Scientia Horticulturae 233 (2018) 238–248

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

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

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A R T I C L E I N F O

Keywords:
Acidity
Color
Ethylene
Maturation
Quality
Soluble solids

A B S T R A C T

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.

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

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https://doi.org/10.1016/j.scienta.2018.01.055
Received 21 July 2017; Received in revised form 16 January 2018; Accepted 19 January 2018

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; Alós 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., 2013a, 2013b).

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 stablish 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) stablished 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.

Table 1
Comparison of the content of selected metabolites in the pulp of orange, mandarin, lemon and grapefruit.

Metabolite	Orange	Mandarin	Lemon	Grapefruit
Sugars (mg/g FW)	100–130	110–140	90–100	60–120
Glucose (mg/g FW)	20–50	10–40	10–20	10–30
Fructose (mg/g FW)	20–50	10–30	10–30	20–30
Sucrose (mg/g FW)	50–60	60–80	5–10	30–70
Citric acid (g/L)	6–12	8–12	40–45	4–9/13–27***
Malic acid (g/L)	1–3	0.8–2	1–3	0.3 – 2
Vitamin C (mg/g)	0.46–0.60	0.20–0.57	0.30–0.57 1.9–3.0*	0.30–0.42
Carotenoids (μg/g FW)	4–38	12–40	0.3–6.0	0.4–2–0^{w} 2.0–53^{r}
Flavonoids (mg/g FW)	0.65–0.75	0.20–1.0	nd	0.6–0.9**
Phenolic acids (mg/g FW)	0.90–1.0	0.50 – 0.85	nd	60–72**

Sources: Alquezar et al., 2008a; De Ancos et al., 2017; Emmanouilidou and Kyriacou, 2017; Goulas and Manganaris, 2012; Lado et al., 2016; Liu et al., 2012; Martí et al., 2009; Qin et al., 2015; Sdiri et al., 2012a; Zheng et al., 2016.*lemon Meyer, *d.w., ***mg/g Ff.w; ^{w}white varieties, ^{r}red varieties.

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 to 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