chemical control is the control method against harmful organisms that cause economic loss in plants, by using synthetic or naturally derived chemicals that have a killing effect (toxic effect). These products are called pesticides, synthesized substances or biological agents used to attract, seduce, destroy or mitigate any pest [14]. In addition to the benefits of these chemicals, it is known that they can create extremely important human, animal, plant and environmental health risks. For this reason, these chemicals are produced and sold subject to the most advanced control and inspection systems worldwide. Pesticide is defined by the FAO as a matter or mixture which is used to prevent, repel or destroy organisms such as animal and human vectors and unwanted plants and animals that cause damage in horticultural production.

Besides, FAO defines it as otherwise interfering with the processing, storage, production, transportation or marketing of products such as agricultural crops and animal foods [7]. Historically, in the 1930s, DDT was widely accepted as a pesticide that significantly conduced to the enhancement in the turnover of agricultural crops, especially food products, but then fell out of favor in the 1960s as a result of its different effects than usual [1]. The extent of the damage caused by the pests on agricultural products is high. Problems such as the overuse of pesticides used to minimize this damage and the environment, food poisoning and food insecurity are of great concern. However, insects and other species that cause damage to agricultural production and infect humans or animals have therefore become pests that need to be controlled as much as possible [4]. Pesticides are grouped in many different ways according to their appearance, physical structure and formulation, the pest and disease group they affect and their biological period, the type and group of the active substance they contain, the degree of toxicity and the technique of use. The most commonly used classification forms are the classifications made according to the harmful groups they are used and the active matter group in their structure. Pesticides are grouped by pest species or target organism. In this grouping, there are three main groups of pesticides. These are insecticides, fungicides and herbicides. The most important classifications of pesticides according to their chemical structures are organic chlorine pesticides, phosphorus, carbamates, natural and synthetic prethyroids [15]. The most important way to increase agricultural production; it is to get more products from the unit area, that is, to increase the yield. One of the most significant factors in enhancing the yield is to manage harmful organisms that limit plant production. Pesticide applications are intensively applied in fruit growing because it is easy to apply and effective in a short time.

2.2 Biological method

Biological method is the whole of the measures taken to use natural enemies, entomopathogenic microorganisms or to make them more effective against pests, diseases and weeds that damage crop plants. In other words, the agricultural control activity carried out by using natural enemies to suppress pests in agricultural areas and keep them below the level of economic damage is called biological control [16]. In the "Regulation on the Import and Release of Exotic Biological Control Agents" issued by FAO in 1996, biological control is defined as "a pest control strategy using living natural enemies, antagonists, competitors and other self-reproducing biological entities. This sentence can be said as the definition that best describes the biological method. Predator, parasitoid and entomopathogens are used as biological method agents. Predators live freely and directly feed on large numbers of prey during their lifetime. Parasites are organisms that live and consume or on a larger host [17]. Insect parasites (more precisely called parasitoids) are smaller than their hosts and develop inside or adhere to the outer part of the body of their hosts [18]. Predator insects lay their eggs next to their prey, and the hatched larvae consume their prey by stinging, sucking or chewing. Generally, predatory insects are polyphagous and therefore they are the most important agents which are used in biological control. Parasitoid insects, on the other hand, usually lay their eggs on the pest itself or its eggs. Parasitoid insect larvae emerging from the eggs cause the death of the pest's egg or itself, and in this way, they suppress the pest population and increase their own population. Entomopathogens include bacteria, viruses, fungi and nematodes used against harmful insects. Naturally occurring entomopathogens attack harmful insects, making them sick and sometimes killing them.

The best example of entomopathogens is the beneficial bacteria named "*Bacillus thuringiensis*", which is known as "Bt spray" in the agricultural companies. This bacterium can also be called a biological insecticide. Natural enemies have been used as a pest control method for centuries. However, in the last 100 years, there has been a significant increase in the understanding of humans and especially producers and the use of these biological control agents about how biological control agents, which are part of safe and effective pest control methods, can better manipulate pests [19]. Since the biological control method does not have negative effects on nature, the environment, in short on biodiversity and human health, it is a control method that should be used predominantly in agricultural production and especially in fruit growing. There are three types of biological control strategies implemented in pest control programs. These are importation (sometimes called classical biological control), augmentation and conservation. Classical biological control is defined as the deliberate introduction of an exotic (non-natural), often co-developed biological control agent for the permanent establishment and long-term pest control [18]. When a new pest enters from one country to another and there are no natural enemies of that pest in the country, its population increases in a short time and causes economic damage. To prevent this damage, natural enemies of the pest are imported from the country of origin and tried to be placed in the fauna where the pest is found. The need to re-establish interactions between harmful organisms and their natural enemies is based on the principle of importing and planting beneficial insects in locations where pests generally have no natural enemies or where the population of existing natural enemies is lower than the pest population. Within the scope of classical biological control against pests, 2000 biological control agent species and more than 5000 placement applications were made in 196 different countries. No negative effects of these practices, which have been carried out for years, have been detected [4]. Augmentation contains the additional release of natural enemies, rising the population which is found naturally. At a crucial stage of the growing period, few amount of beneficial insects can be extricated (inoculative release) or millions can be extricated (inundative release) [18]. The most commonly used biological control agents in this application are entomopathogens. Predator and parasitoid agents are more difficult and expensive to produce. Producing and multiplying predators and parasitoid agents in artificial media is less costly. However, the main problem here is to investigate whether these beneficial insects produced in artificial nutrient media are effective in nature, and accordingly, nutrient media should be prepared and produced. In addition, the production and release of predators and parasitoids into nature must be at certain standards. For example, an egg parasitoid should be made during the period when the pest egg is found in nature and at times of the day that are suitable for the parasitoid. For this reason, the production and release of predators and parasitoids are mostly applied in crops with high economic value and in greenhouses [4]. Conservation of natural enemies in an environment is the third method of biological pest control. Natural enemies are already adapted to the habitat and target pest and their protection through vegetation manipulation can be simple and cost-effective, while Classical Biological Control provides control of both primary and secondary pests, reducing the likelihood of pest outbreaks and resurrections [18]. This type of biological method can be reached in two ways: changing pesticide use and manipulating the growing environment in favor of natural enemies [20]. If natural enemies are adversely affected and their population declines, the pests get rid of the pressure of natural enemies and multiply in a short time and rise above the economic damage threshold.

Biological control, carried out by protecting and supporting native beneficial insects, gives more successful results in large areas than conventional and replicated biological control applications [17].

2.3 Biotechnical method

Biotechnical Control aims to prevent or control the normal biological or physiological activities of pests by using some artificial or natural compounds. That is, it interferes with the behavior and development of pests in their natural life processes such as feeding, mating, laying eggs, and flying. Some substances such as pheromone, attractant, antifeedant, kairomone, insect growth regulator, repellant, oviposition deterrent and chemosterilant are applied on biotechnical control. This management method can not pollute the environment and is compatible with other control methods and does not cause residue problems in foods. The compounds used in this method specifically target only the harmful organism and ensure the preservation of the natural balance. The biotechnical control method can be used in harmony with Organic Agriculture and Integrated Pest Methods [21]. The most commonly used pheromones for biotechnical control methods used within the scope of agricultural pest control are sexually attractive pheromones, which are secreted by females and invite males to mate, and aggregation pheromones that inform a food source or places suitable for nesting. In general, pheromones can be used for four different purposes; Use in combination with a trap for pest population monitoring (Monitoring), likewise combined with a trap for use in a mass trapping technique to reduce pest populations (Mass Trapping), inhibiting mating by emitting an intense signal, preventing males and females from finding each other and preventing them from mating and the use in the technique of mating (Mating Disruption) and finally, the use of pull and kill (Attract & Kill) technique by using it with an insecticide. Monitoring purposes are mostly aimed at determining the population development such as whether there is a pest, if it is, the first adult emergence, the periods when the population is dense, how long the pest is in the nature, when it goes to winter, and the flight period. The utilization of pheromones for monitor purposes is used in population monitoring of many pests [3]. Attractive traps such as McPhail, yellow sticky traps and delta traps are used against harmful insects belonging to the Tephritidae family such as Mediterranean fruit fly and Olive fruit fly, which cause product losses in fruit growing. However, due to the high cost of this type of traps, alternative traps can be used. These types of traps can be prepared by opening holes in the 1 lt. plastic bottles we use in our house, and putting apple juice + sugar mixture in them, and they can be used against these harmful insects, which belong to this family and cause significant yield losses in fruit growing.

2.4 Cultural method

One of the oldest methods of pest control in agricultural production is the cultural control method. However, with the development of synthetic pesticides, cultural control methods were quickly abandoned or not focused on, and research on them was largely stopped. The emergence of synthetic pesticides was effective in stopping these studies, as well as the fact that the cultural control method depends on preventive and long-term planning rather than an effective application method. It is applied as a pre-control method because it is less effective than other control methods.

There are many applications such as site selection, planting design and management (crop rotation, planting trap plants, planting and planting timing, placement of alternative hosts, etc.), plowing, irrigation, drainage, fertilization, removal of plant residues, mulching, adjustment of harvest time among cultural control methods [22]. The general principle in the processes considered as "Cultural Control" in the management against diseases, pests and weeds is to reduce the reproduction, shelter and living opportunities of harmful organisms by changing the environment in which they live in a way that is not suitable for the harmful organism. For successful cultural control, the most sensitive periods of harmful organisms should be determined, information about the interaction of host plant, harmful organism and environmental conditions should be learned to prevent the attacks of harmful organisms, to destroy them or to reduce the rate of reproduction, and cultural processes should be changed or developed accordingly. Cultural measures that have been applied for centuries from the past to the present are still important and up-to-date, as they are generally the sum of this knowledge and practices that have been experienced and adopted before, with positive results [23].

2.5 Physical method

The physical method of pests in fruit production has come to the fore in recent times because of the resistance development of pesticides avoidance from residue which causes pesticide and economic causes [24]. In physical control methods, the physical environment of the pest is changed in such a way that the insects no longer pose a threat to the agricultural crop. This can be achieved by creating stress levels ranging from agitation to death, or by using devices such as physical barriers that protect products or pants from invasion. While many physical control methods target a whole range of physiological and behavioral processes, chemical methods have well-defined and limited modes of action [25]. Physical control practices include repelling pests or restricting the accession of pests to plants, distorting the behavior of insects. In addition to this, this method includes the death of insects directly [4]. Physical methods are divided into two main groups. The name of these groups is active and passive [25]. Active methods include picking up the larvae of harmful insects, pruning of damaged or infected plant tissues, and removal of heavily damaged and infected plants. Generally, passive methods consist of the use of a tool or device to remove pests from a product. This equipment acts as a barrier between plants and pests, protecting plants from damage caused by insects. Other passive tools include repellants and traps [20]. Chemical and biological methods are often inharmonious; however, there is a harmony between cultural, biological, and physical methods and when used together they can be more effective against pests than chemical method.

2.5.1 Sterile insect technique

The idea of sterilizing insects' dates back to earlier than the invention and use of modern insecticides. Sterilization was first tried on *Lasioderma serricorna* (F.) (Tobacco beetle) in 1916, and the insect produced sterile eggs. However, an American scientist, Dr. E.F.Knipling started to work on this subject since 1937 and investigated the possibilities of management with insects by sterilizing them or making some changes in their genetic structures. As a result of long studies, Knipling was able to make his first publication on this subject in 1955. There are mainly two sources of insect sterilization. The first is radiation, and the second is some chemical substances called chemosterilants.

Cobalt-60 and Cesium-137 are the most common sources used for this purpose. Radiation produces dominant lethal mutations in the gametes of insects. These lethal mutations actually do not adversely affect the maturation of the sex cells or the formation of the zygote, and they prevent the maturation of the zygote. Radiation, by interrupting the spermatogenesis in male, stops the formation of sperm (aspermia) and reduces the activity of the sperms or causes the loss of mating power. In this case, the male does not mate or fertilization does not occur because it cannot stay in the mating position long enough. In females, on the other hand, egg formation decreases or does not occur at all, since it damages the organia or the nutrient cells or both [26]. This method is applied effectively against pests such as Olive fruit fly and Mediterranean fruit fly and gives positive results as a management method against these harmful insects.

2.6 Integrated Pest management method

End of the 19th century, the idea of Integrated Pest Management began to emerge and some applications were seen in the early 20th century. It is noteworthy that in these first applications, only biological control was considered and applied besides chemical control. However, the concept of Integrated Pest Management in its current sense was first put forward in 1954 and its principles were determined in the symposium held in Rome in 1965 by the Food and Agriculture Organization (FAO). It is considered to be the most modern application developed in the field of plant protection. This practice, which is commonly known as integrated pest management and integrated pest control in English, has been defined in previous years with names such as Complementary Control, Complementary Pest Control, Integrated Control, Integrated Pest Management. This management method is defined by the FAO as a control method of pests that takes into account population fluctuations of pest species and their relationship with environment, and keeps their populations below the level of economic damage by using all appropriate control methods and techniques appropriately and this definition is accepted in the world. The aim of this method is expressed as the use of multifaceted tactics in good coordination to ensure balanced crop production, to keep the losses caused by pests at the level that will provide the highest economic gain, to meet the other goals of the farmers, to minimize the risks of pesticides on humans, animals and the environment [27]. Integrated pest management aims not only to suppress or eliminate the population of pests, but also seeks solutions that combine viable, economically acceptable, effective and environmentally friendly, sustainable ways. Integrated pest management aims not only to suppress or eliminate the population of pests, but also seeks solutions that combine viable, economically acceptable, effective and environmentally friendly, sustainable ways. Regular inspection of agricultural lands is always critical and must be done in an integrated management programme. Without control, the information needed to decide whether action should be taken in the first place and how severe the pest population is may not be gathered. Without all this information and properly determining how high and how widespread a pest is in the field, it may not be possible to make the right interventions at the correct time. Therefore, the population and spread of a pest should always be known before taking comprehensive action and planning. The basic principle of the integrated control method is to apply the control method when the pest population rises above the economic damage threshold, not as a routine method that always exists. A carefully planned integrated control program aims to adjust the terrain to prevent the emergence of the pest in the first place and to completely destroy the pest itself or reduce its population if the pest is present in the land [4].

2.7 Plant Defense chemicals

Plants produce defensive metabolites that do not affect normal vegetative growth and development but reduce the palatability of the tissues in which they are produced [28]. In other words, plants have a variety of inducible and constitutive defense mechanisms to defend themselves against attack. These include structural defenses such as spines and waxy cuticles, as well as protein-based and chemical defenses [29]. Plants respond to herbivory through a variety of molecular mechanisms, biochemical and morphological and exhibit multifactorial traits that are constitutively expressed against herbivory or induced upon attack. Plant defenses activated in herbivores are a complex network of different pathways of direct and indirect defenses. Direct defense compounds such as glucosinolates or protease inhibitors directly affect insect performance and feeding behavior, while indirect defenses, such as the emission of volatile organic compounds after herbivore attack, act as attractants for the parasitic wasp that precedes the attacker. As plants develop new defense compounds or mechanisms, resistance to herbivores, their attackers find new ways to bypass or detoxify them [28]. Plant defense chemicals consist of secondary metabolites whose core structures are predominantly terpenes, benzenoids, phenylpropanoids, flavonoids or N-containing compounds. Plant defense chemicals can be classified according to their inducible or structural production. Initially, these classes were grouped according to their responses to pathogens were called phytoalexins and phytoanticipins. These have been defined as low molecular weight phytoalexins, "antimicrobial compounds that are both synthesized and accumulated in plants after exposure to microorganisms, and phytoanticips" as "low molecular weight antimicrobial compounds that are present in plants before they are threatened by microorganisms or are produced only after infection from pre-existing components". Defense compounds (phytoalexins) induced by these insects may have important functional roles as nutritional deterrents. The disadvantage of inducible defense systems is the delay in the synthesis of new compounds. An alternative strategy is to constitutively produce the compounds in tissues susceptible to attack. The disadvantage of phytoanticipins is the metabolic energy required to produce compounds even in the absence of insect threat and the active form of certain compounds being toxic not only to insects but also to the plant itself a common alternative approach to circumventing the toxicity problem is to store compounds as readily activated non-toxic forms and activate them upon insect attack. These compounds are known as phytoanticipins because they are produced in anticipation of a threat [29].

3. Conclusion

Global climate change and urbanization have increased the pressure on water, soil and climate, which are the natural resources of agricultural production. As a result of these pressures, existing breeding systems have also been damaged. Urgent measures are required to reduce the increasing pressure and to deliver natural resources suitable for agriculture to future generations. With the increase in the world population, the production areas are decreasing. However, to meet the needs of people with agricultural products produced in these declining areas, the amount of agricultural products produced per unit area should be increased. It is not possible to increase agricultural production only with plant nutrition. It is imperative that plant protection measures are also fully implemented in agricultural products. Due to living and non-living effects, regressions occur in the growth and development of plants. There are some signs of disease in plants. The severity and intensity of these symptoms indicate the extent of the disease. Therefore, these symptoms in plants are very important to find the source of the problem by detecting it well and taking measures in terms of agricultural management. With this information obtained, methods that cause the least harm to the environment and nature should be applied to combat diseases. These management practices are a physical method, cultural method, biological method, biotechnical method, integrated pest management and sterile insect technique. Time is important in the fight against diseases. When the right time is not selected, the success of the control method applied decreases. It is a costly and challenging process for a diseased plant to become healthy. Therefore, it is important to prevent the plant from contracting the disease. Taking precautionary measures to prevent the plant from contracting the disease will provide longer-term gains. The most effective methods should be used without harming the living creatures in nature. When the 7 control methods, we mentioned above are used in the right time and manner, the yield, quality and pest protection methods in agricultural production will become easier. In order to, we can say that all of the methods we mentioned are used properly in sustainable agriculture. Pest control methods in agricultural areas will be much easier if sustainable farming methods are adopted and used appropriately.

Conflict of interest

The author has no conflict of interest.

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Chapter 6

Aphid on Almond and Peach in Tunisia: Species, Bioecology, Natural Enemies and Control Methods

Lassaad Mdellel, Rihem Adouani and Monia Ben Halima Kamel

Abstract

Aphids are among the most obnoxious pests of almond and peach in Tunisia. Accurate control of these insect pests requires the determination of their major species as well as the thorough understanding of the biology and identification of their major natural enemies. The scope of this chapter is to identify the main aphid species infesting almond and peach in Tunisia, to describe their biology, to determine their natural enemies and to study their efficiency as biological agents. A field survey was carried out during 2007–2016 period at Almond and Peach orchards in Tunisia. Results demonstrated the presence of *Hyalopterus pruni* Geoffroy, *Hyalopterus amygdali* Blanchard, *Brachycaudus amygdalinus* Schouteden, *Myzus persicae* Sulzer, *Brachycaudus schzartwi* Borner and *Pterochloroides persicae* Cholodkovsky. Biological study of recorded species demonstrated the presence of holocyclic and anholocyclic life cycle depending on host trees and aphid species. For predators, four families (Coccinellidae, Syrphidae, Chrysopidae, Cecidomyiidae) and one parasitoid and two entomopathogenic fungi species were identified. For control of *Pterochloroides persicae*, results showed that *Pauesia antennata* Mukergi was more efficacy than *Coccinella algerica* Kovar. This parasitoid should be reared and used in future integrated pest management program in almond and peach orchard in Tunisia.

Keywords: almond, peach, aphids, biology, predators, parasitoids

1. Introduction

Peach and almond are being considered as the most important fruit trees in Tunisia covering more than 22714.5 and 22139.9 hectares, respectively [1]. These fruit trees are tolerant to stress conditions (salinity, water deficiency) and still bear good yields. Nevertheless, a wide range of insect pests infest almond and peach trees reducing yield's quantity and quality. Among them, *Ceratitis capitata* Wieddeman (Diptera; Tephritidae), *Ruguloscolytus amygdali* Guerin (Coleoptera; Scolytidae) and aphids are considered as the major insect pests that affect almond and peach [2–8]. Of them, aphids are considered as the most destructive [3–5]. There are sap-sucking insects, which feed in colonies, cause

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yellow leaf spots and deformity in leaves and flowers, transmit viruses, exude honeydew upon which sooty mold grows, but it also attracts ants. The ants, in return for the honeydew, they facilitate dissemination of aphids and carry wingless form to the trees carried the aphids to the trees when they are wingless [9–11]. In Tunisia, *Myzus persicae* Sulzer, *Hyalopterus pruni* Geoffroy, *Brachycaudus amygdalinus* Schouteden and *Pterochloroides persicae* Cholodokovsky are the most common aphid species that infest peach and almond [5–7, 12, 13]. Currently, protection of peach and almond orchards is mainly achieved by preventive and intensive chemical control. However, excessive pesticide misuse and selection of inappropriate active ingredients result in more crop diseases, auxiliary fauna destruction and environmental pollution. For that reason, selection of resistant cultivars and use of aphids' natural enemies (predators, parasitoids, entomopathogens) as pest-control alternatives probably provide the best long-term solution for aphid pest control [14–16]. Aphid biological control programs need the choice of natural enemy (predator, parasitoid, entomopathogen) based on their efficacy and climate adaptation and specificity. Some species of ladybird, hoverfly, ladybird, hover fly, green lacewing, true bugs and wasps are known as aphid natural enemies and considered as potential biological agents.

In Tunisia, extensive traditional growth of almond and peach trees in large cultivated areas can result in a flourishing habitat for attracting several aphid species and their natural enemies. In this chapter, we define the composition of aphid fauna and their natural enemies on almond and peach in Tunisia, and describe bioecology of defined aphid species and control methods of *P. persicae* using *Coccinella algerica* (Coleoptera; Coccinellidae) and *Pauesia antennata* (Hymenoptera; Lachninae).

2. Survey of aphid species in almond and peach trees

This study was held in 11 sites of north, center and south of Tunisia, where wild almonds and peach distributed there. This study lasted ten years: 2006 until 2016,

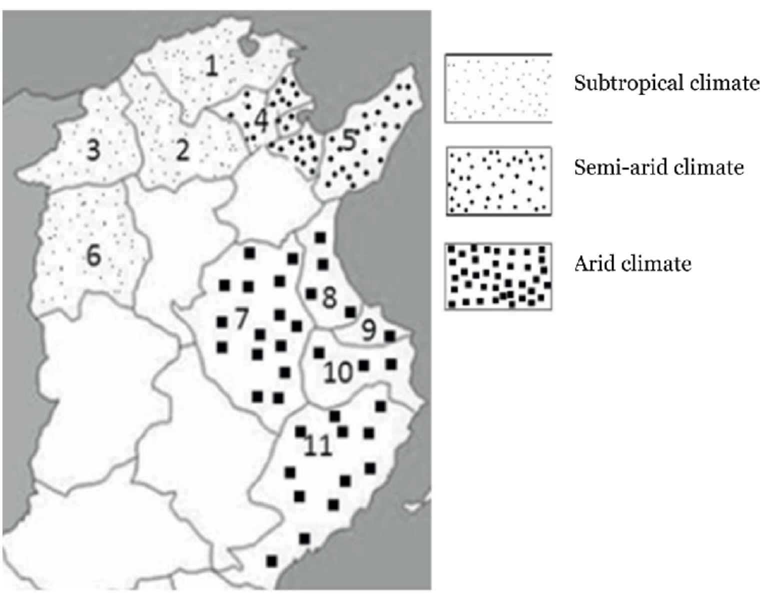

Figure 1.
Tunisia map representing sites of study.

throughout the aphid injury presence on almonds and peach. Several almond and peach varieties have been chosen (**Figure 1**).

3. Aphids species on almond and peach in Tunisia

Aphid species were identified according to Blackman and Eastop and using taxonomy keys [17–19]. Our results demonstrated the presence of six species that belonged to the Aphidinae and Lachninae subfamilies. For the Aphidinae, species *Hyalopterus pruni* Geoffroy (**Figure 2A** and **B**), *Hyalopterus amygdali* Blanchard (**Figure 2C** and **D**), *Brachycaudus amygdalinus* Schouteden (**Figure 2E** and **F**), *Brachycaudus schwartzi* Borner (**Figure 2G** and **H**) and *Myzus persicae* Sulzer (**Figure 2K** and **L**) were identified. These species usually feed on the young leaves almond and peach causing a stunted growth [20]. For Lachninae, we identified only the *Pterochloroides persicae* Kolodkovsky species that attacks the bark and trunk of almond and peach trees (**Figure 2I** and **J**) [20–24]. Of them, *H. pruni*, *M. persicae* and *P. persicae* are the most abundant species causing extensive damages on peach and almond [5, 6, 7, 13]. In Egypt and Syria, similar studies on almond and peach demonstrated the presence of the same species that were identified in this work [25–27]. Other aphid species (*Aphis gossypii* Glover, *Macrosiphum rosae* L., *Brachycaudus prunicola* Kaltenbach, *Aphis spiraecola* Patch, *Brachycaudus helichrysi*

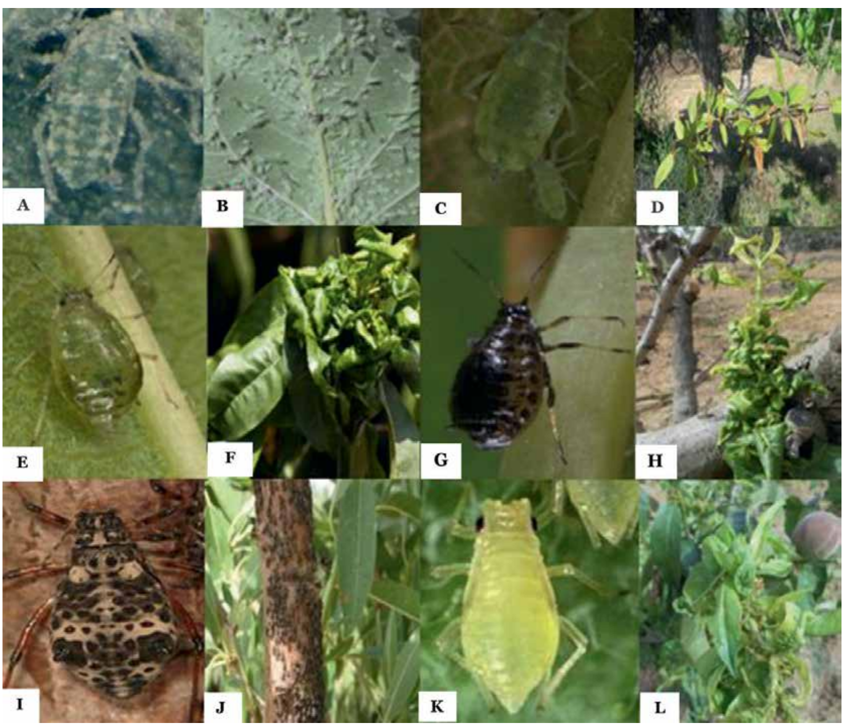

Figure 2.
Aphid species on almond and peach in Tunisia. (a: Hyalopterus pruni, *b:* Hyalopterus pruni *on almond leaf, c:* Hyalopterus amygdali, *d: Symptoms of* Hyalopterus amygdali *attack on almond, e:* Brachycaudus amygdali, *f: Symptoms of* Brachycaudusamygdalinus *attack on almond, g:* Brachycaudus schwartzi, *h: Symptoms of* Brachycaudus schwartzi *attack on peach, i:* Pterochloroides persicae, *j:* Pterochloroides persicae *population on peach trunk, k:* Myzus persicae, *l: Symptoms of* Myzus persicae *attack on peach).*

Kaltenbach, *Brachycaudus persicae* Passerini, *Brachycaudus schwartzi* Borner, *Hysteroneura setariae* Thomas, *Macrosiphum euphorbiae* Thomas, *Myzus cerasi* Fabricius, *Myzus varians* Davids and *Hyalopterus persikonus* M. were also observed on peach and almond) could be observed on almond and peach and classified as rare [13, 28–30].

4. Aphids bioecology infesting peach and almond trees in Tunisia

Biology of infestation of different species that were identified in this study was recorded during the four seasons of each year. For *Hyalopterus* species, an ovoid green egg (**Figure 3**) was observed around dormant buds of almond and peach during November, December and January [20]. *Hyalopterus* was also observed on herbaceous plant *Phragmites spp* (Poales; Poaceae) in the rivers. This indicated that *Hyalopterus* species were dioeciously holocyclic, colonizing peach and almond as primary hosts and *Phragmites spp.* as secondary host. For the green peach aphid (*M. persicae*), ovoid and white eggs were found around dormant buds and the trunks of peach (**Figure 4**). The presence of eggs of *M. percicae* on dormant buds and trunks proved their holocyclic life cycle. Results considering egg-laying period were similar to those of the Jerraya's [4, 5]. However, Hulle et al. [31] showed that eggs of *M. persicae* were shiny black. However, Strathdee et al. [32] demonstrated that color of fertilized eggs can change. Holocyclic life cycle of *M. persicae* was demonstrated in several others studies [4, 5, 31]. In contrast, on herbaceous plants, only viviparous parthenogenetic females of *M. percicae* are present throughout the year (anholocyclic life cycle) [33, 34]. It is also an heteroecious holocyclic specie [35]. The study on *B. amygdalinus* bioecology showed that almond is the preferential host for this aphid species compared with the peach tree ones. This aphid is holocyclic dioecic, which was

Figure 3.
Hyalopterus pruni *egg.*

Figure 4.
Myzus persicae *egg.*

observed on different spontaneous plants such as *Polygonum persicaria* (Caryophyllales; Polygonaceae) [31]. *B. schwartzi* was observed infesting both almond and peach without preference. *P. persicae* was observed on different parts of peach and almond (root, trunk, branch), and it is a parthenogenetic species in temperate regions and holocyclic species in cold regions [21]. Anholocyclic cycle of *P. persicae* in Tunisia was demonstrated in several studies [6, 13, 36]. In other countries, the anholocyclic cycle of this species was demonstrated [26, 37, 38]. The holocyclic cycle was also demonstrated [19, 39, 40].

5. Aphids natural enemies

Our survey on aphid taxonomy infestating almond and peach orchards in Tunisia revealed the co-existence of a wide range of natural enemies living in the same habitat. Insect natural enemies were collected and identified in laboratory according to Le Monnier and Livory [41], Chandler [42], Rotheray [43], Stary [44] and Lawrence [45]. Our results demonstrated the presence of four families of predators (Coccinellidae, Cecidomyiidae, Syrphidae and Chrysopidae). For Coccinellidae, we identified the following species *Coccinella algerica* Kovar (Coleoptera; Coccinellidae) (**Figure 5**), *Hyppodamia variagata* Goeze (Coleoptera; Coccinellidae) and *Scymnus apetzi* Mulsant (Coleoptera; Coccinellidae). Concerning population abundance, *C. algerica* is the most popular predator of the lady beetle species observed near all aphid colonies [20]. For Syrphidae family, *Episyrphus balteatus* De Geer (Diptera; Syrphidae) larvae (**Figure 6**) and adults (**Figure 7**) and *Metasyrphus carollae* Fabricius adult (**Figure 8**) were the two identified species. Larvae of *Aphidoletes aphidimyza* (Diptetra, Cecidomyiidae) (**Figure 9**) were the observed ear populations of *Hyalopterus species*, *M. persicae* and *P. pericae*. *Chrysoperla carnea* Stephens eggs and larvae were observed on aphid colonies at the end of April, May and June (**Figure 10**). *Aphidius transcaspicus* Telenga (Hymenoptera: Braconidae) (**Figure 11**) was the only identified parasitoid species on *Hyalopterus* species.

Figure 5.
Coccinella algerica *Kovar.*

Figure 6.
Syrphid larva on Pterochloides persicae *population.*

Figure 7.
Episyphus balteatus *Degeer.*

Figure 8.
Metasyrphus carollae *Stephens.*

Figure 9.
Aphidoletes aphidimiza *larva.*

Figure 10.
Chrysoperla carnea *larva.*

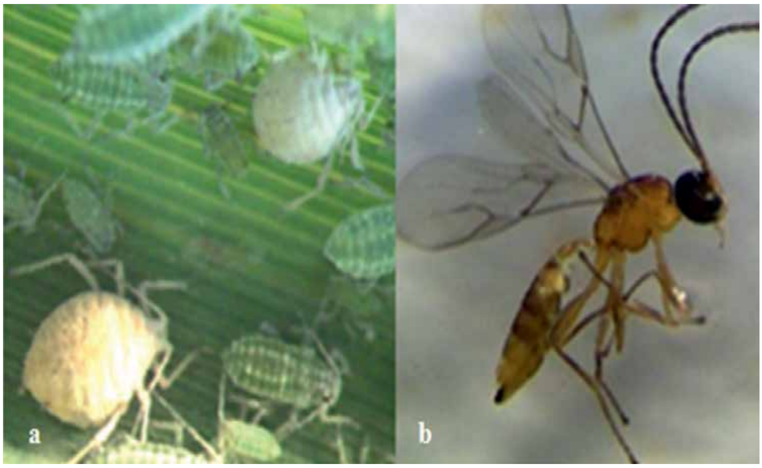

Figure 11.
Aphidius transcaspicus *Telenga. a): mummies, b) adult.*

Entomopathogenic fungi naturally infecting *P. persicae* were collected and identified according to Humber [46] and Barnett and Hunter [47]. Two entomopathogenetic fungus were identified: *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales, *Cordycipitaceae*) and *Metacordyceps liangshanensis* (Ascomycota: Hypocreales, *Clavicipitaceae*) [48]. In the word, *Capnodium spp*. in Central Asia and *Entomophthora thaxteriana* (Entomophthorales; Entomophthoraceae) were also identified on *P. persicae* population [47].

6. Control methods

6.1 Efficiency of *Coccinella algerica* Kovar

Efficiency of *C. algerica* to control *P. persicae* under laboratory conditions was studied. *C. algerica* eggs were collected. Emerged larva was separated and placed in test tube. Each larva instar was fed with *P. percicae* adults. Results demonstrated that the mean predation rate of *C. algerica* larvae during larval development time (9.8 ± 4.8 days) was of 30.13 ± 1.65 individuals of adult *P. persicae*. Of them, 72.3% were consumed by the first and second instar. Adults consumed daily 9.18 ± 0.088 *P. persicae* individuals. As for the efficiency of natural enemies, the predation of *P. persicae* by fourth instar larvae and adults of *C. algerica* demonstrated that both larvae and adults feed successfully on *P. persicae*. Several works demonstrated that predation rate of *C. septempunctata*, which is similar to *C. algerica* in morphology and biology [49], reared on *A. gossypii* in the same conditions of temperature and photoperiod was 9.7 aphids per day [50].

6.2 Efficiency of *Pauesia antennata* Mukerji (Hymenoptera, Braconidae, Aphidiinae)

P. persicae mummies were collected at May/2011 from almond trees from Iran and imported to entomology laboratory of Higher Agronomic Institute of Chott Mariem, Chott Mariem, Sousse, 4042, Tunisia. Emerged parasitoids were reared and efficiency was studied. Results demonstrate that longevity of adult parasitoids is of 3 to 4 days. Cross and Poswal [51] showed that *P. antennata* has a very short life span (5–6 days). Longevity of *P. antennata* seems much shorter than that of *Aphidius ervi* Haliday, which was 12.29 ± 0.43 days at 20°C [52]. Parasitism and emergence rates were of 40.5 ± 12.4% and 36.4 ± 17.2%, respectively. The study of impact of aphid density on parasitism and emergence rates demonstrated that parasitism and emergence rates decreased by increasing aphid densities (45 ± 16.1, 36.4 ± 9.9 and 27.5 ± 8.1, for the three densities of *P. persicae*, D1 (50 aphids), D2 (100 aphids), and D3 (150 aphids), respectively). Similarly, emergence rate decreased when aphid density increased (40.8 ± 1.6, 31.2 ± 11.2 and 27.3 ± 12.2 on D1, D2 and D3 densities respectively). The study of aphid's population effect on *P. antennata* parasitism rate demonstrated that, upon introduction of one couple of *P. antennata*, parasitism and emergence rates decreased when the aphid population densities were high (D2 and D 3). Similar results were demonstrated for *Aphidius ervi* when the mean number of parasitized aphids and laid eggs during *A. ervi* female's life time increased with the increase of host density and the daily parasitism rate decreased when the host density increased to 50/cylinder [53]. These results indicate that the parasitoid can adjust the oviposition strategy in response to host density. Effect of parasitoid number on parasitism rate increased when the number of released parasitoids increases. This is demonstrated also after using *Lysiphlebus testaceipes* parasitoid. Parasitism rate of this parasitoid species increased after release of eight *L. testaceipes* (four males and four females) for a density of 80 individuals of *A. gossypii* compared to parasitism rate after release of four parasitoid individuals [54].

7. Conclusions

This chapter highlighted the major aphid on almond and peach in Tunisia (species, bioecology, natural enemies and control methods). Among six aphid identified species, *H. pruni, M. persicae* and *P. persicae* were the most damaged species. These species can be multiplied either by parthenogenesis or by sexual form. For natural enemies, six predator's species, one parasitoid and two entomopathogenic fungus are identified. Among predators, *C. algerica* is the most widespread. However, this ladybird (larva and adult) is inefficient to control *P. persicae*. The introduction of specific parasitoid *P. antennata* and its use to control *P. persicae* showed efficiency. It can be used in future program for control of aphid on almond and peach. Future studies should focus on efficiency of *Aphidius transcaspicus* to control *Hyalopterus pruni* and on pathogenicity of *Beauveria bassiana* and *Metacordyceps liangshanensis* to *M. persicae* and *P. persicae* must have realized and used in integrated pest management program.

Conflict of interest

No conflict of interest to declare.

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Section 4

Fruit Composition

Chapter 7

Biochemical Composition of Japanese Quince (*Chaenomeles japonica*) and Its Promising Value for Food, Cosmetic, and Pharmaceutical Industries

Ieva Urbanavičiūtė and Pranas Viškelis

Abstract

Japanese quince (*Chaenomeles japonica*) is one of the most underutilized plant species that have high nutrient value and a positive impact on human health. Due to the high content of bio-compounds, such as phenols, vitamin C, triterpenes, fibers, essential amino acids, and microelements, the fruits, leaves, and seeds are excellent raw materials for functional food production. In addition, their biochemical composition and anti-inflammatory, anticancer, and antibacterial properties expanded their uses in the pharmaceutical field. Moreover, it was demonstrated that quince waste after industrial processing is still valuable and suitable for remanufacturing and developing innovative high value-added products, which can provide economic and ecological benefits. This chapter presents the biochemical composition and possible application of *C. japonica* cultivars Rasa, Darius, and Rondo. The optimization of processing and extraction parameters was evaluated to increase the extraction efficiency of biologically active compounds and to reduce the extraction time and cost of electricity and environmentally harmful solvents. Moreover, the detailed nutritional and pharmacological value of Japanese quince can help for more selective plant organs application. Our study revealed that cultivars Rasa, Darius, and Rondo are very valuable with many new options for utilization, including food, cosmetic, and pharmaceutical industries.

Keywords: Japanese quince, fruit, leafs, seeds, phenols, triterpenes, by-products, oil, antioxidant activity, anticancer, antibacterial

1. Introduction

The *Chaenomeles* genus is one of the oldest cultivated plants from the *Rosaceae* family, a subgenus of *Maloideae*, phylogenetically very close to the genera *Cydonia*, *Pyrus*, and *Malus* [1–3].) *Chaenomeles japonica* named Japanese quince (JQ) is a dwarf shrub from East Asia (**Figure 1**), was used in Chinese medicine around 3000 years

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Figure 1.
Chaenomeles japonica *cultivar rondo growing in the garden of Babtai, Lithuania, on the left – in may, and on the right – in September. Photo I. Urbanavičiūtė.*

ago [3]. Among several species of the *Chaenomeles* genus, JQ is the most suitable to the North European climate. JQ propagation by seeds is undesirable and causes high genetic variation in morphological and quality characteristics. JQ breeding program funded by the European Research Project "Japanese quince (*C. japonica*)*–a new European fruit crop for the production of juice, flavor, and fiber* (FAIR5-CT97-3894, 1998-2001)," resulted in new improved JQ cultivars. Three needleless cultivars have been released and registered in the European Union after passing the DUS (distinctness, uniformity, and stability) tests. Cultivars Darius, Rondo, and Rasa were released in the Baltic region with the quality and desirable characteristics of fruits and products [4, 5]. JQ is a cross-pollinated crop, and a minimum of three different cultivars should be planted in the same garden to ensure a highly successful fruit set and yield [6]. Nowadays, cultivation and scientific research on JQ are mostly concentrated in the North part of Europe, in Poland, Finland, Sweden, Latvia, and Lithuania.

Properties of JQ fruit such as firmness, and a high amount of juice and fibers characterized them as a potential crop in horticulture [7–9]. The knowledge about the biochemical characteristics of the JQ fruit recently increased, and their bioactive compound quantity and composition highlighted them as promising raw materials with a positive influence on human health [3, 10, 11]. The abundance of compounds with strong antioxidant properties, such as phenols, triterpenes, and vitamin C, can contribute to chronic disease prevention [2, 12–14]. Phenols as natural antioxidants are well-known with a positive effect on human health as anti-inflammatory [15], neuroprotective [16], anticancer [17], antiviral [18], improving cardiovascular activity [19], antidiabetic [20], and blood cholesterol and triglyceride-lowering [21, 22].

JQ fruits are also rich in fibers, as well organic acids, mainly malic and citric acids at levels of 10 times that of apple fruits [8, 23]. However, due to their characteristics, such as firmness, sourness, and astringency they are not suitable for fresh consumption, so mostly used for the production of juices, jams, syrups, alcoholic and non-alcoholic beverages, etc. [7, 24–27]. If JQ fruits are not immediately processed or to be frozen, and appropriate storage should be considered to ensure minimum losses in quality parameters and biochemical composition. Several studies have shown that JQ fruits could be stored without significant losses in quality under a controlled atmosphere system or at 1°C and 85% relative humidity [9, 28]. Moreover, an unavoidable big amount of waste is generated during processing, mostly consisting of seeds [7, 29]. Even so, recent studies showed that seeds left after industrial manufacturing can be used for oil recovery, mucilage extraction, and as a source of organic acids, polyphenols, triterpenes, and microelements [29–32]. Another residual, such as pomace, also is suitable for secondary uses, for example, pectin and fiber extraction [33, 34].

Significant amounts of biologically active compounds, including phenols and triterpenes, were detected also in JQ leaves [11, 35, 36]. Since the branches of the wild JQ plant are mostly prickly, collecting their leaves is a hard and complicated process.

The new cultivars Darius, Rondo, and Rasa are needleless, which can enrich the use-value of the whole plant. In order to expand the utilization of these new cultivars, it was necessary to determine their biochemical value. Moreover, the optimization of process parameters will help increase the extraction efficiency, as high temperature, oxidation, and other decomposition processes have negative effects on sensitive biocompounds. Adjusting extraction parameters such as polarity of the solvent and its ratio to water, time, temperature, additional energy source (ultrasound, microwave, etc.), have a crucial impact on the efficiency of phenols extraction [37–40]. This chapter aims to determine the impact of genotype and extraction methods on quince *C. japonica* biochemical composition and to evaluate possible applications of three cultivars Rasa, Darius, and Rondo cultivated in Lithuania.

2. Materials and methods

2.1 Chemicals

All the solvents, reagents, and standards were used of analytical grade. The following substances were used in the study: Ethanol 96% (*v*/*v*) (AB Strumbras, Kaunas, Lithuania), procyanidin C1, procyanidin B2, quercetin, hyperoside, avicularin, quercitrin, kaempferol 3-O-glucoside, luteolin 7-O-glucoside, phloridzin, formic acid, acetonitrile, (+)-catechin, (−)-epicatechin, rutin, isoquercitrin, chlorogenic acid, p-coumaric acid, caffeic acid, hydrochloric acid (Sigma-Aldrich, Steinheim, Germany). Where used purified de-ionized water was prepared with the Milli–Q® (Millipore, Bedford, MA, USA) water purification system.

2.2 Plant material

Fruits, leaves of the Japanese quince cultivars, Darius, Rondo, and Rasa were collected from test gardens belonging to the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai (55° 60′ N, 23° 48′ E). The fruits were harvested from late August to mid-September, depending on the technical maturity, for each cultivar randomly from five shrubs in September. Leaves were collected for each cultivar randomly at different seasons, spring (May), summer (August), and autumn (October). Fruits and leaves were frozen (at −40°C) in a freezer with air circulation, and then lyophilized with a sublimator Zirbus 3 × 4 × 5 (ZIRBUS technology GmbH, Bad Grund, Germany), at a pressure of 0.01 mbar (temperature of condenser −85°C) for 24 h. The lyophilized fruits, leaves were grounded to a fine powder with a knife mill GM (Retsch GmbH, Haan, Germany). The seeds were removed and dried in a convection dryer for 24 hours at 60°C, after seeds were crushed and divided into five

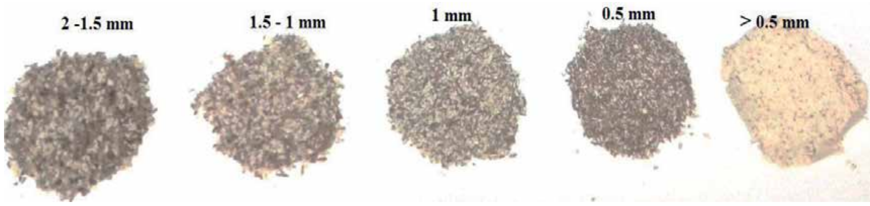

Figure 2.
Different fractions of grounded Japanese quince seeds.

fractions according to seeds particle size: 2–1.5 mm, 1.5 mm–1 mm, 1 mm, 0.5 mm, > 0.5 mm (**Figure 2**).

2.3 Phenolic compounds extraction optimization

First, the influence of different solvents on the extraction efficiency was initially determined, 0.5 g of the seeds or leaves powder with 10 mL solvent in different concentrations (ratio 1:20, w/v) were mixed and left at room temperature 22°C in the dark for 24 h. After extraction, the samples were centrifuged and filtered through a Whatman filter paper. For phenolic compounds extraction efficiency analysis, three solvents (ethanol, methanol, and acetone) and three concentrations (100%, 70%, and 50%) were chosen. After selecting the most efficient solvent system, ultrasound extraction (UE) of phenolic compounds was carried out with Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany). Response surface methodology (RSM) was used to examine the influence of UE processing variables on phenols extraction, and three parameters were selected for optimization—temperature, extraction time, and ultrasonic power regarding methodology described by Urbanavičiūte et al. [10].

2.4 Determination of dry matter (DM), total soluble solids (TSS), firmness, sugar, and fiber content

Dry matter content was determined after forced air convention drying at 105°C to a constant weight. The total soluble solids were determined using a digital refractometer (ATAGO PR-32, Atago Co., Ltd., Tokyo, Japan). Fruit firmness was determined by the texture analyzer TA.XTPlus (Stable Micro Systems, UK) using the P/2 probe. Total sugar content was determined using the Bertrand method. Fiber content was determined using.

Fiber analyzer Ankom 2000 (Ankom Technologies, Madison NY, USA) and expressed as a percentage.

2.5 Determination of biochemical composition, antioxidant and antimicrobial activities

Spectrophotometric measurements were performed using a Genesys-10 UV/Vis spectrophotometer (Thermo Spectronic, Rochester, NY, USA). The total amount of phenols was assessed using Folin–Ciocalteu method according [41] at 765 nm wavelength, and was expressed in mg 100 g^{-1}, the equivalent of gallic acid. The antiradical activity was determined using two methods—the DPPH (515 nm) method described by Brand-Williams et al. [42], and ABTS (734 nm) assay was applied according to the methodology described by Re et al. [43]. Antiradical activities were expressed in μmol of Trolox equivalents in g^{-1} dry extracts. Total proanthocyanidins (640 nm) were determined using the technique described by Heil et al. [44]. According to the methodology described by Liaudanskas et al. [45], the high-performance liquid chromatography (HPLC) method was used for the determination of phenolic compounds. The antimicrobial activity against three Gram-positive and three Gram-negative bacteria was evaluated by the agar well diffusion method described by Urbanavičiūte et al. [10].

2.6 Statistical analysis

Data collected are expressed as mean ± standard deviation. The results of three replicates were presented and univariate analysis of variance (ANOVA) was applied. Tukey's HSD (honest significant difference test) was used for multiple mean comparisons. Statistical significant differences were considered at $p < 0,05$. The statistical analysis was performed using Statistica 10 software (StatSoft, Inc., Tulsa, OK, USA).

3. Results and discussion

3.1 Japanese quince fruit characteristics and biochemical composition

The cultivars Darius, Rondo, and Rasa are characterized by the absence of needles, their leaves are resistant to blemishes, the whole plant is resistant to frost and the fruit to rot. The fruits finish ripening evenly in early September. Rondo is characterized by large shrubs with orange flowers. Shrubs of Darius are small, with several main branches, blooming in orange-dense inflorescences. Shrubs of the Rasa are of medium size, the flowers are pink and not dense. The characteristics of the fruit listed in **Table 1**, may help for better adoption of quince processing techniques, and for farmers to select cultivars with desired properties.

Significant differences were found between the three cultivars in fruit size and their number of seeds. Rasa had the largest fruit and highest number of ripening seeds, almost twice as much as others (**Table 1**). The skin and flesh firmness were not significantly different between cultivars, but the fruits of the Rondo were slightly softer. Moreover, Rasa had the lowest amount of sugars and sucrose content.

Our previous studies have shown that the biochemical composition significantly varied between genotypes. Rasa had the highest amount of rutin and lowest vitamin C, while Rondo and Darius had higher catechin and chlorogenic acid, respectively [10]. Genotypic differences in the biochemical composition of JQ juices were also reported [46].

Moreover, it was found that different solvents and their ratio to water had a significant impact on phenols extraction efficiency using the simple maceration method from freeze-dried JQ fruit powder [10]. Besides, using ultrasound power decreased extraction time while increasing phenol yield from JQ fruit by 14.5% [10]. Five phenols (−)-epicatechin, (+)-catechin, chlorogenic acid, rutin, and isoquercitrin were identified in all the three cultivars Rasa, Darius, and Rondo, using the HPLC method [10]. The predominant phenols in all cultivars were flavan-3-ols (catechin and epicatechin), which account for around 94% of the total amount [10]. It was also reported that all cultivars have accumulated high levels of proanthocyanidins [10]. Similar results were demonstrated where 11 phenols were determined with a distribution of procyanidins (57.8%), (−)-epicatechin (33%), and chlorogenic acid (4.4%) [47]. In addition, 24 phenols were identified in the study of five *Chaenomeles* species including

Characteristic	*C. japonica*		
	Darius	**Rondo**	**Rasa**
Average yield, kg/shrub	9 ± 0.6^{a}	10 ± 0.5^{a}	8 ± 0.8^{a}
Fruit weight, g	46.2 ± 8.3^{a}	75.7 ± 13.1^{b}	97.7 ± 21.2^{b}
Diameter of fruit, mm	44.4 ± 2.1^{a}	53.0 ± 2.7^{b}	58.7 ± 3.4^{b}
Seeds weight, g	2.5 ± 0.8^{a}	3.4 ± 0.9^{a}	7.8 ± 1.9^{b}
Number of seeds	59.2 ± 19.9^{a}	62.0 ± 15.6^{a}	127.2 ± 36.3^{b}
Flesh thickness, mm	11.6 ± 1.3^{a}	13.5 ± 0.6^{a}	12.0 ± 1.9^{a}
Diameter of the core, mm	22.6 ± 1.7^{a}	24.8 ± 2.9^{a}	39.6 ± 5.0^{b}
Dry Matter of fruit, %	9.2 ± 0.1^{a}	9.6 ± 02^{a}	9.2 ± 0.1^{a}
Dry Matter of leaves, %	46.1 ± 0.1^{a}	43.1 ± 0.1^{a}	43.7 ± 0.1^{a}
Dry Matter of seeds, %	60.2 ± 0.1^{a}	53.8 ± 0.1^{b}	54.1 ± 0.3^{b}
Skin firmness, N cm^{-2}	329.3 ± 31.1^{a}	315.5 ± 14.3^{a}	324.3 ± 38.6^{a}
Flesh firmness, N cm^{-2}	180.6 ± 13.3^{a}	150.8 ± 12.2^{a}	171.2 ± 20.1^{a}
Total sugar content, %	3.45 ± 0.02^{a}	4.0 ± 0.34^{a}	2.69 ± 0.22^{b}
Sucrose, %	1.08 ± 0.07^{a}	1.05 ± 0.04^{a}	0.77 ± 0.03^{b}
Fiber content, %	19.3 ± 2.3^{a}	17.9 ± 3.1^{a}	21.1 ± 2.1^{a}
Dry soluble solids, %	9.9 ± 0.2^{a}	9.4 ± 0.3^{a}	8.1 ± 0.1^{b}

Table 1.
The characteristics of Chaenomeles japonica cultivars. Different letters (a, b, and c) in the same row indicate significant differences between samples ($p < 0.05$).

JQ, and they reported variations in their antioxidant activity and the quantity of compounds, such as chlorogenic acid, catechin, epicatechin, procyanidins B1 and B2 [2].

Moreover, phenols' quantity and their biological activity expand the uses of JQ fruit as a promising substitute for chemical preservatives in the food and cosmetic industry due to demonstrated antibacterial activity [48]. The cultivars Rasa, Darius, and Rondo showed antimicrobial activity against three Gram-positive and three Gram-negative bacteria, in a concentration-dependent manner [10]. However, they have not shown antifungal activity against *Candida albicans* yeast [10].

Nevertheless, it was mentioned that phenol-rich extracts obtained from JQ fruit could replace aggressive synthetic drugs with side effects through demonstrated anticancer activity [13, 14, 49, 50]. Also, JQ fruit extracts can be used as an antioxidant drug for the prevention of diseases caused by inflammation or oxidative stress [47, 51]. Moreover, JQ phenols-rich extracts as modulators of carbohydrates metabolism showed a promising hypoglycemic effect and decreased intracellular ROS accumulation [52]. Despite all these health benefits and pharmaceutical properties of JQ fruits, till now it is mostly used for the food industry. Moreover, exploiting significant unavoidable amounts of waste left after industrial processing can offer potential economic and ecological benefits.

3.2 Japanese quince by-products biochemical composition and possible utilizations

Due to the firmness, sourness, and astringency of JQ fruit, they are not suitable for fresh consumption, mostly used for syrup and candied production. The JQ

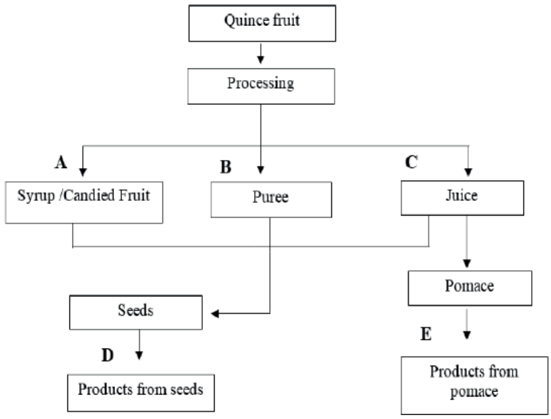

Figure 3.
General scheme of quince processing.

by-products after processing were received from Puree/juice manufacturer, collected after three different processing methods—syrup and candied fruits, puree, and juice (**Figure 3**). The seeds separation and preparation for analysis were performed following methods described by Urbanavičiūtė et al. [29]. The amount of by-products after JQ fruits processing depended on the manufacturing technology, ranged from 20–40%, and consisted mostly of pomace and seeds [29].

Most of the by-products remain after juicing (**Figure 2C**), range 40–60% of fresh fruit weight [7, 29]. The pomace left after juicing showed a significant amount of phenols, even 13 times more than juice (**Table 2**). Juicing is a rapid process that releases only a fraction of the biologically active compounds from the fruit, so most of them remain in the pomace. The JQ pomace also showed strong antioxidant activity and high amounts of proanthocyanidins.

Numerous studies have reported an important role of phenols in inhibiting the growth of microorganisms [53–55]. Our results showed that JQ pomace extracts had antimicrobial activity against three Gram-positive and three Gram-negative bacteria in a concentration-dependent manner, where the strongest inhibition effect was obtained using 5% concentration pomace extracts (**Figure 4**).

The greatest inhibitory effect of pomace extracts was found on the gram-positive *Enterococcus faecalis* (ATCC 29212) strain. In general, this strain was most sensitive for treatment with JQ pomace extracts, using all concentrations (**Figure 4**). Moreover, dried pomace is a promising source of pectin and an excellent raw material for fiber-rich products [33, 34].

Parameters	Juice	Pomace
Total Phenols, mg 100 g^{-1}	488 ± 11^{b}	6645.6 ± 211^{a}
RSA (DPPH), μmol TE 100 g^{-1}	14.7 ± 1.1^{b}	152.2 ± 13^{a}
RSA (ABTS), μmol TE 100 g^{-1}	69.7 ± 2.1^{b}	938.2 ± 33^{a}
Content of proanthocyanidins, mg 100 g^{-1}	218.5 ± 7.1^{b}	1368.7 ± 55.4^{a}

Table 2.
The total phenols, proanthocyanidins, and RSA - Radical scavenging activity DPPH, and ABTS of JQ juice and pomace. Different letters (a, b, and c) in the same row indicate significant differences between samples ($p < 0.05$).

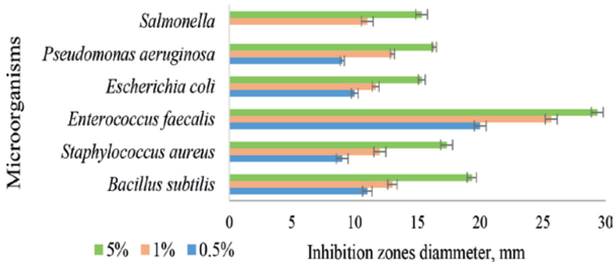

Figure 4.
Antibacterial activity of Chaenomeles japonica *pomace extract.*

In the food industry, JQ seeds are mostly discarded as waste, while new utilization can reduce losses for producers, especially when the seeds accounted for more than 30% of the total waste [29]. Moreover, it was reported that seeds from different species of the *Chaenomeles* genus including JQ are potential phenols sources, especially proanthocyanidins, triterpenes, essential amino acids, K, and microelements such as Fe, Cu, Zn, and Mn [56].

Before determining the content and diversity of phenolic compounds in Rasa, Darius, and Rondo seeds, the impact of different extraction parameters on their efficiency was performed. To determine the most efficient solvent system for phenols extraction, three solvents in different concentrations were used. After maceration with methanol, the highest content of phenolic compounds was extracted with a solvent concentration of 70% (**Figure 5**).

Pure ethanol has been shown to be the wrong choice for extracting phenols from quince seeds, and no significant differences between fractions were found (**Figure 6**). Extraction efficiency with ethanol was the highest when the ratio of water/alcohol was 1:1 (**Figure 6**).

The extraction of crushed JQ seeds with pure acetone also was not appropriate, while 70% concentration showed the highest amount of extracted phenols, five and six times more than using ethanol and methanol, respectively (**Figure 7**).

Our results showed that solvent type and its concentration had significant effects on phenol extraction efficiency from JQ seeds. Moreover, our results agreed with previously reported studies that dual solvent systems are more efficient for phenols extraction than with pure solvents [37, 57, 58]. Besides, phenols dissolved differently

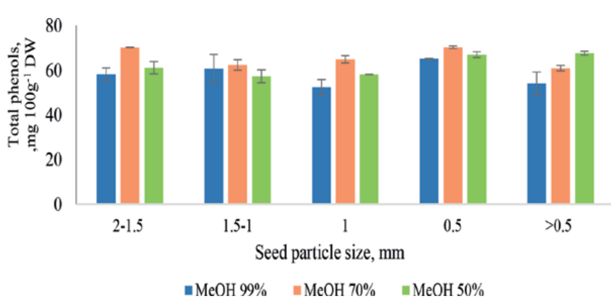

Figure 5.
Impact of methanol concentration and seeds particle size on the total content of total phenols compounds in quince seeds.

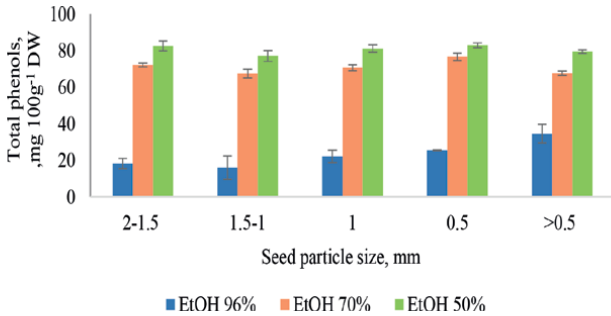

Figure 6.
Impact of ethanol concentration and seeds particle size on the total content of phenolic compounds in quince seeds.

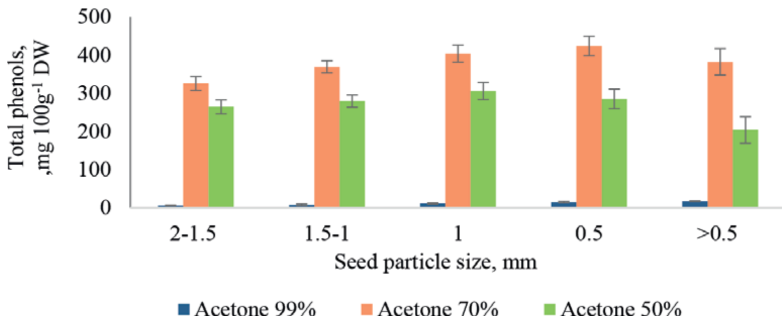

Figure 7.
Impact of acetone concentration and seeds particle size on the total content of phenolic compounds in quince seeds.

regarding the solvent system, for example, it was reported that methanol is the best for extraction of catechin, epicatechin, and epigallocatechin, then 70% acetone provides the highest content of proanthocyanidins and total phenolic compounds, while the highest content of gallic acid was extracted with 75% ethanol [59].

The most efficient solvent system (70% acetone) was used for ultrasound extraction of phenolic compounds with an ultrasonic bath. Response surface methodology (RSM) was used to examine the influence of ultrasound processing variables on phenols extraction, and three parameters were selected for optimization—temperature (15–50°C), extraction time (15 min–60 min), and ultrasonic power (48 W–480 W) following the methodology described by Urbanavičiūte et al. [10].

Using RSM, the highest amount of phenols was obtained when the samples were extracted for 60 min, at 50°C with 480 W ultrasonic power (**Figure 8**).

The temperature had the highest impact on phenols extraction, the efficiency increased around 80%, and time was reduced to 23 hours in comparison with simple maceration. Such a strong effect may have been due to the ultrasound power ability to destroy complexes of mucus with phenols, which makes their extraction more difficult from seeds [60]. Using the previously determined optimal parameters for phenol extraction, qualitative and quantitative analysis for Rasa, Rondo, and Darius seeds extracts were performed. The highest total phenolic compounds and antioxidant activity in seed extracts were found in Rondo and the lowest in Darius (**Table 3**).

Twelve phenolic compounds in Rasa, Rondo, and Darius seeds extracts using high-performance liquid chromatography (HPLC) were identified (**Table 4**).

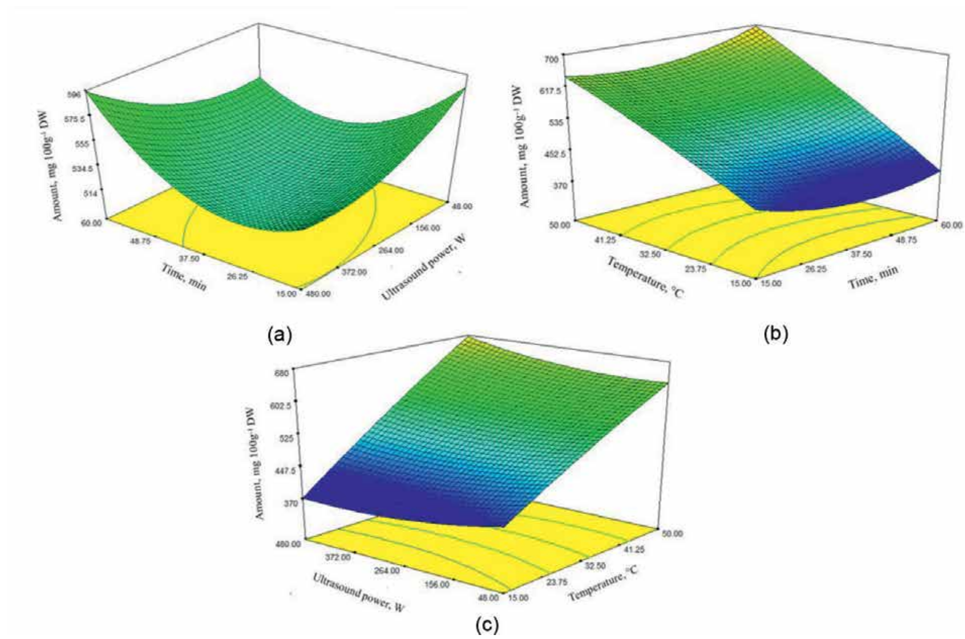

Figure 8.
The influence of (a) – Time and ultrasound power, (b) – Temperature and time, (c) – Ultrasound power and temperature on extraction efficiency of phenols from JQ seeds.

Cultivars	Total phenols mg/100 g	RSA (DPPH) µmol TE g^{-1}	RSA (ABTS) µmol TE g^{-1}
Rasa	427.6 ± 13.2^{b}	20.4 ± 1.4^{b}	41 ± 2.1^{b}
Rondo	499.6 ± 14.5^{a}	26.4 ± 1.2^{a}	50.9 ± 2.4^{a}
Darius	283.5 ± 9.4^{c}	14.7 ± 0.8^{c}	27.7 ± 1.1^{c}

Table 3.
The antioxidant activity, and total phenols of JQ seeds cultivars cultivated in Lithuania. Different letters (a, b, and c) in the same column indicate significant differences between samples ($p < 0.05$).

The predominant compounds were procyanidin B2, procyanidin C1, and chlorogenic acid, their amounts varied between genotypes. Rasa and Darius had a higher amount of chlorogenic acid than Rondo, while Rasa with Rondo had a higher amount of procyanidins than Darius (**Table 4**).

Phenols quantitative analysis using HPLC was applied for seeds left after different industrial processing. Seeds, which were left after puree production had significantly the highest amount of phenols (**Table 5**). From 12 detected phenols, procyanidin B2 and C1, and (−)-epicatechin were predominant. Epicatechin levels could be explained as a consequence of the production method, as JQ fruit (reach in epicatechin) was treated without removing the seeds.

3.3 Japanese quince leaves biochemical composition and possible utilization

To optimize the extraction of phenolic compounds from JQ leaves, three solvents and five concentrations were selected. The lowest concentration of phenolic compounds in quince leaves was determined by the maceration with pure solvents (**Figure 9**). The

Compound, μg g^{-1} DW	*Chaenomeles japonica*		
	Darius	**Rondo**	**Rasa**
Rutin	6.0 ± 0.3^a	6.7 ± 0.4^a	6.5 ± 0.3^a
(+)-Catechin	4.2 ± 0.2^b	4.2 ± 0.1^b	7.5 ± 0.5^a
Chlorogenic acid	20.7 ± 1.5^b	17.2 ± 1.3^a	24.2 ± 2.3^b
Caffeic acid	9.7 ± 0.7^a	9.4 ± 0.5^a	9.3 ± 0.6^a
Syringic acid	3.9 ± 0.2^a	3.9 ± 0.2^a	4.3 ± 0.5^a
Hyperoside	5.3 ± 0.5^a	5.1 ± 0.4^a	ND
Procyanidin B2	22.5 ± 1.5^b	41.5 ± 3.5^a	45.0 ± 1.4^a
Procyanidin C1	29.1 ± 2.1^b	41.0 ± 3.2^a	37.4 ± 1.8^c
(−)-Epicatechin	14.1 ± 0.7^b	19.8 ± 0.5^a	16.7 ± 1.3^c
Isoquercitrin	10.4 ± 1.5^a	9.7 ± 1.2^a	9.7 ± 1.5^a
Quercitrin	4.4 ± 0.2^a	4.6 ± 0.2^a	4.6 ± 0.1^a
p-Coumaric acid	6.4 ± 0.5^a	5.8 ± 0.6^a	6.8 ± 0.7^a
Total	136.8 ± 9.7^b	169.1 ± 4.5^a	172.1 ± 3.7^a

Table 4.
The quantitative composition (HPLC) of phenolic compound in JQ seeds. Different letters (a, b, and c) in the same row indicate significant differences between samples ($p < 0.05$).

Compound, μg g^{-1} DW	Seeds left after different JQ processing		
	After juicing	**After puree**	**After syrup**
Rutin	5.6 ± 0.3^a	8.4 ± 0.4^a	6.6 ± 0.3^a
(+)-Catechin	13.9 ± 0.2^a	4.2 ± 0.1^a	7.5 ± 0.5^b
Chlorogenic acid	20.7 ± 1.5^a	25.0 ± 1.3^b	8.8 ± 2.3^b
Caffeic acid	9.1 ± 0.7^a	10.3 ± 0.5^a	9.6 ± 0.6^a
Syringic acid	5.7 ± 0.2^a	8.2 ± 0.2^b	4.3 ± 0.5^a
Hyperoside	5.0 ± 0.5^a	8.2 ± 0.4^b	ND
Procyanidin B2	24.6 ± 1.5^a	790.9 ± 43.5^b	62.0 ± 1.4^c
Procyanidin C1	27.6 ± 2.1^a	317.6 ± 23.2^b	65.8 ± 4.8^c
(−)-Epicatechin	14.4 ± 0.7^a	709.8 ± 36.5^b	43.3 ± 2.3^c
Isoquercitrin	9.7 ± 1.5^a	14.3 ± 1.2^b	10.0 ± 1.5^a
Quercitrin	4.6 ± 0.2^a	7.6 ± 0.2^b	4.1 ± 0.1^a
p-Coumaric acid	8.0 ± 0.5^a	21.9 ± 0.6^a	6.8 ± 0.7^a
Total	146.2 ± 9.7^a	2041.4 ± 84.5^b	250.7 ± 13.7^c

Table 5.
Impact of JQ processing on the quantitative composition of phenols in seeds. Different letters (a, b, and c) in the same row indicate statistically significant differences between the individual compounds ($p < 0.05$).

proportion of water in solvents had no significant effect on the extraction efficiency. Significant higher content of phenolic compounds was obtained by maceration of quince leaves with 50% acetone (**Figure 9**).

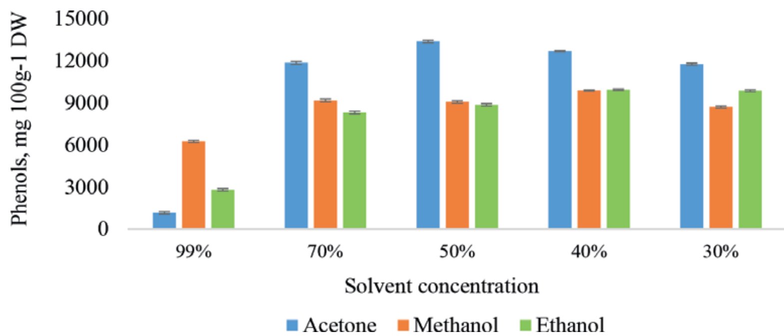

Figure 9.
Impact of solvents and their concentration on the total content of phenolic compounds in quince leaf.

The following ranges of independent variables were chosen for the extraction of phenolic compounds from quince leaves in the ultrasonic bath—ultrasonic power 48 W–480 W, temperature 30–60°, and time 20–60 minutes (**Figure 10**).

Based on the developed model, the obtained results showed that the effect of all the three variables was not significant and had no effect on the extraction efficiency, except that the process was shortened by 23 hours compared to simple maceration. The highest extraction yield of phenolic compounds was obtained when the samples were extracted with 480 W ultrasonic power for 60 min, at 60°C temperature (**Figure 10**).

Our previous studies revealed that leaves of Rasa, Darius, and Rondo cultivated in both Latvia and Lithuania were rich in biologically active compounds [11, 35]. The highest amount of phenols were found in Darius and the lowest in Rondo leaves. The identified compounds belong to three main groups—hydroxycinnamic acid derivatives, flavonols, and flavan-3-ols. The chlorogenic acid was the most common

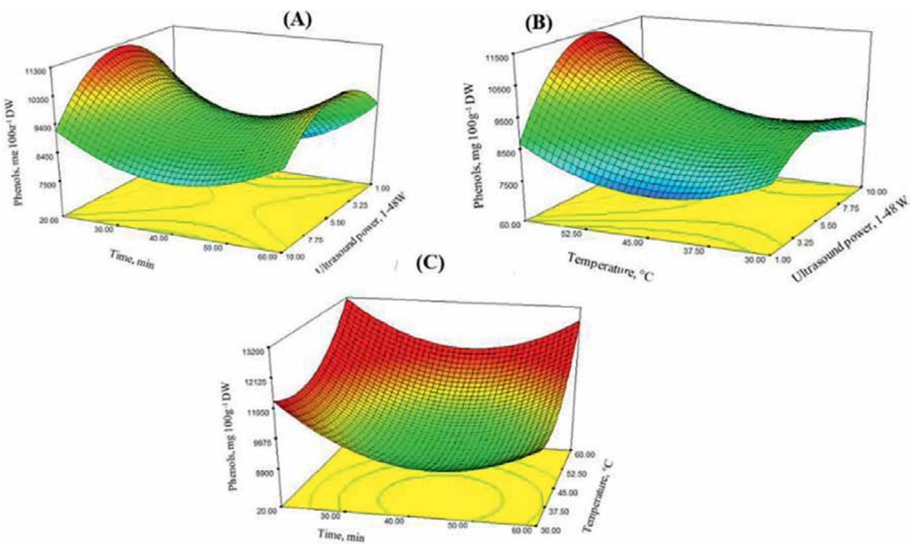

Figure 10.
The influence of (A) – time and ultrasound power, (B) – temperature and ultrasound power, (C) – time and temperature on extraction efficiency of phenols from JQ leaves.

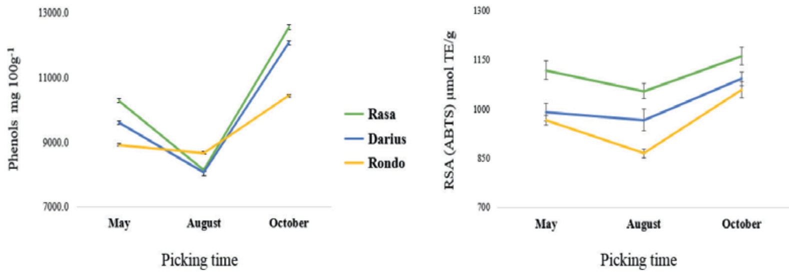

Figure 11.
Effect of cultivar and climate conditions on the accumulation of phenolic compounds and radical scavenging activity in quince leaves.

in leaves of all three cultivars, which accounts for about 80% of the total phenolic compound [11, 35].

The environmental conditions in different seasons also significantly influenced the total amount of phenolic compounds and antiradical activity in leaves. They accumulate higher levels of phenolic compounds in spring and autumn in adverse weather conditions such as frosts and temperature fluctuations, and less levels in summer under more favorable conditions (**Figure 11**). To summarize, the development of new needleless cultivars Rasa, Darius, and Rondo has facilitated collecting promising and long-undervalued raw materials, such as *Chaenomeles japonica* leaves. Moreover, the maximum phenols content and the best time to collect was at a very convenient time after harvesting fruits without affecting the quality or yield parameters.

Due to the significant amount of phenols in JQ leaves, the antimicrobial activity against three Gram-positive and three Gram-negative bacteria, and one yeast strain, in Rasa, Darius, and Rondo was identified. JQ leaves have shown antibacterial activity against microorganisms, the strongest inhibition effect was obtained for the *Enterococcus faecalis* (ATCC 29212) strain using all cultivars extracts (**Table 6**). Extracts of Rasa leaves had a stronger inhibitory effect on *Bacillus subtilis* and *Pseudomonas aeruginosa*, while Rondo inhibited *Staphylococcus aureus* stronger. However, extracts have not shown antifungal activity against *Candida albicans* (ATCC 10231) yeast strain.

A previous study has been reported that *C. japonica* leaves extracts with similar predominant phenolic compounds had antibacterial activity against the same four strains, however, antifungal activity against yeast strain *C. albicans* (ATCC 10231) was demonstrated [48].

Although no significant differences were detected between cultivars in fiber content, leaves had a higher amount than their fruits (**Figure 12**).

3.4 Japanese quince applications and health benefits

Recent research on *Chaenomeles japonica* and cultivars Rasa, Darius, and Rondo have extended their range of applications, which are summarized in **Table 7**. Fruits of these cultivars are promising for pharmaceutical industries due to their biochemical composition, anticancer, antibacterial, anti-inflammation properties, and hypoglycaemic effect.

JQ fruit extracts rich in proanthocyanidins showed proapoptotic activity in Caco-2 cells [13]. Their extracts rich in flavanols had an antiproliferative effect against various cancer cells, decreased their invasiveness by regulating several genes involved in apoptosis, angiogenesis, and metastasis [14, 49]. Their extracts also demonstrated

	Microorganism	Extract Conc., %	C. japonica		
			Rasa	Darius	Rondo
			Inhibition zone size, mm		
Gram-positive	*Bacillus subtilis* (ATCC 6633)	0.5	10.0 ± 0.0[a]	11.3 ± 0.5[a]	11.0 ± 0.0[a]
		1	15.0 ± 0.0[a]	15.3 ± 0.5[a]	12.0 ± 0.0[b]
		5	18.3 ± 0.5[a]	17.0 ± 0.0[a]	14.7 ± 0.5[b]
	Enterococcus faecalis (ATCC 29212)	0.5	17.0 ± 0.0[b]	17.3 ± 0.5[b]	20.0 ± 0.0[a]
		1	20.0 ± 0.0[b]	20.3 ± 0.5[b]	23.0 ± 0.0[a]
		5	25.3 ± 0.5[a]	24.0 ± 0.0[a]	25.7 ± 0.5[a]
	Staphylococcus aureus (ATCC 25923)	0.5	9.0 ± 0.0[a]	9.3 ± 0.5[a]	0.0
		1	14.0 ± 0.0[a]	10.3 ± 0.5[b]	11.0 ± 0.0[b]
		5	16.3 ± 0.5[b]	16.0 ± 0.0[b]	17.7 ± 0.5[a]
Gram-negative	*Escherichia coli* (25922 ATCC)	0.5	9.0 ± 0.0[a]	9.3 ± 0.5[a]	10.0 ± 0.0[a]
		1	10.0 ± 0.0	10.3 ± 0.5	11.0 ± 0.0
		5	15.3 ± 0.5	15.0 ± 0.0	15.6 ± 0.5
	Pseudomonas aeruginosa (27853 ATCC)	0.5	0.0	0.0	9.0 ± 0.0[a]
		1	11.7 ± 0.8[a]	10.3 ± 0.5[a]	11.0 ± 0.0[a]
		5	16.3 ± 0.5[a]	15.0 ± 0.0[a]	12.7 ± 0.5[b]
	Salmonella Typhimurium (ATCC 14028)	0.5	9.0 ± 0.0[a]	0.0	0.0
		1	10.0 ± 0.0[a]	9.3 ± 0.5[a]	9.0 ± 0.0[a]
		5	14.3 ± 0.5[a]	15.0 ± 0.0[a]	12.7 ± 0.5[b]
	Candida albicans (ATCC 10231)	0.5	0	0	0
		1	0	0	0
		5	0	0	0

Table 6.
Antibacterial activity of Chaenomeles japonica cultivars leaves extracts. Different letters (a, b, and c) in the same row indicate statistically significant differences between the individual compounds ($p < 0.05$).

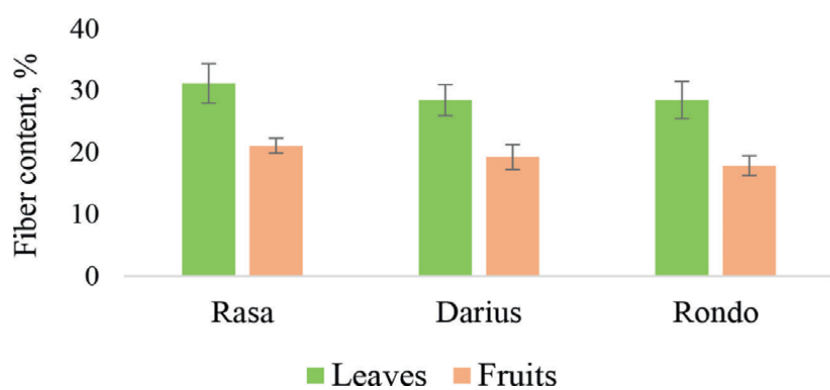

Figure 12.
The total fiber content in Japanese quince leaves and fruit.

Source	Bio compounds	Possible utilization	References
Fruits	Phenols, triterpenes	Functional food, Cosmetic	[2, 10, 26]
		Pharmaceutical (Anti-inflammation)	[47, 51]
		Pharmaceutical (Anticancer)	[14, 49, 50]
		Pharmaceutical (Antibacterial)	[10, 48]
		Pharmaceutical (hypoglycaemic)	[52]
	vitamin C	Functional food, cosmetic	[3, 7, 9]
	Fibers	Functional food	[8, 23]
By-Products			
Pomace	Phenols, fibers	Functional food, Cosmetic	(**Table 2**), [33]
		Pharmaceutical (Antibacterial)	(**Figure 4**), [48]
Seeds	Phenols	Functional food, Cosmetic	(**Tables 3–5**), [56]
	Tocopherols, carotenoids, squalene, phytosterols	Cosmetic	[29, 31, 61]
	Mucilage	Food safety	[62, 63]
		Pharmaceutical (wound healing)	[64–67]
		Cosmetic (emulsion stabilization)	[68]
Leaves	Phenols, triterpenes	Functional food, Cosmetic	[11, 35]
		Pharmaceutical (Anti-inflammation)	[69]
		Pharmaceutical (Anticancer)	[11, 70]
		Pharmaceutical (Antibacterial)	(**Table 6**)
	Fibers	Functional food	(**Figure 12**)

Table 7.
Promising utilisation of quince Chaenomeles japonica plant.

an inhibitory effect on the MMP-2 and MMP-9 enzymes activity and can be used in cancer chemoprevention [50]. Besides, they showed the ability to protect biological membrane lipids from oxidation, and usage of those extracts as an antioxidant drug can be a prevention for diseases caused by inflammation or oxidative stress [47, 51]. Moreover, the human cells of hepatoma HepG2 pretreated with JQ phenols-rich extracts as modulators of carbohydrates metabolism showed a promising hypoglycemic effect and decreased intracellular ROS accumulation [52].

Due to the high content of bio-compounds with positive effects on human health, fruits, leaves, and by-products of cultivars Rasa, Darius, and Rondo are excellent raw materials for functional food, which allows increasing antioxidants in final products (**Table 7**). Moreover, extracts from fruit and their pomace, leaves, seeds, could be used as an antibacterial agent and a substitute for chemical preservatives in both the food and cosmetic industry. The importance of antioxidants for skincare is as essential as their internal consumption, so all JQ organs are a great source for cosmetic products as plant-based raw material.

Several studies showed that seeds of JQ are suitable for obtaining the oil rich in α-tocopherol, carotenoids, squalene, phytosterols, and phenols [29, 31, 61]. It was reported that seeds remaining after industrial manufacturing are still suitable for oil recovery. The yield and biochemical composition of oils depended on both the processing of seed pretreatment and the oil extraction method [29]. The biochemical composition varied between cultivars, especially the profile of fatty acids and accumulation of bioactive compounds, while oil yield was affected by the extraction technique [71]. The lowest oil yield was obtained using the cold-press method from Rondo seeds, and the largest from Rasa using ultrasonic extraction [71]. Despite the fact that JQ seed oils contain strong antioxidants it is not recommendable for the food industry regarding a very high omega-6/omega-3 ratio [29, 61, 72]. However, JQ seed oil is well suited for skincare products regarding the high content of linoleic acid and perfect fitness of linoleic acid/oleic acid ratio [73]. In addition, studies have shown that quince seeds are suitable for other products, such as mucilage preparation, which have various biological activities and possible applications [32, 64]. Quince seed mucilage has been used successfully for wounds and toxin-damaged skin treatment [64–67]. Quince seed's ability to form edible films together with oregano or thyme essential oil can be used to ensure food safety, and extend the shelf life of food products [62, 63]. Ethanol extract from quince seeds protected skin from allergen-induced Th2-type inflammation and reduced the effects of atopic dermatitis [74]. In addition, quince seed can be used for skincare products as an excellent emulsifier and stabilizer [68].

The same as fruits and seeds of these cultivars, leaves are promising raw material due to the high amount of chlorogenic acid that has shown many biological properties, including antibacterial, antioxidant, anticarcinogenic activities, hypoglycaemic and hypolipidemic effects [75–77]. The compounds with strong biological activity such as triterpenes have been detected in these three cultivar leaves as well [35]. Among the four identified triterpenes, ursolic and oleanolic acid were predominant, whose functions for human health have been investigated including cardioprotective [78], strong immunomodulatory [79], suppress tumorigenesis [80], valuable antimetastatic agent [81], and prevention or alleviation of glycation-associated renal diseases [82]. Finally, other *C. japonica* species leaves extracts, and extracts from Rasa, Darius, and Rondo demonstrated anticancer and anti-inflammatory activities [11, 69, 70].

4. Conclusion

This work about *C. japonica* and new cultivars Rasa, Darius, and Rondo will expand the knowledge about their new possible application in the food, cosmetic and pharmaceutical industries. As well as the utilization of additional materials, such as waste (pomace and seeds) and leaves, could provide economic and ecological advantages.

Conflict of interest

The authors declare no conflict of interest.

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Fruits contain important vitamins, minerals, phytochemical compounds, and dietary fiber. The phytochemicals in fruit can reduce the risk of diseases such as cancer, heart disease, diabetes, and hypertension. Fruits play an important role in not only human health but also in the world economy. As the global population continues to grow, there is an urgent need to ensure all people have access to healthy foods, thus the fruit industry is integral to ensuring food security and sustainability. This book provides a comprehensive overview of the fruit industry and highlights new topics for future research.

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