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A comparative analysis of the volatile profiles in the pulp of red-fleshed and standard orange varieties during fruit maturation

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ABSTRACT

Kirkwood Navel and Ruby Valencia red-fleshed sweet oranges accumulate high concentrations of the carotenes phytoene, phytofluene and lycopene in the pulp. In order to gain insight into the parameters that influence fruit quality in these varieties, a comparative analysis of volatile organic compounds (VOCs) in the pulp of Kirkwood Navel and Ruby Valencia red-fleshed sweet oranges and their standard blond counterparts, Navel and Valencia, was performed during fruit development and ripening. A total of 95 VOCs were identified by HS-SPME/GC–MS during the ripening process. The observed differences in VOCs between red-fleshed and blond oranges were more closely related to the fruit ripening stage than to the genotype. However, two distinctive common features were identified in both red-fleshed oranges compared to their respective standard varieties. These were lower levels of sesquiterpenes and higher levels of the norisoprenoids 6-methyl-5-hepten-2-one and geranylacetone. These findings indicate that altered carotenoid metabolism in red-fleshed oranges not only affects flesh pigmentation but also leads to changes in specific VOCs that may influence flavor perception.

1. Introduction

Citrus is one of the world's most important fruit crops in terms of production and consumption due to its attractive color, pleasant taste and aroma, and nutritional properties. All these factors contribute to the quality of the fruit and define its potential for the fresh market and the juice industry. Citrus flavor, as with many other fruits, is a combination of flavor and aroma. Taste is primarily influenced by sugars and organic acids, while citrus aroma largely arises from a complex mixture of specific volatile organic compounds (VOCs), with a smaller contribution from non-volatile compounds. Among the different VOCs identified in orange fruit, 20–40 compounds are generally recognized as important contributors to fruit aroma, including esters, aldehydes, monoterpenes, sesquiterpenes, alcohols and ketones (Plotto et al., 2004; Baxter et al., 2005; Arena et al., 2006; Pérez-Cacho and Rouseff, 2009). The most diverse and abundant VOCs in orange fruit are monoterpenes and sesquiterpenes, but they are responsible for limited odor activity due to their high odor thresholds (Plotto et al., 2004; Pérez-Cacho and Rouseff, 2009). In addition, other less abundant oxygenated monoterpenes, especially those of alcohols and aldehydes, actively contribute to citrus aroma (Salvatore et al., 2022). Non-terpenoid compounds represent a small fraction of the total VOCs, but can have a major impact on citrus aroma. Among them, aldehydes such as octanal, nonanal, decanal or (*Z*)-3-hexenal have been described as important contributors to orange aroma (Pérez-Cacho and Rouseff, 2009). Aliphatic esters, such as ethyl butanoate and hexanoate, are also among the most intense odorants in orange juice with fruity and floral flavors (Pérez-Cacho and Rouseff, 2009; Ren et al., 2015). Apocarotenoid volatiles or norisoprenoids are a family of terpenoid compounds (C9–C13) including citral, β-ionone, β-damascenone, β-cyclocitral, and geranylacetone, derived from the oxidative cleavage of carotenoids, which have extremely low odor thresholds. This oxidative cleavage often occurs at specific double bonds of the carotenoid structure and is catalyzed by a family of enzymes called carotenoid cleavage dioxygenases (Winterhalter and Rouseff, 2002; Vogel et al., 2008). Therefore, content of carotenoids may have a strong impact on volatile norisoprenoids affecting sweet orange fruit

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aroma, as has been described for other fruit (Lewinsohn et al., 2005a,b; Vogel et al., 2010; Brandi et al., 2011; Wei et al., 2018).

Fruits of different *Citrus* species and varieties show a wide variability in their volatile profiles, which is often qualitative, but mostly quantitative in nature (González-Mas et al., 2011; Rambla et al., 2014; Ren et al., 2015; González-Mas et al., 2019; Zhang et al., 2017). In addition to the genetic basis of VOCs biosynthesis (Buettner and Schieberle, 2001; Brandi et al., 2011; Ren et al., 2015; Wei et al., 2018) many other factors can affect the composition of VOCs in citrus fruit such as the maturity stage (Bai et al., 2016; Hijaz et al., 2020; Hou et al., 2020), environmental conditions, agronomic practices (Salvatore et al., 2022) (e.g., rootstocks, irrigation, conventional or organic farming) as well as postharvest storage (Sdiri et al., 2017; Habibi et al., 2020).

The information available in the literature makes it difficult to compare the profiles of VOCs in fruit among citrus species and varieties, mainly due to the diversity of techniques and methodologies used for the detection and quantification of these compounds. Different studies have addressed these comparisons on the basis of qualitative similarities/differences regarding the VOCs profiles. Oranges and grapefruits seem to be richer in diversity of sesquiterpene hydrocarbons than other citrus species such as mandarins and lemons (Ren et al., 2015; González-Mas et al., 2019). The biosynthesis of volatile organic compounds (VOCs) in fruit is a genetically regulated process that is finely modulated during ripening (Defilippi et al., 2009; Granell and Rambla, 2013). In this context, only a few studies have evaluated the dynamic changes in the volatile profiles in the juice sacs/pulp of the most commonly cultivated citrus species, including oranges (Obenland et al., 2009; Bai et al., 2016; Wei et al., 2018; Hou et al., 2020), mandarins (Hijaz et al., 2020) or lemons (Li et al., 2022).

Lycopene is a red carotene which only accumulates in the fruit of specific varieties of some citrus species such as grapefruit (*Citrus paradisi*) (Rouseff et al., 1992) pummelo (*Citrus grandis*) (Xu et al., 2006), lemon (*Citrus limon*) (Lana et al., 2020) and sweet orange (*Citrus sinensis*) (Zacarías-García et al., 2022a). The accumulation of lycopene in the pulp of these red-fleshed varieties provides not only an attractive pigmentation but also may be associated with relevant health-promoting effects (Khan et al., 2021). In addition to the interest in red-fleshed citrus fruit, there is a scarcity of information regarding potential differences in volatile profiling when compared with standard colored varieties. Previous studies have characterized the composition of volatile compounds in fruit of different lycopene-accumulating citrus mutants, including the sweet orange Red Anliu (Liu et al., 2019; Liu et al., 2022), the red-fleshed Guanxi and Chuhong pummelos (Liu et al., 2019; Liu et al., 2013) and pink grapefruit (Zheng et al., 2016). Recently, two novel red-fleshed sweet orange varieties named as Kirkwood Navel (Kirkwood or K) and Ruby Valencia (Ruby or R), originating from the blond varieties Palmer Navel and Olinda Valencia, respectively, have been described (Zacarías-García et al., 2022a). The characterization of K and R oranges during fruit maturation revealed that the pulp of K and R fruit contained very high levels of the linear carotenes phytoene, phytofluene and moderate content of lycopene from the early stages of fruit development to the maturation time (Zacarías-García et al., 2022a). Moreover, previous analysis of VOCs in the pulp of these varieties during postharvest cold storage (28 days at 2 °C) and shelf-life (5 days at 20 °C) revealed a higher abundance of monoterpenes and volatiles derived from carotenoids over time, in comparison with standard orange varieties (Zacarías-García et al., 2023). However, the dynamics of VOCs profiles in the pulp during development and ripening have not been yet investigated. Therefore, the objective of this study was to characterize the VOC profile in the pulp of the red-fleshed oranges Kirkwood Navel and Ruby Valencia during fruit development and maturation and compare it with those occurring in the reference varieties Foios Navel and Midknight Valencia. Moreover, the impact of the distinctive carotenoid profile of red-fleshed oranges on their volatile composition will be examined.

2. Material and methods

2.1. Plant material

Fruit were harvested from adult trees of the two blond sweet orange (*Citrus sinensis*) genotypes, Washington Navel (cv. Foios) (N) and Valencia late (cv. Midknight) (V) as reference varieties, and the two red-fleshed genotypes Kirkwood (K) and Ruby (R), planted in experimental orchards of the ANECOOP Foundation (Museros, Valencia, Spain; 39º34'10.8"N 0º21'28.8"W). All genotypes were grafted onto Citrange Carrizo rootstock (*Citrus sinensis* x *Poncirus trifoliata*), and trees were located in contiguous rows under identical agronomical and environmental conditions. N and K oranges are mid-season maturity varieties, with the period of harvesting in Valencia (Spain) extending until February. In contrast, V and R are late-maturing varieties, with harvesting generally occurring between April and May (Zacarías-García et al., 2022a). Considering the developmental and ripening periods for each variety, fruit from the four varieties were harvested at four stages in season 2019–2020, covering the entire process of development and maturation. Fruit from varieties N and K were harvested at different stages: in September (mature green), November (breaker), December (mature), and January (full mature). In contrast, fruit from varieties V and R were harvested in September (immature green), November (mature green), January (breaker), and April (full mature). The criteria for categorizing the maturity stages of the fruit were based on a combination of external and internal color index, as well as the maturity index, as described by Zacarías-García et al. (2022a,b). For each variety and sampling time 20–30 fruit were harvested from the external canopy of three trees, and two biological replicates were collected. Fruit were immediately delivered to the laboratory and inspected for uniformity of color and size. Each fruit was sliced into halves and pulp tissue was obtained by excising small pieces of approximately 1 cm^3 containing the juice vesicles free of segment membranes. Tissue was immediately frozen in liquid nitrogen, ground to fine powder with an electric grinder and stored at –80 °C until analysis.

2.2. Carotenoids extraction and analysis by HPLC–DAD

The extraction of carotenoids from pulp tissue was essentially carried out following the protocol described by Zacarías-García et al. (2022c). The analysis of the carotenoids content and composition was performed by a Waters liquid chromatography system (HPLC) equipped with a 600E pump and a photodiode array detector (DAD) model 2998, and Empower3 software (Waters Cromatografía, Barcelona, Spain). Carotenoids separation was done using a C30 column (250 × 4.6 mm, 5 μm) coupled to a guard column cartridge C18 (10 × 4.3 mm, 5 μm) (IDEX, Teknokroma, Barcelona, Spain) and chromatographic conditions described in Zacarías-García et al. (2022). The identification and quantification of carotenoids was achieved by comparison of absorbance spectra and retention time, and using the appropriate calibration curves, as described in Zacarías-García et al. (2022). Carotenoid contents are expressed as μg g^{-1} of fresh weight. Results are the mean of two biological replicates (mean ± SD).

2.3. Analysis of VOCs by headspace-solid phase microextraction (HS-SPME)/GC–MS

Gas chromatography–mass spectrometry (GC–MS) analysis after volatile extraction by headspace solid-phase microextraction (HS-SPME) was conducted essentially as described in González-Mas et al. (2011) with minor modifications. For VOCs analysis 500 mg of frozen pulp sample were introduced into a 10-mL screw-cap headspace vial. Then, 500 μL of $CaCl_2$ 5 M and 250 μL of EDTA 500 mM pH 7.5 were added, mixed gently and incubated at 25 ºC for 5 min in a water bath. Volatiles were extracted from the headspace by means of an SPME fiber (50/30 μm DVB/CAR/PDMS; Supelco, Bellefonte, PA). Vials were first

incubated for 10 min at 40 ºC (to prevent thermal degradation of carotenoids) with 500 rpm agitation. Then, volatiles were captured by introducing the SPME fiber into the vial headspace, which was exposed for 20 min under the same conditions of temperature and agitation. Fiber desorption was performed for 1 min in the GC injection port at 250 ºC, in splitless mode. Analyses were performed by GC–MS using a COMBI-PAL autosampler (CTC Analytics, Zwingen, Switzerland), a 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA) and an Agilent 5975B Inert XL MSD, equipped with a DB-5MS fused silica capillary column (5 % phenyl/95 % dimethylpolysiloxane as stationary phase; 60 m length, 0.25 mm i.d., and 1 mm film thickness) (Agilent J&W Scientific). Oven ramp was 40 ºC for 2 min, then the temperature was increased at 5 ºC/min until 250 ºC, then maintained at 250 ºC for 5 min. Helium was used as carrier gas at 1.2 mL/min constant flow. Detection was obtained by an Agilent mass spectrometer operating in EI mode (ionization energy, 70 eV; source temperature 230 ºC). Data acquisition was performed in scan mode (mass range *m/z* 35–250; six scans per second). Chromatograms and mass spectra were recorded using Enhanced ChemStation E.02.02 software for GC–MS (Agilent). RSD of each volatile compound determined is shown in Table S1.

2.4. VOCs identification and quantification

Identification and quantification of VOCs were carried out as described in González-Mas et al. (2011) for citrus fruit. Compound identification was based on the comparison between the MS for each compound with those of the NIST 2005 Mass Spectral library and Kovats retention indices calculated on DB-5 column and compared with those of different databases (Flavournet database, www.flavournet.org; Pherobase database, www.pherobase.com) (Table S1). Additionally, the identification of all compounds was confirmed through comparison with authentic standards provided by Sigma-Aldrich (Barcelona, Spain) and Extrasynthèse (Genay, France) except for sesquiterpenes 1–8 that were tentatively identified based on mass spectra and retention time. For compound quantification, a specific ion (*m/z*) was chosen for each compound based on ion specificity and the highest signal-to-noise ratio (Table S1), and the corresponding peak area was integrated. A reference sample was prepared by combining equal quantities of each sample from the experiment and injected daily. The reference sample was employed for normalization purposes, serving to correct for variations in the detector and the aging of the fibers over the course of the experiment. Finally, the results were normalized for each compound, expressed as the relative levels in the sample to the corresponding levels found in the reference sample.

2.5. Statistical analysis

Results are presented as the mean of two biological replicates ± standard deviation (SD). Significant differences between ripening stages (harvest month) for the same variety were determined by one-way ANOVA ($p < 0.05$) whereas significant differences between the red-fleshed and the corresponding standard blond variety for each ripening stage were determined by *t*-test ($p < 0.05$). Statistical analysis, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were conducted by XLSTAT software (Addinsoft, Paris, France).

3. Results

3.1. Carotenoids content and composition in the pulp of red-fleshed Kirkwood Navel and Ruby Valencia oranges during fruit development and maturation

The fruit of the Kirkwood Navel and Ruby Valencia genotypes are distinguished by the reddish coloration of the pulp, in comparison with the reference orange genotypes selected in this study, Foios Navel and Midknight Valencia (Figure S1). The most striking feature of Kirkwood and Ruby oranges is the higher levels of total carotenoids in the pulp compared with the blond varieties Navel and Valencia, throughout the entire process of fruit development and maturation (Fig. 1). The pulp of Navel and Valencia oranges contains very low levels of carotenoids (less than 1 μg g^{-1} FW) at early stages of development and reach the maximum content (nearly 12 μg g^{-1} FW) at full maturity. In both blond Navel and Valencia oranges xanthophylls comprised the majority of the carotenoid content. In contrast, the pulp of Kirkwood and Ruby accumulated extraordinary levels of the colorless carotenes phytoene (40.50–84.85 μg g^{-1} FW in Kirkwood and 68.13–146.80 μg g^{-1} FW in Ruby) and phytofluene (8.83–13.96 μg g^{-1} FW in Kirkwood and 10.06–20.40 μg g^{-1} FW in Ruby) at all stages of development, accounting for 85–99 % of the total carotenoid content. Furthermore, the red carotene lycopene, which is not present in standard oranges, is detectable in Kirkwood and Ruby after September, with an increase in its content during the maturation of the fruit from 1.99 to 8.17 μg g^{-1} FW in Kirkwood and from 0.9 to 9.5 μg g^{-1} FW in Ruby.

3.2. Identification of VOCs in the pulp of blond and red-fleshed oranges during fruit development and maturation

A total of 95 volatile compounds were identified by HS-SPME/GC–MS, 87 of which were unambiguously identified by comparing their mass spectra in NIST 2005 Mass Spectral Library and GC retention time with commercially available standards (Table S1). Volatiles were grouped into two main categories: 50 terpenoids and 45 non-terpenoids. Among the terpenoids, 12 compounds were classified as monoterpene hydrocarbons, 16 as monoterpenoids, and 12 as sesquiterpenes. In all samples, eight sesquiterpenes, designated as sesquiterpene 1–8, were inferred from their mass spectra and retention time, although unequivocal identification was not possible. Within the group of terpenoids, 10 compounds were classified as norisoprenoids, based on the nature of the precursors rather than the chemical composition. The non-terpenoid VOCs were grouped into seven chemical groups, including 5 alcohols, 17 aldehydes, 3 ketones, 14 esters, 4 furans, and 2 acids.

As a preliminary step in characterizing the VOCs composition of the pulp, a principal component analysis (PCA) was performed to examine the evolution of the volatile profile of the Navel and Kirkwood (Fig. 2A, B) oranges, as well as the Valencia and Ruby (Fig. 2C, D) oranges. The red-fleshed oranges and their corresponding standard varieties showed a parallel evolution during maturation (Fig. 2). N and K fruit were rich in fatty acid derivatives such as alcohols, aldehydes, and ketones in samples of September and November while V and R fruit were characterized by the large number of monoterpenes. However, as the maturation progressed, fruits of the four varieties underwent an increase in the proportion of norisoprenoids, sesquiterpenes and esters.

The PCA score plot of N and K demonstrated that the first two principal components (PCs) were capable of differentiating samples according to the stage of maturity, rather than by variety. This is evidenced by the clustering of N and K samples near one another across the four stages of development (Fig. 2A, B). These two PCs collectively explained 72.2 % of the total variance of the data (PC1 = 46.6 %, PC2 = 25.6 %). The scores plot indicates that the VOC profiles of fruit harvested in September and November are comparable to one another, in contrast to the profiles of fruit harvested in December and January, which are positioned independently. A PCA was also performed to study the distribution of VOCs during the development and maturation of V and R fruit (Fig. 2C, D). The two PCs explained 55.9 % of the total variance of the data (PC1 = 35.1 %, PC2 = 20.8 %). The evolution of the volatile profile in V and R oranges followed a similar pattern during fruit maturation. Nevertheless, the red-fleshed and blond Valencia oranges exhibited notable differences between them after November. The scores plot (Fig. 2C) demonstrated that fruit harvested at intermediate stages, specifically in November (mature green) and January (breaker), exhibited a greater degree of similarity in VOCs than those harvested in

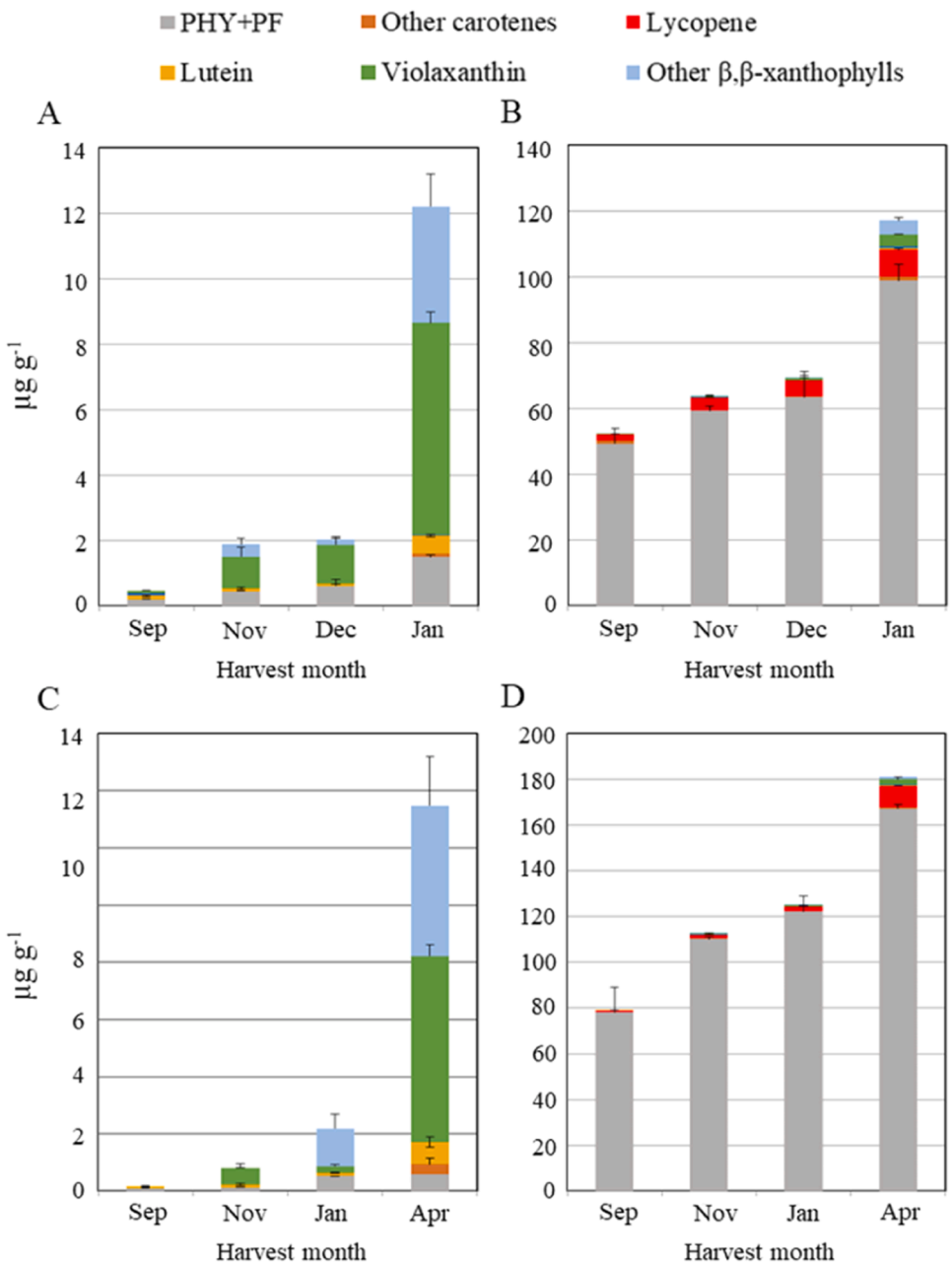

Fig. 1. Stacked bar plot representing the content (µg g^{-1} fresh weight) of carotenoids in the pulp of Foios Navel (A), Kirkwood Navel (B), Midknight Valencia (C) and Ruby Valencia (D) orange varieties at four stages of fruit development and maturation. PHY: phytoene; PF: phytofluene. Other carotenes is the sum of β-carotene, neurosporene and δ-carotene. Violaxanthin is the sum of 9-*Z*-violaxanthin and all-trans-violaxanthin isomers. Other β,β-xanthophylls is the sum of β-cryptoxanthin, zeaxanthin and antheraxanthin. Sep: September; Oct: October; Nov: November; Dec: December; Jan: January; Apr: April. Results are the mean of two biological replicates ± standard deviation.

September (immature green) and April (mature).

3.3. Changes in VOCs in the pulp of the standard Navel and the red-fleshed Kirkwood orange during fruit development and maturation

A total of 94 volatile organic compounds (VOCs) were identified in the pulp of N and K fruit (Table S2). The mature green (September) and color break (November) fruit exhibited the highest diversity and relative content of volatiles derived from fatty acids, including four aliphatic alcohols (1-penten-3-ol, 1-hexanol, 1-octen-3-ol and 1-octanol), seven short-chain aldehydes (C5–C8) (pentanal, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, heptanal, (*E*)-2-heptenal and (*E*)-2-octenal), three ketones (1-penten-3-one, 3-pentanone and 1-octen-3-one), three furans (2-methylfuran, 2-ethylfuran and 2-pentylfuran), and several monoterpenes such as camphene, β-pinene and nerol (Table S2). In contrast, fruit harvested in December exhibited a notable increase in the relative content of most monoterpenes, the norisoprenoids (neral, geranial, neryl acetate, and geranyl acetate), and the long-chain fatty acid-derived aldehydes (C8–C12: octanal, nonanal, undecanal, (*E,E*)-2,4-decadienal, and dodecanal). Conversely, β-ionone, ethanol, acetaldehyde, 3-methylfuran, and esters (predominantly those with an ethyl moiety) were the compounds that contributed most to the observed differences in the mature fruit of January.

In mature fruit, only 12 VOCs showed significant differences between K and N (Table 1). These VOCs belong to the groups of sesquiterpenes (9) and norisoprenoids (3). The relative content of these

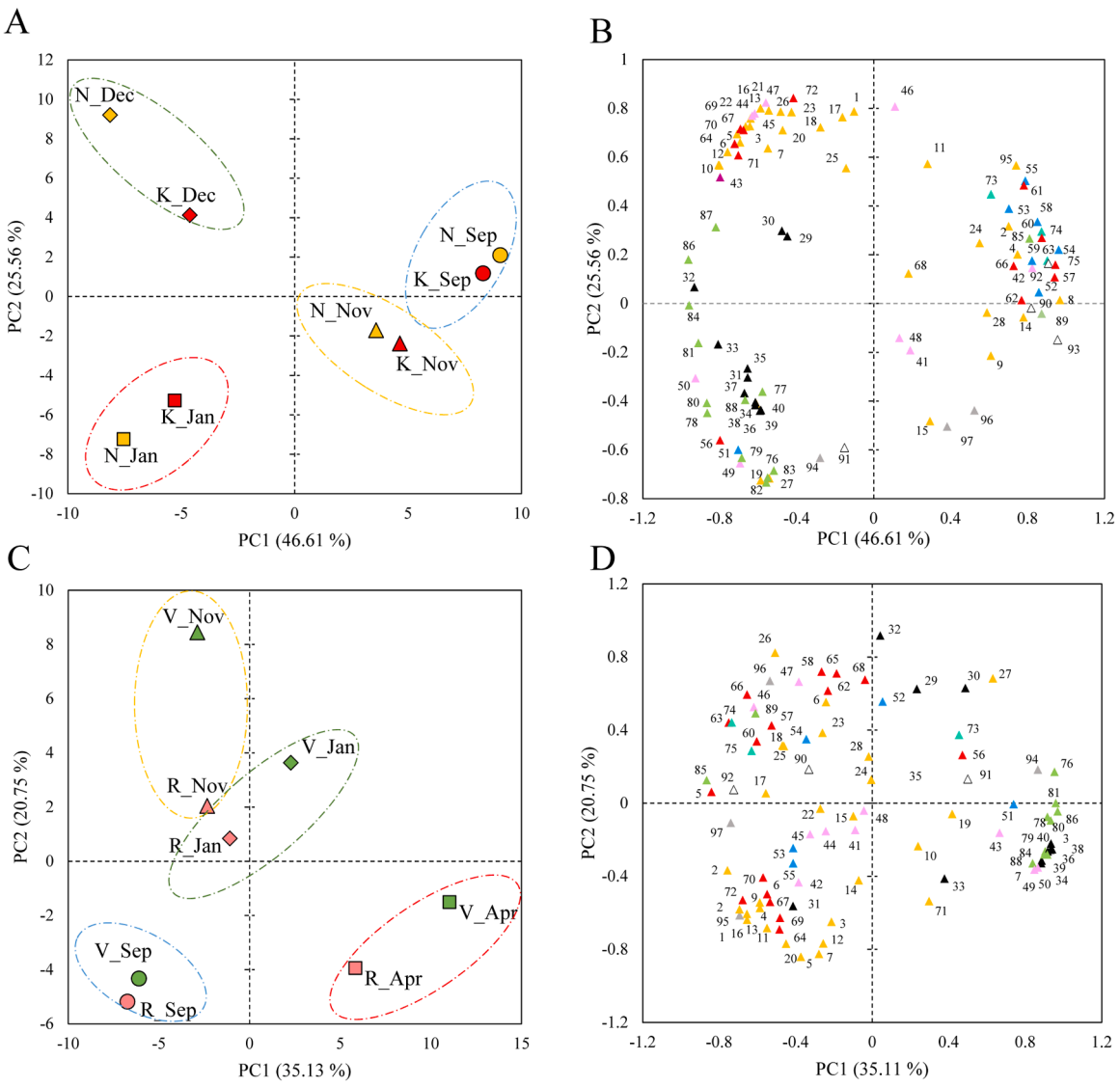

Fig. 2. Principal component analysis (PCA) score plot (A, C) and loading plot (B, D) of the volatile compounds detected in Foios Navel (N) and Kirkwood Navel (K), and Midknight Valencia (V) and Ruby Valencia (R) orange fruit, respectively, at four stages of fruit development and maturation. Sep: September; Oct: October; Nov: November; Dec: December; Jan: January; Apr: April. Different chemical families of volatiles are colored as follow: yellow, monoterpenes (1–28); black, sesquiterpenes (29–40); purple, norisoprenoids (41–50); blue, alcohols (51–55); red, aldehydes (56–72); blue-green, ketones (73–75); green, esters (76–89); white, furans (90–93); grey, acids (94,95).

sesquiterpenes was between 3 and 10 times lower in mature red-fleshed K oranges compared to blond N oranges (Table 1). Furthermore, at intermediate stages (November and December), their relative levels were already lower in K. However, in green fruit (September) most of these sesquiterpenes were not detected in both genotypes and three of them (sesquiterpene 2 and 6, and α-humulene) exhibited higher relative levels in K than in N (Table 1). The norisoprenoids MHO and geranylacetone showed between 10 and 45 higher relative levels in K compared to N during maturation, and the maximum differences between genotypes were observed at intermediate stages (November and December) (Table 1). In contrast, the norisoprenoid β-ionone showed an opposite trend to the other two norisoprenoids, and the relative levels in mature fruit of K were approximately 50 % lower than those of N (Table 1).

3.4. Changes in VOCs in the pulp of the standard Valencia and the red-fleshed Ruby oranges during fruit development and maturation

The analysis of VOCs in the pulp of V and R fruit identified 90 different compounds (Table S3). In general, the immature fruit (September) of V and R exhibited the greatest relative levels for most monoterpenes and monoterpene alcohols, and several fatty acid-derived alcohols (1-hexanol and 1-octanol) and aldehydes ((*Z*)-3-hexenal, (*E*)-2-hexenal, octanal, nonanal, decanal, undecanal and dodecanal). Fruit harvested in the intermediate stages of maturation (November or January) showed the highest relative levels in several monoterpenes (limonene oxide, β-citronellol, carvone, perillaldehyde, (*Z*)-carvyl acetate and citronellyl acetate), fatty acid-derived aldehydes (hexanal, (*E*)-2-heptenal, heptanal, benzaldehyde, (*E*)-2-octenal and (*E*)-2-nonenal), alcohols (1-hexanol and 1-octanol) and the norisoprenoids neryl acetate and geranyl acetate. The V and R mature fruit (April) are characterized by the highest diversity and relative levels of two group of compounds, sesquiterpenes and esters, but also by relative high levels of ethanol, β-citronellal, β-cyclocitral, and β- and α-ionone. On the other hand, several compounds remained constant during the whole process of ripening in fruit of both V and R, such as limonene, terpinolene, dihydrocarvone, α-humulene, 1-penten-3-one and 3-methylfuran (Table S3). It is interesting to note that the MHO norisoprenoid in the blond V orange showed its lowest relative level in full mature stage (April) while in

Table 1
Relative levels (fold change) in selected VOCs during maturation of Navel Foios and the red-fleshed Kirkwood oranges. VOCs listed are those showing significant differences ($p < 0.05$) between mature fruits (February) of Navel Foios and Kirkwood. Levels are normalized to the values of green fruits of Navel Foios (September). When a VOC was not detected in Navel Foios fruits of September, November was then set to 1. Code indicates the VOC number in Table S1.

		Foios Navel									Kirkwood Navel								
Code	VOCs	September		November		December		February			September		November		December		February		
Sesquiterpenes																			
31	Sesquiterpene 2[a]	1.00	±	0.01*	±	0.06	±	0.76*	±	16.53	±	0.76*	1.97	±	0.12	2.11	±	0.35	3.63	±	0.12	2.56	±	0.18
33	β-caryophyllene	1.00	±	0.01	±	0.50	±	1.08*	±	36.00	±	1.08*	1.25	±	0.01	2.23	±	0.92	11.43	±	0.42	8.60	±	1.75
34	Sesquiterpene 4[a]	nd	±	0.00	±	0.07*	±	0.33*	±	27.44	±	2.26*	nd			0.31	±	0.04	1.92	±	0.48	3.00	±	0.15
35	α-humulene	1.00	±	0.12*	±	0.32*	±	0.28*	±	9.92	±	0.04*	1.81	±	0.08	2.24	±	0.76	3.96	±	0.04	2.30	±	0.36
36	Sesquiterpene 5[a]	nd			±	0.11*	±	0.11*	±	21.58	±	0.36*	nd	±	0.01	0.43	±	0.08	1.77	±	0.01	2.52	±	0.53
37	Valencene	nd		1.00	±	0.01*	±	0.34*	±	14.43	±	0.17*	0.01	±	0.01	0.35	±	0.01	1.47	±	0.01	2.35	±	0.34
38	Sesquiterpene 6[a]	1.00	±	0.01*	±	2.00*	±	1.00*	±	368.00	±	14.50*	1.35	±	0.01	8.58	±	1.50	28.35	±	1.00	39.92	±	7.50
39	Sesquiterpene 7[a]	nd			±	0.19	±	0.38*	±	39.43	±	0.67*	nd			0.41	±	0.05	2.20	±	0.01	3.84	±	0.90
40	Sesquiterpene 8[a]	nd		1.00	±	0.03*	±	0.21*	±	24.61	±	0.27*	nd			0.39	±	0.09	1.69	±	0.12	2.53	±	0.58
Norisoprenoids																								
41	MHO	1.00	±	0.05*	±	0.05*	±	0.01*	±	0.59	±	0.05*	3.86	±	0.14	10.01	±	0.73	4.77	±	0.01	5.08	±	0.27
48	Geranylacetone	1.00	±	0.01*	±	0.01*	±	0.17*	±	1.67	±	0.01*	19.33	±	3.67	90.00	±	8.83	55.10	±	10.17	34.16	±	1.33
50	β-ionone	1.00	±	0.07	±	0.71	±	0.34*	±	11.17	±	1.90*	1.32	±	0.27	2.79	±	1.05	2.69	±	0.20	6.95	±	1.39

Asterisk in Foios Navel indicates significant differences ($p<0.05$) compared to Kirkwood Navel at the same maturity stage. MHO, 6-methyl-5-hepten-2-one. nd, indicates VOC not detected. [a]Sesquiterpene 2, 4, 5, 6, 7 and 8 were tentatively identified based on mass spectra and retention time.

the red-fleshed R increased during maturation and the maximum accumulation was reached in January.

A total of 33 volatile organic compounds (VOCs) were identified as differentially accumulated between the mature fruit R and V genotypes (Table 2). Five of these VOCs belong to the group of monoterpenes, ten to sesquiterpenes, four to norisoprenoids, nine to alcohols and aldehydes, three to ethyl esters, and one to acids. The relative levels of these five monoterpenes at the mature and full-mature stage (January and April) were between 2- and 4-fold higher in R than in V. Furthermore, most of them exhibited significant differences between genotypes at the green and breaker stages. The sesquiterpene group exhibited the greatest number of differentially accumulated compounds between R and V. In R fruit, the relative levels of sesquiterpenes were reduced by 50 % compared to V, but these differences were observed only at the full mature stage. The relative levels of norisoprenoids were between 4- and 40-fold higher in R than in V along fruit development and maturation. However, the maximum differences in norisoprenoid levels between genotypes were observed in the fruit harvested in January (mature). Two alcohols, one acid, and seven aldehydes exhibited relative levels that were between 2- to 8-fold higher in R than in V at the mature stage. Moreover, for the majority of the alcohols and aldehydes, the differences in the relative content between V and R were observed in all ripening stages. Significant differences in the relative levels of three ethyl esters were observed between both varieties since January and their content were diminished by approximately 50 % in the red-fleshed R compared to V at the full mature stage (April).

4. Discussion

The accumulation of lycopene and other colorless carotenes in red-fleshed sweet orange varieties represents an exceptional feature with potential interest to the citrus industry and consumers. However, the volatile composition of lycopene-accumulating citrus fruits has been relatively understudied. In this context, K and R are two novel red-fleshed orange varieties recently characterized under Spanish Mediterranean conditions (Zacarías-García et al., 2022a; Zacarías-García et al., 2023). These varieties are differentiated from the standard oranges by the markedly elevated concentration of the colorless carotenes phytoene and phytofluene, as well as the accumulation of lycopene (Fig. 1). Given the interest in these red-fleshed varieties for fresh consumption and the juice industry, this study has, for the first time, evaluated the changes in VOCs in the pulp of these varieties during fruit development and maturation, comparing them with the standard Navel and Valencia varieties. The VOC profiles of the four varieties analyzed in the current study are comparable to those previously described for other sweet orange genotypes (Tables S2 and S3) terpenes, aldehydes, alcohols, esters, and ketones being the most representative groups, and likely the main contributors to their characteristic aroma (Buettner and Schieberle, 2001; Baxter et al., 2005; Pérez-Cacho and Rouseff, 2009). The PCA analysis showed that the VOC profiles of the red-fleshed and their corresponding standard varieties exhibited similar patterns at each stage of development (Fig. 2). This suggests that the maturity stage of the fruit is the primary factor contributing to the observed variability in the samples, rather than the genotype and, consequently, the degree of similarity in VOC profiling between each mutant and its corresponding standard variety is greater than between the two mutants and the two blond varieties (Fig. 2, Tables S2 and S3). Monoterpenes represent the most abundant class of aroma compounds in citrus fruits during development and ripening. Among this group, limonene, the predominant terpene in sweet orange fruit and responsible for the citrus-like aroma (Arena et al., 2006; Pérez-Cacho and Rouseff, 2009; Rodríguez et al., 2017), exhibits similar levels in red-fleshed oranges compared with their corresponding blond counterparts (Table S2). Other monoterpene hydrocarbons and monoterpene alcohols detected in this study, such as α-pinene, β-myrcene, p-cymene, linalool, α-terpineol, and terpinen-4-ol, have also been described as aroma-active compounds in citrus juice

Table 2
Relative levels (fold change) in selected VOCs during maturation of Midknight Valencia and the red-fleshed Ruby oranges. VOCs listed are those showing significant differences ($p < 0.05$) between mature fruits (April) of Midknight Valencia and Ruby. Levels are normalised to the values of green fruits of Midknight Valencia (September). When a VOC was not detected in Midknight Valencia fruits of September, April was then set to 1. Code indicates the VOC identification number in Table S1.

		Midknight Valencia												Ruby Valencia											
Code	VOCs	September			November			January			April			September			November			January			April		
Monoterpene																									
6	3-carene	1.00	±	0.38	2.09	±	0.06*	0.69	±	0.20*	0.69	±	0.05*	0.57	±	0.06	2.69	±	0.01	1.86	±	0.00	1.23	±	0.03
13	eucalyptol	1.00	±	0.18	0.49	±	0.01	0.63	±	0.06*	0.46	±	0.09*	1.32	±	0.02	0.74	±	0.01	1.38	±	0.12	0.88	±	0.09
16	linalool	1.00	±	0.05*	0.43	±	0.02*	0.24	±	0.04*	0.24	±	0.03*	1.61	±	0.07	0.78	±	0.04	0.94	±	0.05	0.87	±	0.11
26	perillaldehyde	1.00	±	0.08	2.75	±	0.06*	1.19	±	0.05	0.21	±	0.04*	0.84	±	0.03	1.58	±	0.15	1.24	±	0.04	0.54	±	0.02
28	citronellyl acetate	1.00	±	0.05*	3.10	±	0.15*	1.51	±	0.12*	1.78	±	0.26*	1.77	±	0.17	5.91	±	0.04	6.54	±	0.15	3.95	±	0.20
Sequiterpene																									
29	sesquiterpene 1[a]	1.00	±	0.16	2.20	±	0.09*	0.90	±	0.11	2.01	±	0.08*	0.90	±	0.04	1.68	±	0.04	1.49	±	0.47	0.88	±	0.04
30	α-copaene	1.00	±	0.26	2.22	±	0.24*	1.47	±	0.19	2.54	±	0.08*	0.94	±	0.01	2.00	±	0.02	1.65	±	0.46	1.09	±	0.01
33	β-caryophyllene	1.00	±	0.16*	0.37	±	0.04	0.30	±	0.06	0.90	±	0.03*	0.19	±	0.01	0.24	±	0.00	0.37	±	0.06	0.64	±	0.04
34	sesquiterpene 4[a]	nd			nd			nd			1.00	±	0.05*	nd			nd			nd			0.64	±	0.10
35	α-humulene	1.00	±	0.29	1.01	±	0.04	0.62	±	0.05	1.24	±	0.09*	0.74	±	0.02	1.03	±	0.01	0.81	±	0.21	0.71	±	0.02
36	sesquiterpene 5[a]	nd			Nd			0.18	±	0.05	1.00	±	0.05*	nd			nd			0.09	±	0.00	0.58	±	0.04
37	valencene	1.00	±	0.27	1.00	±	0.07	17.00	±	4.01*	96.00	±	8.00*	1.00	±	0.13	nd			7.00	±	0.08	53.00	±	0.59
38	sesquiterpene 6[a]	1.00	±	0.11	0.47	±	0.11	1.47	±	0.24	7.24	±	1.13*	0.46	±	0.03	0.54	±	0.01	0.78	±	0.08	3.60	±	0.12
39	sesquiterpene 7[a]	Nd			Nd			0.12	±	0.00	1.00	±	0.01*	nd			nd			nd			0.55	±	0.03
40	sesquiterpene 8[a]	1.00	±	0.28*	1.14	±	0.03	3.00	±	0.61*	14.71	±	0.43*	0.43	±	0.05	1.00	±	0.10	1.71	±	0.21	8.14	±	0.30
Norisoprenoid																									
41	MHO	1.00	±	0.16*	0.71	±	0.07*	0.68	±	0.04*	0.43	±	0.07*	1.86	±	0.19	2.75	±	0.02	6.00	±	1.59	3.46	±	0.11
44	neral	1.00	±	0.03*	0.99	±	0.10*	1.12	±	0.20*	0.68	±	0.11*	1.68	±	0.00	1.82	±	0.04	2.58	±	0.07	1.86	±	0.17
45	geranial	1.00	±	0.01*	0.93	±	0.03*	1.16	±	0.22*	0.51	±	0.01*	2.07	±	0.13	2.25	±	0.06	2.91	±	0.27	1.97	±	0.25
48	geranylacetone	1.00	±	0.35*	1.50	±	0.24*	1.17	±	0.08*	1.67	±	0.16*	8.00	±	1.63	26.67	±	2.02	47.50	±	9.18	21.67	±	4.45
Alcohol																									
53	1-hexanol	nd			1.23	±	0.01	nd			1.00	±	0.11*	4.71	±	0.20	1.26	±	0.21	0.34	±	0.50	nd		
55	1-octanol	nd			4.26	±	0.95*	nd			1.00	±	0.06*	19.89	±	3.02	10.42	±	2.07	8.84	±	0.04	8.47	±	2.37
Aldehyde																									
64	octanal	1.00	±	0.01*	0.28	±	0.02*	0.32	±	0.01*	0.44	±	0.07*	1.85	±	0.06	0.60	±	0.03	1.02	±	0.04	1.07	±	0.06
67	nonanal	1.00	±	0.04*	0.52	±	0.01*	0.70	±	0.04	0.52	±	0.06*	1.89	±	0.02	1.20	±	0.06	1.70	±	0.07	1.44	±	0.00
68	(*E*)−2-nonenal	1.00	±	0.11	1.76	±	0.17*	1.43	±	0.03	0.76	±	0.05*	0.79	±	0.08	0.89	±	0.04	1.45	±	0.15	1.35	±	0.13
69	decanal	1.00	±	0.12*	0.33	±	0.02*	0.30	±	0.01*	0.39	±	0.04*	2.52	±	0.09	0.84	±	0.05	0.90	±	0.07	1.22	±	0.02
70	undecanal	1.00	±	0.20*	0.79	±	0.03*	0.71	±	0.10*	0.39	±	0.01*	1.94	±	0.05	1.53	±	0.02	1.46	±	0.08	1.40	±	0.02
71	(*E*,*E*)−2,4-decadienal	1.00	±	0.31*	Nd			3.86	±	0.73	3.24	±	0.48*	6.43	±	0.12	3.48	±	0.03	5.76	±	0.29	10.48	±	0.18
72	dodecanal	1.00	±	0.22*	0.45	±	0.04*	0.32	±	0.06*	0.26	±	0.03*	2.02	±	0.17	1.24	±	0.01	0.78	±	0.08	0.76	±	0.02
Ester																									
78	ethyl butanoate	nd			0.03	±	0.00	0.42	±	0.08*	1.00	±	0.06*	nd			nd			0.08	±	0.01	0.31	±	0.04
79	ethyl 2-methylbutyrate	nd			Nd			0.08	±	0.01	1.00	±	0.12*	nd			nd			0.06	±	0.00	0.58	±	0.06
86	ethyl octanoate	nd			0.15	±	0.04	0.59	±	0.05*	1.00	±	0.07*	nd			nd			0.22	±	0.03	0.67	±	0.03
Acid																									
95	dodecanoic acid	1.00	±	0.05	0.26	±	0.24	nd			0.09	±	0.06*	1.37	±	0.30	0.25	±	0.23	nd			0.28	±	0.01

Asterisk in Midknight Valencia indicates significant differences ($p < 0.05$) compared to Ruby Valencia at the same maturity stage. MHO, 6-methyl-5-hepten-2-one. nd, indicates VOC not detected. [a]Sesquiterpene 1, 4, 5, 6, 7 and 8 were tentatively identified based on mass spectra and retention time.

(Plotto et al., 2004; Arena et al., 2006; Pérez-Cacho and Rouseff, 2009), but none of these VOCs showed significant differences between N and K (Table S2). However, the relative level of five monoterpenes, including linalool, which is described as a floral note in orange juice (Pérez-Cacho and Rouseff, 2009), was higher in R fruit than in V (Table 2; Table S3). The higher level of linalool in R fruit, which is one of the more odor-active volatiles in freshly squeezed juice (Pérez-Cacho and Rouseff, 2009), may exert a considerable influence on the aroma of this variety.

In citrus fruit the accumulation of ethanol and acetaldehyde has been associated to fruit over-ripening and both volatiles are indicators of anaerobic metabolism (Hijaz et al., 2020). Furthermore, the build-up of these compounds may have an unfavorable influence on the flavor and acceptance of fresh citrus fruit and juice. In this sense, our results show that the red-fleshed oranges accumulate similar levels of ethanol and acetaldehyde than the standard Navel and Valencia varieties during maturation (Tables S2 and S3).

The catabolism of fatty acids through β-oxidation, α-oxidation, or lipoxygenase pathways results in the production of a variety of volatile compounds, including aliphatic aldehydes, alcohols, carboxylic acids, esters, lactones, and ketones (Gonzáles-Mas et al., 2011; Wang et al., 2001; Granell and Rambla, 2013). Our results show that most of these compounds accumulate at early and intermediate stages of fruit maturity in the four genotypes analyzed in this study (Fig. 2, Tables S2 and S3). It is noteworthy that the relative levels of these VOCs were comparable between K and N fruit at the mature stage. However, R fruit exhibited significantly elevated levels of C8–C12 aldehydes in comparison to V, suggesting differences in the oxidative activity of long-chain fatty acid between these varieties (Table 2; Table S3). These variations in fatty acid aldehydes between R and V may impact the orange flavor in mature fruit, as these VOCs are associated with fatty, green, and citrus-like flavors (Pérez-Cacho and Rouseff, 2009).

On the other hand, our findings align with those of Bai (2016) and Hijaz et al. (2020), which describe an increase in aliphatic esters in mature fruit. It is noteworthy that the mature fruit of the V variety contains higher levels of ethyl esters than the R mutant (Table 2). These compounds are considered as the primary contributors to the fruity aroma of citrus fruits, with ethyl butanoate being particularly noteworthy. This ester is described as one of the most intense odorants in orange juice and is considered the single most important ester (Arena et al., 2006; Pérez-Cacho and Rouseff, 2009; Obenland et al., 2009). These variations between varieties are likely to be regulated by either alcohol acyltransferase activity and/or substrate availability. Given that no differences in ethanol content were observed between R and V fruit, it is reasonable to assume that these changes could be related to a lower alcohol acyltransferase activity in the pulp of V, as has been postulated in fruits of different citrus varieties to explain the differences in esters contents (González-Mas et al., 2011).

The comparative analysis of VOCs in the pulp of red-fleshed varieties with the corresponding blond oranges revealed two common differences: the levels of sesquiterpenes were found to be lower in the red-fleshed oranges, while the levels of the norisoprenoids MHO and geranylacetone were found to be higher (Tables 1 and 2). A PLS-DA (partial least squares discriminant analysis) provided further confirmation that the norisoprenoids MHO and geranylacetone, along with specific sesquiterpenes, are significant discriminators between the red-fleshed orange varieties (K and R) and the traditional varieties (N and V). This reinforces the role of these volatiles in differentiating between the two groups of oranges (Figure S2). It has been proposed that the norisoprenoid geranylacetone is derived from the enzymatic or chemical oxidative breakdown of the linear carotenoids phytoene, phyophene, neurosporene, and ζ-carotene while lycopene would give rise to MHO, neral, and geranial (Lewinsohn et al., 2005b; Vogel et al., 2008; Walter and Strack, 2011). A number of studies have demonstrated that the content of norisoprenoids in fruit of tomato (Vogel et al., 2010), peach (Brandi et al., 2011) and sweet orange (Wei et al., 2018) changes in a comparable manner to the relative accumulation of the carotenoid precursors. In citrus fruits, the potential correlation between the content of lycopene and norisoprenoids has been investigated in the red-fleshed orange Anliu and pummelo Guanxi, showing a positive relationship between the concentration of the norisoprenoids neral and geranial, and the carotene lycopene (Liu et al., 2013; Liu et al., 2019; Liu et al., 2022). The levels of MHO and geranylacetone in the K and R red-fleshed oranges were found to be considerably higher than those of the corresponding standard varieties (Tables 1 and 2). These results are consistent with the very high content of colorless carotenes and the accumulation of lycopene in the pulp of K and R fruit (Fig. 1) (Zacarías-García et al. 2022, 2023). Furthermore, two additional norisoprenoids (neral and geranial) accumulated in higher proportions in R fruit than in V. Therefore, the higher levels of norisoprenoids in K and R are likely due to the large amounts of their carotenoid precursors in the pulp of the red-fleshed varieties. Given the high sensitivity of the human olfactory system to norisoprenoids (Mahattanatawee et al., 2005), the elevated relative levels of these volatiles in the pulp of red-fleshed oranges in comparison to their standard varieties may contribute significantly to their flavor, as has been described in tomato fruit and orange juices (Winterhalter and Rouseff, 2002; Mahattanatawee et al., 2005; Tieman et al., 2017).

The second common trait that distinguishes K and R volatile composition from that of standard blond oranges is the lower levels of sesquiterpenes, particularly in mature fruit. In general, the content of sesquiterpenes has been reported to increase with fruit maturation in different varieties of sweet orange (Bai et al., 2016; Hou et al., 2020) and lemon fruit (Li et al., 2022). However, few studies have addressed the analysis of these terpenes in other red-fleshed citrus. Liu et al. (2022) observed that the leaves of the red-fleshed Cara Cara orange exhibited a significantly reduced content of sesquiterpenes in comparison with the standard Navel orange 'Seiku'. Similarly, the pulp of the red Anliu orange exhibited lower amounts of sesquiterpenes in comparison to its parental genotype (Liu et al., 2019). In the four genotypes analyzed in this study, the relative levels of most of the sesquiterpenes were low or non-detectable at early stages of development and increased with maturity. However, in the red-fleshed oranges K and R, the magnitude of the increment during ripening was lower than in the blond orange counterparts (Tables 1 and 2; Tables S2 and S3). Of particular interest is the lower valencene content observed in the pulp of the red varieties K and R since this volatile has been proposed to be the most abundant sesquiterpene and an important contributor to orange fruit flavor (Ren et al., 2015; Zhang et al., 2017; Hou et al., 2020).The relationship between the high accumulation of carotenes and the low relative level of sesquiterpenes may be explained by metabolic crosstalk between the mevalonate (MVA) and the methyl-D-erythritol phosphate (MEP) pathways at the isopentenyl diphosphate (IPP) level, the central intermediate in the biosynthesis of isoprenoids (Gutensohn et al., 2014). The formation of IPP from the MVA pathway is localized in the cytosol and produces farnesyl diphosphate (FPP), the precursor of sesquiterpenes. The MEP pathway, which operates in the plastids, provides geranyl diphosphate (GPP), the precursor of monoterpenes, diterpenes, and carotenoids. Although the subcellular compartmentalization allows the MVA and MEP pathways to operate independently, a metabolic crosstalk between both pathways has been reported at the IPP level, particularly in the flow from plastids to the cytosol (Bick and Lange, 2003; Hemmerlin et al., 2003). Thus, it is reasonable to hypothesize that the over-accumulation of carotenoids in K and R oranges from early stages of fruit development may involve a high demand of IPP supply for the MEP pathway in plastids, which may affect the flux of the MVA pathway in the cytosol through co-regulatory mechanisms, and hence decreasing the availability of precursors for sesquiterpenes.

5. Conclusions

The availability of two independent spontaneous mutations of sweet orange, Kirkwood and Ruby, in two genetic backgrounds, Navel and

Valencia, which result in similar alterations in the content of carotenes, represents a valuable tool for investigating the effect of this alteration on the fruit VOCs. Overall, the changes in volatiles in the pulp of Kirkwood and Ruby fruit during development and ripening are similar to those in standard blond oranges. However, the increased levels of carotenoids in the red-fleshed varieties appear to be closely associated with changes in specific VOCs, resulting in higher levels of specific linear carotenoid-derived norisoprenoids and lower levels of sesquiterpenes. Future studies should aim to investigate the impact of these changes in VOCs on the flavor and consumer acceptance of red-fleshed citrus fruit.

Abbreviations

K, Kirkwood Navel; R, Ruby Valencia; N, Foios Navel; V, Midknight Valencia. HS-SPME/GC-MS, headspace-solid phase microextraction / gas chromatography couple mass spectrometry. MHO, 6-methyl-5-hepten-2-one.

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CRediT authorship contribution statement

Jaime Zacarías-García: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Jose Luis Rambla:** Writing – review & editing, Validation, Methodology, Formal analysis. **Lorenzo Zacarias:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Maria J. Rodrigo:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization. **Antonio Granell:** Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106821.

References

Arena, E., Guarrera, N., Campisi, S., Asmundo, C.N., 2006. Comparison of odour active compounds detected by gas-chromatography–olfactometry between hand-squeezed juices from different orange varieties. *Food Chemistry* 98, 59–63. https://doi.org/10.1016/j.foodchem.2005.04.03.

Bai, J., Baldwin, E.A., McCollum, G., Plotto, A., Manthey, J.A., Widmer, W.W., Luzio, G., Cameron, R., 2016. Changes in volatile and non-volatile flavour chemicals of "Valencia" orange juice over the harvest seasons. Foods 5 (1), 4. https://doi.org/10.3390/foods5010004.

Baxter, I.A., Easton, K., Schneebeli, K., Whitfield, F.K., 2005. High pressure processing of Australian navel orange juices: Sensory analysis and volatile flavour profiling. Innov. Food Sci. Emerg. Technol. 6 (4), 372–387. https://doi.org/10.1016/j.ifset.2005.05.005.

Bick, J.A., Lange, B.M., 2003. Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membrane. Arch. Biochem. Biophys. 415 (2), 146–154 https://doi.org/1010.1016/s0003-9861(03)00233-9.

Brandi, F., Bar, E., Mourgues, F., Horváth, G., Turcsi, E., Giuliano, G., Liverani, A., Tartarini, S., Lewinsohn, E., Rosati, C., 2011. Study of 'Redhaven' peach and its white-fleshed mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism. BMC Plant Biol. 11, 24. https://doi.org/10.1186/1471-2229-11-24.

Buettner, A., Schieberle, P., 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavour reconstitution experiments. J. Agric. Food Chem. 49 (5), 2387–2394. https://doi.org/10.1021/jf001363l.

Defilippi, B.G., Manriquez, D., Luengwilai, K., Gonzalez-Aguero, M., 2009. Aroma Volatiles: Biosynthesis and Mechanisms of Modulation during Fruit Ripening. Adv. Bot. Res. 50, 1–37.

González-Mas, M.C., Rambla, J.L., Alamar, M.C., Gutiérrez, A., Granell, A., 2011. Comparative analysis of the volatile fraction of fruit juice from different citrus species. PLoS One 6 (7), e22016. https://doi.org/10.1371/journal.pone.0022016.

González-Mas, M.C., Rambla, J.L., López-Gresa, M.P., Blázquez, M.A., Granell, A., 2019. Volatile Compounds in Citrus Essential Oils: A Comprehensive Review. Front. Plant Sci. 10, 12. https://doi.org/10.3389/fpls.2019.00012.

Granell, A., Rambla, J.L., 2013. Biosynthesis of volatile compounds. In: Seymour, In.G.B., Tucker, G.A., Poole, M., Giovannoni, J.J. (Eds.), Molecular Biology and Biochemistry of Fruit Ripening. Wiley-Blackwell, pp. 135–161.

Gutensohn, M., Nagegowda, D.A., Dudareva, N., 2014. Involvement of compartmentalization in monoterpene and sesquiterpene biosynthesis in plants. In: Bach, In.T.J., Rohmer, M. (Eds.), Isoprenoid Synthesis in Plants and Microorganisms. Springer, pp. 155–170.

Habibi, F., Ramezanian, A., Guillen, F., Serrano, M., Valero, D., 2020. Effect of various postharvest treatment on aroma volatile compounds of blood orange fruit exposed to chilling temperature after long-term storage. Food Bioprocess Technol. 13, 2054–2064. https://doi.org/10.1007/s11947-020-02547-1.

Hemmerlin, A., Hoeffler, J.F., Meyer, O., Tritsch, D., Kagan, I.A., Grosdemange-Billiard, C., Rohmer, M., Bach, T.J., 2003. Cross-talk between the cytosolic mevalonate and the plastidial methyl erythritol phosphate pathways in tobacco bright yellow-2 cells. J. Biol. Chem. 278 (29), 26,666–26,676. https://doi.org/10.1074/jbc.M302526200.

Hijaz, F., Gmitter, F.G., Bai, J., Baldwin, E., Biotteau, A., Leclair, C., McCollum, T.G., Plotto, A., 2020. Effect of fruit maturity on volatiles and sensory descriptors of four mandarin hybrids. J. Food Sci. 85 (5), 1548–1564. https://doi.org/10.1111/1750-3841.15116.

Hou, J., Liang, L., Wang, Y., 2020. Volatile composition changes in navel orange at different growth stages by HS-SPME-GC-MS. Food Res. Int. 136, 109333. https://doi.org/10.1016/j.foodres.2020.109333.

Khan, U.M., Sevindik, M., Zarrabi, A., Nami, M., Ozdemir, B., Kaplan, D.N., Selamoglu, Z., Hasan, M., Kumar, M., Alshehri, M.M., Sharifi-Rad, J., 2021. Lycopene: Food Sources, Biological Activities, and Human Health Benefits. Oxid. Med. Cell. Longev., 2713511 https://doi.org/10.1155/2021/2713511.

Lana, G., Zacarias-Garcia, J., Distefano, G., Gentile, A., Rodrigo, M.J., Zacarias, L., 2020. Transcriptional analysis of carotenoids accumulation and metabolism in a pink-fleshed lemon mutant. Genes 11 (11), 1294. https://doi.org/10.3390/genes11111294.

Lewinsohn, E., Sitrit, Y., Bar, E., Azulay, Y., Meir, A., Zamir, D., Tadmor, Y., 2005a. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. J. Agric. Food Chem. 53, 3142–3148. https://doi.org/10.1021/jf047927t.

Lewinsohn, E., Sitrit, Y., Bar, E., Azulay, Y., Ibdah, M., Meir, A., Yosef, E., Zamir, E., Tadmor, Y., 2005b. Not just colors- carotenoid degradation as a link between pigmentation and aroma in tomato and watermelon fruit. Trends Food Sci. Technol. 16, 407–415. https://doi.org/10.1016/j.tifs.2005.04.004.

Li, C., Li, X., Liang, G., Xiang, S., Han, G., 2022. Volatile composition changes in lemon during fruit maturation by HS-SPME-GC-MS. J. Sci. Food Agric. 102 (9), 3599–3606. https://doi.org/10.1002/jsfa.11706.

Liu, C., He, M., Xu, J., 2022. The constitutions of terpenoids and hormones of leaves in red-flesh citrus mutants and their wild types. Hortic. Plant J. 8 (3), 297–310. https://doi.org/10.1016/j.hpj.2021.11.006.

Liu, C., He, M., Wang, Z., Xu, J., 2019. Integrative analysis of terpenoid profiles and hormones from fruits of red-flesh citrus mutants and their wild types. Molecules 24, 3456. https://doi.org/10.3390/molecules24193456.

Liu, C., Yan, F., Gao, H., He, M., Wang, Z., Cheng, Y., Deng, X., Xu, J., 2013. Features of citrus terpenoid production as revealed by carotenoid, limonoid and aroma profiles of two pummelos (*Citrus maxima*) with different flesh color. J. Sci. Food Agric. 95, 111–119. https://doi.org/10.1002/jsfa.6689.

Mahattanatawee, K., Rouseff, R., Valim, M.F., Naim, M., 2005. Identification and aroma impact of norisoprenoids in orange juice. J. Agric. Food Chem. 53 (2), 393–397. https://doi.org/10.1021/jf049012k.

Obenland, D., Collin, S., Mackey, B., Sievert, J., Fjeld, K., Arpaia, M.L., 2009. Determinants of flavour acceptability during the maturation of navel oranges. Postharvest Biol. Technol. 52, 156–163. https://doi.org/10.1016/j.postharvbio.2009.01.005.

Pérez-Cacho, P.R., Rouseff, R.L., 2009. Fresh Squeezed Orange Juice Odor: A Review. Crit. Rev. Food Sci. Nutr. 48 (7), 681–695. https://doi.org/10.1080/10408390701638902.

Plotto, A., Margaría, C.A., Goodner, K.L., Goodrich, R., Baldwin, E.A., 2004. Odour and flavour thresholds for key aroma components in an orange juice matrix: Terpenes and aldehydes. Flavour Fragrancy J. 19, 491–498. https://doi.org/10.1002/ffj.1470.

Rambla, J.L., González-Mas, M.C., Pons, C., Bernet, G.P., Asins, M.J., Granell, A., 2014. Fruit volatile profiles of two Citrus hybrids are dramatically different from those of their parents. J. Agric. Food Chem. 62, 11312–11322. https://doi.org/10.1021/jf5043079.

Ren, J.N., Tai, Y.N., Dong, M., Shao, J.H., Yang, S.Z., Pan, S.Y., Fan, G., 2015. Characterisation of free and bound volatile compounds from six different varieties of citrus fruits. Food Chem. 185, 25–32. https://doi.org/10.1016/j.foodchem.2015.03.142.

Rodríguez, A., Peris, J.E., Redondo, A., Shimada, T., Costell, E., Carbonell, I., Rojas, C., Peña, L., 2017. Impact of d-limonene synthase up- or down-regulation on sweet orange fruit and juice odor perception. Food Chem. 217, 139–150. https://doi.org/10.1016/j.foodchem.2016.08.076.

Rouseff, R.L., Sadler, G.D., Putnam, T.J., Davis, J.E., 1992. Determination of β-Carotene and Other Hydrocarbon Carotenoids in Red Grapefruit Cultivars. J. Agric. Food Chem. 40, 47–51.

Salvatore, M.M., Nicoletti, R., Andolfi, A., 2022. Essential Oils in Citrus Fruit Ripening and Postharvest Quality. Horticulturae 8 (5), 396. https://doi.org/10.3390/horticulturae8050396.

Sdiri, S., Rambla, J.L., Besada, C., Granell, A., Salvador, A., 2017. Changes in the volatile profile of citrus fruit submitted to postharvest degreening treatment. Postharvest Biol. Technol. 133, 48–56. https://doi.org/10.1016/j.postharvbio.2017.07.001.

Tieman, D., Zhum, G., Resende, M.F.J., Lin, T., Nguyen, C., Bies, D., Rambla, J.L., Beltran, K.S., Taylor, M., Zhang, B., Ikeda, H., Liu, Z., Fisher, J., Zemach, I., Monforte, A., Zamir, D., Granell, A., Kirst, M., Huang, S., Klee, H., 2017. A chemical genetic roadmap to improved tomato flavour. Science 355 (6323), 391–394. https://doi.org/10.1126/science.aal1556.

Vogel, J.T., Tan, B.C., McCarty, D.R., Klee, H.J., 2008. The carotenoid cleavage dioxygenase 1 enzyme has broad substrate specificity, cleaving multiple carotenoids at two different bond positions. J. Biol. Chem. 283, 11364–11373. https://doi.org/10.1074/jbc.M710106200.

Vogel, J.T., Tieman, D.M., Sims, C.A., Odabasi, A.Z., Clark, D.G., Klee, H.J., 2010. Carotenoid content impacts flavour acceptability in tomato (*Solanum lycopersicum*), 10 J. Sci. Food Agric. 90 (13), 2233–2240. https://doi.org/10.1002/jsfa.4076.

Xu, J., Tao, N., Liu, Q., Deng, X., 2006. Presence of diverse ratios of lycopene/β-carotene in five pink or red-fleshed citrus cultivars. Sci. Hortic. 108 (2), 181–184. https://doi.org/10.1016/j.scienta.2006.01.032.

Walter, M.H., Strack, D., 2011. Carotenoids and their cleavage products: Biosynthesis and functions. Nat. Prod. Rep. 28, 663–692. https://doi.org/10.1039/c0np00036a.

Wang, C., Xing, J., Chin, C.-K., Ho, C.-T., Martin, C.E., 2001. Modification of fatty acids changes the flavour volatiles in tomato leaves. Phytochemistry 58 (2), 227–232. https://doi.org/10.1016/s0031-9422(01)00233-3.

Wei, X., Song, M., Chen, C., Tong, H., Liang, G., Gmitter, F.G., 2018. Juice volatile composition differences between Valencia orange and its mutant Rohde Red Valencia are associated with carotenoid profile differences. Food Chem. 245, 223–232. https://doi.org/10.1016/j.foodchem.2017.10.066.

Winterhalter, P., Rouseff, R., 2002. Carotenoid-Derived Aroma Compounds. ACS. https://doi.org/10.1021/bk-2002-0802.ch001.

Zacarías-García, J., Cronje, P.J., Gianfranco, D., Rodrigo, M.J., Zacarías, L., 2022a. A comprehensive analysis of carotenoids metabolism in two red-fleshed mutants of Navel and Valencia sweet oranges (*Citrus sinensis*). Front. Plant Sci. 13, 1034204. https://doi.org/10.3389/fpls.2022.1034204.

Zacarías-García, J., Cronje, P.J., Gianfranco, D., Rodrigo, M.J., Zacarías, L., 2022b. A comprehensive analysis of carotenoids metabolism in two red-fleshed mutants of Navel and Valencia sweet oranges (Citrus sinensis). Front. Plant Sci. 13, 1034204. https://doi.org/10.3389/fpls.2022.1034204.

Zacarías-García, J., Pérez-Través, L., Gil, J.-V., Rodrigo, M.-J., Zacarías, L., 2022c. Bioactive Compounds, Nutritional Quality and Antioxidant Capacity of the Red-Fleshed Kirkwood Navel and Ruby Valencia Oranges. Antioxidants 11 (10), 1905. https://doi.org/10.3390/antiox11101905.

Zacarías-García, J., Rodrigo, M.J., Ramble, J.L., Granell, A., Zacarías, L., 2023. Physiological responses, nutritional quality and aroma volatiles of the red-fleshed kirkwood navel and ruby valencia oranges during postharvest cold storage. Postharvest Biol. Technol. 199, 112303. https://doi.org/10.1016/j.postharvbio.2023.112303.

Zhang, H., Xie, Y., Liu, C., Chen, S., Hu, S., Xie, Z., Deng, X., Xu, J., 2017. Comprehensive comparative analysis of volatile compounds in Citrus fruits of different species. Food Chem. 230, 316–326. https://doi.org/10.1016/j.foodchem.2017.03.040.

Zheng, H., Zhang, Q., Quan, J., Zheng, Q., Xi, W., 2016. Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps. Food Chem. 205, 112–121. https://doi.org/10.1016/j.foodchem.2016.03.007.