Review

Special Issue: Quality and safety of fruits and vegetables at harvest

Key determinants of citrus fruit quality: metabolites and main changes during maturation

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Abstract

Citrus is one of the main fruit crops in the world and widely recognized by their organoleptic, nutritional and health-related properties of both fresh fruit and juice. The genetic diversity among the genus and the autonomous and independent changes in peel and pulp, make the definition of standard maturity indexes of fruit quality difficult. Commercial maturity indexes in the citrus industry are usually based on peel coloration, percentage of juice, soluble solids/acidity ratio but their relevance may differ among varieties and the specific requirements of the markets. There is also a marked influence of environmental and agronomic conditions such as light and temperature, rootstock selection and plant nutrition, among others. Besides commercial requirements, a more comprehensive definition of fruit quality should also consider organoleptic and nutritional properties that are determined by a complex interaction among a number of bioactive components. Citrus fruit are an excellent source of many phytochemical, including ascorbic acid, carotenoids (antioxidant and pro-vitamin A), polyphenols, flavonoids, limonoids, terpenoids, etc., which greatly contribute to the health-related benefits of these fruits. Criteria and definition of the main maturity indexes for citrus fruit worldwide are described, as well as changes during fruit maturation in key components affecting organoleptic and nutritional properties. Moreover, the involvement of hormonal and nutritional signals and their interaction in the regulation of external and internal maturation of citrus fruit, as well as the influence of environmental and agronomic factors are also critically revised and discussed.

39 *Keywords:* Acidity, color, ethylene, maturation, quality, soluble solids

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43 **Highlights**

- 44 • Maturity indexes of citrus fruit depend on the species and market standards
- 45 • External and internal characteristics define commercial fruit quality
- 46 • Citrus fruit is an excellent source of nutritional and health-related compounds
- 47 • Hormonal and nutritional signals coordinately regulate citrus fruit maturation

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1. Introduction

Citrus is one of the main fruit crops in the world with an estimated production of more than 170 million tm (FAOSTAT, 2015). The attractiveness and high consumption of citrus fruits worldwide are mainly due to the health-related and sensorial attributes of both fresh fruits and juice, and to the consumer perception of the benefits of their composition in primary and secondary metabolites (Lado et al., 2016; Liu et al., 2012; Sidana et al., 2013). The *Citrus* genus is characterized by a great diversity of species and varieties, including mandarins, oranges, lemons, grapefruits, pummelos, citrons, limes, kumquats and different hybrids. This genetic diversity makes it especially complex to define standards for citrus fruit quality since external and internal quality as well as nutritional and nutraceutical properties may vary largely. Moreover, evidence indicates that maturation of the peel and pulp are not fully coordinated and that the natural ripening of both tissues are autonomous and independent processes (Tadeo et al., 2008), making to their understanding more complex. This independent physiological behavior of the peel and pulp has been related, among other, to the lack of vascular connections (Monselise, 1977). Differences in the complex metabolic network operating between peel and pulp are well illustrated by the large variations in carotenoid content and composition, and ascorbic acid, two key metabolites in citrus fruit, that are usually higher in peel than in the pulp (Rodrigo et al., 2013a; Alós et al., 2014).

Furthermore, accumulation of many nutrients and phytochemicals in the fruit are influenced by different factors (genetic, environmental, agronomic and cultural practices) and highly dependent on the fruit maturation program. In this sense, the particular composition of each variety is strongly determined by the stage of maturation at harvest. Commercial maturity indexes in citrus fruit differ depending on the citrus species and varietie, growing regions and the requirements of the destination markets.

In general, peel coloration, soluble solids, acidity and juice content are key parameters for harvesting; however, the relative importance of these parameters depends on the citrus variety and the distance to the market destination (Lado et al., 2014). Other potential uses of citrus fruit such as essential oil extraction from lemon peel can influence the harvest time with the objective of increasing the content and composition of these components. Therefore, in order to maximize the quality of citrus fruits at harvest for fulfilling the commercial and nutritional requirements of the markets, it is critical to understand the evolution of the main phytochemical components during fruit development and maturation in each particular species and variety and also the influence of environmental and cultural factors.

2. Citrus quality at harvest: maturity indexes and key metabolites

Fruit growth and development in Citrus is divided in three stages: first, a cell division phase of slow growth; a second stage of cell enlargement of major increase in size and weight by growth of juice sacs from the pulp, and a third phase during which fruit growth is reduced and take place most of the transformations characteristic of fruit maturation, such as color changes, sugar accumulation, acid degradation, etc. (Bain, 1958). Citrus fruit are recognized as non-climacteric; displaying a progressive reduction in the rate of respiration and low ethylene production during the whole maturation process (Aharoni, 1968; Eaks, 1970; Goldschmidt et al., 1993). Despite this behavior, physiological and molecular evidences indicate the involvement of the hormone in the control of some ripening-associated processes (Porat et al., 1999; Yu et al., 2012; Alos et al., 2013; Ding et al., 2015). Therefore, the changes taking place during maturation that are essential to define maturity indexes are regulated by a complex interplay of endogenous hormonal and nutritional signal, and highly influenced by both

environmental factors and agronomic practices (Manera et al., 2013, 2012; Porras et al., 2014; Rodrigo et al., 2013).

The maturity indexes integrate relevant components to define the standards of citrus fruit quality worldwide but are not sufficient by themselves to fulfill the quality requirements. To establish these requirements other attributes such as fruit shape and size, coloration, incidence of peel disorders (shriveling and dehydration, chilling injury or sun burn), pest damages, wind or hail scars and peel roughness should also be considered in addition to the potential health-related benefits and sensory acceptance, that are mainly influenced by the phytochemical composition (Lado et al., 2014). Thus, the decision of the harvest time is critical, being a compromise between the prevailing climatic and agricultural conditions and the postharvest practices of the producing countries, and the standards established by the demanding countries and costumers.

Defining the criteria to accurately and consistently determine citrus fruit maturation is not an easy task since it involves physiological changes occurring in two different and independent tissues: color transformation taking place in the fruit peel and compositional changes occurring in the flesh. Ordinary citrus fruit as sweet oranges, mandarins, lemons and grapefruits are considered to be mature for fresh consumption when their external coloration, juice content and soluble solids: acidity ratio and other internal constituents reached to a minimum standard of visual acceptance or palatability. In European Union markets, the maturity indexes used to define fruit quality consider juice content (%), total soluble solids (TSS, °Brix), TSS:acidity ratio and the proportion of the fruit surface with a minimum coloration (Lado et al., 2014). Agricultural Quality Standards of the United Nations Economic Commission for Europe (UNECE) established a minimum sugar/acid ratio of 6.5 in Satsuma and oranges, 7.0 in Clementine and 7.5 in other mandarins or hybrids (UNENCE STANDARD FFV-14, 2010). All

these parameters are determined by the accumulation of primary and secondary metabolites during the ripening process on the tree.

Accumulation of soluble sugars and the decline in acid content (mostly citric and malic acids, depending on the different citrus species) are typical changes taking place in the pulp during maturation of citrus fruit. These changes are concomitant with modifications in relevant bioactive components, i.e. carotenoids, flavonoids, polyphenols, limonoids, furocoumarins and volatile terpenoids, which combined determine the final sensory and health-related properties of mature fruits. Following is a summary of the main changes occurring in key components widely used to establish maturity indexes and quality in citrus fruits.

2.1. Key primary metabolites: sugars, acids and vitamins

2.1.1 Sugars and acids

Sugars and organic acids are important components of citrus fruit flesh and their levels are commercially used as a harvest maturity indicator. The taste and the characteristic palatability of citrus fruit is mainly determined by the ratio between sugar and acid levels in the juice sacs and remarkable differences in both parameters may exist among citrus species and varieties (Goldenberg et al., 2014). Carbohydrates are the main soluble components in the pulp of citrus fruit (75-80 %) and hold the key to sweetness of the juice. The major compounds are sucrose, glucose, and fructose, with a general ratio of 2:1:1. In general, sugar levels in mandarin range from 1-2 % glucose, 1-3 % fructose and 2-6 % sucrose but can differ among citrus species (Table 1). During maturation of mandarin fruit, the content of sucrose increases as it becomes the primary non-reducing sugar and the major transported carbohydrate (Ladaniya, 2008). A rise in total sugars during fruit ripening was also described in the pulp of Delta and Lane Late

oranges (Emmanouilidou and Kyriacou, 2017), increasing from 65 to 74 g/L and from 54 to 73 g/L, respectively. Glucose (12-16 g/L), fructose (13-19 g/L) and sucrose (28-41 g/L) were the predominant sugars in both orange varieties. TSS content in the pulp of mature fruit varies notably among the different species ranging from less than 1 % in limes, 8-9 % in lemons, 8-11 % in Satsuma, 8-13 % in some orange varieties such as Navel, Valencia, Lane Late and Delta (Emmanouilidou and Kyriacou, 2017; Goulas and Manganaris, 2012) and in different mandarins or up to 16-18 % in new Uruguayan and Spanish hybrids (Goldenberg et al., 2014; Qin et al., 2015; Rivas et al., 2013; Sdiri et al., 2012a; Xu et al., 2008a). In general higher content occurs in late-ripening varieties. TSS in the pulp of grapefruit also varies between 9 and 13 %, depending on the variety, and is usually higher in red-fleshed varieties (Goulas and Manganaris, 2012; Zheng et al., 2016).

Key genes influencing sugar accumulation and acid degradation during maturation have been studied in citrus fruit. Sucrose synthase (Sus) (EC 2.4.1.13) is a key enzyme for sugar accumulation that catalyzes the reversible reaction of sucrose and UDP into UDP-glucose and fructose, whereas invertase hydrolyzes sucrose into glucose and fructose (Koch, 2004). During fruit development, there is an increase in *CitSus5* transcript levels, being *CitSus1*, 2, 5 and 6 predominantly expressed in fruit juice sacs during ripening (Islam et al., 2014). Gene expression studies indicated that sucrose is synthesized in the juice sacs and that the balance between sucrose synthase/invertase play a key role in the regulation of sucrose content (Katz et al., 2011).

Citrus acidity not only stimulates the sourness perception to consumers, but it is also a key determinant for the commercial acceptability since its balance with appropriate sugar levels provides the delightful and typical citrus taste (Liu et al., 2012). Organic acids composition varies widely in the pulp of the different citrus species or varieties

(citric acid being the predominant in the pulp, representing 70-90 % of total acids) followed by malic acid in oranges and mandarins (Table 1). In orange varieties, citric acid accounted for 8-11 g/L whereas malic and succinic range from 1.3 to 2.0 g/L, in mature fruit showing slight changes during fruit ripening (Emmanouilidou and Kyriacou, 2017). In grapefruit, quinic acid was the second most abundant acid with 15.7–33.4 % of the total organic acids. Tartaric acid and malic acid were the third-most abundant organic acid, accounting between 9.1–13.7 % and 5.3–14.4 % of total organic acids, respectively (Zheng et al., 2016). Other minor acids present in citrus juice are acetic, pyruvic, glutamic, formic and succinic.

Acidity levels at commercial harvest maturity differ notably among mandarin varieties, from as low as 0.3-0.5 % in common mandarins to 0.6-1.0 % in Satsuma Owari and Clementines, or 1-2 % in hybrids of mandarins, oranges or grapefruit or up to 4.5 % in lemons (Chaudhary et al., 2014; Goldenberg et al., 2014; Goulas and Manganaris, 2012; Liu et al., 2012; Qin et al., 2015; Sdiri et al., 2012a; Xu et al., 2008a).

It is well known that accumulation of acids in citrus fruit is initiated during the first phase of stage II to reach a maximum when the fruit has reached 50% of the final size. Accumulation of citric acid in the vacuole of juice sacs induced its acidification and is mediated by a tonoplastic H⁺-ATPase (Shimada et al., 2006). Citrate content declined progressively over the last phase of stage II and continues during fruit maturation. Differences in citrate accumulation between acid and acidless varieties did not correlate with citrate synthase gene expression and activity, indicating that other steps are involved (Sadka et al., 2000; Albertini et al., 2006). Citrate is metabolized to glutamate and then processed through the gamma-aminobutyrate (GABA) shunt (Cercos et al., 2006). The involvement of specific members of the aconitase gene family in the

degradation of acids in the pulp of citrus has been also demonstrated (Sadka et al., 2000; Terol et al., 2010).

2.1.2 Vitamins

The flesh of citrus fruits is an important source of vitamins, with vitamin C (ascorbic +dehydroascorbic acid) being the most important for their contribution to the antioxidant properties and nutritional benefits (Barros et al., 2012; Goldenberg et al., 2014; Zou et al., 2015). Other vitamins present in citrus are folate (mainly as reduced 5-methyl tetrahydrofolate and polyglutamate derivatives), pro-vitamin A compounds (by the presence of the precursors β -carotene and β -cryptoxanthin) and vitamin E (Codoñer-Franch and Valls-Bellés, 2010; Zou et al., 2015).

Ascorbic acid (AsA) is usually higher in oranges than in grapefruits and mandarins, with few exceptions (Alós et al., 2014; Dhuique-Mayer et al., 2005; Martí et al., 2009). In general, AsA is higher in the peel than in the pulp and increased during fruit maturation but declines in the pulp (Alós et al., 2014). The average of AsA in the pulp of oranges is around 0.40-0.50 mg/g FW, while nearly 0.20 mg/g FW was reported in Satsuma Owari mandarin (Alós et al., 2014; Aschoff et al., 2015; Martí et al., 2009). It has been reported that Clemenrubí and Clemenules have 0.60 and 0.39 mg/mL AsA, respectively (Sdiri et al., 2012b). Meyer lemon is one of the citrus varieties with the highest AsA content in the pulp, ranging between 1.9-3.0 mg/g, whereas other lemon varieties contained around 0.30-0.60 mg/g F.W. (Uckoo et al., 2015) (Table 1). Differences in the AsA content in the pulp among red grapefruit varieties may be also noticeable, ranging from 0.30-0.35 mg/g FW in Rio Red (Vanamala et al., 2005) to 1.25 mg/g FW in Star Ruby (Lado et al., 2015b).

Mandarin varieties can be generally ranked with respect to their vitamin C content in the juice (± 0.40 mg/g FW) in the following order: tangor hybrids (*C. reticulata* x *C. sinensis*) > other hybrids (Ortanique, Nova and Ellendale)>common mandarins (*C. reticulata*)> Satsuma mandarin (*C. unshiu*; Dhuique-Mayer et al., 2005; Beltran et al., 2008; Sdiri et al., 2012a).

Different mechanisms and biosynthetic pathways have been proposed to regulate AsA biosynthesis in peel and pulp of oranges and mandarins (Alos et al., 2014). The *L*-Galactose pathway may predominate in the peel and the selective stimulation of GDP-mannose pyrophosphorylase (GMP) and mainly GDP-*L*-galactose phosphorylase (GGP) would contribute to a higher flow of metabolic precursors in the peel of oranges than in mandarins during fruit ripening (Alos et al., 2014; Yang et al., 2011). However, it was also proposed that the D-galacturonic acid pathway, especially *GalUR12* gene, may also contribute to the regulation of AsA concentration in the flavedo of citrus fruits (Lado et al., 2015d)

2.2. Changes in secondary metabolites during fruit maturation

The content of the secondary metabolites, carotenoids and polyphenols as well as vitamin C, are the main determinants of the total antioxidant capacity of citrus fruit (Goldenberg et al., 2014; Goulas and Manganaris, 2012; Gurak et al., 2014; Yoo and Moon, 2016; Zou et al., 2015), and their relative content in the pulp has a direct influence on the nutritional quality of the Citrus species and cultivar.

2.2.1. Carotenoids

Fruit color is an important quality attribute in citrus and directly influences consumer perception and acceptance. Carotenoid content and composition (more than 100

different compounds have been reported in citrus fruit) are responsible for the wide range of color singularities of the different species; from the yellow of lemon and white grapefruits to the red of red grapefruits, and including a wide range of orange tones from oranges, mandarins and hybrids. Some carotenoids also display a high singlet oxygen quenching capacity, as well as pro-vitamin A capacity, being part of the antioxidant and chronic-disease prevention benefits of citrus species (Di Mascio et al., 1992; Liu et al., 2012; Zou et al., 2015). Similar to other bioactive compounds, carotenoid content is much higher in the peel (up to 70% of the total carotenoid content) than in the pulp, with the exception of red-fleshed grapefruits (Alquezar et al., 2008a; Kato, 2012).

Analysis of carotenoid content and composition in different species and varieties suggest that citrus fruits can be categorized into three groups: a) abundant in β -cryptoxanthin, represented by Satsuma (*Citrus unshiu*), Clementines (*Citrus clementine*) and common mandarin (*C. reticulata* Blanco) and as well as a great number of hybrids; b) abundant in violaxanthin, being oranges the main component; and c) carotenoid-poor, including limes, lemons, grapefruits and pummelos (Ikoma et al., 2016).

Total carotenoids in mandarins vary from 25 to more than 300 $\mu\text{g/g}$ FW in the peel, and from 10 to 40 $\mu\text{g/g}$ FW in the pulp tissue. As mentioned, β - β -xanthophylls usually represent between 68-90 % of total carotenoid in the peel (Kato, 2012; Rodrigo et al., 2013), being β -cryptoxanthin (60-80 %) the predominant in the edible pulp (Alquezar et al., 2008a; Matsumoto et al., 2009; Yano et al., 2005). Orange fruit usually account for 4-38 $\mu\text{g/g}$ FW in the pulp and 40-120 $\mu\text{g/g}$ FW in the peel tissues, represented by nearly 80 % 9-*cis*-violaxanthin (Alquezar et al., 2008a; Rodrigo et al., 2004). By contrast, white grapefruits only contained minute amounts of carotenoids, mainly phytoene and violaxanthin, whereas red grapefruits are able to accumulate high amounts of the linear

colorless carotenes phytoene and phytofluene, but also the red lycopene in both peel and pulp tissues (10-30 µg/g FW) (Alquezar et al., 2008a; 2013; Lado et al., 2015b). Certain orange and grapefruit varieties are able to accumulate lycopene in the pulp (i.e. Cara Cara, Hong Anliu oranges or Star Ruby and Rio Red grapefruit) accounting for 15-60 µg/g FW (Alquezar et al., 2009, 2008; Lado et al., 2015a; Lee, 2001; Liu et al., 2007). Mechanisms regulating lycopene accumulation in citrus fruit remain still elusive, although the existence of a partial blockage at the lycopene cyclization due to the presence of defective β -lycopene cyclase allele has been suggested in red grapefruit (Alquezar et al., 2013, 2009). In addition, a higher expression of key genes from the carotenoid MEP-derived precursors pathway was proposed in red orange Cara Cara (Alquezar et al., 2008). Lycopene presence is especially attractive in citrus fruit since it is not only an unusual feature but also provides additional health benefits and antioxidant activity (Di Mascio et al., 1989; Palozza et al., 2012; Rao et al., 2006), having a direct impact on citrus fruit quality and commercial value.

A direct correlation between the external coloration and the presence of carotenoid-derived apocarotenoid C30 (β -citraurin and δ - β -apocarotenal) has been established in orange and mandarin fruit. It has been demonstrated that these apocarotenoids are exclusive to the *Citrus* genus and their content is regulated by a carotenoid-cleavage activity which specifically directs accumulation in the peel and imparting the intense orange-reddish coloration of some varieties of orange, mandarin and hybrids thereof (Ma et al., 2013; Rodrigo et al., 2013b).

2.2.2. Polyphenols (*phenolic acids and flavonoids*)

Polyphenols have at least one aromatic ring with one or more hydroxyl groups attached and are classified as flavonoids and non-flavonoids (phenolic acids). There is a

growing acceptance that polyphenols in fruits and vegetables exert beneficial effects in the prevention of degenerative diseases (Del Rio et al., 2013; Zou et al., 2015).

Flavonoids are important secondary metabolites present at relatively high concentrations in citrus fruit, mainly as conjugated molecules (Khan et al., 2014). Flavonoids content increases during early stages of fruit development to decline at the cell enlargement phase (Ladaniya, 2008). Their concentration in fruits of different citrus species is variable (Table 1), depending on genetic, environmental and agronomic factors. Mandarins are especially rich in flavonoid in the pulp, ranging in five common varieties (Dancy, Beauty, Fairchild, Fizu and Suhugan) from 0.27 to 0.60 mg quercetin equivalents/g FW (Ramful et al., 2011). In other mandarin varieties, the levels of flavonoids vary from 0.11 mg/g FW in Ponkan and Murcott (Wang et al., 2007), 0.19-0.20 mg/g FW in Clementine (De Ancos et al., 2017), and 0.40 mg/g FW in Satsuma Owari mandarin (Ramfull et al., 2011), as well as in juice (10 mg/L in Afourer to up 42 mg/L in Nova mandarin) (Goldenberg et al., 2014). The flavonoid content in the pulp of oranges is slightly higher, accumulating around 0.65-0.75 mg/g FW (De Ancos et al., 2017), whereas levels as high as 2.0-2.4 mg/g FW have been described in the juice of Valencia and Navel sweet oranges (Nagota et al., 2006).

The most common flavonoid in the juice of oranges and mandarin fruit is the flavanone hesperidin, accounting 40-50 % of total content in oranges and 53-75 % in mandarins (Nogata et al., 2006; Sdiri et al., 2012a). Hesperidin can be found in the segment membrane of fresh fruits in the form of white spots crystals in freeze-damaged mandarins (Ladaniya, 2008). The pulp of white grapefruits contains high content of flavonoids (about 840 mg/100g FW), all which 70% is naringenin, followed by poncirin (14 %) and narirutin (11 %) (Nogata et al., 2006). Similarly, total flavonoids are also high in red-fleshed grapefruit (11.5 mg/g FW), mainly as naringenin (80 %) (Chaudhary

et al., 2014). The content in lemons and limes are lower, around 1.5 and 1.3 mg/g FW, respectively, predominating hesperidin and eriocitrin (Nogata et al., 2006). It is also noticeable that certain flavonoids are characteristic or particularly abundant in certain citrus species and this property has been used for taxonomical classification of varieties and as molecular markers for verification of fruit juice quality (Abad-García et al., 2014).

Regarding phenolic acids, a total content of 17-22 mg/g DW was described in the pulp of 14 different Chinese mandarin varieties, being chlorogenic and ferulic acid the main components (Xi et al., 2014; Xu et al., 2008a, b). However, total phenolics vary notably among mandarin species and varieties, and concentrations from 380 to 650 mg GA Eq/L (Gallic Acid Equivalents) have been reported (Al-juhaimi and Ghafoor, 2013; Goldenberg et al., 2014; Xu et al., 2008b). In lemon pulp, around 10-18 mg GAE/g DW have been detected, whereas Star Ruby grapefruit accumulated around 8-15 mg catechinE/g DW (Brito et al., 2014; Chaudhary et al., 2012). In Lane Late and Delta oranges, total phenols reached a maximum of 363 and 413 mg/L in December, respectively, slightly decreasing in mature fruit (January) to 330 and 360 mg/L, respectively (Emmanouilidou and Kyriacou, 2017). Total phenolic content (esterified, glycosides or ester-bound) is higher in the peel than in the pulp, and sharply decreased during fruit maturation but increase during postharvest cold storage (Xu et al., 2008b; Quian et al., 2014). In general, phenolic composition is very dependent on the fruit type and also on the growing environmental conditions.

2.2.3. *Limonoids and furocoumarins*

Limonoids, usually found as limonoid aglycones or limonoid glycosides, are triterpenoids present in members of the Rutaceae family, and limonin and nomilin being

the most common bitter-tasting components of citrus fruit (Sidana et al., 2013). Nomilin is the common precursor of all known limonoids in citrus. Several limonoids are extremely bitter at relatively low concentration which may result in consumer rejection. The physical disruption of juice sacs causes the transformation of a tasteless limonoid aglycone (limonate A-ring lactone) into the bitter limonin, a step that is catalyzed by the enzyme limonin D-ring lactone hydrolase at pH 6.5 or lower (Manners, 2007). Total limonoids increase until fruit maturation and decrease thereafter, but a high concentration is always maintained within seeds. The enzyme limonoid glucosyltransferase is responsible for the conversion of limonoid aglycones to glycosides during maturation, consequently reducing the bitterness in juice. Evidences indicate that citrus limonoids are biologically active displaying anticarcinogenic and hypocholesterolemic activity, insecticidal and anti-feedant activity against insects. However, the antioxidant capacity of these compounds is usually very low (Manners, 2007).

Citrus produce other defense compounds such as coumarins and furocoumarins as protection against herbivorous insects and pathogens infection. These compounds are more abundant in grapefruit, in both white and red varieties, being associated with typical bitterness and possible interferences with different pharmaceutical products (Girennavar et al., 2007), a problem known as the 'grapefruit juice effect' (Dugrand-Judek et al., 2015). The peel of citrus fruit contains higher and more diverse concentration of coumarins than the pulp and are more common in pummelo, citron and grapefruit, but can be also detected in sour orange, lemon and lime, whereas mandarins are almost devoid of them (Dugrand-Judek et al., 2015). Bergamottin and 6,7-dihydroxybergamottin may have reached levels as high as 25-90 µg/g DW in the pulp of

grapefruit at harvest (Chaudhary et al., 2014) but declined to 1.2-2.0 µg/mL in juice (Girennavar et al., 2008).

2.2.4. Volatiles terpenoids and norisoprenoids

Limonoids, furocoumarins and volatiles compounds have a direct impact on the sensory quality of citrus fruit, determining the acceptability by consumers (Goldenberg et al., 2014). Fruit and flavor sensations derive from the combination of taste and aroma. Taste is mainly determined by the juice balance between sugars and acids whereas fruit aroma is strongly influenced by the content and composition of aroma volatiles (Tietel et al., 2012).

Citrus oils are complex mixtures of volatile components, mainly terpenic hydrocarbons and oxygenates aldehyde, alcohol, ketone and ester derivatives. The enrichment of genes involved in the biosynthesis of volatile terpenoids and nonvolatile phenylpropanoids in epidermal cells indicates a metabolic specialization in this cell type for the synthesis of essential oils. Moreover, the expression pattern of most biosynthetic genes is consistent with the accumulation of the major essential oils (monoterpenes, prenylated coumarins, and polymethoxylated flavonoids) in the oil glands of the peel (Voo et al., 2012).

The citrus aroma is complex, comprising a heterogeneous mix of different volatiles, most of them belonging to the terpene family. In citrus fruit this group is composed of monoterpenes (C₁₀) and sesquiterpenes (C₁₅) and their derivatives (i.e. alcohols, esters, aldehydes and ketones) (Moshonas and Shaw, 1997; Miyazaki et al., 2011). During fruit maturation, important qualitative and quantitative changes are observed in the aromatic profile, being more abundant and diverse during early stages of fruit development and color change (Ladaniya, 2008). Limonene is the most abundant volatile compound in citrus fruit, reaching up to 90 % of total volatile content; other relevant compounds are

linalool, γ -terpinene, β -myrcene, α -pinene, valencene and octanal (Qiao et al. 2007). During maturation, the relative content of limonene increased from 20 to 87 %, while linalool decreased from 62 to 4.2 % as well as thymol, α - and β -pinene, α - and γ -terpinene and geraniol (Bourgou et al., 2012). A total of 38 volatile compounds were identified in the peel of various mandarin varieties, with most being monoterpenes (95% limonene), followed by β -myrcene, α -pinene, decanal, trans-ocimene and linalool (Simón-Grao et al., 2014). The sesquiterpene nootkatone is a typical compound found in grapefruit varieties and pummelo (Ortuño et al., 1995) and responsible for the typical flavor of these fruits. The volatile fraction has important uses in the flavor, fragrance, aromatherapy, and agro-chemical industries.

3. Endogenous factors affecting citrus quality

Many aspects of fruit quality are the result of complex interaction between hormonal and nutritional signals taking place at different stages of fruit development that are directly or indirectly under hormonal control. Understanding the physiological, biochemical and molecular basis regulating citrus fruit maturation and quality, and how endogenous and exogenous signals interact in the coordination of these processes is still unknown (Cercós et al., 2006; Zhang et al., 2012; Wu et al., 2014). The role and plant hormones, nutrients, and their interaction, as well as other environmental factors on external and internal fruit quality are summarized in the following sections.

3.1. Hormonal changes during fruit growth and their effect on fruit quality

3.1.1. Auxins

Due to their role on cell enlargement during stage II of fruit growth, auxins are closely associated with pulp quality. Pioneering work established high auxin-like activity in citrus ovaries and fruitlets during anthesis and fruit set, declining rapidly thereafter (Igoshi et al., 1971; Goren and Goldschmidt, 1970; Talon et al., 1990). However, a marked increase in Indol-3-Acetic Acid (IAA) concentration during stage II of fruit growth, closely associated with cell enlargement. In oranges, there was a transient increase in IAA in the central axis, suggesting a role of auxin in carbohydrate storage in the pulp (Kojima et al., 1994). Increases in the expression of indole-3-acetyl-alanine hydrolase gene in the pulp during this stage, suggested the release of auxin from conjugated forms as a mechanism of hormone availability during fruit flesh development (Cercós, 2006). Auxins have been proposed to induce acid invertase activity and then, sucrose hydrolysis to hexoses. This osmotic imbalance could promote passive water influx to result in cell expansion (Evans 1985). Carbohydrate accumulation induced by application of 3,5,6-trichloro-2-pyridyl-oxyacetic acid (3,5,6-TPA) in Satsuma mandarin, suggests that stimulation of fruit growth might operate via sink strength (Agustí et al., 2002).

The IAA-induced cell wall degradation is a prerequisite to promote cell enlargement and also up-regulate the expression of expansin genes during fruit growth (Catalá et al., 2000). In citrus fruit, increasing gene expression of an expansin homologue from the beginning of stage II has also been reported (Goudeau et al., 2008). Differences in the expansin gene expression patterns between albedo and flavedo have been related to the specific morphology of citrus rind (Kita et al., 2000). Considering this information, it is likely that auxins and expansins could operate in citrus fruit with a mechanism similar to that suggested for tomato.

3.1.2. *Gibberellins*

Gibberellin acid (GA) levels have been directly related to fruit-set and ovary growth, indeed, the seeded Pineapple orange, with higher GA concentrations, presented increased fruit set than the seedless Washington navel orange (Bermejo et al., 2016). Similarly, parthenocarpic ability has been positively associated with synthesis of GAs in the ovary (Talón et al. 1990; 1992; Bermejo et al., 2015; Mesejo et al., 2013; 2016). During the transition from fruit enlargement to maturation, an increase in GA₁ and GA₄ concentration was found in the flavedo of orange, prior to color break, decreasing as peel coloration progressed. Moreover, in peduncle-girdled fruits, which remained green, GA₁ and GA₄ levels remain high, suggesting that the presence of GA in the peel prevents the onset of fruit color change (Gambetta et al., 2012). In this sense, application of GAs is long recognized to delay chlorophyll degradation and carotenoid biosynthesis in citrus fruit (Lewis and Coggins, 1964; Agustí et al., 1981; García Luis et al., 1986; Alós et al., 2006; Gambetta et al., 2014). Moreover, foliar spray of prohexadione-Ca, a GA biosynthesis inhibitor, before color break, enhanced coloration in Navel orange and increased total carotenoid accumulation in the peel (Barry and van Wyk, 2004). Beside the effect of GAs delaying color changes in the peel, exogenous hormonal treatment has been also reported to reduce fruit drop and delay fruit softening and puffing, and these effects may be related to the anti-senescent effect of GA (Kawase et al., 1981; Ladaniya 2008). Moreover, different observations indicate that GAs may also affect internal fruit quality, delaying acidity loss, increase in SSC, juice content, and also reduced postharvest losses (Ladaniya, 2008; Rokaya et al., 2016). Whether these effects on internal quality may be indirect throughout the GA action on fruit senescence or are a direct GA influence remains to be determined.

The mode of GA's action in regulating peel coloration is not well understood. It was shown that GA₃ up-regulated the transcription of an *Mg*-chelatase gene, increasing chlorophyll synthesis (Fujii et al., 2008), whereas it down regulated a pheophorbide A oxygenase (*PaO*), reducing chlorophyll depletion (Alós et al., 2006). These results support the hypothesis proposed by Jacob-Wilk et al. (1999), that chlorophyll levels were determined by a balance between its synthesis and breakdown. In addition, most of the carotenoid biosynthetic genes are down-regulated by GA₃, reducing the flux through the β,β -branch, and thus slowing down the accumulation of downstream β,β -xanthophylls (Alós et al., 2006; Rodrigo and Zacarías, 2007; Fujii et al., 2008). However, the effects of GA₃ on internal quality are not conclusive. GA₃ does not affect juice percentage, TSS and acidity during fruit maturation, suggesting that action of GAs appears to be restricted to the peel (Coggins, 1969; Davies et al., 1999; Fidelibus et al., 2002).

3.1.3. Absciscic acid

ABA content in the peel of citrus fruit increased during maturation (Goldschmidt et al., 1973; Rodrigo et al., 2003), in accordance with the expression of genes involved in its biosynthesis by 9-cis-epoxycarotenoid dioxygenase (NCED) (Rodrigo et al., 2006; Agustí et al., 2007). Since this hormone is an end product of the carotenoid pathway, its involvement in the regulation of citrus peel coloration remains elusive (Rodrigo et al., 2013). Peduncle-girdled oranges remained greener compared to control fruit, but GA and ABA remained at high concentrations (Gambetta et al., 2012). Additionally, exogenous ABA did not promote color development (Iglesias et al., 2007), and norflurazon application, an inhibitor of PDS activity, did not affect chlorophyll degradation, although it partially blocked carotenoid biosynthesis, and thus transiently

reduced color intensity and ABA concentration in the flavedo (Gambetta et al., 2014). Moreover, it has been determined that in juice sacs cultured *in vitro*, ABA induced its own biosynthesis at the transcriptional level, and this feedback regulation of ABA led to a decrease in carotenoid content (Zhang et al., 2012). Pinalate is a mutant of Navelate orange with a particle blockage in the desaturation of ζ -carotene, and with reduced levels of ABA in both peel and pulp. The rate of fruit degreening was much lower in Pinalate than in the parental oranges, indicating that ABA may be involved in the regulation of fruit coloration (Rodrigo et al. 2003). Thus, the role of ABA in color break is still unknown, but it has been suggested that it would enhance tissue sensitivity to ethylene, as it has been demonstrated in climacteric fruit (Mou et al., 2016). Moreover, ethylene has been demonstrated to stimulate ABA accumulation and expression of the corresponding biosynthesis genes (Rodrigo et al., 2003; Fujii et al., 2007) and GA₃ is well known to counteract ABA content and action (Kurooka et al., 1977; Fujii et al., 2008), indicating a complex hormonal interaction in the regulation of ABA content during fruit maturation.

3.1.4. Ethylene

Citrus are longtime recognized as non-climacteric fruit, as respiration rate declines progressively during fruit ontogeny and mature fruit produce very low and constant amount of ethylene (Aharoni, 1968; Eaks, 1970). However, by particular and transient response of most citrus fruits to exogenous ethylene have been also referred to as pseudoclimacteric. Ethylene biosynthesis is also regulated in citrus fruits in a usual manner, since at early stages of fruit development, ethylene stimulates its own production in an autocatalytic-like system, whereas mature fruits responded as an auto-inhibitory system (Katz et al., 2009; Alos et al., 2013). These results confirm that

ethylene is able to stimulate a myriad of molecular responses in the peel of citrus fruits, but as a non-climacteric fruit, it requires the continuous presence of the hormone to sustain these physiological and molecular responses. Interestingly, molecular evidences indicate that the expression of ethylene receptor genes are coordinated with the sensitivity to the ethylene-induced peel degreening (John-Karuppiah and Burns, 2010; Alos et al., 2014).

The primary effect of ethylene on citrus fruits is the promotion of peel coloration by a concomitant dual action: degradation of chlorophylls (Purvis and Barmore, 198; Fujii et al., 2007) and stimulation of carotenoids pigments (Eilati et al., 1975; Rodrigo and Zacarias, 2007). Thus, application of ethylene is a common practice in many citrus producing-countries specially to stimulate rind coloration and to advance the marketing period in early harvested oranges and mandarin varieties (Porat et al., 2008). Exogenous ethylene increases chlorophyllase activity and gene expression (Trebitsh et al. 1993, Jacob-Wilk et al. 1999) as well as other genes involved in chlorophyll breakdown (Fujii et al., 2007; Alos et al., 2008; 2014). Ethylene also down-regulate chlorophyll biosynthesis, by repressing the gene expression of *Mg*-chelatase and most genes involved in photosynthesis and chloroplast biogenesis (Fujii et al., 2007). Moreover, ethylene stimulated the transcription of most carotenoid biosynthetic genes in the peel of citrus fruits, which is paralleled to the transformation from chloroplast to chromoplast and to the accumulation of xanthophylls and apocarotenoids in the peel of orange and mandarin fruit (Fujii et al., 2007; Rodrigo and Zacarias, 2007; Zhou et al., 2010; Rodrigo et al., 2013). Thus, ethylene accelerates molecular changes related to carotenoid biosynthesis, which naturally occur during citrus fruit maturation, suggesting that changes in the hormone sensitivity may be related to its action in natural fruit degreening (Carmona et al., 2012; Rodrigo et al., 2013).

Experimental evidences accumulated over the years in fruits of different varieties indicate that ethylene action, however, is not directly involved in the regulation the different physiological processes of internal maturation. Ethylene-degreening did not have a significant impact on juice, TSS and acidity in different varieties of mandarin and grapefruits, and had only minor effects on the contents and composition of juice aroma volatiles. This later effect is likely related to long or high-dose ethylene treatment that may be detrimental and overstimulate off-flavors (Mayuoni et al., 2011a). Moreover, degreening with ethylene did not affect the levels of other bioactive compounds (phenolics, carotenoids, flavonoids, ascorbic acid, coumarins), total antioxidant activity and aroma volatiles. Overall, these results indicate that ethylene doesn't affect internal quality, flavor and nutritional properties of citrus flesh, and appears not to be involved in the regulation of internal maturation (Mayuoni et al., 2011a; Chaudhary et al., 2012; Sdiri et al., 2012c). However, ethylene has been shown to modify transcriptomic changes in the pulp of mandarins fruits, activating several metabolic pathways, including secondary metabolism, gibberellins and auxin degradation, and also transcription factors and responses to biotic and abiotic stresses (Mayuoni et al., 2011b).

3.2. Effect of carbohydrates and nitrogen on citrus fruit quality

Carbohydrates are essential for fruit growth: during stage I they are the primary energy source to support cell division and thereafter they are stored in the fruit (Mehouachi et al., 1995). Exported products from leaves are primarily deposited in the pulp, even in periods of fast peel growth, while flavedo photosynthesis generates assimilates that remain almost entirely in this tissue (Yen and Koch, 1990). Molecular data suggest that in the middle and later stages of development, carbohydrate synthesis and catabolism is mostly down-regulated in the pulp, while sugar transport appears to

be rather operative (Cercós et al., 2006). Sink strength in citrus fruits has been associated to sucrose metabolizing enzymes; alkaline invertase and sucrose-phosphate synthase activities are mainly detected in the sink cells, while sucrose synthase activity appears to be associated with vascular bundles (Lowell et al., 1989; Tomlinson et al., 1991), indicating a role of these enzymes in the phloem loading and unloading processes (Nolte and Koch, 1993). Moreover, it has been suggested that thereafter, sucrose phosphate synthase activity may re-synthesize sucrose for further transport to vacuoles (Komatsu et al., 2002). A mechanism allowing direct incorporation of sucrose into the vacuole bypassing membrane transport by H⁺-sucrose symporters has been proposed (Etxeberria et al., 2005).

The physiological roles of organic acids accumulation in the pulp during the first half of stage II are not clear. However, it has been suggested that low pH could result in enhanced sink strength, increasing sugar accumulation (Hockema and Etxeberria, 2001). Branch girdling and defoliation to reduce leaf: fruit ratio after fruit set delayed maturation, reducing TSS in the pulp and increasing organic acids and carotenoid accumulation. Then, carbohydrates availability does not directly determine carotenoid synthesis, but carbohydrate starvation could promote carotenoid accumulation (Poiroux-Gonord et al., 2012). However, carbohydrate availability is one of the main factors controlling citrus peel color development. Soluble sugar concentration increased in flavedo since stage II of fruit growth (Huff, 1984; Fidelibus et al., 2008; Gambetta et al., 2012), and chlorophyll has been negatively correlated to sugar content in this tissue (Huff, 1984). Moreover, exogenous sucrose supplementation promotes peel degreening, through the increase in flavedo sucrose content and the reduction in nitrogen level (Huff, 1983; Iglesias et al., 2001). In maturing mandarin, sucrose translocation (rather than sucrose synthesis) has been proposed as the main mechanism responsible for the

636 maintenance of flavedo sucrose level (Holland et al., 1999). Indeed, while ethylene
637 treatment down-regulates transcription of most genes involved in photosynthesis,
638 chloroplast biogenesis and sugar metabolism, it also up-regulates a sucrose transporter
639 and acidic invertase genes (Fujii et al., 2007) and opposite effects have been reported
640 for GA₃ treatment (Fujii et al., 2008). Exogenous GA₃ reduces flavedo sugar
641 concentration without modifying sucrose:hexoses ratio, suggesting that color delay is
642 partially mediated by fructose and glucose levels (Fidelibus et al., 2008). Thus
643 carbohydrate-induced color break could be also mediated by the action of ethylene and
644 GAs.

645 Nitrogen involvement in peel color development has been studied for decades: late
646 or high doses of N delay color break (Jones and Embleton, 1959; Quiñones et al., 2004)
647 and increase the percentage of green fruits at harvest (Koo and Reese, 1977). On-tree
648 fruit regreening due to excessive N application was also reported. Moreover, N was the
649 only mineral necessary to regreen citrus epicarp discs *in vitro* and high N concentration
650 in the medium reduced sugar-induced chlorophyll degradation (Huff, 1983, 1984). In
651 addition, continuous nitrate applications to fruits previous to color break, delay flavedo
652 chlorophyll loss and slow down the reduction in lutein, neoxanthin and all violaxanthin,
653 maintaining a typical green tissue carotenoid profile (Alós et al., 2006). However,
654 information about N evolution in the flavedo during citrus fruit maturation is
655 conflicting, since no changes (Huff, 1984; Win et al., 2006) or reduction in total N
656 concentration in the same tissue (Iglesias et al., 2001; Gambetta et al., 2012) have been
657 reported.

658 A model integrating the complex interplay between hormonal and nutritional
659 changes has been proposed to explain the transformation during citrus fruit maturation
660 (Rodrigo et al., 2013). The model establishes that N reduction is an essential

requirement to initiate color break than would be followed by a sugar increment. The nutrient unbalance, with respect to previous developmental stages, would induce a concomitant reduction in GAs content together with an ABA increase. This alteration in GAs/ABA ratio may enhance flavedo sensitivity of ethylene action, allowing fruit to stimulate a series of molecular and physiological alterations in which chlorophyll breakdown and accumulation of carotenoid, and peel pigmentation are the most noticeable. It is worth noting that probably the environmental conditions, mainly temperature and light, display a direct impact and may be the triggers to modify the endogenous status that initiates the characteristic transformations of Citrus fruit quality.

4. Environmental and agronomic factors affecting citrus fruit quality

Fruit quality in *Citrus* is highly influenced by many environmental factors and production practices and, moreover, complex interrelationships may exist among many of them to determine the final quality of the fruit. Specific cultural practices may not always be successful in improving fruit quality deepening on the climatic conditions, nutritional status of the tree, water availability, among other factors. Special care should be taken under variable environmental conditions. Moreover, the control of these factors is difficult and probably constitutes the main limitation for the commercial management of citrus fruit quality.

4.1 Environmental factors

Climatic conditions are the most significant external factors affecting fruit quality. Optimum temperature regimes, relative humidity, rainfall, sunshine are essential not only for high productions but also for excellent fruit quality. Citrus species are thought to have originated from tropical climates but due to dispersion and agronomic

domestication most commercial citrus species have been progressively adapted to subtropical and Mediterranean-like climates (Wu et al., 2014). Under tropical conditions with steady temperatures and small variation in day/night temperatures, tree growth and development is fast but fruit quality is poor. Under these conditions, the rate of fruit respiration is high and carbohydrates accumulation is reduced, producing fruits with low sugar content. However, the decline in acid content is fast and the ratio TSS/TA increases rapidly and maturity is advanced, but fruits taste is insipid with very low acids and lacking of the typical citrus flavor. By contrast, Mediterranean climatic conditions are characterized by hot and dry summers, severe to mild winter, occasional frost, and wider differences between day/night temperatures. These environmental conditions reduce vegetative growth respect to tropical regions, but the rate of fruit respiration is lower and the maturation process delayed, producing fruits of improved eating quality, flavor and sensory acceptance (Ladaniya, 2008). It is noteworthy that the climate-induced effects on fruit quality cannot be totally compensated by the cultural practices and this may explain the differences in quality observed in fruits of the same species or variety growing under distinct climatic conditions (Reitz and Emlenton, 1986).

Climatic conditions differently affect the diverse processes related to fruit quality. It is well established that peel degreening in citrus fruit is highly dependent on variations of day/night temperatures. The rate of fruit coloration depends on the reduction and the length of time below a threshold temperature. In general, the conversion of chloroplast to chromoplast during fruit coloration is stimulated at temperatures below 13 °C and day temperatures above 20 °C delay the rate of degreening (Alquezar et al., 2008a). Soil temperature also appears to play a pivotal role in the control of peel coloration, as a positive relation between rind color break and soil temperatures below 20 °C has been observed. Moreover, an earlier reduction of soil temperature may also accelerate fruit

peel coloration changes and harvest time (Mesejo et al., 2012). As a rule, high diurnal temperatures with low day/night differences inhibit fruit degreening (both carotenoid accumulation and chlorophyll degradation), and produce greener fruit, a situation characteristic of tropical climatic conditions. Recently, it has been demonstrated that temperatures above 30 °C inhibit the expression of several carotenoid biosynthetic genes and chlorophyll degradation (Lafuente et al., 2017). By contrast, under Mediterranean condition with low night temperatures and high day/night temperature differences, peel of oranges and mandarin degree and develop bright orange coloration. The response of red grapefruit varieties to heat is an exception to this general effect of temperature, as fruit developed more intense red pigmentation (and lycopene accumulation) at temperatures above 30 °C (Alquezar et al., 2008a). The expression of the gene coding for the cyclization of lycopene to β -carotene is inhibited at temperatures higher than 30 °C and red grapefruit varieties (Star Ruby, Rio Red, among others) have the capability to accumulate lycopene in the peel and pulp (Rodrigo et al., 2013a).

Light exposure has an important influence on citrus fruit quality. Fruit exposed to the sun (located outside the tree canopy) develop brighter orange coloration than fruit non-exposed or partially shaded that are lighter (Cronje et al 2011, 2013); red grapefruit constitute an exception wherein shaded fruit registered higher carotenoid accumulation (Lado et al., 2015a). This general effect of the light appears to be directly related to changes in the expression of genes involved in carotenoid biosynthesis as well as with the process of chromoplast differentiation (Lado et al., 2015a). This effect appears to be dependent on the quantity and quality of the light, and under certain environmental conditions or geographical locations when fruit are exposed to excess of sunlight causing peel burning, partial covering of the tree may modulated to some extent incidence of the damage (Alquezar et al., 2008a). Beside the effect of sunlight on peel

color, fruit growing outside of the tree canopy had less acids, more TSS and advanced maturity, probably by a faster rate of fruit development than those non-exposed growing inside the canopy (Reitz and Emlenton, 1986). Glucose, fructose and sucrose contents were higher in the peel of sun exposed compared to shaded fruit inside the tree (Cronje et al., 2013). Similarly, the content of ascorbic acid in citrus fruits is also highly influenced by sunlight (Magwaza et al., 2017), as light avoidance has been reported to reduce ascorbic acid content in the peel (but not in the pulp) and suppress the expression of specific genes of the biosynthetic pathway (Lado et al., 2015d).

4.2 Agronomic factors

4.2.1. Irrigation effects

Appropriate water regimes are essential for good vegetative growth and development, fruit setting and fruit production. Citrus can be grown under different irrigation patterns and are moderately resistant to water shortage. It is well known that under water stress, fruit set is severely compromised, excessive fruit June drop may occur and final fruit production and fruit size is reduced (Reitz and Emlenton, 1986). Experimental evidences accumulated over the years in many citrus species and varieties indicated that, in general, fruit from irrigated trees had higher juice content but reduced TSS and total acidity than those from unirrigated trees. It is likely that these effects may be due to a dilution factor associated with the increased percentage of extractable juice. The reduction of juice content in water-stressed fruit is also associated with increasing peel thickness and in some instances with advanced peel degreening (Koo and Reese, 1977). The effect of water shortage on external coloration appears to be variable and dependent of the intensity and time of the stress, and in some instances not important differences in the rate of fruit degreening were observed (Grilo et al., 2017). It has been

also observed that partial root zone drying improved juice quality in Valencia orange fruits, since in addition to the effect on TSS and acidity, total flavonoids concentration was also increased (Grilo et al., 2017). Deficit irrigation strategy (40 and 29% reduction in water supply) in Fortune mandarin increased total soluble solids and acidity as well as juice proline content and improved postharvest behavior associated with a thicker skin whereas no differences were observed in fruit firmness, juice content and fruit color (Conesa et al., 2014). An increase in TSS as a result of water deficit applied during stage III of fruit growth was also reported (Pérez-Pérez et al., 2009), while stress applied at earlier stages may induce the opposite effect (Ginestar and Castel, 1996).

4.2.2. Grafting and rootstock-mediated effects

The selection of optimum rootstock is one in the most important decisions of the growers establishing new citrus tree plantations. Rootstocks have a well characterized effect on tree vigor, precocity, production, disease resistance, and various parameters of citrus fruit quality (Emmanouilidou and Kyriacou, 2017). Excellent and comprehensive reviews on the effects of citrus rootstock on fruit quality have been published (Castle, 1987, 1995; Wutscher, 1988). Rootstocks are usually categorized by the effects on vegetative development and disease resistance, but in general these parameters are positively related to fruit quality factors. The consensus concept of the effects on fruit quality established that fruits from varieties budded on vigorous rootstocks (rough lemon, Rangpur lime, *Citrus macrophylla*, Volkameriana, etc.) are larger, with a thicker peel, and with lower concentration of TSS and TA than fruits budded on less vigorous and slow-growing rootstock (as Citrange Carrizo or Cleopatra). A recent study comparing the effects of different rootstocks on the internal quality of Delta and Lane Late orange varieties, revealed lower titratable sugars, ascorbic acid and total phenols in

Lane Late orange grafted on Volkameriana than in Cleopatra. However, sour orange rootstock showed a higher total acidity (8.7 g/L) compared with Cleopatra (8.0 g/L), Volkameriana (7.6 g/L) and GouTou (7.5 g/L) rootstocks (Emmanouilidou and Kyriacou, 2017). The effect of the rootstock on external fruit color appears to be of minor magnitude but more vigorous rootstocks tend to re-green earlier than less vigorous (Wustcher, 1988). Similarly, a slight influence of the rootstock in fruit color have been also detected in Lane Late and Delta oranges (Emmanouilidou and Kyriacou, 2017).

It is interesting to note that in the juice market, high concentration of total soluble solids per tree or per hectare is required. To that end, rootstocks producing more fruits of lower quality may be more beneficial than less productive rootstocks. However, for the fresh fruit market the criterion for rootstock selection is different, and excellent quality fruits are required. How fruit quality is affected by the rootstock is not yet fully understood, but it is generally thought that water relations, mineral nutrition, hormonal contribution are key factors determining the influence of the rootstock on juice quality. A comparative study addressing the effect of different rootstocks on soluble solid content concluded that total production is a function of fruit yield and the distribution of fruit sizes is probably related to the ability of each rootstock for carbohydrate partitioning (Barry et al., 2004). The rootstock effect on the sensory quality of citrus fruits and on the emission of volatile compounds is a matter of recent interest. The interaction of the rootstock on the flavor perception is complex, and more aroma volatile, especially terpenes, were detected in fruits budded on more vigorous rootstocks. The emission of volatiles appears to be also influenced by the vigor of the rootstock and positively correlated with TSS and TA, but the flavor perception of the fruit is dependent on the specify rootstock/scion combinations (Benjamin et al., 2013).

4.2.3. Plant nutrition

Fertilization has a large influence on fruit quality and is usually the preferred technology of the growers to improve fruit quality. Plant nutrition constitutes a sustainable approach for modulating secondary metabolism and improving functional quality since modification of the cationic (K, Ca, Mg) or anionic (N, P, S) composition of the soil solution could impact the fruit final quality (reviewed in Kyriacou and Roupshael, 2017). The response of citrus fruits to N and K is quite rapid and affects fruit quality to different extents. The involvement of nitrogen on external color development and its interaction with carbohydrates and hormones has been examined in section 2.2, and in this section other effects of nitrogen on fruit quality are discussed (Reitz and Embleton, 1986). Nitrogen has large influence on fruit quality and there are evidences indicating that elevated levels of N fertilization negatively impact fruit quality. However, suboptimum levels of N fertilization have an adverse impact on yield, leading to a trade-off between quality and yield ((Reitz and Embleton, 1986). Application of N above a certain threshold produces small fruits, with thick and course peel, reduced juice content and delayed external fruit coloration. However, high N levels produces adverse effects such as a delay in peel degreening and the increase the proportion of green fruits with a lower carotenoid concentration in the peel at harvest together, and also increase the content in soluble solids, acidity and rind thickness (Ladaniya 2008). Lower ascorbic acid concentration has been also reported in pulp of fruits exposed to high N or P rates, whereas a high K fertilization was associated with increased vitamin C content. A reduction in ascorbic acid content has been also observed in fruits exposed to a lower light intensity located inside the tree canopy (reviewed in Magwaza et al., 2017). Micronutrients such as zinc or boron also influenced vitamin C content to

different extent depending species and variety (Magwaza et al., 2017). Modulation of N fertilization may also affect the fruit behavior during storage and, in general, fruit from high-nitrogen fertilized plants displayed a reduced storage potential (Ladaniya, 2008). Special care should be taken manipulating N fertilization on the field, since reduction of nitrogen levels in summer and fall may be beneficial for fruit quality at harvest. The timing and date of nitrogen application are important for fruit quality in most citrus species but only a limited effect has been observed in lemon trees (Reitz and Embleton, 1986).

An accurate K fertilization is consistent with the production of high quality citrus, since K effects on fruit quality are more important than its effects on yield. The application of higher amounts of K reduced the content ascorbic acid but increased the concentration of dehydroascorbic acid, therefore total concentration of vitamin C is not affected (Magwaza et al., 2017). However, other authors reported that fertilization with K and Zn in Kinnow mandarin during fruit growth determined a higher ascorbic acid content in the fruit whereas other quality factors (soluble solids, acidity, total phenols) were not affected at the applied doses (Nasir et al., 2016). Increasing K concentration has also important effects on fruit quality: increase in fruit size, peel thickness and juice content. Moreover, total acidity is also increased and consequently the ratio TSS/TA is reduced and fruit maturity is slightly delayed. It has been also observed K effect is dependent on fruit size (Koo and Reese, 1977). Although fertilization and different mineral elements may adversely affect citrus fruit quality, N and K are key minerals with a major influence in fruit quality development.

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862 **Acknowledgements**

863 The financial support of the research grants AGL-2015-70218R (Ministerio de
864 Economía, Industria y Competitividad, Spain) and PROMETEOII 2014/27 (Generalitat
865 Valenciana) is gratefully acknowledged.

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5. References

- Abad-García, B., Garmón-Lobato, S., Sánchez-Ilárduya, M.B., Berrueta, L. a., Gallo, B., Vicente, F., Alonso-Salces, R.M., 2014. Polyphenolic contents in citrus fruit juices: authenticity assessment. *Eur. Food Res. Technol.* 238, 803–818.
- Agustí, J., Zapater, M., Iglesias, D.J., Cercós, M., Tadeo, F.R., Talón, M. 2007. Differential expression of putative 9-cis-epoxycarotenoid dioxygenases and abscisic acid accumulation in water stressed vegetative and reproductive tissues of citrus. *Plant Sc.* 172, 85-94.
- Agustí, M., Almela, V., Guardiola, J.L., 1981. The regulation of fruit cropping in mandarins through the use of growth regulators. *Proc. Int. Soc. Citric.* 1, 216–220.
- Agustí, M.; Zaragoza, S.; Iglesias, D.J.; Almela, V.; Primo-Millo, E.; Talón, M. 2002. The synthetic auxin 3,5,6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. *J. Plant Growth Regul.* 36, 141–147.
- Aharoni, Y., 1968. Respiration of oranges and grapefruit harvested at different stages of development. *Plant Physiol.* 43, 99–102.
- Al-juhaimi, F.Y., Ghafoor, K., 2013. Bioactive compounds, antioxidant and physico-chemical properties of juice from lemon, mandarin and orange fruits cultivated in Saudi Arabia. *Pak. J. Bot.*, 45, 1193-1196
- Albertini, M.V., Carcouet, E., Pailly, O., Gambotti, C., Luro, F. And Berti, L. (2006) Changes in organic acids and sugars during early stages of development of acidic and acidless citrus fruit. *J. Agric. Food Chem.* 54, 8335-8339.
- Alós, E., Cercós, M., Rodrigo, M.J., Zacarias L., Talon, M., 2006. Regulation of color break in citrus fruits. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. *J. Agric. Food Chem.* 54:4888-4895.
- Alós, E., Roca, M., Iglesias, D.J., Mínguez-Mosquera, M.I., Damasceno, C.M., Thannhauser, T.W., Rose, J.K., Talón, M., Cercós, M, 2008. An evaluation of the basis and consequences of a stay-green mutation in the navel negra citrus mutant using transcriptomic and proteomic profiling and metabolite analysis. *Plant Physiology* 147, 1300–1315.
- Alós, E., Distefano, G., Rodrigo, M.J., Gentile, A., Zacarías, L. 2013. Altered sensitivity to ethylene in ‘Tardivo’, a late-ripening mutant of Clementine mandarin. *Physiol. Plant.* 147, 1300-1315.
- Alós, E., Rodrigo, M.J., Zacarías, L., 2014. Differential transcriptional regulation of l-ascorbic acid content in peel and pulp of citrus fruits during development and maturation. *Planta* 239, 1113-1128

Alquezar, B., Rodrigo, M.J., Lado, J., Zacarías, L., 2013. A comparative physiological and transcriptional study of carotenoid biosynthesis in white and red grapefruit (*Citrus paradisi* Macf.). *Tree Genet. Genomes* 9, 1257–1269.

Alquezar, B., Rodrigo, M.J., Zacarías, L., 2008a. Carotenoid biosynthesis and their regulation in citrus fruits. *Tree For. Sci. Biotechnol.* 2, 23–35.

Alquezar, B., Rodrigo, M.J., Zacarías, L., 2008b. Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochem.* 69, 1997–2007.

Alquezar, B., Zacarías, L., Rodrigo, M.J., 2009. Molecular and functional characterization of a novel chromoplast-specific lycopene beta-cyclase from citrus and its relation to lycopene accumulation. *J. Exp. Bot.* 60, 1783–97.

Aschoff, J.K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R., Schweiggert, R.M., 2015. In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices (*Citrus sinensis* (L.) Osbeck). *J. Agric. Food Chem.* 63, 578–587.

Bain, J.M. 1958. Morphological, anatomical and physiological changes in the developing fruit of the Valencia orange *Citrus sinensis* (L.) Osbeck. *Australian J. Bot.* 6, 1-24.

Barros, H.R.D.M., Ferreira, T.A.P.D.C., Genovese, M.I., 2012. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chem.* 134, 1892-1898.

Barry, G.H., van Wyk, A.A. 2004. Novel approaches to rind colour enhancement of citrus. *Proc. Int. Soc. Citric.* III, 1076-1079.

Barry, G.H., Castle, W.S. Davies, F.S. 2004. Soluble solids accumulation in Valencia sweet oranges as relate to rootstock selection and fruit size. *J. Amer. Soc. Hort. Sci.* 129, 594-598.

Beltran, F., Perez-Lopez, a. J., Lopez-Nicolas, J.M., Carbonell-Barrachina, a. a., 2008. Effects of Mandarin Cultivar on Quality of Mandarin Juice. *Food Sci. Technol. Int.* 14, 307–313.

Benjamin, G., Tietel, Z., Porat, R. 2013. Effects of the rootstock/scioncombination on the flavor of citrus fruits. *J. Agric. Food. Chem.* 61, 11286-11294.

Bermejo, A., Martínez-Alcántara, B., Martínez-Cuenca, M-R., Yuste, R., Mesejo, C, Reig, C., Agustí, M., Primo-Millo, E., Iglesias, D.J. 2016. Biosynthesis and contents of gibberellins in seeded and seedless sweet orange (*Citrus sinensis* L. Osbeck) cultivars. *J. Plant Growth Regul.* 35, 1036-1048.

Bermejo, A., Primo-Millo, E., Agusti, M., Mesejo, C., Reig, C. Iglesias D.J., 2015. Hormonal

933 profile in ovaries of mandarin varieties with differing reproductive behavior. J. Plant
934 Growth Regul. 34, 584-594

935 Bourgou, S., Rahali, Z.R., Ourghemmi, I., Tounsi, M.S., 2012. Changes of peel essential oil
936 composition of four Tunisian citrus during fruit maturation. The Scientific World Journal.
937 Article ID 528593, 10 pages

938 Brito, A., Ramirez, J., Areche, C., Sepúlveda, B., Simirgiotis, M., 2014. HPLC-UV-MS profiles
939 of phenolic compounds and antioxidant activity of fruits from three citrus species
940 consumed in northern Chile. Molecules 19, 17400–17421.

941 Catalá, C., Rose, J.K., Bennett, A.B., 2000. Auxin-regulated genes encoding cell wall-
942 modifying proteins are expressed during early tomato fruit growth. Plant Physiol. 22, 527-
943 534.

944 Carmona, L., Rodrigo, M.J., Zacarías, L., 2012. Exploring the involvement of ethylene in the
945 regulation of colour changes in citrus fruit. Acta Hort. 934, 879-886.

946 Castle, S.W. 1987. Citrus rootstock. In R.C. Rom and R.F. Carlson (Eds.). Rootstock for fruit
947 crops. Wiley, New York, pp 361-399.

948 Castle, S.W. 1995. Rootstock as a fruit quality factor in citrus and deciduous fruit crops. New
949 Zealand J. Crop Hort. Sci., 23, 383-394.

950 Cercós, M., Soler, G., Iglesias, D.J., Gadea, J., Forment, J., Talón, M., 2006. Global analysis of
951 gene expression during development and ripening of citrus fruit flesh. A proposed
952 mechanism for citric acid utilization. Plant Mol. Biol. 62, 513–527

953 Chaudhary, P., Jayaprakasha, G.K., Porat, R., Patil, B.S., 2012. Degreening and postharvest
954 storage influences “Star Ruby” grapefruit (*Citrus paradisi* Macf.) bioactive compounds.
955 Food Chem. 135, 1667–1675.

956 Chaudhary, P., Jayaprakasha, G.K., Porat, R., Patil, B.S. 2012. Degreening and postharvest
957 storage influences ‘Star Ruby’ grapefruit (*Citrus paradisi* Macf.) bioactive compounds.
958 Food Chem. 135, 1667-1675

959 Chaudhary, P.R., Jayaprakasha, G.K., Porat, R., Patil, B.S., 2014. Low temperature conditioning
960 reduces chilling injury while maintaining quality and certain bioactive compounds of “Star
961 Ruby” grapefruit. Food Chem. 153, 243-249.

962 Codoñer-Franch, P., Valls-Bellés, V., 2010. Citrus as functional foods. Curr. Top. Nutraceutical
963 Res. 8, 173–184.

964 Coggins, C.W. 1969. Gibberellin research on citrus rind aging problems. Proc. First Int. Citrus
965 Symp. 3, 1177-1185.

966 Conesa, M.R., García-Salinas, M.D., de la Rosa, J.M., Fernández-Trujillo, J.P., Domingo, R.,
967 Pérez-Pastor, A., 2014. Effects of deficit irrigation applied during fruit growth period of
968 late mandarin trees on harvest quality, cold storage and subsequent shelf-life. *Sci. Hortic.*
969 165, 344–351.

970 Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011. Postharvest rind breakdown of ‘Nules
971 Clementine’ mandarin is influenced by ethylene application, storage temperature and
972 storage duration. *Postharvest Biol. Technol.* 60, 192–201.

973 Cronje, P.J.R., Barry, G.H., Huysamer, M., 2013. Canopy position affects pigment expression
974 and accumulation of flavonoid carbohydrates of ‘Nules Clementine’ mandarin fruit, thereby
975 affecting rind condition. *J. Am. Soc. Hortic. Sci.* 138, 217–224.

976 Davies, F.S., Campbell, C.A., Zalman, G. 1999. Gibberellic acid application timing effects on
977 juice yield and peel quality of ‘Hamlin’ oranges. *Proc. Florida State Hortic. Soc.* 112, 22-
978 24.

979 De Ancos, B., Cilla, A., Barberá, R., Sánchez-Moreno, C., Cano, M.P., 2017. Influence of
980 orange cultivar and mandarin postharvest storage on polyphenols, ascorbic acid and
981 antioxidant activity during gastrointestinal digestion. *Food Chem.* 225, 114–124.

982 Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G., Crozier, A.,
983 2013. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of
984 protective effects against chronic diseases. *Antioxid. Redox Signal.* 18, 1818–92.

985 Dhuique-Mayer, C., Caris-Veyrat, C., Ollitrault, P., Curk, F., Amiot, M.-J., 2005. Varietal and
986 interspecific influence on micronutrient contents in citrus from the Mediterranean area. *J.*
987 *Agric. Food Chem.* 53, 2140–2145.

988 Di Mascio, P., Kaiser, S., Sies, H., 1989. Lycopene as the most efficient biological carotenoid
989 singlet oxygen quencher. *Arch. Biochem. Biophys.* 274, 532–538.

990 Di Mascio, P., Sundquist, A.R., Devasagayam, T.P.A., Sies, H., 1992. Assay of lycopene and
991 other carotenoids as singlet oxygen quenchers. *Methods Enzymol.* 213, 429–438.

992 Ding, Y., Chang, J., Ma, Q., Chen, L., Liu, S., Jin, S., Han, J., Xu, R., Zhu, A., Guo, J., Luo, Y.,
993 Xu, J., Xu, Q., Zeng, Y-L., Deng, X., Cheng, Y., 2015. Network analysis of postharvest
994 senescence process in citrus fruits revealed by transcriptomic and metabolomic profiling.
995 *Plant Physiol.*, 168, 357–376

996 Dugrand-Judek, A., Olry, A., Hehn, A., Costantino, G., Ollitrault, P., Froelicher, Y., Bourgaud,
997 F., 2015. The Distribution of Coumarins and Furanocoumarins in Citrus Species Closely
998 Matches Citrus Phylogeny and Reflects the Organization of Biosynthetic Pathways. *PLoS*
999 *One* 10, e0142757.

1000 Eaks, I.L., 1970. Respiratory response, ethylene production, and response to ethylene on citrus
1001 fruit during ontogeny. *Plant Physiol.* 45, 334–338.

1002 Eilati, S.K., Budowski, P., Monselise, S.P., 1975. Carotenoid changes in the Shamouti orange
1003 peel during chloroplast-chromoplast transformation on and off the tree. *J. Exp. Bot.* 26,
1004 624-632.

1005 Emmanouilidou, M.G., Kyriacou, M.C., 2017. Rootstock-modulated yield performance, fruit
1006 maturation and phytochemical quality of 'Lane Late' and 'Delta'sweet orange. *Sci. Hortic.*
1007 225, 112–121.

1008 Etxeberria, E., González, P., Pozueta-Romero, J. 2005. Sucrose transport into citrus juice cells.
1009 Evidence for an endocytic transport system. *J. Amer. Soc. Hortic. Sci.* 30, 269-274.

1010 Evans, M.L. 1985. The action of auxin on plant cell elongation. *Critical Rev. Plant Sci.* 2, 213–
1011 265.

1012 Fidelibus, M.W., Koch, K.E., Davies, F.S. 2008. Gibberellic acid alters sucrose, hexoses, and
1013 their gradients in peel tissues during color break delay in 'Hamlin' orange. *J. Amer. Soc.*
1014 *Hortic. Sci.* 133, 760-767

1015 Fujii, H., Shimada, T., Sugiyama, A., Nishikawa, F., Endo, T., Nakano, M., Ikoma, Y., Shimizu,
1016 T., Omura, M., 2007. Profiling ethylene-responsive genes in mature mandarin fruit using a
1017 citrus 22K oligoarray. *Plant Sci.* 173, 340–348.

1018 Fujii, H., Shimada, T., Sugiyama, A., Endo, T., Nishikawa, F., Nakano, M., Ikoma, Y., Shimizu,
1019 T., Omura, M., 2008. Profiling gibberellin (GA₃)-responsive genes in mature mandarin
1020 fruit using a citrus 22K oligoarray. *Sci. Hortic.* 116, 291-298.

1021 Gambetta, G., Martínez-Fuentes, A., Bentancur, O., Mesejo, C., Reig, C., Gravina, A., Agustí,
1022 M., 2012. Hormonal and nutritional changes in the flavedo regulating rind colour
1023 development in sweet orange [*Citrus sinensis* (L.) Osb.]. *J. Plant Growth Regul.* 31, 273-
1024 282.

1025 Gambetta, G., Mesejo, C., Martínez-Fuentes, A., Reig, C., Gravina, A., Agustí, M. 2014.
1026 Gibberellic acid and norflurazon affecting the time-course of flavedo pigment and abscisic
1027 acid content in 'Valencia' sweet orange. *Sci. Hortic.* 180, 94-101.

1028 García-Luis, A., Fornés, F., Guardiola, J.L., 1986. Effects of gibberellin A₃ and cito-quinins on
1029 natural and post-harvest, ethylene-induced pigmentation of Satsuma mandarin peel.
1030 *Physiol. Plant.* 68, 271-274.

1031 Ginestar, C., Castel, J.R., 1996. Utilización de dendrómetros como indicadores de estrés hídrico
1032 en mandarinos jóvenes regados por goteo. *Riegos y Drenajes XXI* 89,40-46.

- 1033 Girennavar, B., Jayaprakasha, G.K., Jadegoud, Y., Nagana Gowda, G. a, Patil, B.S., 2007.
1034 Radical scavenging and cytochrome P450 3A4 inhibitory activity of bergaptol and
1035 geranylecoumarin from grapefruit. *Bioorg. Med. Chem.* 15, 3684–91.
- 1036 Girennavar, B., Jayaprakasha, G.K., Mclin, S.E., Maxim, J., Yoo, K.S., Patil, B.S., 2008.
1037 Influence of electron-beam irradiation on bioactive compounds in grapefruits (*Citrus*
1038 *paradisi* Macf.). *J. Agric. Food Chem.* 56, 10941–10946.
- 1039 Goldenberg, L., Yaniv, Y., Kaplunov, T., Doron-Faigenboim, A., Porat, R., Carmi, N., 2014.
1040 Genetic diversity among mandarins in fruit-quality traits. *J. Agric. Food Chem.* 62, 4938–
1041 4946.
- 1042 Goldschmidt, E.E., Goren, R., Even-Chen, Z., Bittner, S., 1973. Increase in free and bound
1043 abscisic acid during natural and ethylene-induced senescence of citrus fruit peel. *Plant*
1044 *Physiol.* 51, 879-882.
- 1045 Goldschmidt, E.E., Huberman, M., Goren, R., 1993. Probing the role of endogenous ethylene in
1046 the degreening of citrus fruit with ethylene antagonist. *J. Plant Growth Regul.* 12, 325-329.
- 1047 Goren, R., Goldschmidt, E.E., 1970. Regulative systems in the developing citrus fruit. I. The
1048 hormonal balance in orange fruit tissues. *Physiol. Plant.* 23, 937-947.
- 1049 Goudeau, D., Uratsu, S.L., Inoue, K., Goes da Silva, F., Leslie, A., Cook, D., Reagan, R.L.,
1050 Dandekar, A.M. 2008. Tuning the orchestra: Selective gene regulation and orange fruit
1051 quality. *Plant Sci.* 174, 310-320
- 1052 Goulas, V., Manganaris, G.A., 2012. Exploring the phytochemical content and the antioxidant
1053 potential of citrus fruits grown in Cyprus. *Food Chem.* 131, 39–47.
- 1054 Grilo, F.S., Di Stefano, V, Lo Bianco, R., 2017. Deficit irrigation and maturation stage
1055 influence quality and flavonoid composition of ‘Valencia’ orange fruit. *J. Sci. Food Agric.*
1056 97, 904–1909.
- 1057 Gurak, P.D., Mercadante, A.Z., González-Miret, M.L., Heredia, F.J., Meléndez-Martínez, A.J.,
1058 2014. Changes in antioxidant capacity and colour associated with the formation of β -
1059 carotene epoxides and oxidative cleavage derivatives. *Food Chem.* 147, 160–169.
- 1060 Hockema, B.R., Etxeberria, E. 2001. Metabolic contributors to drought-enhanced accumulation
1061 of sugars and acids in oranges. *J. Amer. Soc. Hortic. Sci.* 126, 599-605.
- 1062 Holland, N., Sala, J.M., Menezes, H.C., Lafuente, M.T. 1999. Carbohydrate content and
1063 metabolism as related to maturity and chilling sensitivity of cv. Fortune mandarins. *J.*
1064 *Agric. Food Chem.* 47, 2513-2518.
- 1065 Huff, A. 1983. Nutritional control of regreening and degreening in citrus peel segments. *Plant*

1066 Physiol. 73, 243-249.

1067 Huff, A. 1984. Sugar regulation of plastid interconversions in the epicarp of citrus fruit. Plant
1068 Physiol. 76, 307-312.

1069 Iglesias, D.J., Cercós, M., Colmenero-Flores, J.M., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz-
1070 Rivero, O., Lliso, I., Morillon, R., Tadeo, F.R., Talón, M., 2007. Physiology of citrus
1071 fruiting. Braz. J. Plant Physiol. 19, 333–362.

1072 Iglesias, D.J., Tadeo, F.R., Legaz, F., Primo-Millo, E., Talon, M., 2001. In vivo sucrose
1073 stimulation of colour change in citrus fruit epicarps: interactions between nutritional and
1074 hormonal signals. Physiol. Plant. 112, 244–250.

1075 Igoshi, M., I.; Yamaguchi, N.; Takahashi; Hirose, K. 1971. Plant growth substances in the
1076 young fruit of *Citrus unshiu*. Agric. Biol. Chem. 35, 629-631.

1077 Ikoma, Y., Matsumoto, H., Kato, M., 2016. Diversity in the carotenoid profiles and the
1078 expression of genes related to carotenoid accumulation among citrus genotypes. Breed. Sci.
1079 66, 139–147.

1080 Islam, M.Z., Hu, X., Jin, L., Liu, Y., 2014. Genome-wide identification and expression profile
1081 analysis of citrus sucrose Synthase genes : investigation of possible roles in the regulation
1082 of sugar accumulation. PLoS One 1–16.

1083 Jacob-Wilk, D., Holland, D., Goldschmidt, E.E., Riov, J., Eyal, Y., 1999.
1084 Chlorophyllbreakdown by chlorophyllase: isolation and functional expression of the
1085 Chlase1 gene from ethylene-treated citrus fruit and its regulation during development. Plant
1086 J. 20, 653–661.

1087 Jones, W.W., Embleton, T.W., 1959. The visual effect of nitrogen nutrition on fruit quality of
1088 ‘Valencia’ oranges. Proc. Amer. Soc. Hort. Sci. 73, 234-236.

1089 John-Karuppiah, K., Burns, J.K., 2010. Degreening behavior in ‘Fallglo’ and ‘Lee×Orlando’ is
1090 correlated with differential expression of ethylene signaling and biosynthesis genes.
1091 Postharvest Biol. Technol. 58, 185–193.

1092 Kato, M., 2012. Mechanism of carotenoid accumulation in citrus fruit. J. Japan. Soc. Hortic.
1093 Sci. 81, 219–233.

1094 Katz, E., Hwan-Boo, K., Kim, O.Y., Eigenheer, R.A., Phinney B.S., Shulaev, V., Negre-
1095 Zakharov, F., Sadka, A., Blumwald, E., 2011. Label-free shotgun proteomics and
1096 metabolite analysis reveal a significant metabolic shift during citrus fruit development. J.
1097 Exp. Bot. 62, 5367- 5384

1098 Katz, E., Martínez-Lagunes, P., Riov, J., Weiss, D., Goldschmidt, E.E., 2004. Molecular and

1099 physiological evidence suggests the existence of a system II-like pathway of ethylene
1100 production in non-climacteric citrus fruit. *Planta* 219, 243-252.

1101 Kawase, K., Susuki, K. and Hirose, K., 1981. Use of growth regulators to control rind puffing of
1102 Satsuma mandarin fruit. *Proc. Int. Soc. Citric.* 1, 237-239

1103 Khan, M.K., Zill-E-Huma, Dangles, O., 2014. A comprehensive review on flavanones, the
1104 major citrus polyphenols. *J. Food Compos. Anal.* 33, 85–104.

1105 Kita, M., Hisada, S., Endo-Inagaki, T., Omura, M., Moriguchi, T., 2000. Changes in the levels
1106 of mRNAs for putative cell growth-related genes in the albedo and flavedo during citrus
1107 fruit development. *Plant Cell Rep.* 19, 582–587

1108 Koch, K., 2004. Sucrose metabolism: Regulatory mechanisms and pivotal roles in sugar sensing
1109 and plant development. *Curr. Opin. Plant Biol.* 7, 235–246.

1110 Kojima, K., Yamada, Y., Yamamoto, M., 1994. Distribution of ABA and IAA within a
1111 developing Valencia orange fruit and its parts. *J. Japan Soc. Hortic. Sci.* 63, 335-339.

1112 Komatsu, A., Moriguchi, T., Koyama, K., Omura, M., Akihama, T., 2002. Analysis of sucrose
1113 synthase genes in citrus suggests different roles and phylogenetic relationships. *J. Exp. Bot.*
1114 53, 61-71.

1115 Koo, R.C.J., Reese, R.L., 1977. Influence of nitrogen, potassium, and irrigation on citrus fruit
1116 quality. *Proc. Int. Soc. Citric.* 1, 34-38.

1117 Kuraoka, T., Iwasaki, K., Ishii, T., 1977. Effects of GA₃ on puffing and levels of GA₃-like
1118 substances and ABA in the peel of Satsuma mandarin (*Citrus unshiu* Marc.). *J. Amer. Soc.*
1119 *Hortic. Sci.* 102, 651-654.

1120 Kyriacou, M.C., Rouphael, Y., 2017. Towards a new definition of quality for fresh fruits and
1121 vegetables. *Sci. Hortic.* In press.

1122 Ladaniya, M.S., 2008. Citrus fruit, Biology, Technology and Evaluation. Ladaniya M.S.
1123 (Editor). USA: Academic Press.

1124 Lado, J., Rodrigo, M.J., Zacarías, L., 2014. Maturity indicators and citrus fruit quality. *Stewart*
1125 *Postharvest Rev.* 2, 1–6.

1126 Lado, J., Cronje, P., Alquezar, B., Page, A., Manzi, M., Gómez-Cadenas, A., Stead, A.D.,
1127 Zacarías, L., Rodrigo, M.J., 2015a. Fruit shading enhances peel color, carotenes
1128 accumulation and chromoplast differentiation in red grapefruit. *Physiol. Plant.* 154, 469–
1129 484.

1130 Lado, J., Pascual, M., Zacarías, L., Rodrigo, M.J., 2015b. Effect of storage temperature on
1131 carotenoid and vitamin C content in red grapefruit. *Agrociencia Uruguay* 19, 48–56.

1132 Lado, J., Zacarías, L., Gurrea, A., Page, A., Stead, A.D., Rodrigo, M.J., 2015c. Exploring the
1133 diversity in Citrus fruit colouration to decipher the relationship between plastid
1134 ultrastructure and carotenoid composition. *Planta* 242, 645–661.

1135 Lado, J., Alos, E., Rodrigo, M.J., Zacarias, L. 2015d. Light avoidance reduces ascorbic acid
1136 accumulation in the peel of citrus fruit. *Plant Sci.* 231, 138-147

1137 Lado, J., Cuellar, F., Rodrigo, M.J., Zacarías, L., 2016a. Nutritional Composition of Mandarins,
1138 In: Simmonds, M.S.J., Preedy, V.R. (Eds.), *Nutritional Composition of Fruit Cultivars*.
1139 Academic Press, pp. 419–443.

1140 Lafuente, M.T., Establés-Ortíz, E., González-Candelas, L. 2017. Insights into the molecular
1141 events that regulate heat-induced chilling tolerance in citrus fruits. *Frontier Plant Sci.* 8.

1142 Lee, H.S., 2001. Characterization of carotenoids in juice of red navel orange (Cara Cara). *J.*
1143 *Agric. Food Chem.* 49, 2563–8.

1144 Lewis, L.N., Coggins, C.W., 1964. The inhibition of carotenoid accumulation in navel oranges
1145 by gibberellin A3, as measured by thin layer chromatography. *Plant and Cell Physiol.* 5,
1146 457–463.

1147 Liu, Q., Xu, J., Liu, Y., Zhao, X., Deng, X., Guo, L., Gu, J., 2007. A novel bud mutation that
1148 confers abnormal patterns of lycopene accumulation in sweet orange fruit (*Citrus sinensis*
1149 L. Osbeck). *J. Exp. Bot.* 58, 4161-4171.

1150 Liu, Y., Heying, E., Tanumihardjo, S.A., 2012. History, Global Distribution, and Nutritional
1151 Importance of Citrus Fruits. *Compr. Rev. Food Sci. Food Saf.* 11, 530-545.

1152 Lowell, C.A., Tomlinson, P.T., Koch, K.E., 1989. Sucrose metabolizing enzymes in transport
1153 tissues and adjacent sink structures in developing citrus fruit. *Plant Physiol.* 90, 1394-1402.

1154 Ma, G., Zhang, L., Matsuta, A., Matsutani, K., Yamawaki, K., Yahata, M., Wahyudi, A.,
1155 Motohashi, R., Kato, M., 2013. Enzymatic Formation of β -Citraurin from β -Cryptoxanthin
1156 and Zeaxanthin by Carotenoid Cleavage Dioxygenase4 in the Flavedo of citrus Fruit. *Plant*
1157 *Physiol.* 163, 682–95.

1158 Magwaza, S.L., Mditshwa, A., Tesfay, Z.S., Opara, L.U., 2017. An overview of preharvest
1159 factors affecting vitamin C content of citrus fruit. *Sci. Hortic.* 216, 12–21.

1160 Manera, F.J., Brotons, J.M., Conesa, A., Porras, I., 2012. Influence of temperature on the
1161 beginning of degreening in lemon peel. *Sci. Hortic.* 145, 34–38.

1162 Manners, G.D., 2007. Citrus limonoids: analysis, bioactivity, and biomedical prospects. *J.*
1163 *Agric. Food Chem.* 55, 8285–8294.

1164 Martí, N., Mena, P., Cánovas, J.A., Micol, V., Saura, D., 2009. Vitamin C the role of citrus

1165 juices as functional food. Nat. Prod. Commun. 4, 677–700.

1166 Matsumoto, H., Ikoma, Y., Kato, M., Nakajima, N., Hasegawa, Y., 2009. Effect of postharvest
1167 temperature and ethylene on carotenoid accumulation in the flavedo and juice sacs of
1168 Satsuma mandarin (*Citrus unshiu* Marc.) fruit. J. Agric. Food Chem. 57, 4724–4732.

1169 Mayuoni, L., Tietel, Z., Patil, B.S., Porat, R. 2011a. Does ethylene degreening affect internal
1170 quality of citrus fruit?. Postharvest Biol. Technol. 62, 50-58.

1171 Mayuoni, L., Sharabi-Schwager, M., Feldmesser, E., Porat, R., 2011b. Effects of ethylene
1172 degreening on the transcriptome of mandarin flesh. Postharvest Biol. Technol. 60, 75–82

1173 Mehouachi, J., Serna, D., Zaragoza, S., Agustí M., Talón, M., Primo-Millo, E., 1995.
1174 Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits
1175 and woody tissues of *Citrus unshiu*. Plant Science 107, 189-197.

1176 Mesejo, C., Gambeta, G., Gravina, A., Martínez-Fuentes, A., Reig, C., Agustí, M. 2012.
1177 Relationship between soil temperature and fruit colour development of ‘Clemenpons’
1178 Clementine mandarin (*Citrus clementina* Hort ex. Tan). J. Sci. Food Agric. 92: 520-525.

1179 Mesejo, C., Yuste, R., Martínez-Fuentes, A., Reig, C., Iglesias, D.J., Primo-Millo, E., Agustí,
1180 M., 2013. Self-pollination and parthenocarpic ability in developing ovaries of self-
1181 incompatible Clementine mandarins (*Citrus clementina*). Physiol. Plant. 148, 87–96.

1182 Mesejo, C., Yuste, R., Reig, C., Martinez-Fuentes, A., Iglesias, D.J., Muñoz-Fambuena, N.,
1183 Bermejo, A., Germana, M.A., Primo-Millo, E., Agustí M., 2016. Gibberellin reactivates
1184 and maintains ovary-wall cell division causing fruit set in parthenocarpic citrus species.
1185 Plant Sci. 247:13-24

1186 Miyazaki, T., Plotto, A., Goodner, K., Gmitter, F.G., 2011. Distribution of aroma volatile
1187 compounds in tangerine hybrids and propose inheritance. J. Sc. Food Agric. 91, 449-460.

1188 Monselise, S.P. 1977. Citrus fruit development, endogenous systems, and external regulation.
1189 Proc. Inter. Soc. Citric. 2, 664-668.

1190 Moshonas, M.G., Shaw, P.E., 1997. Quantitation of volatile constituents in mandarin juice and
1191 its use for comparison with orange juices by multivariate analysis. J. Agric. Food Chem.
1192 45: 3968-3972.

1193 Mou, W., Li, D., Bu, J., Jiang, Y., Khan, Z.U., Luo, Z., Mao, L., Ying, T. 2016. Comprehensive
1194 analysis of ABA effects on ethylene biosynthesis and signaling during tomato fruit
1195 ripening. PlosOne 10.1377.1-30

1196 Nasir, M., Khan, A.S., Basra, S.M.A., Malik, A.U., 2016. Foliar application of moringa leaf
1197 extract, potassium and zinc influence yield and fruit quality of ‘Kinnow’ mandarin. Sci.

- 1198 Hort. 210, 227–235.
- 1199 Nishikawa, K., Okabayashi, H., Mitiku, S.B., Sawamura, M., 2002. Bitter and volatile
1200 compounds in ethylene-treated *Citrus grandis* [L.] Osbeck fruit. J. Jap. Soc. Hort. Sci. 71,
1201 292-296.
- 1202 Nogata, Y., Sakamoto, K., Shiratsuchi, H., Ishii, T., Yano, M., Ohta, H., 2006. Flavonoid
1203 composition of fruit tissues of citrus species. Biosc. Biotechnol. Biochem. 70, 178–192.
- 1204 Nolte, K.D., Koch, K.E., 1993. Companion cell specific localization of sucrose synthase in
1205 zones of phloem loading and unloading. Plant Physiol. 101, 899-905.
- 1206 Ortuño, A., García-Puig, D., Fuster, M.D., Pérez, M.L., Sabater, F., Porras, I., García-Lidón, A.,
1207 Del Río, J.A., 1995. Flavannone and nootkatone levels in different varieties of grapefruit
1208 and pummelo. J. Agric. Food Chem. 43, 1-5.
- 1209 Palozza, P., Catalano, A., Simone, R., Cittadini, A., 2012. Lycopene as a guardian of redox
1210 signalling. Acta Biochim. Pol. 59, 21-5.
- 1211 Pérez-Pérez, J.G., Robles, J.M., Botía, P., 2009. Influence of deficit irrigation in phase III of
1212 fruit growth on fruit quality in ‘lane late’ sweet orange. Agric. Water Manage. 96, 969-974.
- 1213 Poiroux-Gonord, F., Fanciullino, A.L., Bert, L., Urban, L., 2012. Effect of fruit load on maturity
1214 and carotenoid content of Clementine (*Citrus clementina* Hort. ex Tan.) fruits. J.Sci. Food
1215 Agric. 92, 2076–2083.
- 1216 Porat, R., 2008. Degreening of citrus fruit. Tree Forest. Sci. Biotech. 2, 71–76.
- 1217 Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., Droby, S., 1999. Effects of ethylene and 1-
1218 methylcyclopropene on the postharvest qualities of ‘Shamouti’ oranges. Postharvest Biol.
1219 Technol. 15, 155–163.
- 1220 Porras, I., Brotons, J.M., Conesa, A., Manera, F.J., 2014. Influence of temperature and net
1221 radiation on the natural degreening process of grapefruit (*Citrus paradisi* Macf.) cultivars
1222 Rio Red and Star Ruby. Sci. Hort. 173, 45-53.
- 1223 Purvis, A.C., Barmore, C.R., 1981. Involvement of ethylene in chlorophyll degradation in peel
1224 of citrus fruits. Plant Physiol. 68, 854–856.
- 1225 Qian, Z., Wang, H., Liu, T., Jia, Y., Prasad, K.N., Qu, H., Duan, X., Jiang, Y., 2014. Changes in
1226 quality attributes of mandarin with and without leaf during refrigerated storage. J. Food
1227 Process. Preserv. 38, 11–20.
- 1228 Qiao, Y., Xie, B.J., Zhang, Y., Zhou, H.Y., Pan, S.Y., 2007. Study on aroma components in
1229 fruit from three different Satsuma mandarin varieties. Agric. Sc. China 6, 1487-1493.

- 1230 Qin, Y., Li, G., Wang, L., Teixeira Da Silva, J.A., Ye, Z., Feng, Q., Hu, G., 2015. A
1231 comparative study between a late-ripening mutant of citrus and its original line in fruit
1232 coloration, sugar and acid metabolism at all fruit maturation stage. *Fruits* 70, 5-11
- 1233 Quiñones, A., González, M.C., Montaña, C., Primo-Millo, E., Legaz, F., 2004. Fate and uptake
1234 efficiency of ¹⁵N applied with different seasonal distributions in citrus trees. *Proc. Inter.*
1235 *Soc. Citric.* 2, 587-592.
- 1236 Ramful, D., Tarnus, E., Aruoma, O.I., Bourdon, E., Bahorun, T., 2011. Polyphenol composition,
1237 vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Res. Int.*
1238 44, 2088-2099.
- 1239 Rao, V, Ray, M.R., Rao, L.G., 2006. Lycopene. *Adv. Food Nutr. Res.* 51, 99-164.
- 1240 Reitz, H.J. Embleton T.W. 1986. Production practices that influence fresh fruit quality. In
1241 “Fresh Citrus Fruits”, W. F. Wardowski, W. M. Miller, D. J. Hall and W. Grierson (Eds.),
1242 Avi Pub. 49-77
- 1243 Rivas, F., Laxage, J., Suarez, D., Menes, R., Spina, M., Luque, E., Pintos, P., Varela, P.,
1244 Vignale, B., 2013. Mejoramiento genético de cítricos: nuevas alternativas promisorias en
1245 mandarinas. *Rev. INIA* 33, 44–48.
- 1246 Rodrigo, M.J., Marcos, J.F., Alférez, F., Mallent, D., Zacarías, L., 2003. Characterization of
1247 Pinalate, a novel *Citrus sinensis* mutant with a fruit-specific alteration that results in yellow
1248 pigmentation and decreased ABA content. *J. Exp. Bot.* 54, 727–738.
- 1249 Rodrigo, M.J., Marcos, J.F., Zacarías, L., 2004. Biochemical and molecular analysis of
1250 carotenoid biosynthesis in flavedo of orange (*Citrus sinensis* L.) during fruit development
1251 and maturation. *J. Agric. Food Chem.* 52, 6724–31.
- 1252 Rodrigo, M.J., Alquezar, B., Zacarías, L., 2006. Cloning and characterization of two 9-cis-
1253 epoxycarotene dioxygenase genes, differentially regulated during fruit maturation and
1254 under stress conditions, from orange (*Citrus sinensis* L. Osbeck). *J. Exp. Bot.* 57, 633-643.
- 1255 Rodrigo, M.J., Zacarías, L., 2007. Effect of postharvest ethylene treatment on carotenoid
1256 accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange
1257 (*Citrus sinensis* L. Osbeck) fruit. *Postharvest Biol. Technol.* 43, 14-22.
- 1258 Rodrigo, M.J., Alquézar, B., Alós, E., Lado, J., Zacarías, L., 2013a. Biochemical bases and
1259 molecular regulation of pigmentation in the peel of citrus fruit. *Sci. Hortic.* 163, 46-62
- 1260 Rodrigo, M.J., Alquézar, B., Alós, E., Medina, V., Carmona, L., Bruno, M., Al-Babili, S.,
1261 Zacarías, L., 2013b. A novel carotenoid cleavage activity involved in the biosynthesis of
1262 citrus fruit-specific apocarotenoid pigments. *J. Exp. Bot.* 64, 4461-4478.

1263 Rokaya, P.R., Baral D.R., Gautam, D.M., Shrestha, A.K., Paudyal, K.P., 2016. Effect of pre-
 1264 harvest application of gibberellic acid on fruit quality and shelf life of mandarin (*Citrus*
 1265 *reticulata* Blanco). Amer. J. Plant Sci. 7, 1033-1039

1266 Sadka, A., Dahan, E., Cohen, L. and Marsh, K. B., 2000. Aconitase activity and expression
 1267 during the development of lemon fruit. Physiol. Plant. 108, 255-262.

1268 Sdiri, S., Bermejo, A., Aleza, P., Navarro, P., Salvador, A., 2012a. Phenolic composition,
 1269 organic acids, sugars, vitamin C and antioxidant activity in the juice of two new triploid
 1270 late-season mandarins. Food Res. Int. 49, 462–468.

1271 Sdiri, S., Navarro, P., Monterde, A., Benabda, J., Salvador, A., 2012b. Effect of postharvest
 1272 degreening followed by a cold-quarantine treatment on vitamin C, phenolic compounds and
 1273 antioxidant activity of early-season citrus fruit. Postharvest Biol. Technol. 65, 13-21

1274 Sdiri,S., Navarro, P., Monterde, A., Benabda, J., Salvador, A., 2012c. New degreening
 1275 treatments to improve the quality of citrus fruit combining different periods with and
 1276 without ethylene exposure. Postharvest Biol. Technol. 63, 25–32

1277 Shimada, T., Nakano, R., Shulaev, V., Sadka, A., Blumwald, E., 2006. Vacuolar citrate/H⁺
 1278 symporter of citrus juice cells. Planta 224, 472-480.

1279 Sidana, J., Saini, V., Dahiya, S., Nain, P., Bala, S., 2013. A review on citrus-“The boon of
 1280 nature”. Int. J. Pharm. Sci. Rev. Res. 18, 20–27.

1281 Simón-Grao, S., Gimeno, V., Simón, I., Lidón, V., Nieves, M., Balal, R.M., Carbonell-
 1282 Barrachina, A.A., Manera, F.J., Hernández, F., García-Sánchez, F., 2014. Fruit quality
 1283 characterization of eleven commercial mandarin cultivars in Spain. Sci. Hortic. 165, 274-
 1284 280

1285 Tadeo, F.R., Cerco, M., Flores, M.C., Iglesias, D.J., Naranjo, M.A., Ri, G., Morillon, L.,
 1286 Ollitrault, P., Talon, M., 2008. Molecular physiology of development and quality of Citrus.
 1287 Adv. Bot. Res. 47, 147-223.

1288 Talón, M., Zacarías, L., Primo-Millo, E. 1990. Hormonal changes associated with fruit set and
 1289 development in mandarins differing in their parthenocarpic ability. Physiol. Plant. 79, 400-
 1290 406.

1291 Talón, M., Zacarías, L., Primo-Millo, E. 1992. Gibberellins and parthenocarpic ability in
 1292 developing ovaries of seedless mandarins. Plant Physiol. 99, 1575-1581

1293 Terol, J., Soler, G., Talon, M. and Cercos, M., 2010. The aconitase hydrolase family from
 1294 Citrus. BMC Plant Biol. 10, 222.

1295 Tietel, Z., Lewinsohn, E., Fallik, E., Porat, R., 2012. Importance of storage temperatures in

1296 maintaining flavor and quality of mandarins. *Postharvest Biol. Technol.* 64, 175–182

1297 Tomlinson, P.T., Duke, E.R., Nolte, K.D., Koch, K.E., 1991. Sucrose synthase and invertase in
1298 isolated vascular bundles. *Plant Physiol.* 97, 1249-1252.

1299 Trebitsh, T., Goldschmidt, E.E., Riov, J., 1993. Ethylene induces de novo synthesis of
1300 chlorophyllase, a chlorophyll degrading enzyme, in citrus fruit peel. *Proc. Nat. Acad. Sci.*
1301 USA 90, 9441–9445.

1302 Uckoo, R.M., Jayaprakasha, G.K., Patil, B.S., 2015. Phytochemical analysis of organic and
1303 conventionally cultivated Meyer lemons (*Citrus meyeri* Tan.) during refrigerated storage. *J.*
1304 *Food Compos. Anal.* 42, 63-70

1305 UNECE, 2010. UNECE STANDARD FFV-14 Citrus Fruit. Available in:
1306 https://www.unece.org/fileadmin/.../2010/INF2e_CitrusFruit.pdf

1307 Vanamala, J., Cobb, G., Turner, N.D., Lupton, J.R., Yoo, K.S., Pike, L.M., Patil, B.S., 2005.
1308 Bioactive compounds of grapefruit (*Citrus paradisi* cv . Rio Red) respond differently to
1309 postharvest irradiation, storage, and freeze drying. *J. Agric. Food Chem.* 53, 3980-3985.

1310 Voo, S.S., Grimes, H.D., Lange, B.M., 2012. Assessing the biosynthetic capabilities of
1311 secretory glands in citrus peel. *Plant Physiol.* 159, 81–94

1312 Wang, Y-C., Chuang, Y-C., Ku, Y-H., 2007. Quantitation of bioactive compounds in citrus
1313 fruits cultivated in Taiwan. *Food Chem.* 102, 1163–1171.

1314 Win, T.O., Srilaong, V., Kyu, K.L., Poomputsa, K., Kanlayanarat, S., 2006. Biochemical and
1315 physiological changes during chlorophyll degradation in lime (*Citrus aurantifolia* Swingle
1316 cv. ‘Paan’). *J. Hort. Sci. and Biotech.* 81, 471-477.

1317 Wu, J., Xu, Z., Zhang, Y., Chai, L., Yi, H., Deng, X., 2014. An integrative analysis of the
1318 transcriptome and proteome of the pulp of a spontaneous late-ripening sweet orange mutant
1319 and its wild type improves our understanding of fruit ripening in citrus. *J. Exp. Bot.* 65,
1320 1651-1671.

1321 Wu, G. A., Prochnik, P., et al. 2014. Sequencing of diverse mandarin, pummelo and orange
1322 genomes reveals complex history of admixture during citrus domestication. *Nature*
1323 *Biotech.* 32, 656-662.

1324 Wutscher, H.K, 1988. Rootstock effects on fruit quality. In J.J. Ferguson, and W.F. Wardowski
1325 (eds.) *Factors affecting fruit quality*. University of Florida, pp 24-34.

1326 Xi, W., Zhang, Y., Sun, Y., Shen, Y., Ye, X., Zhou, Z., 2014. Phenolic composition of Chinese
1327 wild mandarin (*Citrus reticulata* Blanco) pulps and their antioxidant properties. *Ind. Crops*
1328 *Prod.* 52, 466-474.

- 1329 Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y., Shi, J., 2008a. Juice components and antioxidant
1330 capacity of citrus varieties cultivated in China. *Food Chem.* 106, 545-551.
- 1331 Xu, G., Ye, X., Liu, D., Ma, Y., Chen, J., 2008b. Composition and distribution of phenolic acids
1332 in Ponkan (*Citrus poonensis* Hort. ex Tanaka) and Huyou (*Citrus paradisi* Macf.
1333 Changshanhuoyou) during maturity. *J. Food Compos. Anal.* 21, 382–389.
- 1334 Yang, X-Y., Xie, J-X., Wang, F-F., Zhong, J., Liu, Y-Z., Li, G-H., Peng, S-A., 2011.
1335 Comparison of ascorbate metabolism in fruits of two citrus species with obvious difference
1336 in ascorbate content in pulp. *J. Plant Physiol.* 168, 2196-2205.
- 1337 Yano, M., Kato, M., Ikoma, Y., Kawasaki, A., Fukazawa, Y., Sugiura, M., Matsumoto, H.,
1338 Oohara, Y., Nagao, A., Ogawa, K., 2005. Quantitation of carotenoids in raw and processed
1339 fruits in Japan. *Food Sci. Technol. Res.* 11, 13-18.
- 1340 Yen, Ch-R., Koch, K.E., 1990. Developmental changes in translocation and localization of ¹⁴C-
1341 labeled assimilates in grapefruit: light and dark CO₂ fixation by leaves and fruit. *J. Amer.*
1342 *Soc. Hort. Sc.* 115, 815-819.
- 1343 Yoo, K.M., Moon, B., 2016. Comparative carotenoid compositions during maturation and their
1344 antioxidative capacities of three citrus varieties. *Food Chem.* 196, 544-549
- 1345 Yu, K., Xu, Q., Da, X., Guo, F., Ding, Y., Deng, X., 2012. Transcriptome changes during fruit
1346 development and ripening of sweet orange (*Citrus sinensis*). *BMC Genomics* 13, 10
- 1347 Zhang, L., Ma, G., Kato, M., Yamawaki, K., Takagi, T., Kiriwa, Y., Ikoma, Y., Matsumoto,
1348 H., Yoshioka, T., Nesumi, H., 2012. Regulation of carotenoid accumulation and the
1349 expression of carotenoid metabolic genes in citrus juice sacs *in vitro*. *J. Exp. Bot.* 63, 871-
1350 886
- 1351 Zheng, H., Zhang, Q., Quan, J., Zheng, Q., Xi, W., 2016. Determination of sugars, organic
1352 acids, aroma components, and carotenoids in grapefruit pulps. *Food Chem.* 205, 112-121
- 1353 Zhou, J.Y., Sun, C.D., Zhang, L.L., Dai, X., Xu, C.J., Chen, K.S., 2010. Preferential
1354 accumulation of orange-colored carotenoids in Ponkan (*Citrus reticulata*) fruit peel
1355 following postharvest application of ethylene or ethephon. *Sci. Hortic.* 126, 229-235.
- 1356 Zou, Z., Xi, W., Hu, Y., Nie, C., Zhou, Z., 2015. Antioxidant activity of Citrus fruits. *Food*
1357 *Chem.* 196, 885–896