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The quality and yield of essential oils from sour orange (*Citrus aurantium*) and sweet orange (*C. sinensis*) are challenged by pre- and postharvest factors

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Abstract

The composition of essential oils (EO) varies according to the species and cultivars, the cultivation techniques, the environment, modifying their aroma and the quality of the processed product. We evaluated the impact of rootstock, ploidy, stage of fruit development, and of mutation versus hybridization (varietal diversity) on the chemical composition and aroma profile of orange and sour orange EO. The peel and leaf essential oils (PEO and LEO) were extracted by hydro distillation, and analysed by gas chromatography coupled with a flame ionization detector and a mass spectrometry. In parallel, their aromas were characterized by different sensory analysis techniques (discriminant and quantitative). Genetic markers (SSR and SNP) were used to assess the genetic diversity of sweet and sour oranges cultivars. A SNP genetic map of orange was built via a genotyping sequencing approach (GBS) to locate the quantitative trait loci (QTLs) of aromatic compounds. Hybridization is obviously the most disturbing factor because sweet orange and sour orange are two interspecific hybrids. Very few clementine/orange hybrids reproduce an orange aromatic profile. Furthermore, mutation-based diversity generates very little variation, either aromatic or chemical composition. The stage of fruit development has also a strong influence but only when the fruits are immature (June-September). The rootstock and the ploidy level have only a weak influence on the composition and aromatic profile of orange PEO. In postharvest, the drying method induced, under the action of heat, the disappearance of compounds of the family of oxygenated monoterpenes and aliphatic aldehydes, which leads to the deterioration of the aromatic profile. All these studied factors significantly modify the oil yield. Genetic mapping located 20 quantitative trait loci of 15 compounds and EO yield, some of which were positioned in the same linkage groups. This work points to the main factors of variation and suggests improvements for better control and preservation of aroma quality.

Keywords: fruit maturity, rootstock, ploidy, drying process, storage conditions, GC

INTRODUCTION

Sweet orange (*Citrus sinensis*) and sour orange (*Citrus aurantium*) have been frequently studied due to their respective economic importance, and several literature reviews have been published (Dugo, 2010; González-Mas et al., 2019). Monoterpenes represent the overwhelming majority of compounds, with limonene accounting for over 90% of compounds in both species. The other monoterpenes, myrcene, sabinene, α-pinene and linalool, account for around 1% of compounds in sweet orange (Mitiku et al., 1999; Sawamura et al., 2005). In sour orange, myrcene, β-pinene, α-pinene, linalool and linalyl acetate are found (Lota et al., 2001; Kirbaslar and Kirbaslar, 2003). Both species also contain numerous monoterpenes in low or trace proportions. The oils of these two citrus fruits are also composed of sesquiterpenes, with clearly different compositions, but in both cases representing less than 1% of the total composition. The main orange sesquiterpenes are α- and β-sinensal, valencene

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and δ-cadinene, while the sour orange sesquiterpenes are (E)-nerolidol, germacrene-D, nootkatone and β-caryophyllene. There are also numerous trace sesquiterpene compounds in both species. Still in percentages below 1%, there are also aliphatic compounds. Octanal, decanal, nonanal and dodecanal are the main aldehydes in orange peel essential oil. In sour orange, the same aldehydes are present but in lower proportions, and esters such as octyl acetate and decyl acetate have been identified. In summary, the proportions of monoterpene esters (much higher in sour orange) and aliphatic esters, as well as sesquiterpene composition, are the two main differences in zest EOs between sour orange and sweet orange (Kostadinovic et al., 2005).

The relative aromatic contribution (RAAC) of a compound can be calculated from the ratio of the compound's concentration in the product and its detection threshold (Brattoli et al., 2013). The detection threshold represents the lowest concentration at which a pure compound can be detected. The detection threshold is influenced by the environment in which the compound is found and by the olfactory mode (Buettner and Schieberle, 2001; Plotto et al., 2008; Tamura et al., 2008).

GC-O studies have been carried out on sweet orange peel using various analytical methods (Qiao et al., 2008; Xiao et al., 2016; Gonçalves et al., 2018). This shows that aliphatic aldehydes play a major role in aroma, mainly octanal, nonanal and decanal. The same applies to monoterpene aldehydes (citronellal, neral/geranial). Linalool appears to be an essential compound, while the other monoterpene alcohols contribute very little to the aroma of sweet orange zest. Finally, a few hydrocarbon monoterpenes contribute to the aromatic profile, namely limonene, myrcene and α-pinene. It is important to note even though limonene accounts for over 90% of the total composition, its aromatic contribution is probably minor (Rodríguez et al., 2017). Indeed, the latter co-elutes with 1,8 cineole, potentially misleading experimenters. It is likely that its role is more physical, carrying other aromatic compounds with it to the mucosa, like ethanol in wine (Perez-Cacho and Rouseff, 2008). Finally, β-sinensal seems to be the main sesquiterpene compound contributing to aroma. These results are in line with the two studies whose objectives were to reconstitute orange zest aroma from pure products (Chida et al., 2006). Most of the compounds presented above have been identified as contributors to orange juice aroma (Plotto et al., 2008; Arena et al., 2006; Perez-Cacho and Rouseff, 2008; Deterre et al., 2016).

The citrus processing industry, particularly those involved in the production of spirits, uses mainly citrus peels from different countries. The quality and quantity of the essential oil extracted vary according to the geographical origin (Luro et al., 2020). Many factors challenge the EO quality such as cultivation and drying methods and the maturity of harvested fruit. We present here a bibliographical review of some of the factors influencing citrus yield and quality at the level of fruit production, fruit peel treatment and storage.

FRUIT DEVELOPMENT STAGE

The composition of the essential oil in orange and sour orange peel evolves during the 3 phases of fruit development (Ferrer et al., 2022a) (Figure 1). In sweet orange, limonene increases during fruit development and then stabilizes, while linalool decreases and valencene increases in the later stages of fruit development (Phase III) (Goh et al., 2021). According to the literature, it is not possible to identify other clear trends in sweet oranges apart from the stability of chemical composition during Phase III (Trozzi et al., 1999).

The evolution of the EO composition of sour orange zest follows similar trends to those of sweet orange, with a low limonene content during the immature stages (Rowshan and Najafian, 2015; Azhdarzadeh and Hojjati, 2016). Linalool also appears to decrease during fruit development, as do most other monoterpenes (Boussaada and Chemli, 2007; Rowshan and Najafian, 2015). The proportion of sesquiterpenes also seems to increase during the ripening phase (Boelens and Jimenez, 1989). As with sweet oranges, the composition stabilizes when the fruit is ripe (Ferrer et al., 2022a). Finally, the evolution of oil yield per fruit in these two species is conditioned by the number of oil glands per fruit and their oil content (Knight et al., 2001; Voo et al., 2012). Since the maximum amount of oil is reached when the fruit has reached its maximum size, it seems obvious that if maximum yield of essential oil is to be achieved, it is necessary to wait for the fruit to reach its maximum size. However, 1 kg of immature fruit can contain up to twice as much essential oil as one kg of mature fruit (Havkin-Frenkel and Dudai, 2016). Since essential oil is often extracted from dried zest, it seems appropriate to study the evolution of EO yield in relation to zest dry mass during fruit development. This variable is little or not at all documented (Boussaada and Chemli, 2007; Bourgou et al., 2012; Azhdarzadeh and Hojjati, 2016).

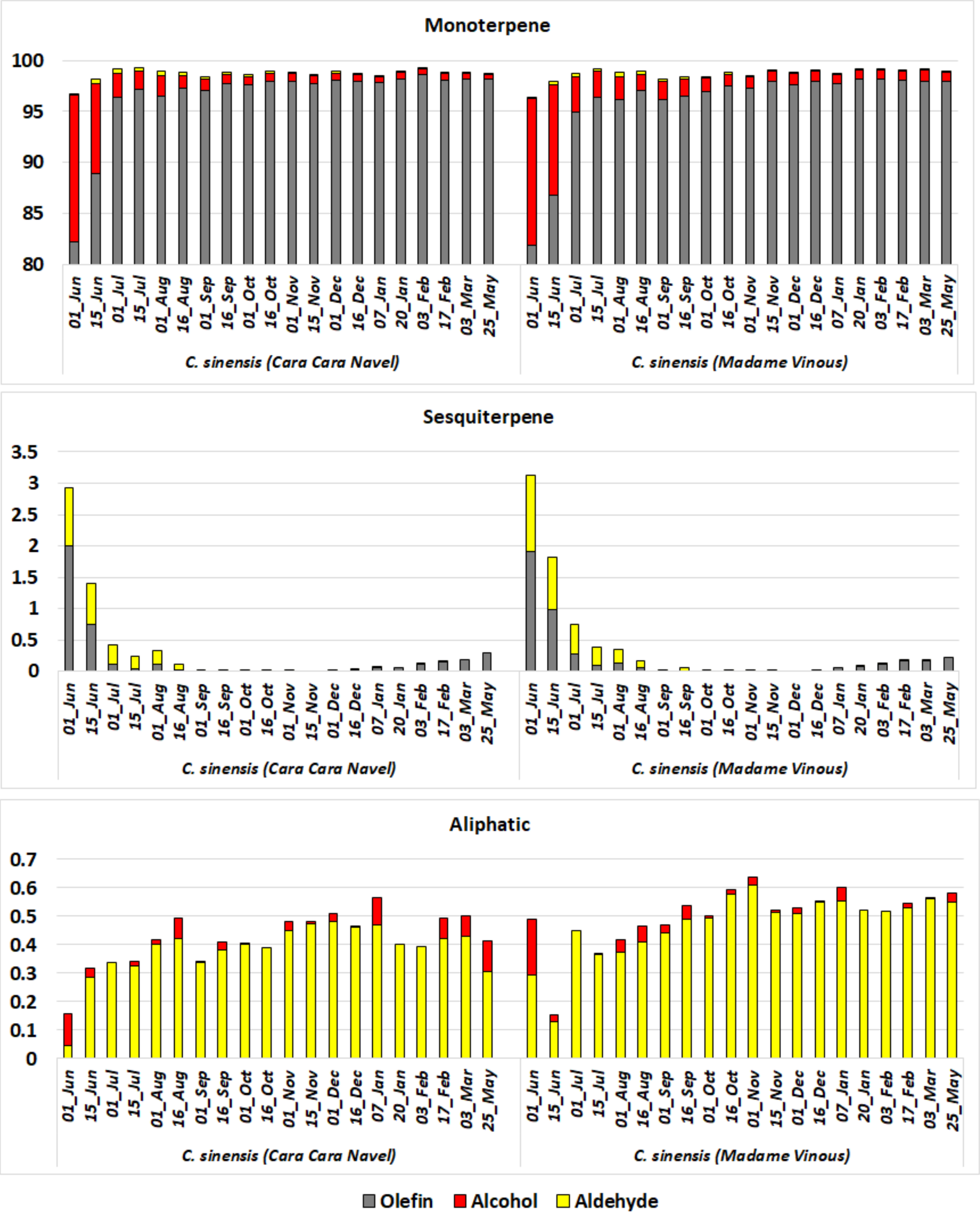

Figure 1. Stacked bar plot representing the mean proportion value of main chemical family of both *Citrus sinensis* cultivars during fruit development process (from June 1 to May 25).

ROOTSTOCK

Citrus is grown as an association between a productive cultivar called “scion” and a rootstock cultivar chosen for its adaptation to the biotic and abiotic constraints of the growing area. The influence of the rootstock on sweet orange PEO has been studied. Most authors report little or no effect of rootstock on essential oil composition and yield in sweet orange (Kesterson et al., 1980; Cano and Bermejo, 2011). The same trend is found in lemon, kumquat and bergamot (Verzera et al., 2003; Darjazi, 2017; Aguilar-Hernández et al., 2020). However, that while the rootstock has little or no influence on the oil content of the peel, it can significantly influence the quantity of fruit per tree and therefore the yield per production unit area (Hussain et al., 2013; Emmanouilidou and Kyriacou, 2017; Caruso et al., 2020). Often grown free-standing (on its own roots) because it is itself used as a rootstock, there are no studies of the relationship between the rootstock and the sour orange tree when the latter is used as a producing cultivar. A study carried out on sweet orange peel EO showed that the rootstock had an influence on yield and composition (α-pinene, neral, geranial, linalool and sabinene) (Figure 2) and, less conclusively, on the aromatic profile (Ferrer et al., 2022b).

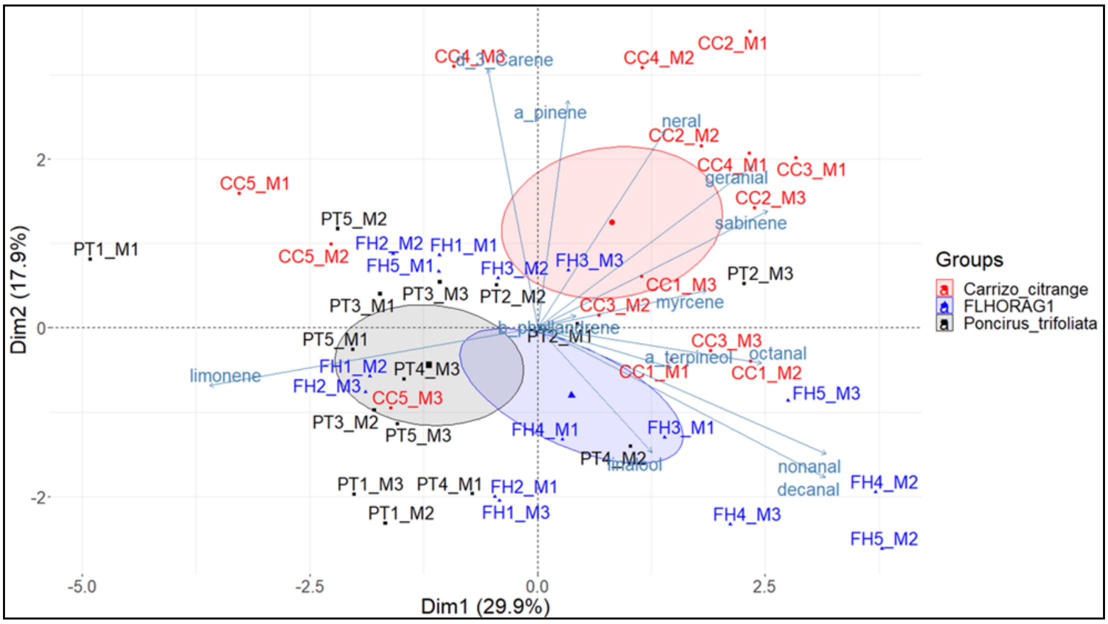

Figure 2. Principal component analysis representing the PEO diversity of sweet orange ‘Navelina’ grafted on three rootstocks (CC: Carrizo citrange; PT: trifoliate orange and FH: *P. trifoliata* + *C. reticulata* somatic hybrid) based on the 13 main compounds of the peel essential oil. Three replicates were analysed for each 5 trees per rootstock/scion combination. The ellipses represent the probable position of the gravity centre with a 0.95 probability.

PLOIDY

With the exception of citron, grapefruit and certain mandarin hybrids such as clementine, the majority of citrus plants have partial apomictic reproduction, producing somatic embryos supernumerary to the zygotic embryo (Aleza et al., 2011). During cell division, chromosomal doubling can be quite frequent in nucellar embryos, depending on the species and temperature during flowering. Ploidy variation in orange results in a 25% decrease in octanal and linalool (Cameron and Scora, 1968). The 2*x* and 4*x* combinations of scion (Hamlin sweet orange) and rootstock (Citrumelo 4475) demonstrated that scion ploidy variation had a strong influence on PEO composition: PEOs from 2*x* oranges were richer in oxygenated compounds (aldehydes and alcohols) than their 4*x* counterparts, and therefore discriminated aromatically (Ferrer et al., 2022a) (Figure 3). However, no variation in yield was observed with ploidy variation.

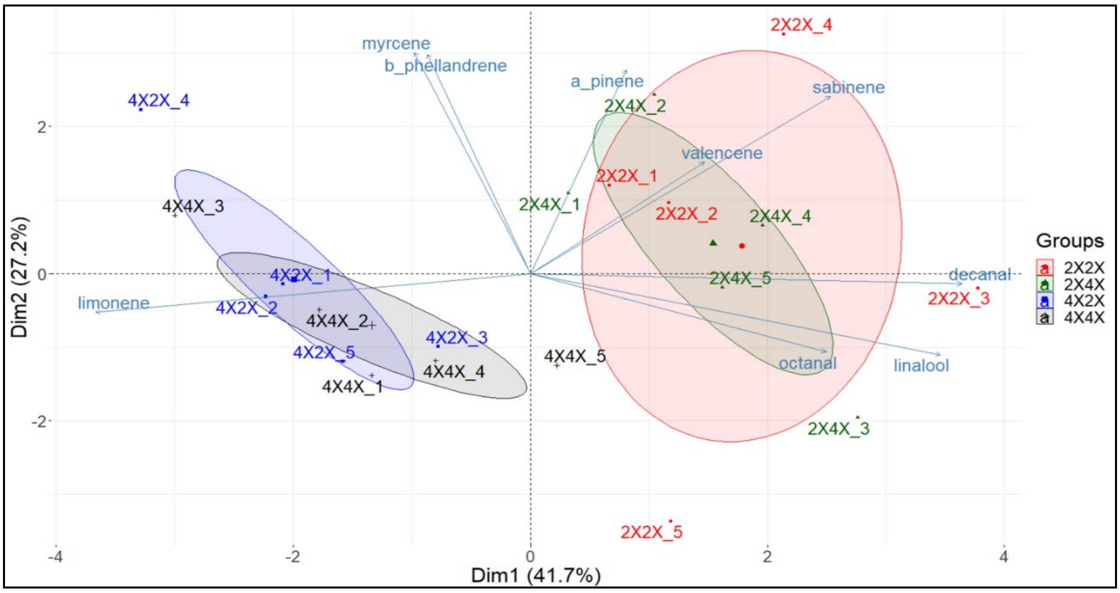

Figure 3. Principal component analysis based on the nine main compounds, representing the diversity of the 'Pineapple' sweet orange PEO according to the ploidy level (2*x* or 4*x*) of the scion (first) and the rootstock (second). Five replicates (trees) were analysed for each four combinations. The ellipses represent the probable position of the gravity centre with a 0.95 probability.

ZEST DRYING

Drying reduces the proportion of free water, i.e., water that microorganisms can use to grow. To achieve this, the moisture content of zests must be reduced to 10% or less, for optimum preservation (Carsky, 2008). In order to dry a product, it must be subjected to relatively high temperatures or air flow and relatively low ambient humidity. Several authors have studied the influence of drying methods on the aromatic composition of citrus zests. While high-temperature thermal drying methods have a strong impact on composition, with the appearance/disappearance of a number of compounds (Zhang et al., 2018; Farahmandfar et al., 2020; Zheng et al., 2021), low-temperature methods have very little effect on composition compared with fresh zest. Oxygenated compounds are the most affected by temperature, and their proportion decreases with increasing drying temperature. These transformations can certainly be explained by various oxidation reactions, isomerization, dehydration, polymerization and thermal rearrangements (Turek and Stintzing, 2013; Sun et al., 2014; Calandra et al., 2015, 2016). These modifications are certainly not without consequences for the aromatic profile. Publications coupling GC-O and sensory analysis on the influence of ultraviolet light, the presence of oxygen and temperature on grapefruit essential oil are available (Sun et al., 2014, 2018). The authors conclude that temperature and oxygen have a strong impact on the aroma profile reducing pleasant descriptors and increasing those of defects. In the case of whole zests subjected to different drying conditions, heat is likely to have the greatest impact, as zests represent a physical barrier to ultraviolet light and oxygen, preventing the formation of limonene and linalool oxides and their derivatives such as carvone and carveol, as well as degradation reactions (Calandra et al., 2015, 2016).

There is no consensus on the influence of drying on oil yield due to the different ways of measuring it. Yields are often calculated from material still containing water and not in relation to dry mass. If we examine yield in relation to a dry mass of zest (g 100 g^{-1} dry zest) the EO yield decreases with increasing drying temperature (Marey and Shoughy, 2016; Zhang et al., 2018; Bechlin et al., 2020).

ZEST STORAGE LIFE

After drying, zests are generally stored in the dark at room temperature before use.

There are few studies on the effect of citrus zest storage on oil composition (Benjamin et al., 2007; El-Sawi and Ibrahim, 2018; Chao et al., 2022). Most studies test the effect of various parameters on the storage of already extracted zest essential oil or on the whole fruit (Njoroge et al., 2003; Nguyen et al., 2009; Sun et al., 2014, 2018; Strano et al., 2014). Once extracted, the oils are exposed to oxygen, light and traces of solvent or water, whereas enclosed in the skin glands they are partially protected but can still evolve physiologically (Turek and Stintzing, 2013; Sun et al., 2014, 2018; Calandra et al., 2015, 2016). Chao et al. (2022) observed a decrease in oxygenated compounds (alcohols, esters, aldehydes) over the storage life of mandarin peel, while the proportion of hydrocarbon monoterpenes increased. Finally, the oil yield calculated from orange dry mass appears to decrease by 20% after one year of zest storage (El-Sawi and Ibrahim, 2018).

CONCLUSIONS

Many factors can affect the yield, composition and aromatic quality of orange peel and sour orange essential oils. Standardization of drying and storage processes can be considered to reduce fluctuations in essential oil quality. The choice of rootstock adapted to environmental conditions is crucial for the cultivation of these varieties but can also modified the PEO of fruit scion. Tetraploid rootstocks will certainly be used in the future to better tolerate certain constraints (such as water stress or HLB) without altering the essential oil quality of the orange peel. As the fruit matures, essential oil quality is stable. On the other hand, fruits at stage I of their development, at the moment of their physiological fall, present very different aromatic profiles that could potentially be exploited to generate new aromas or fragrances in the processing industry.

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