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Advance in Citrus Postharvest Management: Diseases, Cold Storage and Quality Evaluation

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Abstract

Citrus is a fruit crop grown in different Mediterranean countries. Generally, harvested fruits are used for fresh consumption or are processed (mainly to produce juices). In this chapter, the authors discuss the state of art on citrus postharvest with a scientific approach, evaluating the current knowledge about the physiology and pathology of citrus fruits and the main causes of deterioration. In addition, the authors explain the main facilities for the cold storage of citrus fruit with particular reference to the rapid-cooling techniques and treatments needed prior to shipment of citrus fruits (refer shipment). In the last part of the chapter, the non-destructive methods for the quality evaluation are presented.

Keywords: citrus fruit, physiological disorders, postharvest treatments, non-destructive methods

1. Introduction

In 2015, the world citrus production was around 121 million tons, with about 24 million tons in the Mediterranean Region (primarily Spain, Egypt, Turkey, Italy, Morocco and Greece), mainly destined for the fresh fruit market [1]. In Italy, the areas of citrus cultivation are mostly in the Southern regions, characterized by a Mediterranean climate where oranges (*Citrus sinensis* (L.) Osbeck), clementines (*C. clementina* Hort. ex Tan.), mandarins (*C. reticulata* L.) and lemons (*C. limon* (L.) Burm.) are the most cultivated varieties. The Eastern coast of Sicily, in the foothills of the Etna volcano (**Figure 1**), has a long-standing tradition in pigmented oranges

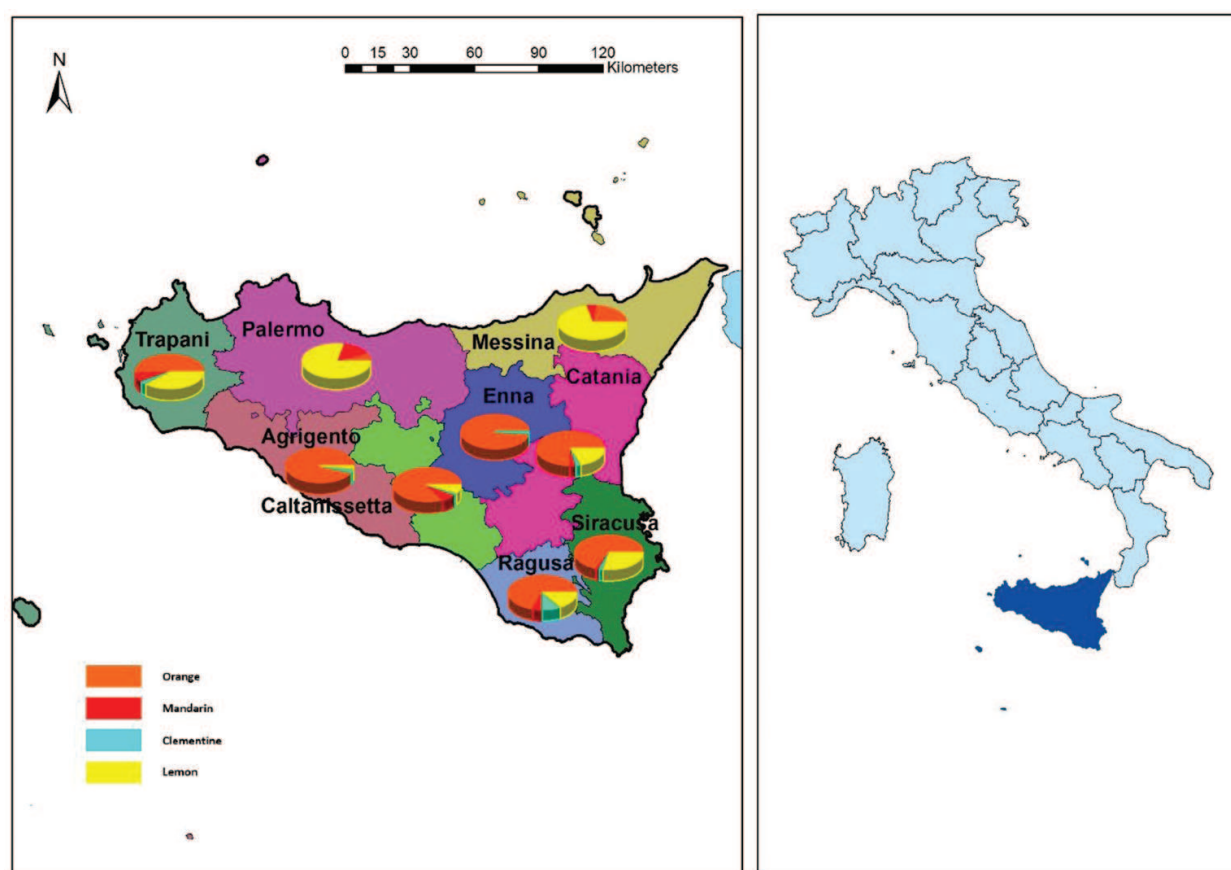


Figure 1. Citrus production is a fundamental sector of agriculture in Sicily, the largest of the Italian islands, located in the South of Italy.

growing, such as 'Tarocco', 'Moro' and 'Sanguinello'; along the coast is located the 90% of the Italian lemon production, based on three major cultivars: 'Femminello', 'Monachello' and 'Interdonato'.

Different species of citrus are cultivated all over the world, especially oranges, mandarins, lemons, grapefruits (*C. paradisi* Macf.), limes (*C. aurantifolia* Swing), pummelos (*C. maxima* (Burm.) Merrill), kumquats (*Fortunella* spp.), citrons (*C. medica* L.) and many other hybrids also commercially important.

During the last decade, citrus production increased significantly, which oriented the commercialization of the fruit towards distant markets. Therefore, increased consumers' concern regarding fungicides residues in the fruit has prompted the research into finding safer and effective alternative strategies as well as the development of adequate postharvest-handling technologies. New eco-friendly solutions would offer the opportunity to eliminate chemically synthesized fungicides currently used with the aim to preserve the natural qualities of fresh citrus fruits after harvest and to extend their shelf-life.

In addition, further development of non-hazardous postharvest management practises and technologies to preserve the quality of freshly harvested citrus fruit would also permit to obtain a high value-added product, with the possibility to reach distant markets.

2. Postharvest issues

Citrus fruits (genus *Citrus*; family *Rutaceae*) are specialized form of berry, named hesperidium, characterized by a juicy pulp made of vesicles within segments. The size range varies from nearly 2.25 cm for kumquats to more than 20 cm in diameter for pummelo. The shape is also variable going from spherical to oval in sweet oranges, oblate in grapefruit and mandarins, and oblong in lemons. Citrus rind (pericarp) comprises two regions: the exocarp or flavedo and the mesocarp or albedo. The flavedo is the outer, pigmented part containing oil glands; the albedo is the inner white section. The outermost constituent of the flavedo is the cuticle, a thin continuous polymer comprising waxes that plays an important role as the primary barrier between the fruit and its environment, before postharvest wax application. Moreover, it is mainly responsible for gas exchange and helps in maintaining high water content within tissues necessary for normal metabolism [2].

Citrus fruits are highly appreciated by consumers not only for their taste but also for the positive health values, representing a rich source of bioactive substances that include vitamin C, phenolic compounds such as hydroxycinnamic acids and flavonoids. The major classes of flavonoids in citrus are flavanones and anthocyanins, which are largely present in the pigmented varieties. The anthocyanins give an important sensory input and they have a very important role for their pharmacological and antioxidants properties. The advantage of fresh fruit consumption is due to some salutistic properties of these components, associated with the reduction of cardiovascular, stroke and cancer risks [3].

Citrus fruits are non-climateric, and thus different from climateric fruit (e.g. apple, pear, melon, tomato), are characterized by the lack of a ripening-associated increase in respiration and in ethylene production. They have a relatively long shelf-life compared to other tropical fruits; however, if not properly handled and stored, the fruit would become unsuitable for marketing. The perishability of fruits is generally proportional to their respiration rate and energy released as heat affects postharvest technology considerations such as estimations of refrigeration and ventilation requirements. Transpiration is a physical process characterized by the evaporation of water from fruit tissues, having as consequence fruits deterioration because of the loss in appearance (wilting and shrivelling), textural (softening) and nutritional quality [2]. Transpiration rate is influenced by rind injuries, maturity stage and environmental factors, such as temperature, relative humidity and air movement. The application of surface coatings and the manipulation of the storage environment (low temperatures, high relative humidity levels and control of air circulation) allow the management of the process. Diseases, physiological disorders, fruit senescence and physical damages represent the major causes of postharvest losses. Postharvest citrus decay is the most severe cause of wastage and quality deterioration since it renders fresh fruit unsuitable for consumption, causing consequently heavy economic losses. Losses can reach 30% of the total production and 50% in less developed countries. Physiological disorders and fruit senescence depend on inadequate temperatures used for citrus fruit storage, high rate of respiration and transpiration, and the stress caused by harvesting and postharvest handling. Rind injuries and impact bruising are the major contributors to fruit deterioration, since they accelerate water loss, stimulate higher respiration and ethylene production rates and help pathogens development [4].

2.1. Diseases and physiological disorders

Fungal pathogens are considered the main cause of citrus diseases, severely affecting post-harvest management. Pre-harvest infections include Brown rot (*Phytophthora* spp.), Alternaria rot (*Alternaria* spp.), stem-end rot (*Diplodia natalensis* Pole-Evan, *Phomopsis citri* Fawcett), Grey mould (*Botrytis cinerea* Pers.), Anthracnose (*Colletotrichum gloeosporioides* Penz.), whilst post-harvest infections include Green mould (*Penicillium digitatum* Sacc.), Blue mould (*P. italicum* Weh.) and Sour rot (*Geotrichum candidum* Link). Green and blue moulds represent the most common and serious diseases, and cause significant economic losses during fruit storage and marketing. *P. digitatum* and *P. italicum* particularly attack blood oranges, and infection takes place only through rind wounds, where nutrients are available to stimulate spore germination. The incidence of other pathogens is generally low but become a serious problem in warm, wet years [5].

Postharvest physiological disorders are a consequence of environment, handling, treatments, and storage conditions to which fruits are subjected. In particular, losses of citrus fruits stored for several months or shipped for long distances are a consequence of the low temperatures required for cold storage. Chilling injury (CI) represents the major disorder of citrus fruit that occurs when fruits are held at low non-freezing temperature storage (0–10°C) and it depends on species, cultivars, season, maturity stage and location of the crop. The severity of CI is related to the temperature and time of exposure. Recent findings indicate that oxidative stress is related to CI [6]. This physiological disorders manifested in a variety of symptoms, most often characterized by areas of the rind that collapse and darken to form pit (not targeted to the oil glands) and brown colour areas. As a consequence of CI, it is possible to observe an increase in mould development on fruit surface. Ageing is a physiological disorder indicated by the shrivelling and collapse of the button tissue, caused by prolonged storage at low temperature.

2.2. Sanitation

As studied [7], population of *Penicillium* spp. on fruit surface and in the packinghouse atmosphere fluctuated along the packingline, reaching a peak at “bin emptying” step. An accurate sanitation of packingline and environment is provided to reduce the inoculum density of the spore, with positive consequences for fruits.

Sanitizers, used for fruit surface sterilization, have the aim to reduce the initial high level of inoculum present on the products. Chlorine solutions are the current products used in packinghouses. Cleaning is usually achieved by spraying sodium hypochlorite solutions (100–150 ppm) and washing on brushes followed by a potable water rinse [7]. However, this method has some negative aspects due to the constant adjustment of the available chlorine and the monitoring of pH of the solution for a correct treatment. Moreover, chlorine-releasing compounds can produce toxic breakdown by-products (halogenated) when reacting with organic materials [2].

The use of different sanitizers (peroxyacetic acid (PAA), ozone and electrolyzed water (EW)) has been evaluated, for water disinfection in packinghouses operations of citrus fruit [8].

Peroxyacetic acid is a strong oxidizer formed from hydrogen peroxide and acetic acid, effective against a wide range of microorganisms. Decomposition products of PAA are acetic acid and oxygen, which are not toxic and no rinse step is necessary. PAA applications at 800 µg/ml for 1 min significantly reduced green mould incidence on lemon [9, 10]. Ozone is a strong naturally occurring oxidizing agent, known for its efficacy to kill various microorganisms (bacteria, cysts of protozoa, viruses and fungal spores). Moreover, a significant advantage of ozone in water is related to the quick decomposition of ozone molecule to oxygen, with no residues. Electrolyzed water, generated by passing a diluted salt solution through an electrolytic cell, has shown a reduction of the microbial pathogen population in citrus process water [11].

2.3. Conventional and alternative treatments

Current postharvest decay control strategies are based on the application of synthetic chemical fungicides which are relatively inexpensive, easy to apply, with preventive and curative action against established and new infections, respectively. The most widely used methods of application are by adding fungicides [e.g. imazalil (IMZ)] to liquid commercial wax that is sprayed onto the fruit, before fruit storage. This method simplifies packinghouse operations, because the improvement in the packing line is inexpensive and straightforward, and, unlike dipping, there are no problems due to drainage and water treatment. Moreover, several problems arise when spraying IMZ in mixture with wax, as obstruction of nozzles with reduction in treatment efficacy and uniformity of distribution; dispersion and loss of fungicide and wax on brushes and along the packing line; accumulation of fungicide on brushes leading to waste treatment problems; accumulation of spores in the brushes requiring their regular cleaning [8]; and the chemical composition of wax, as it contains several alkaline soluble materials which may cause more IMZ partition in the wax and hence a reduced amount in the aqueous phase to penetrate the peel. Consequently, all these problems require an increase in fungicide concentrations.

Fruit dipping is more effective than wax spraying with regard to decay control, because pathogens basically develop in small punctures on the fruit's peel where the water solution penetrates better than the more viscous wax. Moreover, the fungicide concentration gradually decreases [12] and the number of spores increases in water emulsion, with possible problems related to the uniformity of treatment, the cross contamination of fruit and the stability of fungicide in the presence of sanitizing agents. IMZ, thiabendazole (TBZ) and sodium-ortho-phenylphenate (SOPP) are the only authorized fungicides for postharvest applications, and their use is regulated by government authorities and differs from one country to another [5]. These treatments, although carefully tested for side effects and tightly regulated, leave a residue on fruit as well as in the environment. For these reasons, there is an increasing interest to develop and implement alternative eco-friendly, nontoxic strategies effective and not reliant on conventional fungicide applications, for postharvest diseases control of fresh commodities.

In recent years, several low-risk fungicides (Trifloxystrobin, Azoxystrobin, Fludioxonil, Cyprodinil and Pyrimethanil) classified as a minimal risk to human and environmental health have been registered, also for the control of citrus postharvest decay. Effectiveness of

Fludioxonil tested on Tarocco orange fruit inoculated 24 h before with *P. digitatum* showed high curative activity in the fruit of various cultivars, harvested at different degrees of maturity [13].

Finding alternatives that are widely accepted and commercially viable has been a challenge [14]. Alternative methods to fungicide treatments include the application of physical treatments, generally recognized as safe (GRAS) compounds, biological control agents and natural antimicrobial compounds. Their inhibitory effect on decay control is mainly a consequence of direct inhibition of pathogens. However, some of these strategies have shown the ability to enhance defence mechanisms on citrus fruit tissues, inducing disease resistance and making a significant contribution to *Penicillium* decay control. Nevertheless, due to low persistence and lack of preventive effect on pre-existent diseases, the alternative strategies should be used in combination, as part of an integrated management programme, able to further minimize postharvest decay and to extend the fruit's shelf-life.

2.3.1. Physical treatments

Physical treatments are considered an interesting and feasible alternative to chemicals, because of the total lack of residues on treated products and the potential to develop induction of resistance against future infections. They appear as a cheap solution, when compared to other heat treatments, for organic crops and for markets requirement of minimal or no chemical postharvest fruit treatments. Moreover, an increase of resistance to chilling injury during storage was observed in some fruits. However, some negative effects could occur affecting fruit quality, when not properly applied and technological problems can be better studied to permit applications on a commercial scale. Heat is the physical treatment most employed in postharvest applications, used in the form of hot water dip (HWD), short hot water brushing (SHWB), curing, hot dry air and vapour heat [15].

HWD is based on the use of water at temperature above 40°C, and consists in a complete immersion of fruit for 2–3 min. Many authors found that HWD on organic citrus fruits reduced rot development, without detrimental effects on fruit appearance and quality traits [16]. However, the period of fruit immersion represents an obstacle to adopting this method in packinghouses, where it is necessary to process large volumes of product quickly. Treatments at 56°C for 20 s are shown to have higher effectiveness in inhibiting *P. digitatum* spore germination than at 52°C for longer exposure time [17].

SHWB treatment consists on the employment of tap water using a spray nozzle system. Water is sprayed above fruits rolling over brushes on sorting line, followed by pressurized hot water rinse at higher temperature (60°C) and short exposure time (20–60 s), and final forced-air drying [15]. It provides a more effective cleaning than HWD, thanks to its capacity to remove heavy dirt and fungal spores on fruit pericarp, while maintaining fruit quality. SHWB is commercially adopted in Israel, Egypt, Indonesia and Morocco, for various commodities, such as melon (*Cucumis melo* L.), mango (*Mangifera indica* L.), grapefruit (*Citrus paradisi* M.) and pepper (*Capsicum annum* L.) [18]. In Europe, the use of hot water treatments currently regards organic apples (*Malus domestica* Borkh.). The application of SHWB at 60°C for 20 s has been reported to reduce consistently green mould on citrus fruit [8, 10].

Curing is a treatment consisting on the exposure of fruits for several days to an air atmosphere at 30–36°C and high relative humidity (RH > 90%). As reported by different authors, the exposure of citrus fruit to this treatment evidenced the healing of rind wounds. The application of curing against citrus green and blue moulds has shown satisfactory disease reductions in a variety of citrus cultivars [10, 19], less for blue mould when the fruit was cold stored for long periods after treatment [20]. As reported by Pérez et al. [21], an intermittent curing treatment of two cycles of 18 h at 38°C completely controls *P. italicum* on mandarin stores under ambient conditions.

In recent years, different promising technologies have acquired increasing interest for the control of fruit postharvest diseases as the use of the radio frequency or microwave heating, hypobaric and hyperbaric pressure and ultraviolet-light therapy (UV-C irradiation) that have also shown the potential in inducing resistance in the fruit [22–25]. Some studies have been carried out to control the most common pathogens of stone fruit, small fruit and berries, obtaining variable results. Further studies are carried out to evaluate the influence of these treatments on fruit quality.

2.3.2. Microbial biocontrol agents

In the past 30 years, impressive progress has been achieved since the first publication on biological control by bacteria [26]. Different products reached advanced stages of development and commercialization to manage key postharvest pathogens. Aspire™ (*Candida oleophila*), Pantovital™ (*Pantoea agglomerans*) and Biosave™ (*Pseudomonas syringae* Van Hall) were originally registered in the USA and Spain as commercial products for the control of several postharvest diseases of pome and citrus fruit, such as *Penicillium* rot of apples (*P. expansum*), Green mould (*P. digitatum*), Blue mould (*P. italicum*), Rhizopus rot (*Rhizopus stolonifer*) and Grey mould (*Botrytis cinerea*). Aspire is not currently commercialized and Biosave was later extended to cherries (*Prunus avium* L.), potatoes (*Solanum tuberosum* L.) and sweet potatoes (*Ipomoea batatas* (L.) Lam.). Shemer™ (*Metschnikowia fructicola*), initially registered in Israel for both pre- and postharvest application on various fruits and vegetables, was later acquired by Bayer CropScience (Germany) and recently sublicensed to Koppert (Netherlands) [27].

Research work is currently carrying out to develop new microbial antagonists that occur naturally on fruit surfaces, able to avoid infections in wounded fruits, also studying the several possible mechanisms involved in a tritrophic host-pathogen-antagonistic interaction system [27, 28].

Despite the progress obtained with microbial biological control agents (BCAs), their application in packinghouses is still limited, mainly due to the difficulty in obtaining adequate results under commercial conditions.

Among the antagonistic microorganisms used as BCAs, for postharvest applications, yeasts resulted the most effective on fresh fruits, due to their ability to adapt and to grow on particular fruit micro-environment and environmental postharvest conditions and to compete with the fungal pathogen for nutrients and space at the wound site. Penicilli and minor wound pathogens, parasite the citrus fruit throw rind wounds, where nutrients are more available and utilized for germination and host colonization. Yeasts need the same environmental

conditions, and if present before pathogen infections are able to immediately develop and colonize the wound, by formation of an extracellular polysaccharide capsule that can promote adhesion to fruit surface forming biofilms covering the entire wound area. However, competition becomes an effective biocontrol mechanism when the antagonist is present in sufficient amounts at the right time. In the case of pre-established infections, the efficacy of antagonistic microorganisms is lower. Often, pathogens' growth is inhibited, but they leave alive [29].

Results obtained on citrus fruits applications of yeasts showed no commercially acceptable value, when antagonist stand-alone treatments were carried out [9]. For this reason, the integration of antagonists with different alternative methods has been proposed regarding different combined application of antagonists followed by generally recognized as safe (GRAS, for food contact applications) [30], hot water treatments [31] and elicitors of resistance. Most of the substances used (sodium bicarbonate) resulted in a delay in spore pathogen germination, with a competitive advantage obtained for antagonistic development. Recent study about mechanisms of action of microbial antagonists is a promising chance for a better utilization of microbial antagonists for postharvest treatments [27, 32, 33]. They effectively can be useful in improving knowledge about the quadrifrophic interactions taking place among the antagonist, the pathogen, the host and the resident epiphytic microflora, on wound site, offering the opportunity to improve yeast's efficacy, when applied on fruits under commercial conditions [34, 35].

2.3.3. *Generally recognized as safe*

Owing to rapid degradation on the host surface, GRAS leaves low or no detectable residues in the commodity.

Carbonate and bicarbonate salts resulted as the most effective substances, on reducing *Penicillium* rots on citrus fruit immersed in 3% (wt/vol.) of sodium carbonate and bicarbonate solutions at ambient or at high temperature [36]. Ozone has successfully proven to be suitable for fruits and vegetable preservation, due to its antimicrobial and antioxidant activity (increased vitamin C and phenolic content). In addition, ozone has been used for the reduction of volatile compounds and ethylene present in the storage room or during shipping, in order to slow the senescence of the fruit, to reduce the incidence of rots conferring greater resistance to some physiological disorders, and it is fundamental to extend the shelf-life of citrus fruit during long-term storage [37].

During the postharvest treatments of fresh fruit and vegetable, ozone can be used for short periods in air or washing water, or it can be added continuously or intermittently into the environment during the storage period. Ozone diluted in water can be used as a hypochlorite substitute for disinfection and sanitation purpose.

Di Renzo et al. [12, 38] studied a prototype in order to control ozone/air mixture during washing and storage of citrus fruits. A feedback control system, equipped with high-precision-measuring sensors, was set up to control the active concentration. The obtained results showed a low efficacy of control when using ozone in the washing water, probably due to the variable level of contamination due to the impurities deriving from citrus fruits. Whangchai et al. [39] demonstrated the synergistic effect of ultrasonic irradiation in combination with

ozone in reducing residual ethion of tangerine (*C. reticulata* Blanco cv. Sai Nam Pung) fruit after harvest. Ethion concentration was reduced to 75.43% after ultrasonic irradiation at 1000 kHz and ozone exposure for 60 min. Palou et al. [40] studied the ozone gas penetration and its effectiveness in controlling the sporulation of *P. digitatum* and *P. italicum* within commercial packages of oranges (cv. 'Lanelate') during cold storage at 12.8°C. However, discrepancies in results were found in the literature [41] due to variables that may influence ozone efficacy (O_3 generation and application methods, O_3 concentration and duration time, method of O_3 exposure, storage conditions and varieties). In recent years, a large variety of natural compounds with antifungal activity from plants, animal-derived materials and microorganisms have been evaluated, and much literature have been reported [42, 43]. The effectiveness at low concentrations of some aroma components was found of particular interest. In vitro fungal inhibition was obtained by some isothiocyanates, trans-2-hexenal, carvacrol, thymol, citral and trans-cinnamaldehyde [44]. An obstacle to their practical application has been the off-odours absorbed by commodities, able to alter their flavour, the phytotoxicity and humans' safety issues, when used at high concentrations and the possible spore germination stimulation when used at low concentrations [45, 46]. Recent studies have been carried out employing a pomegranate peel extract (PGE) for the control of postharvest rots [47].

2.3.4. Integrated treatments

Effectiveness of alternative methods can be improved by the combination of different approaches, such as GRAS, physical methods, biocontrol agents and ultraviolet light [20, 40, 48]. This strategy could result in a synergic effect on disease control and two or more alternative approaches need to be combined to reach commercially acceptable effectiveness for postharvest decay control comparable to the synthetic fungicide treatments.

2.3.5. Coatings

Synthetic coatings are generally anionic microemulsions containing resins and/or waxes (shellac, wood rosin, candelilla, carnauba, beeswax, polyethylene and petroleum). The main purpose of coatings is to reduce fruit weight loss, and chilling injuries and shrinkage by reducing transpiration and respiration, and improve fruit appearance, providing gloss. Alternative coatings known as edible coatings and films have been developed as an eco-friendly technology. Located on food surface as a coating or placed on the environment of a packaged food, edible coatings or films provide a semi-permeable barrier to water vapour, oxygen and carbon dioxide between the food and the surrounding atmosphere, prevent physical damages, chemical and microbiological deteriorations, and prolong the shelf-life of products [49]. Due to the high economical value of worldwide citrus trade, the development of novel antifungal edible coatings for citrus fruit is a very active research field and a considerable number of studies are available, including biopolymers, cellulose derivatives with generally regarded as safe (GRAS) salts and plant, pectin and commercial waxes with essential oils [40, 50–52]. Chitosan, the cationic deacetylated derivative of chitin, is a biopolymer owing to biocompatibility, biodegradability and absence of toxicity, studied for its antimicrobial activity against a variety of bacteria and fungi and as chemical elicitor, able to enhance the protection of host plant tissue through induced/acquired resistance.

Despite the substantial progress that has been accomplished in evaluating new antifungal edible coatings, their implementation is still limited mainly because of general limitations associated to the edible nature of food-grade- coating components.

3. Cold storage

Temperature and relative humidity are considered the key factor in the control of deterioration rate during the postharvest-handling chain of citrus fruit. Several authors have shown that the maintenance of an optimal temperature level during the postharvest storage is the main strategy in order to extend the shelf-life. Optimal temperature and high relative humidity levels (RH 90–95%) represent the best strategy for citrus fruit storage. RH prevents moisture loss from the host tissues and consequent shrivelling.

The optimal storage temperature is variable with reference to citrus species and varieties. Most citrus cultivars are able to tolerate low-temperature levels during prolonged storage while other cultivars such as 'Fortune' and 'Nova' mandarins, lemons and grapefruit are most sensitive to low temperature so values above 9°C are recommended to avoid the insurgence of chilling injuries [53]. Common chilling injuries are the formation of brown pitlike depression in the flavedo (mandarins and grapefruits) and superficial scald in the flavedo of some oranges ('Navelate').

Rapid cooling allows lowering the temperature to the levels applicable during storage, cold treatment and shipment to market, resulting in a substantial reduction in both weight loss and decay. Different systems for rapid cooling are available, in relation to the heat-removal system: room cooling (by chilled air), forced air cooling, hydrocooling (by chilled water) and vacuum cooling. The air-cooling system (especially using a forced airflow through the fruits stacked in a pallet) is the most efficient method for the removal of heat in citrus fruits [54].

In the 'room-cooling' system, the heat exchange between fruits (packed in cartons, sacks, or bins) and cold air takes place directly in cold storage rooms, by means of air fans blowing the cold air (air speed between 1 and 2 m/s). Once the final temperature has reached (depending on species requirements), the air velocity is reduced to about 0.05–0.1 m/s. The room cooling system is still the most widely adopted system, mainly for economic reasons. However, this system leads to both inefficient cooling (very slow) and excessive water loss (associated with an over-drying of citrus skin). The space between stacks of boxes inside the storage room is fundamental in order to reduce the cooling time. A distance of about 10–15 cm (4–6 inches) is enough to allow cold air to circulate around the boxes or pallets. Furthermore, using vented boxes leads to a more efficient cooling process with respect to unvented boxes. For these reasons, it is clear that the use of traditional cold storage room is adequate only for the storage of fruits already chilled, because the mechanism of cooling is too slow and it is not suitable for the quick removal of the 'fruit heat' (thermal level of fruit when harvested in field). Therefore, in order to quickly cool fruit after harvesting specific equipment (pre-cooling plant) is needed, characterized by very high cooling capacity to make possible the removal of the 'field heat'

in medium-short times. In order to improve the uniformity of the temperature and cooling in citrus storage rooms, the forced ventilation system was developed. The rapid cooling with forced air provides for the removal of heat by means of an airflow which, chilled on an evaporator of a mechanical refrigeration system, is forced through the mass of the produce by means of a fan, working generally in pressure. Examples include a fixed unit equipped with a fan housed inside the wall of a cold room, or a portable fan unit that can be moved around inside the cold room. Furthermore, the alignment of boxes vents is fundamental in order to allow the cold airflow to pass through the pallets. Forced air cooling is able to reduce the cooling time from more days (room cooling) to few hours, depending from the availability of adequate cooling power.

After pre-cooling, it is important to maintain high relative humidity levels around the fruits. Among the techniques applicable in commercial distribution and for the preservation of citrus fruit, the use of plastic films is particularly considerable. Such films are able to lead to an accumulation of carbon dioxide and a reduction of oxygen, slowing both the respiratory activity and degradation of the reserve substances. Furthermore, the transpiration of the fruits favours the establishment of high moisture conditions, which typically slows down the ageing phenomena and the weight losses, restricting the production of ethylene and extending the “shelf-life” [54].

3.1. Citrus shipment

The global citrus fruit market had recently seen a great development in export activity, hence requiring both the respect of fruit quality standards and the restriction of the parasites spread (Mediterranean fruit fly, *Ceratitis capitata* Wiedemann; Mexican fruit fly, *Anastrepha ludens* (Loew)) in fruits. With this aim, it is mandatory for the importer to verify the thermal history (in terms of temperature variations) of fruits during the shipment, before the produce acceptance, and the respect of the cold treatment.

The cold treatment consists in low-temperature storage of fruits for a specific time (**Table 1**), carried out in cold storage rooms prior to shipping or during shipping (‘in transit’). After a partial abandonment in favour of the methyl bromide fumigation, cold treatment was definitively introduced once the effect of methyl bromide (phased out on January 2005) was demonstrated on the atmospheric ozone layer depletion [55].

Core temperature (°C)	Treatment time (days)
0°C or less	12
0.6°C or less	13
1.1°C or less	14
1.6°C or less	16
2.2°C or less	18

Table 1. Protocol for the cold treatment adopted in the United States (USDA – APHIS 2002) for the control of the Mediterranean fly (*Ceratitis capitata*) spread during import of citrus fruit (orange, clementine, grapefruit and lime).

Both empirical and theoretical studies have been carried out to study the thermal variations and airflow during perishable goods shipping, showing that, in almost any transport situation, local temperature deviations are always present. From the literature reports, deviations of roughly 5°C or more depending on the transport conditions [56] are indicated.

Tauriello et al. [57] carried out experimental trials to simulate a cold treatment during a refrigerated transport of citrus fruits at industrial scale, monitoring the fruit temperature distribution inside the reefer in order to reduce the temperature difference in the load.

Several approaches have been used to predict both the airflow rate and temperature distribution, nevertheless giving only qualitative information on the air circulation rate. For this reason, computational fluid dynamics (CFD) tools are widely used [58]. CFD is a method that using numerical, physical analysis and computational software solves and analyses problems related to fluid flows (air mixtures and liquids). Recently, Defraeye et al. [59] suggested an innovative and promising cold-chain protocol alternative to the commonly used forced-air pre-cooling (FAC) of citrus fruit prior to shipping. The theoretical approach, by means of computational fluid dynamics (CFD), explored ambient (warm) loading of citrus fruit into refrigerated containers (reefer) for cooling by vertical airflow during marine transport. Results suggested that the optimization of ambient-loading protocol is strictly related to the improvement of both box design and stacking on the pallet, in order to reduce the airflow circuits between the pallets, but authors concluded that a synergy between numerical and full-scale experiments could contribute to improve further model.

4. Non-destructive methods for fruit quality evaluation

Fresh citrus fruit required external and internal quality from the harvest until reaching the consumer. The fruit quality could be defined as the combination of fruit attributes or characteristics that have significance in determining the degree of consumer acceptance. For the sake of quality control, fresh citrus fruits are generally inspected for adherence to both minimum maturity (internal quality) and grade (external quality). Minimal acceptable grade standards are prescribed in various laws and minimum maturity standards are specific for each citrus type. Based on these standards, quality inspectors judge the quality and decide its utility and marketability. Quality evaluation and control is also essential for deciding fruit price. Quality attributes can be evaluated by both subjective and objective methods. The objective methods are precise and involve the use of instruments, while subjective methods make use of human senses. In addition, most acceptable citrus fruit quality classification systems, whether manually operated or automated, are based on some traditional techniques and methods well established for evaluating fruit quality. These methods are normally sample-destructive, laborious and time-consuming, thereby limiting their utility in online/in-line quality monitoring. Moreover, industry demand increased for innovative tools: rapid with cost-effective for the evaluation and monitoring of citrus fruit quality. Therefore, several researches were focused on the application of new non-destructive methods in citrus fruit quality detection, including hyperspectral imaging (HSI), electronic noses (e-noses) techniques and nuclear magnetic resonance (NMR) [60–62].

Consequently, the advantage of non-destructive methods is that they can be used after harvest to make sure that every fruit sent to the market meets the quality norms, in contrast to the destructive methods, in which a representative sample is lost during analysis. Furthermore, citrus fruit quality attributes may significantly vary among citrus species and cultivars within the same species or even within the same cultivar grown in diverse climatic conditions or under different cultural practices.

4.1. Measurement of external quality

4.1.1. Firmness

Fruit firmness is roughly estimated by consumers' touch in the supermarket or by a Magness-Taylor (MT) tester in the laboratory [63]. However, MT testers tend to cause operation; consequently, such tests were replaced with others capable of assessing the mechanical properties of citrus fruits in a more objective and reproducible way [64]. A universal testing machine (UTM) [65] was used to sort on-line only the fruits with tension in the range of 375–445 N m⁻¹, in order to limit or vanish Sicilian Tarocco orange fruit rejection after long shipping in foreign markets and guaranteeing their longer shelf-life. In addition, the firmness of two kinds of orange was estimated using an HIS system [66], with utilization of partial least square regression (PLSR) to build prediction model of orange firmness [67].

4.1.2. Detection of contaminants and defects of fruit

The presence of common defects or disorders is insufferable for consumers and the presence of skin defects is one of the most influential factors in the price of fruit. The challenge is significant regarding citrus rind disorders that do not manifest during harvest grading and postharvest treatments but is developed 1–5 weeks after harvest. Consequently, the main task is to develop non-destructive technology to determine rind quality in the packing line to assist in sorting and segregation of fruit into quality grades.

4.1.3. Fruit contaminants

Various non-invasive and rapid technologies were investigated for the automatic detection of decay in citrus fruit as alternatives to human inspection. The great potential of HSI technique was evidenced to detect an early-infested mandarin with *P. digitatum* from sound ones [68]. Moreover, the potential of Near InfraRed (NIR) reflectance spectroscopy in combination with linear discriminant analysis was proved for the automatic detection of the early symptoms of decay caused by *P. digitatum* fungus in mandarins [69]. Furthermore, e-nose was able to identify different volatile organic compounds (VOCs) surrounding oranges infected by *P. digitatum*, representing an early indication of the up-coming deterioration [4]. In addition, several researches reported that a commercial e-nose was able to differentiate between lemon and oranges non-contaminated and contaminated with *P. digitatum* spores [70, 71]. Moreover, the high specificity and sensitivity of e-nose sensors in combination with a PLS discriminant analysis was evidenced for the early detection of low VOCs production in infected citrus fruit placed in controlled environment [62].

4.1.4. Fruit defects

In general, fruit defects can be divided into two categories. The first occur before the harvest without degeneration after the harvest, while the second occur in the whole process from fruit growth to their postharvest marketing. Traditional detection methods of fruit defects such as visual inspection, computer vision and spectroscopic technique are only able to detect defects occurring on fruit surface or under peel [72]. HSI technique is increasingly introduced to simultaneously estimate defects on fruit surface and under the peel. In addition, Near InfraRed (NIR) region of the spectrum can improve the inspection by detecting specific defects or allowing the detection of non-visible damages. NIR in the absorbance/reflectance mode has been used successfully to detect drying internal disorder in Tangerine citrus [73], and to predict oleocellosis sensitivity in citrus fruit [74]. Furthermore, multi-spectral inspection system was developed to detect and sort citrus fruits according to 11 different types of external defects including some morphological features of defects [75]. In addition, species and cultivars of citrus present a high rate of unpredictability in texture and colour, which makes it difficult to develop a general unsupervised method able to perform this task. In this context, a novelty detection technique was performed by using unsupervised method, based on a multivariate image analysis strategy in combination with PCA for the detection of new unpredictable defects in oranges and mandarins [76]. This unsupervised method needs only a few samples to be trained and could be suitable for the task of citrus inspection.

4.1.5. Measurement of internal quality

Citrus internal quality parameters can be assessed using NIR methods by determining the concentration of organic molecules on citrus fruit. Many studies have been focused on evaluating the internal quality attributes using reflectance measurements acquired with visible NIR spectroscopy technology on citrus fruit [77, 78]. Currently, the NIR technique has significantly greater accuracy for determining solid soluble content (SSC represents the amount of solids present in fruits, and soluble in tannin or water extracts, correlated to sugar content) in citrus than any other quality parameters such titratable acidity (TA represents the acidity of fruits due to the presence of organic acids, mainly citric acid). Consequently, the low success for the remaining internal parameters could be attributed to the fact that organic acids concentration on intact fruit is relatively low (about 10%); for that reason, calibration of this attribute is likely to represent secondary correlations to the parameters related to fruit maturity [79].

5. Conclusions

Citrus fruits are cultivated all over the world due to their positive health values, especially bio-active substances, vitamin C and phenolic compounds. The perishability of fruits is generally due to wrong temperature and relative humidity, responsible for increased respiration rate, physiological disorders and fungal pathogens. In order to optimize temperature and relative humidity during cold storage and shipping of citrus fruits, both experimental and theoretic-

cal methods are used, also by means of numerical methods (computational fluid dynamics). Fungal pathogens affect severely the postharvest life of citrus fruits, especially if stored for several months or shipped for long distances. Sanitizers (chlorine solutions) and fungicides [imazalil (IMZ), Thiabendazole (TBZ) and sodium-orthophenylphenate (SOPP)] are used for fruit surface treatment. In recent years, several low-risk fungicides (Trifloxystrobin, Azoxystrobin, Fludioxonil, Cyprodinil and Pyrimethanil) classified as a minimal risk to human and environmental health have been proposed.

A fundamental focus in the postharvest sector is the development and application of innovative methods for citrus fruit quality detection. A particular attention is paid to the non-destructive systems, as image vision systems (hyperspectral imaging), spectrophotometric methods (based on visible and infrared light source), sensing technologies (electronic-noses) and nuclear magnetic resonance (NMR).

On the basis of the current knowledge about citrus postharvest and in order to extend the shelf-life of fruits, the following future challenges could be identified: reduction of mechanical damages during handling and packing operations; research to find new alternative methods for fruits treatment to reduce the use of chemical compounds; and optimization of both cold storage room and refrigerated container for citrus shipping.

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