# Influence of the storage temperature on volatile emission, carotenoid content and chilling injury development in Star Ruby red grapefruit

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## Abstract

Grapefruits are sensitive to develop chilling injury (CI) on the peel upon postharvest storage at low temperature. We investigated the influence of the storage at 2 and 12 ºC on CI, carotenoids, and emission of volatiles by intact fruit. CI symptoms at 12 ºC were restricted to green fruit peel sectors but at 2 ºC the CI severity was higher and distributed through the whole fruit surface. Fruit peel coloration and carotenes content increased at 12 ºC whereas experienced minor changes at 2 ºC. At 2 ºC the emission of total volatiles and specific monoterpenes, mainly limonene, but also linalool and α-terpineol was enhanced, while storage at 12 ºC resulted in higher emission and diversity of cyclic sesquiterpenes and aliphatic esters. Results indicate a selective emission of volatiles by intact red grapefruit that appears to be a specific response to the storage temperature or to the cold-induced damage.

## 1. Introduction

Low temperature storage is the most widely used postharvest technology to preserve the quality and to extend storage life of fruits and vegetables. Moreover, fruit exportation of many species, such as Citrus fruits, to USA or Japan requires strict quarantine treatments at low temperature for fruit fly disinfestation (Biolatto, Vazquez, Sancho, Carduza, & Pensel, 2005). However, fruit of certain citrus species are highly susceptible to develop chilling injury (CI) symptoms in the peel upon exposition to low temperatures. Although CI symptoms may vary among susceptible varieties, they are initially manifested as small brown pits on the fruit surface that progressively increase in size forming brown clusters of depressed areas of variable shape, that may affect large surface of the peel under prolonged cold storage periods (Lado, Cronje, Rodrigo, & Zacarías, 2019). CI symptoms are usually restricted to the peel but negatively affect global marketing quality and consumer perception. Experimental evidences accumulated over the years in fruit of cold-sensitive varieties have evidenced that cold itself or the cold-induced damage provoke substantial changes in fruit primary and secondary metabolism as part of the genetic and biochemical responses of the fruit to cope with low temperature stress (Lado et al., 2019).

Previous works showed that storage of orange and mandarin fruit at moderate temperature (12 ºC) stimulates the expression of carotenoid biosynthetic genes, carotenoid accumulation and enhanced peel and pulp coloration, demonstrating an active metabolism of these pigments at this temperature (Carmona, Zacarías, & Rodrigo, 2012). Carotenoids constitute a broad family of isoprenoids that display diverse functions in plants. Citrus fruits are one of most complex sources of carotenoids, with a large diversity among the different species and cultivars in terms of types and amounts (Lado, Zacarías, & Rodrigo, 2016). One of the most important carotenoid

69 properties is their ability to scavenge and quenching reactive oxygen molecules,
70 becoming part of the antioxidant defense system (Krinsky, 1989). Lycopene is a linear
71 carotene and a powerful antioxidant, and its presence in grapefruit peel has been
72 associated with a lower CI incidence, suggesting that this carotene exerts a protective
73 role scavenging singlet oxygen under low temperature stress (Lado, Rodrigo, Cronje, &
74 Zacarías, 2015). Therefore, changes in the profile of these pigments, and particularly
75 lycopene, are important not only to provide the typical red coloration of the peel but
76 also by their influence on grapefruit susceptibility to CI.

77 The emission of volatile compounds by plant tissues has fundamental roles in
78 the communication of plants with the surrounding environment and also in the response
79 to different biotic and abiotic stresses. The response to pathogen attack, as example,
80 drastically modify the volatile profile emission in plants which play key roles in the
81 attraction of natural predators and in the induction of cellular defense mechanisms to
82 cope with pathogenic infection (Rodríguez, Alquézar, & Peña, 2013). Transgenic sweet
83 orange plants with a reduced expression of limonene synthase resulted in fruit with
84 lower limonene but higher content of certain monoterpene alcohols, and showed a
85 higher resistance to pathogens, suggesting a key role of these compounds in citrus fruit
86 interaction with the environment (Rodríguez et al., 2015, 2018). Volatile profile in
87 citrus fruit notably varies during fruit ripening, rootstock and growing conditions
88 (Ortuño et al., 1995) as well as with postharvest practices (curing, degreening and
89 waxing), storage conditions (Biolatto et al., 2005; Obenland, Collin, Sievert & Arpaia,
90 2013; Sdiri, Rambla, Besada, Granell, & Salvador, 2017; Tietel, Lewinsohn, Fallik, &
91 Porat, 2012; Tietel, Plotto, Fallik, Lewinsohn, & Porat, 2011) and physiological
92 disorders (Xie et al., 2018).

Monoterpenes and sesquiterpenes are the predominant volatile compounds identified in fruits of different citrus species and are strongly linked to flavor perception and consumers´ acceptance (Benjamin, Tietel, & Porat, 2013; Goldenberg, Yaniv, Doron-Faigenboim, Carmi, & Porat, 2016; González-Mas, Rambla, López-Gresa, Blázquez, & Granell, 2019; Ren et al., 2015; Tietel et al. 2011). Volatile composition of the juice allowed an accurate discrimination of different citrus species (mandarins, white and red grapefruit and sweet orange) using principal component analysis (Goldenberg et al., 2016; González-Mas, Rambla, Alamar, Gutiérrez, & Granell, 2011; Miyazaki, Plotto, Goodner, & Gmitter, 2011; Rambla et al., 2014; Ren et al., 2015; Xie, Deng, Zhou, Yao, & Zeng, 2018; Zhang et al., 2017). These studies showed that most of the major terpenes (i.e., α-pinene, β-myrcene, *D*-limonene, terpinen-4-ol, α-terpineol, and valencene) and other aldehydes or esters compounds (i.e. 2-hexenal, decanal and ethyl 3-hydroxyhexanoate) are the main contributors to the higher volatile contents in the citrus juices. The volatile emission of different citrus organs and tissues, including fruit, have been monitored in grapefruit (Flamini & Cioni, 2010), showing that the main volatiles emitted by the ripe pericarp were the monoterpenes limonene (68-95 %) α- and β-pinene (0.4-12 %) and myrcene (1.5-2.4 %). Monitoring the changes in volatile emission (mostly ethanol, ethyl butanoate, methyl hexanoate, and ethyl octanoate) in sweet Navel oranges has been proposed as a tool for discriminating freezing damage (Obenland, Aung, Bridges, & Mackey, 2003). Similarly, large changes in the volatile profiles were observed in oleocellosis-damaged peels of sweet orange and mandarin compared to healthy fruit, showing a drastic decrease in volatile compounds such as α-terpinolene, β-caryophyllene, β-terpineol and α-terpineol (Xie et al., 2018).

Based on these studies, we hypothesized that the characterization of the volatile emission profile could be a tool for discriminating postharvest fruit response or

susceptibility to different stresses or physiological disorders. However, scarce information is available regarding changes in volatile emission in citrus fruit during cold stress and the relationship with their sensitiveness to low temperature. Among citrus fruit, grapefruit (*Citrus paradisi* Macf.) are highly susceptible to develop CI during cold transport and storage at temperatures below 8 ºC for prolonged periods (Schirra, 1993). Moreover, we have shown that carotenoids content and composition, in particular lycopene, at harvest time influence tolerance to chilling damage (Lado, Rodrigo, et al., 2015). Grapefruit sensitivity to postharvest CI changes during the growing season, being higher in early and late-season harvests (Schirra, Agabbio, & D`Hallewin, 1998). Therefore, the aim of the present work has been to investigate the changes in the profile of volatiles emitted by intact fruit and in carotenoid composition in the flavedo of the red Star Ruby (SR) grapefruit during postharvest storage at two different temperatures, 2 and 12 ºC. Our hypothesis envisages that changes in the volatile emission profile and/or carotenoids composition may reveal possible markers associated with cold sensitivity, cold responses or CI development in SR grapefruit. Moreover, information generated will provide clues about how postharvest storage temperature may affect these important quality traits in citrus fruit.

## 2. Material and Methods

### *2.1. Fruit material, storage conditions and fruit quality assessment*

Fruit of grapefruit (*Citrus paradisi* Macf.) cv. 'Star Ruby' (SR) were harvested from adult trees cultivated under commercial conditions at the end of November, 240 days after full-bloom under Mediterranean climactic conditions. Fruits were delivered to the laboratory, inspected for free of injuries or defects, selected by uniformity and divided in replicated samples. Replicate lots of fruits were stored at 2 ºC (chilling-

142 temperature) or 12 ºC (non-chilling control temperature) for up to 7 w. At harvest and
143 during postharvest, fruit volatiles emission, carotenoid composition and CI symptoms
144 (% and severity of damaged fruit) were monitored in cold stored and control fruit. Three
145 replicates of 10 fruits per treatment and temperature were used for CI evaluation using a
146 complete randomized design. At harvest time and each sampling date, peel color was
147 measured using a Minolta CR-330 colorimeter on three locations around the equatorial
148 plane of the fruit, using three replicates of 10 fruit each. Color was expressed as the *a/b*
149 Hunter ratio. The *a/b* ratio is negative for green fruit, the zero value corresponds to
150 yellow fruit at color break and is positive for orange to red colored fruit.

151 *2.2. Chlorophyll and carotenoid extraction*

152 Flavedo (outer colored layer of fruit peel) pigments were extracted essentially as
153 described previously (Lado, Cronje, et al., 2015). After Chl measurements, the pigment
154 ethereal solution was dried and saponified using a 10 % methanolic:KOH solution.
155 Carotenoids were kept at -20 ºC until HPLC analysis. All procedures were carried were
156 extracted and the samples dried under $N_2$ and kept out on ice under dim light to prevent
157 possible photo-degradation, isomerization and structural changes of carotenoids.

158 *2.3. Carotenoid analysis by HPLC-PAD*

159 Individual carotenoid composition of each sample was analyzed by HPLC with a
160 Waters liquid chromatography system equipped with a 600E pump and a model 2998
161 photodiode array detector (PAD) and Empower software (Waters). A C30 carotenoid
162 column (250 × 4.6 mm, 5 μm) coupled to a C30 guard column (20 × 4.0 mm, 5 μm)
163 (YMC Europe GmbH) was used. Samples were prepared for HPLC by dissolving the
164 dried carotenoid extracts in $CHCl_3$: MeOH: acetone (3:2:1, v:v:v). A ternary gradient
165 elution with MeOH, water and methyl *tert*-butyl ether (MTBE) was used for carotenoid

166 separation as reported in previous works (Lado, Cronje, et al., 2015). The carotenoid
167 peaks were integrated at their individual maxima wavelength and their content was
168 calculated using calibration curves according to (Lado, Cronje, et al., 2015). Total
169 carotenoid content was calculated as the sum of individual carotenoids. Samples were
170 extracted twice and each analytical determination was replicated three times.

171 *2.4. Fruit volatile emission analysis by GC-MS*

172 The analysis of the volatile compounds emitted by intact fruits of SR grapefruit
173 was performed by the technique headspace solid phase microextraction (HS-SPME).
174 Replicates consisted of three to four fruits that were weighed, placed in glass containers
175 of 4.5 L and closed hermetically during 2 h at the storage temperature to allow volatiles
176 accumulation in the headspace. A fused silica fiber covered with Polydimethylsiloxane
177 (PDMS; Supelco) of 100 μm of thickness was used for volatiles adsorption. As internal
178 standard, 10 μL of 2-Octanol (≥99.5 % GC, Sigma-Aldrich) solution (in ethanol 0.1%)
179 were placed on a 2 $cm^2$ filter paper inside the glass container. Internal standard was used
180 as reference for correction of temporal variations. The fiber was introduced in the
181 containers across a septum and exposed to the headspace during 15 min. Desorption of
182 volatiles from the SPME fiber was performed for 4 min at 220 ºC in the GC injector
183 splitless mode.
184
185 The volatiles were analysed with a Thermo TraceTM GC Ultra equipped with a
186 capillary Innowax column (Agilent Technologies) (30 m x 0.25 mm x 0.25 μm) and
187 coupled to a Thermo DSQ Mass Spectrometer. Helium was used as carrier gas at
188 constant flow of 1.5 mL/min. The oven was programmed at 40 ºC for 5 min and 5
189 ºC/min ramp until 150 ºC, 20 ºC/min ramp until 250 ºC and then held isothermally at
190 250 ºC for 2 min. The MS was operated in the electron ionization mode ($EI^+$) at 70 eV

191 with a transfer temperature of 260 ºC and source temperature of 200 ºC. Data
192 acquisition was performed in scanning mode (mass range 30 to 400 *m/z*). Identification
193 of volatile compounds was obtained by comparison of the mass spectra from Willey6,
194 NIST 2005, REPLIB MANILIB libraries and mass spectra custom library generated
195 using available standards. Chromatograms were recorded and integrated using Xcalibur
196 software 1.4.z.

197 Quantification of the main volatile compounds was conducted using calibration
198 curves with standards obtained from Sigma-Aldrich. If commercial standards were not
199 available, the most structurally similar compound was used for quantification. Each
200 curve was prepared by serial dilution from standard solutions in methanol (HPLC, Carlo
201 Erba).

202 Linear retention index, LRI (formerly, Kovat`s index values) of chromatographic
203 peaks on Innowax column were determined using a series of alkanes (C6-C25) run
204 under identical chromatographic conditions.

205 *2.5. Statistical analysis*

206 For Principal Component Analysis (PCA) all replicates dataset were considered
207 and the program XLSTAT-Pro (Addinsoft, Barcelona, Spain) was used. An ANOVA
208 test was carried out and mean Tukey comparison ($p<0.05$) was included when
209 necessary. Standard deviation was included in volatile data.

210

211 **3. Results and Discussion**

212 *3.1. Effect of temperature storage (2 and 12 ºC) on peel color and chilling injury in red*
213 *Star Ruby grapefruit*

CI is a relevant physiological disorder in citrus due to its impact on external fruit appearance and the detrimental effects on fruit quality during postharvest cold storage (Lado et al., 2019). Grapefruits are among the most sensitive citrus species to develop chilling upon storage at temperatures below 8 °C, that may depend on fruit susceptibility and field conditions (Schirra, 1993). In this work, Star Ruby (SR) grapefruit developed CI symptoms during storage at 2 and 12 °C but the extension, intensity and evolution was different at each temperature, and also dependent of peel color. Fruit were harvested after color-break (late November under Mediterranean conditions) when natural coloration of the peel was progressing (Fig. 1A). Peel color of the fruit was not uniform, showing green sectors of variable size and surrounding extended areas of red and yellow coloration (Fig. 1C). During storage at 2 °C, peel color only experienced minor changes (from an *a/b* value of 0.05 to 0.15 after 8 w, Fig. 1A) and fruit remained with green sectors at the end of the storage period (Fig. 1D). By contrast, external fruit coloration changed at 12 °C, reaching an average *a/b* ratio of 0.42 (Fig. 1A), corresponding to a yellow, pink-red pigmentation at the end of storage (Fig. 1D). Similar changes in fruit coloration have been previously observed in fruit of different citrus species including sweet orange and mandarin, where peel color remained unaltered in fruit stored at cold temperatures (<5 ºC) while coloration was stimulated at moderate temperatures (10-15ºC) (Carmona, Zacarías, & Rodrigo, 2012; Chaudhary, Jayaprakasha, Porat, & Patil, 2014; Tietel et al., 2012).

Under these postharvest conditions, initial CI symptoms appeared in fruit stored at 12 °C for 1 w, which were exclusively restricted to green zones of the peel. Incidence and severity of damage increased moderately thereafter, and at the end of the storage period 30 % of the fruits were slightly affected (CI index around 0.7) (Fig. 1B). Chilling damage initiated between 3-4 w at 2 ºC and latter than at 12 ºC, but sharply progressed

239 in both severity and number of fruit affected, and after 8 w at 2 ºC more than 50 % of
240 the fruit showed chilling damage (CI index of 1.5) (Fig. 1B). It is worth to note that
241 green peel sectors showed a special sensitivity to cold damage since CI symptoms were
242 more intense and developed earlier in these areas compared to yellow or pink zones
243 (Fig. 1D and E).

244 Evidence from different studies indicates that the sensitivity of different Citrus
245 fruit species to postharvest CI is highly dependent on the ripening stage at harvest (Lado
246 et al., 2019). In grapefruit, fruit harvested early and late on the season are more
247 susceptible to develop cold damage in the peel than mid-season fruits (Schirra et al.,
248 1998). Contrastingly, under Mediterranean growing conditions, the cold sensitive
249 'Fortune' mandarin hybrid was more susceptible to CI during mid-season, coincident
250 with the coldest field temperatures, and more resistant when still green (Lafuente,
251 Martínez-Téllez & Zacarías, 1997). Thus, it appears that besides the genetically-derived
252 susceptibility to CI, the environmental conditions during the growing season and at
253 harvest are key determinants for the development of postharvest CI. Mature fully-
254 colored grapefruit usually did not develop CI at temperatures below 10 ºC (Chaudhary
255 et al., 2014; Lado, Rodrigo, et al., 2015; Schirra, 1993) but in this work we detected CI
256 symptoms during the first 2 w of storage at 12 ºC exclusively restricted to green sectors
257 of the fruit (Fig. 1D). This observation suggests that the presence of chlorophyll in the
258 peel of fruit is playing a role in the development of CI. Then, we measured chlorophyll
259 concentration in the peel of SR grapefruit and found that at harvest it was 23 µg/g FW
260 and remained almost unchanged at 2 ºC (25 µg/g FW) while decreased to 9 µg/g FW at
261 12 ºC. Green peel areas of SR grapefruit at this ripening stage are rich in chloroplasts
262 which are cell organelles especially sensitive to low temperatures due to the high
263 generation of reactive oxygen species during photosynthesis (Lado, Cronje, et al.,

264 2015). Then, the ripening stage of grapefruit approaching full maturity and probably the
265 presence of chlorophyll in the peel, predispose its sensitivity to develop chilling damage
266 even at less extreme temperatures. The former may explain the observation that
267 grapefruits at breaker with green blotches harvested early in the season are highly
268 susceptible to CI.

269 *3.2. Effect of temperature storage (2 and 12 ºC) on carotenoid content and composition*
270 *in the peel of red Star Ruby grapefruit*

271 Carotenoid content in the peel of SR fruit at harvest was nearly 28 µg/g FW and
272 analysis of the composition showed a high proportion of colorless carotenes (phytoene
273 and phytofluene) which accounted for 77% of total carotenoid, whereas colored
274 lycopene (1.6 µg/g FW) and β-carotene (1.7 µg/g FW) represented nearly 6 % each
275 (Table 1). During storage at 12 ºC, total carotenoid concentration in the peel increased
276 to 55.5 and 98.5 µg/g FW after 3 and 7 w, respectively, while at 2 ºC only experienced a
277 slight rise up to 33.6 µg/g FW (Table 1). A similar effect of the storage temperature has
278 been also observed in the peel of Navelina orange where total carotenoids increase 2-3
279 times at 12 ºC while a minor or not enhancement in carotenoids was detected in the
280 flavedo of fruits at 2 ºC (Carmona et al., 2012). In Satsuma mandarin (Matsumoto,
281 Ikoma, Kato, Nakajima, & Hasegawa, 2009) there was a higher carotenoid
282 accumulation in fruit peel during storage at 20 ºC compared with fruit maintained below
283 5 ºC. These results reinforce and extend the concept that Citrus fruit, in general,
284 irrespectively of their peel coloration and particular carotenoid complement, are able to
285 stimulate carotenoid biosynthesis after tree detachment and during postharvest storage
286 at intermediate temperatures.

Regarding carotenoid composition, accumulation of linear carotenes was notably stimulated at 12 ºC, representing more than 90 % of total carotenoids compared to 77-79% in the peel of fruit at 2 ºC (Table 1). After 7 w storage at 12 ºC the content of phytoene, phytofluene and lycopene increased 3.6, 9.4 and 4.5-times, respectively, compared to freshly harvested fruit, whereas in fruit stored at 2 ºC only experienced a minor increase or even declined (Table 1). As expected, β-carotene and xanthophylls were in low concentration in the peel of the different SR samples analyzed, which is in good agreement with previous works (Lado, Cronje, et al., 2015; Lado, Rodrigo, et al., 2015). The content of these minor carotenoids was reduced or not modified at 12 ºC while at 2 ºC remained nearly constant or increased (Table 1). It is interesting to mention that the halt of chilling symptoms development observed after 3 w at 12 ºC (Fig. 1B) was associated with the increase in linear carotenes. These results agree with observations in which a higher accumulation of linear carotenes in the red peel of SR grapefruit prevented cold damage and enhanced antioxidant protection (Lado, Rodrigo, et al., 2015). In particular, lycopene, but also phytoene and phytofluene, have been shown to exert important free radical scavenging properties, protecting plastid structures from reactive oxygen species (Krinsky, 1989). Therefore, enhanced peel coloration and accumulation of lycopene and other colorless carotenes (phytoene and phytofluene) during storage of SR grapefruit at intermediate temperature (12 ºC) may help to minimize development of CI in red grapefruit (Lado, Rodrigo, et al., 2015).

### *3.3. Effect of temperature storage (2 and 12 ºC) on volatile emission in the red Star Ruby grapefruit*

Information about volatile emission in citrus fruit under different postharvest conditions causing physiological disorders is scarce and may provide relevant insights concerning fruit physiology and their responses to environmental conditions. Most of

312 the works have been focused on content and composition of volatile in the peel
313 (essential oils) or the volatile profile in juice of different citrus species (recently
314 reviewed by González-Mas et al., 2019), but studies on volatile emission from intact
315 fruit are very limited. Therefore, the emission of volatile by intact SR grapefruit during
316 storage at 2 °C and 12 °C was monitored by HS-SPME-GC-MS. Analysis of the
317 emission profile revealed a total of 38 different volatile compounds which is a relatively
318 low number in comparison with the volatiles extracted from citrus peel or pulp tissues
319 (González-Mas et al. 2019; Miyazaki et al., 2011; Ren et al., 2015; Zhang et al., 2017).
320 In general, great diversity of volatile compounds are present in the peel of citrus fruit,
321 mostly characterized by non-terpenoid ester and aldehyde in the specific case of
322 grapefruit, a total of 67 different constituents have been identified at harvest with a high
323 contribution of monoterpene hydrocarbons (>90% of total compounds) (González-Mas
324 et al., 2019; Njoroge, Koaze, Karanja & Sawamura, 2005). Our results highlight not
325 only the relevance of the moderated number of volatiles emitted by the intact grapefruit
326 at harvest but also in the response to the different storage temperatures. Total volatiles
327 emitted by SR grapefruit can be clustered in four principal groups: monoterpenes
328 (hydrocarbons, alcohols, aldehydes and ketones), sesquiterpenes (cyclic and linear),
329 aliphatic esters, aldehydes and alcohols (Table 2). Total volatiles emission (as the sum
330 of individual compounds identified) decreased by about 3 times after 1 w of storage
331 irrespective of the temperature (Fig.2). Upon 3 w of storage important differences were
332 observed in the total volatiles emitted by fruit depending on the storage temperature:
333 whereas at 2 ºC the emission of volatiles experienced a progressive increase until the
334 end of the storage (7 w), at 12 ºC remained constant during the whole storage period
335 (Fig. 2). Thus, the concentration of total volatiles in the headspace was 5 and 9-times
336 higher at 2 ºC than a 12 ºC after 3 and 7 weeks of storage, respectively (Fig. 2). This

337 difference is mainly explained by the increment in the emission of monoterpenes at 2
338 ºC, which are the most abundant group of volatiles emitted by grapefruit at this
339 temperature (Fig. 3) and specifically limonene, that its emission increased from 4 ng/g
340 FW at 1 w of storage to 64 ng/g FW at 7 w (Fig. 3A). At 12 ºC the emission of
341 monoterpenes remained constant at very low levels (less than 1 ng/g FW) during the
342 whole storage period. By contrast, sesquiterpenes were the most abundant compounds
343 among the volatiles emitted at 12 ºC with a maximum at 1 w (8 ng/g FW), declining (4-
344 5 ng/g FW) thereafter up to the end of storage (Fig. 3B). At 2 ºC, sesquiterpenes
345 emission was gradually reduced reaching the minimum levels (below 2 ng/g FW) after 5
346 w of storage (Fig. 3B). It is interesting to remark that the detection of aliphatic esters in
347 the headspace of fruits at harvest and during storage at 2 ºC was almost negligible but at
348 12 ºC their emission increased after 5 w, reaching at the end of storage levels 50-times
349 higher than the initial (Fig. 3C). The group of aliphatic aldehydes and alcohols
350 experienced a transient increase in their emission with a maximum at 3 w and 5 w in
351 fruits stored at 12 ºC and 2 ºC, respectively (Fig. 3D).

352 The emission of individual volatiles was followed in SR fruit stored at both
353 temperatures in order to detect specific changes that may involve a response to the
354 storage temperature (cold-response) and/or to the development of CI (chilling-response)
355 (Table 2). At harvest, the most abundant volatile emitted was limonene (23.3 ng/g FW)
356 (Fig. 3A), representing almost 80 % of the total identified compounds, followed by α-
357 farnesene (16 %), β-caryophyllene (2.7 %) and β-myrcene (1.5 %). These results are in
358 agreement with previous works where limonene represented more than 90 % of the total
359 volatile compounds present in the peel of different grapefruit varieties (González-Mas et
360 al., 2019; Njoroge et al., 2005; Zhang et al., 2017). Therefore, it seems that limonene is
361 not only the most abundant compound in the oil glands of flavedo tissue but also the

362 main volatile emitted by freshly harvested SR grapefruit. Other compounds emitted at
363 harvest represented less than 1 %, being α-copaene, linalool, valencene, decanal and
364 octanol the more relevant (around 0.1-0.2 % each; Table 2), which also agrees with
365 previous reports about volatile emission in grapefruit (Flamini & Cioni, 2010).

366 Monoterpenes emission was notably stimulated in fruit stored at 2 ºC and in
367 particular, the emission of limonene sharply increased its relative abundance,
368 representing at least 90 % of the total compounds emitted after 3 w of storage.
369 Contrastingly after harvested and storage at 12 ºC, monoterpenes emission was rapidly
370 reduced to near 10 % of the total volatiles, remaining unchanged during the whole
371 storage (Table 2). Different works have monitored the changes in limonene content in
372 fruit of different Citrus species under cold storage (Biolatto et al., 2005; Herrera, Gil,
373 Rodrigo & Zacarías, 2007; Obenland et al., 2003; Obenland, Margosan, Houck, &
374 Aung, 1997). It has been claimed that under low temperature there is an increment in
375 the emission of limonene in cold-sensitive fruit due to a massive release from the oil
376 glands of damaged peel tissue (Obenland et al., 1997). Moreover, the absence of CI
377 symptoms in quarantine-treated grapefruits was related to unchanged limonene content
378 (Biolatto et al., 2005). In agreement with previous works, our results show that
379 limonene emission increased only in fruit stored at 2 ºC, suggesting that it may be a
380 response associated with low temperature exposure and with the cold-induced oil-
381 glands disruption. We have previously observed important alterations in the structure of
382 cell wall and plasmatic membrane in cold-stored grapefruits that may favored the
383 release of limonene to the environment from injury fruit (Lado, Rodrigo, et al., 2015). It
384 should be noted that during storage at 12 ºC SR grapefruit rapidly developed cold
385 damage of small-mild severity but the emission of limonene decreased and remained at
386 very low levels. These results may indicate that the damage induced at 12 ºC is not

sufficient to increase limonene emission or that the emission of limonene may be a specific cold-induced response. This monoterpene has been demonstrated to be crucial in the citrus fruit response to different biotic stresses since the resistance to different pathogens or pests attraction (bacteria, mold and fruit fly) is inversely related to the accumulation of limonene in the oil gland of sweet orange peel (Rodríguez et al., 2015).

Other monoterpene, β-myrcene, was detected in the volatile emitted after 5 and 7 w at 2 ºC, but not at 12 ºC, representing nearly 2 % of the total emission at the end of storage (Table 2). This compound has been shown to be negatively linked to consumers' likeliness of citrus juices, providing a musty and wet soil descriptions (Tietel et al., 2011). Limonene oxide was only detected at 2 ºC after 3 w of storage and stimulated by 5-times as cold storage progressed, being most likely associated with the high emission of limonene under this storage condition (Table 2). Similarly, an increase in limonene oxide (300-750 %) has been also described in the peel of Navel orange showing oleocellosis symptoms (Xie et al., 2018), that could suggest that the appearance of this compound is linked to a stress-related response of citrus fruit with high release of limonene. During fruit storage at 2 ºC, the presence of monoterpene alcohols, such as linalool (from 0.1 to 0.4 %) and L-α-terpineol (from 0.07 to 0.2 %) increased gradually while at the end of storage, β-citronellol (0.07 %) and nerol (0.02 %) were also detected. All these monoterpenes were not detected in fruit stored at 12 ºC (Table 2) and therefore their emissions should be linked to low temperature responses, but their temporal emission patterns indicate that may not be strictly associated with chilling injury development. It is worth noting that linalool is specially abundant in sweet orange and mandarin juices (Zhang et al., 2017) and during cold storage (Obenland et al., 2013) whereas it was extracted from the peel of mandarin hybrids (Li et al., 2017) and linked to citric, floral and green flavors (Tietel et al., 2011). L-α-

412 terpineol, a compound likely derived from limonene, followed a similar trend of
413 emission, coinciding with previous reports in mandarin juice (Obenland et al., 2013). It
414 has been shown that this volatile is specially stimulated under biotic stress (*Penicillium*
415 *digitatum*) in citrus fruit (Badee, Helmy, & Morsy, 2011), suggesting that could be part
416 of the fruit response to stress. This compound has been identified in the juice of sweet
417 oranges and mandarins (Obenland et al., 2013) where it provides a floral and lilac-like
418 description of the mandarin flavor (Tietel et al., 2011), but it has been considered less
419 relevant in grapefruit (Ren et al., 2015). It is interesting to note that transgenic citrus
420 fruit with enhanced content of linalool and other monoterpenes alcohols in the peel
421 showed an induced resistance against fungi pathogen attack (Rodríguez et al., 2018).

422 The emission of certain volatile compounds could be tightly related to degradation
423 or modification of its direct precursors. In this sense, norisoprenoid and some
424 monoterpene volatiles compounds, including geranial, neral, geranyl acetone and β-
425 ionone may be derived from the degradation of specific carotenoids (Lewinsohn et al.,
426 2005). In particular, the presence of geranyl acetone in tomato and watermelon color
427 mutants suggested that this volatile can be a breakdown product from phytoene,
428 phytofluene or ζ-carotene. Hence the emission of geranyl acetone in SR grapefruit
429 stored at 12 ºC for 7 w (0.46 %; Table 2) could be explained by the significant increase
430 in phytoene and phytofluene in the peel of SR grapefruit under this storage condition
431 (Table 1).

432 Cyclic sesquiterpenes were the most diverse group of volatiles and their emissions
433 were predominant at 12 ºC (Table 2). Among the 17 cyclic sequiterpenes detected in the
434 SR grapefruit headspace, only α-copaene, β-caryophyllene and valencene were
435 identified in fruit stored at 2 ºC and all of them followed a decreasing trend during cold
436 storage (Table 2). By contrast, all the cyclic sesquiterpenes identified were emitted by

fruits stored at 12 ºC although their profile and abundance were variable. At this temperature, the main cyclic sesquiterpene emitted was β-caryophyllene, accounting for 36 % of total compounds after 3 w and decreasing to 8 % by the end of the storage (Table 2). Valencene was the second most abundant and increased from 0.18 % at harvest to nearly 19 % after 7 w. Other relevant compounds from this group that increased in their relative abundance at 12 ºC were δ-, α- and 7-epi selinene, representing between 5 and 9 % of the total volatile compounds. The presence of minor proportions of α-gurunjene, β-elemene, aromadendrene, α-humulene, γ-gurunjene, β-charmigrene, β-selinene and δ-elemene were only detected at 12 ºC (Table 2). It is worth to mention that sesquiterpenes are less abundant than monoterpenes in the peel of fruit of nearly 100 accessions of sweet orange, mandarin, grapefruit, pummelo, papedas, lemon and fortunella (González-Mas et al. 2019, Zhang et al., 2017), being their content in the juice modified during postharvest storage (Obenland et al., 2013; Tietel et al., 2012) or after ethylene treatment (Sdiri et al., 2017). The cyclic sesquiterpene ketone nootkatone, a characteristic volatile compound present in ripe grapefruit and pummelo (Ortuño et al., 1995; Ren et al., 2015; Zhang et al., 2017) was only detected at 12 ºC and its relative abundance increased during storage, representing from 0.2 to 8.3 % of the total emitted compounds. This compound is part of the valuable and characteristic 'grapefruit flavor' perception and its emission is enhanced by storage at intermediate but not low temperatures (Ortuño et al., 1995).

It is interesting to remark that the emission of the linear sesquiterpene α-farnesene showed a transient increase during the first week of storage at 12 ºC, accounting for 66 % of total compounds but it was not detected afterwards (Table 2). Similarly, after 1 w at 2 ºC there was a transient increase in the relative emission of α-farnesene, accounting for 50 % of the total of this volatile group, which was subsequently reduced to reach

462 minimum values (about 2 %) at 5 and 7 w (Table 2). The transient increase in α-
463 farnesene observed at both temperatures after 1 w (3 and 4 times at 2 ºC and 12 ºC,
464 respectively) compared to the emission in freshly-harvested fruit could be part of an
465 initial temperature-stress response. In this sense, a higher content of this compound in
466 the peel of different cold-sensitive citrus species (Tahitian lime > Marsh grapefruit =
467 Emperor mandarin > Valencia orange) was associated with their susceptibility to
468 developing CI at 0 ºC. Internal levels of α-farnesene increased during early cold storage
469 in these four species, being also stimulated by exogenous ethylene (Yuen, Tridjaja,
470 Wills, & Wild, 1995). In the present study, we found a complete halt in the emission of
471 this compound after 3 w at 12 ºC, concomitant with the recession in the development of
472 CI, whereas it was detected during the whole storage period at 2 ºC (Table 2) in parallel
473 with the progression of CI symptoms (Fig.1). These results suggest a positive
474 relationship between the emission of the sesquiterpene α-farnesene and the development
475 of CI symptoms in SR grapefruit.

476 The number and relative abundance of emitted aliphatic esters were very low in
477 both fruit at harvest and during storage at 2 ºC, and only hexyl hexanoate and hexyl 2-
478 methyl butyrate were detected (Table 2; Fig. 3C). Prolonged storage at 12 ºC stimulated
479 the emission of aliphatic esters and by 7 w up to 6 esters were detected: hexyl
480 butanoate, hexyl hexanoate, hexyl caprylate and hexyl 2-methyl butyrate, and butyl
481 caprylate and caproate. The most abundant aliphatic ester was hexyl hexanoate,
482 followed by hexyl butanoate and butyl caproate, and for all of them the higher emission
483 was attained at 7 w of storage at 12 ºC, accounting for 6.2, 2.3 and 2.0 % of relative
484 abundance, respectively (Table 2). A stimulation in esters abundance has been also
485 described in the juice (Sdiri et al., 2017) and intact ethylene-treated mandarin fruits
486 (Herrera et al., 2007) or after shelf-life period (20 ºC) in W. Murcott Afourer mandarin

fruit (Obenland et al., 2013), suggesting that in *Citrus* fruit, ethylene and other conditions accelerating ripening and senescence may also stimulate the emission of aliphatic esters similarly to what has been observed in climacteric-like fruit (Rambla and Granell, 2013).

The relative abundance of aliphatic aldehydes and alcohols in the volatiles during storage at 2 ºC was lower than at 12 ºC. There was also a marked specificity of the emitted compounds at both storage temperatures since decanal and octanol were only detected in the headspace of fruit at 2 ºC while pentadecane at 12 ºC, and 2-ethyl-1-hexanol was detected at both temperatures (Table 2). At 2 ºC the most abundant compound emitted from this group was octanol, representing between 0.9 and 2.5 % (Table 2) while at 12 ºC was the aldehyde pentadecane which showed a notable transient increase up to 21 % after 3 w at 12 ºC and decreasing to 4 % at the end of storage (Table 2; Fig. 3D). The proportion of 2-ethyl-1-hexanol was transiently increased at 1 w of storage at 2 ºC representing about 0.24 % of the total emitted volatiles while the emission remained stable at similar percentage during storage at 12 ºC (Table 2).

The emission of 4,8 dimethyl nonatriene (4,8 DMNT) was stimulated only at 12 ºC being especially relevant after 5 and 7 w at 12 ºC, representing 7-10 % of total emitted compounds (Table 2). The 4,8 DMNT is an unusual acyclic homoterpene emitted by many plants in response to herbivorous attack, that can be also an attractant for insects and then provides a good strategy for biological control, promoting the attraction of predators (Kappers, Aharoni, Van Herpen, Luckerhoff, Dicke & Bouwmeester, 2005). The presence of 4,8 DMNT in the headspace only after prolonged storage at 12 ºC suggest that its emission is not associated with peel damage symptoms, being more likely a response related to fruit ripening or senescence (Table 2). It is worth

noting that methyl dihydrojasmonate (MDHJ), a volatile likely involved in fruit cold response, was not detected in freshly-harvested fruit and appeared toward the end of storage at both temperatures (Table 2). The implication of MDHJ in chilling tolerance has been explored (Cao, Zheng, Wang, Jin, & Rui, 2009) and its postharvest application has been shown to increase cold tolerance in lemon (Siboza & Bertling, 2013). Based on these observations, the emission of this compound could be a consequence of the development of peel damage or part of key signaling mechanisms triggered in response to chilling injury.

Finally, PCA analysis allowed to compare the profiling of volatile emission in grapefruit stored at 2 and 12 ºC and 74 % of the total variability could be explained by the first two components: PC1=49.19 % and PC2=23.31 % (Fig. 4A). The score plot showed that samples can be grouped in two defined clusters: the first cluster grouped samples at harvest and stored at 2ºC while the second included fruit samples at 12 ºC (Fig. 4A). Loadings plot (Fig. 4B) revealed that the emission of monoterpenes was associated with the lowest storage temperature whereas most cyclic sesquiterpenes and aliphatic esters were associated with 12 ºC.

## 4. Conclusions

CI was observed during SR grapefruit storage at 2 °C while at 12 °C symptoms were restricted to green chlorophyll-containing blotches, which resulted very sensitive tissue to this disorder. Storage at 12 °C stimulated coloration and pigment changes (chlorophyll degradation and linear carotenes accumulation) in SR grapefruit peel as well as the emission of cyclic sesquiterpenes and aliphatic esters. Contrastingly at 2 °C, monoterpenes, aldehydes and alcohols predominated. As hypothesized, the increases in the emissions of certain compounds (limonene, linalool, L-α-terpineol) appear to be part

of the fruit response to cold stress. A selective emission of different volatile compounds has been observed in the response of grapefruit to chilling and non-chilling temperature, and to the chilling-induced damage, that may be used as biomarkers for the grapefruit response to these postharvest conditions.


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## Conflict of interest statement

The authors declare that there are no conflicts of interest.

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## Figures legends

**Fig. 1**. Evolution of peel color index (Hunter ratio *a/b*) (**A**) and CI Index (0-3) (**B**) in Star Ruby grapefruit stored at 2 ºC (●) or 12 ºC (○). Images illustrating external appearance and chilling symptoms in SR grapefruit at harvest (**C**) and during storage at 2 ºC (**D**) or 12 °C (**E**). Data are means ± standard error.. Asterisk indicates statistical significance ($p < 0.05$) between fruit at 2 ºC and 12 ºC at each storage time.

**Fig. 2**. Quantification of total volatiles (ng/g FW) emitted by Star Ruby grapefruit at harvest and during storage at 2 °C (black bars) or 12 °C (white bars). Data are means ± standard error.

**Fig. 3.** Quantification of the main groups of volatiles (ng/g FW): monoterpenes (A), sesquiterpenes (B), aliphatic esters (C) and aliphatic aldehydes and alcohols (D) emitted by Star Ruby grapefruit at harvest and during storage at 2 °C or 12 °C. Insert panel in (A) shows changes in emission of limonene (ng/g FW) during storage at 2 °C or 12 °C. Data are means ± standard error.

**Fig. 4**. Principal component analysis (PCA) score plot (**A**) and loadings plot (**B**) of the volatiles analyzed in the headspace of Star Ruby grapefruit at harvest (H) and after 1, 3, 5 and 7 w of storage at 2 ºC and 12 ºC. In the loadings plot, each number corresponds to a volatile compound as indicated in Table 2 and different groups of volatiles are represented as follow: blue circle corresponds to monoterpene hydrocarbons; green circle to monoterpene alcohol, aldehyde or ketone; white circle to cyclic sesquiterpenes; blue star to linear sesquiterpene; green triangle to aliphatic esters; blue triangle to aliphatic aldehyde and alcohols; green diamond to `others´ group. Loadings in the blue ellipse correspond to some monoterpenes while in dotted line ellipse corresponds to some cyclic sesquiterpenes and aliphatic esters.

**Table1**

**Table 1.** Carotenoid profile in the peel of Star Ruby grapefruits stored at 2 and 12 ºC. Asterisks indicate differences in individual and total carotenoids between fruit at 2 ºC and 12 ºC, being common to 3 and 7 weeks storage times. The data are means of three determinations ± SE. Asterisks indicate significant differences between 2 and 12 ºC within storage time (Tukey $p<0.05$; ns: non-significant differences).

| | | Storage time | | | |
|---|---|---|---|---|---|
| | | 3 weeks | | 7 weeks | |
| | Harvest | 2ºC | 12ºC | 2ºC | 12ºC |
| Phytoene | 20.1± 0.1 | 23.0 ± 3.1 | 41.0 ± 4.1* | 22.0 ± 0.5 | 72.2 ± 9.0* |
| Phytofluene | 1.6 ± 0.1 | 2.4 ± 0.4 | 5.7 ± 0.5* | 2.2 ± 0.3 | 15.1 ± 1.5* |
| Lycopene | 1.6 ± 0.2 | 1.7 ± 0.4 | 3.7 ± 0.7* | 1.1 ± 0.1 | 7.3 ± 0.5* |
| β-carotene | 1.7 ± 0.1 | 2.6 ± 0.3 | 2.7 ± 0.7ns | 2.9 ± 0.1 | 1.5 ± 0.5ns |
| Lutein | 0.9 ± 0.4 | 1.4 ± 0.04 | 0.7 ± 0.02* | 1.3 ± 0.2 | 0.7 ± 0.1* |
| *9-Z*-violaxanthin | 0.9 ± 0.3 | 1.1 ± 0.02 | 0.9 ± 0.07ns | 1.1 ± 0.5 | 1.1 ± 0.1ns |
| *All*-E-violaxanthin | 1.1 ± 0.1 | 1.4 ± 0.04 | 0.8 ± 0.01ns | 1.5 ± 0.3 | 0.6 ± 0.1ns |
| TOTAL | 27.9 ± 1.3 | 33.6 ± 4.2 | 55.5 ± 6.1* | 32.1 ± 0.1 | 98.5 ± 22.0* |

Table2

**Table 2.** Individual volatiles as percentage of the total volatiles emitted by Star Ruby grapefruit at harvest and during storage for 1, 3 5 and 7 weeks at 2 °C or 12 °C. Data indicates mean ± SE.

| | | | | 2 ºC | | | | 12 ºC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Id** | Compound | LRI | **Harvest** | **1w** | **3w** | **5w** | **7w** | **1w** | **3w** | **5w** | **7w** |
| | ***Monoterpene hydrobarbons*** | | | | | | | | | | |
| 1 | β-Myrcene | 1075 | 1.49±0.13 | - | - | 1.90 ±0.41 | 1.85±0.12 | - | - | - | - |
| 2 | Limonene | 1114 | 78.10±13.13 | 45.90±2.70 | 89.88±8.98 | 91.68±13.16 | 93.7±10.13 | 9.58±1.31 | 10.21±0.10 | 8.57±0.19 | 9.20±0.14 |
| 3 | Limonene oxide | 1465 | - | - | 0.12±0.01 | 0.39±0.02 | 0.53±0.01 | - | - | - | - |
| | **Total** | | **79.60± 13.00** | **45.90±2.70** | **90.00±8.90** | **93.97±13** | **96.11±10.11** | **9.58±1.31** | **10.21±0.10** | **8.57±0.19** | **9.20±0.14** |
| | ***Monoterpene alcohols aldehydes and ketones*** | | | | | | | | | | |
| 4 | Linalool | 1603 | 0.10±0.01 | 0.20±0.05 | 0.20±0.10 | 0.37±0.10 | 0.18±0.01 | - | - | - | - |
| 5 | L-α-Terpinol | 1802 | 0.07±0.01 | 0.14±0.01 | 0.13±0.10 | 0.22±0.12 | 0.12±0.01 | - | - | - | - |
| 6 | β-Citronellol | 1904 | - | - | - | - | 0.07±0.01 | - | - | - | - |
| 7 | Nerol | 1951 | - | - | - | 0.02±0.01 | 0.01±0.01 | - | - | - | - |
| 8 | Geranyl acetone | 2030 | - | - | - | - | 0.07±0.01 | - | - | - | 0.46±0.01 |
| | **Total** | | **0.21±0.003** | **0.34±0.08** | **0.33±0.09** | **0.61±0.1** | **0.44±0.06** | | | | **0.46±0.01** |
| | ***Cyclic Sesquiterpenes*** | | | | | | | | | | |
| 9 | α-Copaene | 1514 | 0.21±0.01 | 0.17±0.04 | 0.07±0.01 | 0.07±0.01 | 0.01±0.002 | 1.32±0.02 | 1.30±0.08 | 0.52±0.06 | 0.21±0.01 |
| 10 | α-Gurjunene | 1565 | 0.04±0.01 | - | - | - | - | 0.14±0.01 | 0.28±0.01 | 0.13±0.01 | - |
| 11 | α-Cubebene | 1579 | 0.07±0.01 | - | - | - | - | - | - | - | - |
| 12 | β-Elemene | 1636 | - | - | - | - | - | - | - | | 1.95±0.01 |
| 13 | Caryophyllene | 1655 | 2.72±0.21 | 0.80±0.02 | 0.31±0.01 | 0.36±0.1 | 0.07±0.01 | 16.53±0.12 | 36.00±0.64 | 22.28±5.69 | 8.45±0.07 |
| 14 | Aromadendrene | 1724 | | - | - | - | - | - | - | 0.38±0.01 | 0.43±0.01 |
| 15 | α-Humulene | 1754 | 0.05±0.01 | - | - | - | - | 0.13±0.01 | 0.39±0.01 | 0.34±0.01 | 0.24±0.02 |
| 16 | δ-Selinene | 1785 | 0.08±0.01 | - | - | - | - | 0.40±0.01 | 4.29±0.01 | 6.68±0.01 | 7.15±0.02 |
| 17 | r-Gurjunene | 1796 | - | - | - | - | - | - | - | 0.33±0.02 | 0.43±0.02 |
| 18 | β-Chamigrene | 1891 | - | - | - | - | - | - | - | 1.66±0.01 | 0.36±0.01 |
| 19 | β-Selinene | 1817 | - | - | - | - | - | - | - | - | 0.49±0.01 |
| 20 | Valencene | 1851 | 0.18±0.03 | 0.50±0.02 | 0.12±0.04 | 0.06±0.04 | 0.09±0.007 | 0.97±0.08 | 6.43±0.76 | 14.08±0.04 | 18.60±2.12 |
| 21 | α-Selinene | 1832 | - | - | - | - | - | 0.42±0.01 | 5.63±0.01 | 7.06±0.01 | 8.79±0.02 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | δ-Elemene | 1844 | - | - | - | - | - | - | 0.70±0.02 | 0.58±0.01 | 0.28±0.02 |
| 23 | 7-epi-selinene | 1885 | - | - | - | - | - | - | 4.78±0.02 | 6.43±0.02 | 6.97±0.03 |
| 24 | Juniper camphor | 2323 | - | - | - | - | - | - | 1.17±0.01 | 1.30±0.02 | 1.83±0.02 |
| 25 | Nootkatone | 2438 | | | | | | 0.16±0.01 | 6.42±0.02 | 8.34±0.03 | 7.58±0.03 |
| | **Total** | | **3.40±0.27** | **1.47±0.34** | **0.50±0.09** | **0.49±0.1** | **0.18±0.01** | **20.07±0.08** | **67.31±0.12** | **70.12±5.70** | **63.79±2.20** |
| | ***Sesquiterpene linears*** | | | | | | | | | | |
| 26 | α-Farnesene | 1882 | 16.25±2.26 | 50.78±5.82 | 7.80±0.23 | 2.35±0.18 | 1.74±0.09 | 66.49±4.20 | - | - | - |
| | ***Aliphatic esters*** | | | | | | | | | | |
| 27 | Butyl caproate | 1416 | - | - | - | - | - | - | - | 0.50±0.02 | 2.34±0.15 |
| 28 | Hexyl butanoate | 1419 | - | - | - | - | - | - | - | 0.53±0.20 | 2.09±0.30 |
| 29 | Hexyl 2-methyl butyrate | 1435 | 0.02±0.01 | - | - | - | - | - | - | - | 0.19±0.01 |
| 30 | Hexyl hexanoate | 1688 | 0.03±0.01 | 0.08±0.01 | 0.02±0.01 | 0.01±0.01 | 0.01±0.01 | - | 0.42±0.04 | 2.06±0.10 | 6.18±0.10 |
| 31 | Butyl caprylate | 1691 | - | - | - | - | - | - | - | - | 0.66±0.02 |
| 32 | Hexyl carpylate | 1970 | - | - | - | - | - | - | - | - | 0.37±0.01 |
| | **Total** | | **0.05±0.01** | **0.08±0.03** | **0.02±0.01** | **0.01±0.01** | **0.01±0.01** | **-** | **0.42±0.04** | **3.09±0.30** | **11.84±1.20** |
| | ***Aliphatic aldehydes and alcohols*** | | | | | | | | | | |
| 33 | 2-Ethyl-1-hexanol | 1521 | 0.05±0.01 | 0.24±0.01 | 0.04±0.01 | 0.02±0.01 | 0.02±0.01 | 0.16±0.01 | 0.25±0.01 | 0.16±0.01 | 0.29±0.02 |
| 34 | Decanal | 1532 | 0.19±0.01 | 0.14±0.01 | 0.13±0.01 | 0.06±0.01 | 0.06±0.01 | - | - | - | - |
| 35 | Pentadecane | 1546 | - | - | | - | - | 3.69±0.01 | 21.30±0.01 | 11.10±0.01 | 4.38±0.01 |
| 36 | Octanol | 1615 | 0.24±0.01 | 0.96±0.01 | 1.17±0.62 | 2.51±0.09 | 1.27±0.03 | - | - | - | - |
| | **Total** | | **0.48±0.12** | **1.34±0.34** | **1.34±0.09** | **2.58±0.07** | **1.35±0.04** | **3.85±0.76** | **21.55±1.35** | **11.26±0.13** | **4.67±1.11** |
| | ***Others*** | | | | | | | | | | |
| 37 | 4,8DMNT | 1273 | - | - | - | - | - | - | - | 6.55±0.02 | 8.71±0.03 |
| 38 | MDHJ | 2347 | - | - | - | - | 0.23±0.01 | - | 0.51±0.01 | 0.42±0.08 | 1.34±0.05 |
| | **Total** | | - | - | **-** | **-** | **0.23±0.01** | **-** | **0.51±0.01** | **6.97±0.05** | **10.05±0.04** |

**Figure1**

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Figure 1. Lado et al.

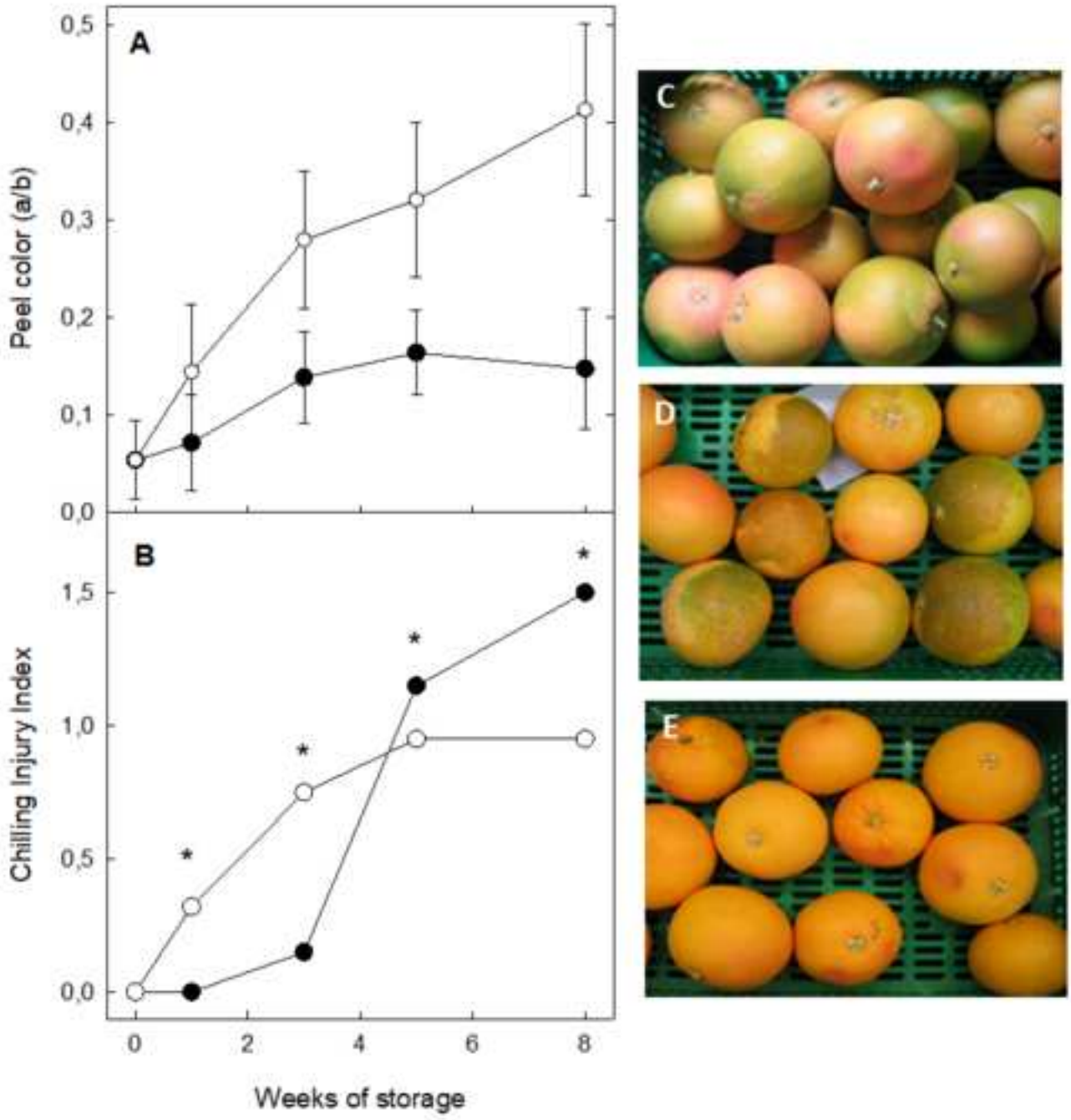

**Figure2**
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Figure 2. Lado et al.

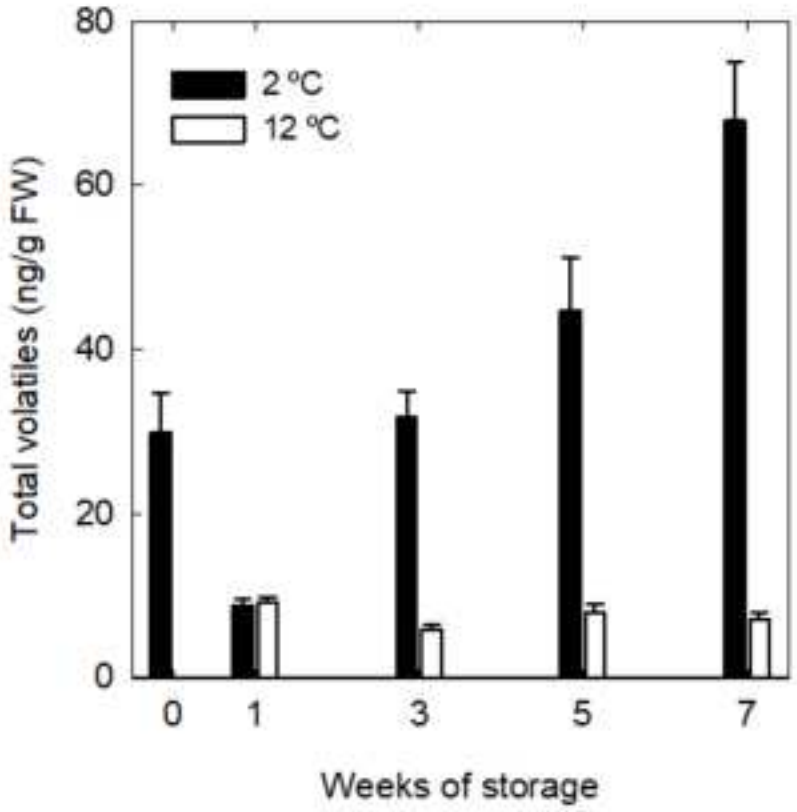

Figure3

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Figure 3. Lado et al.

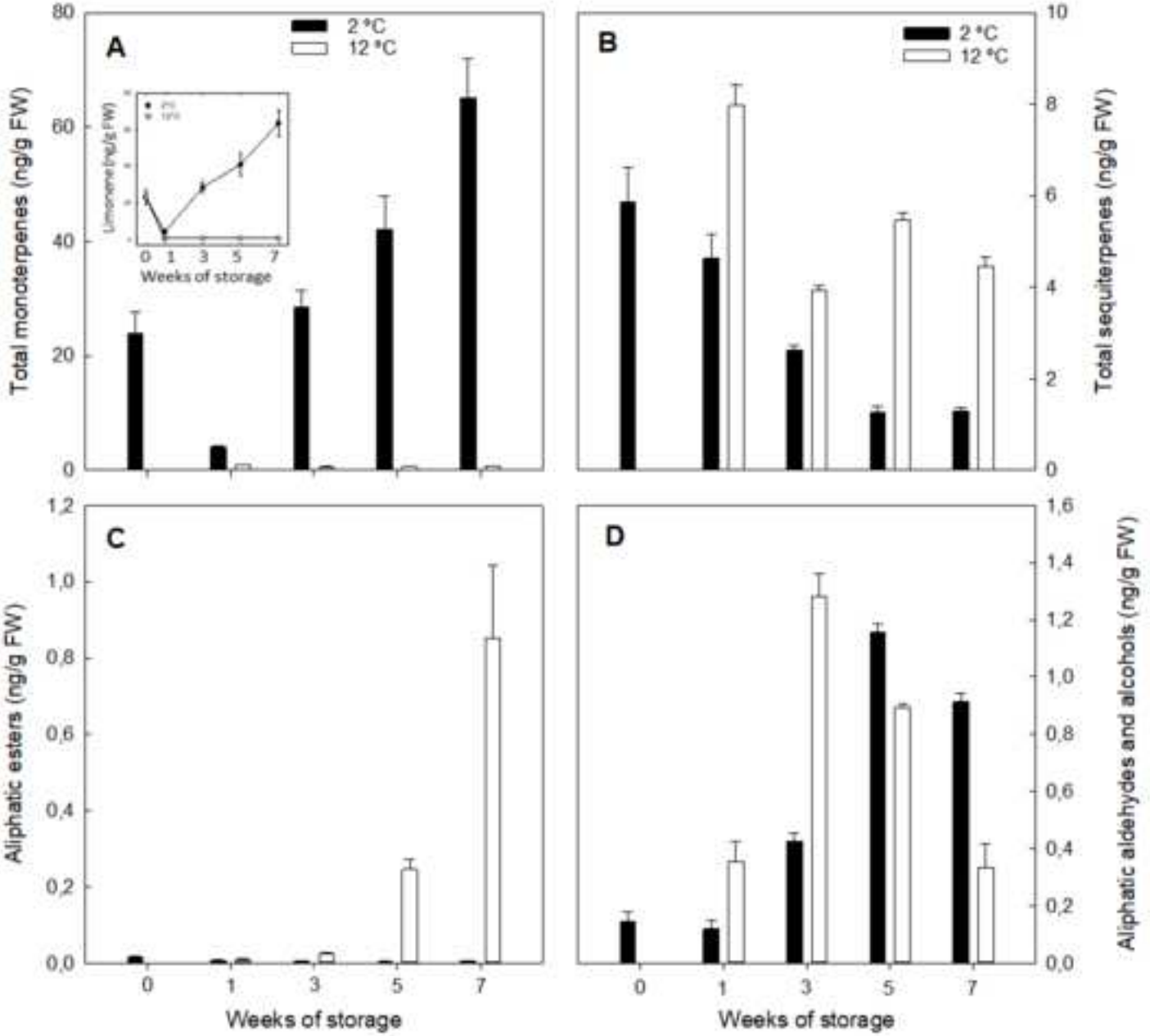

**Figure4**
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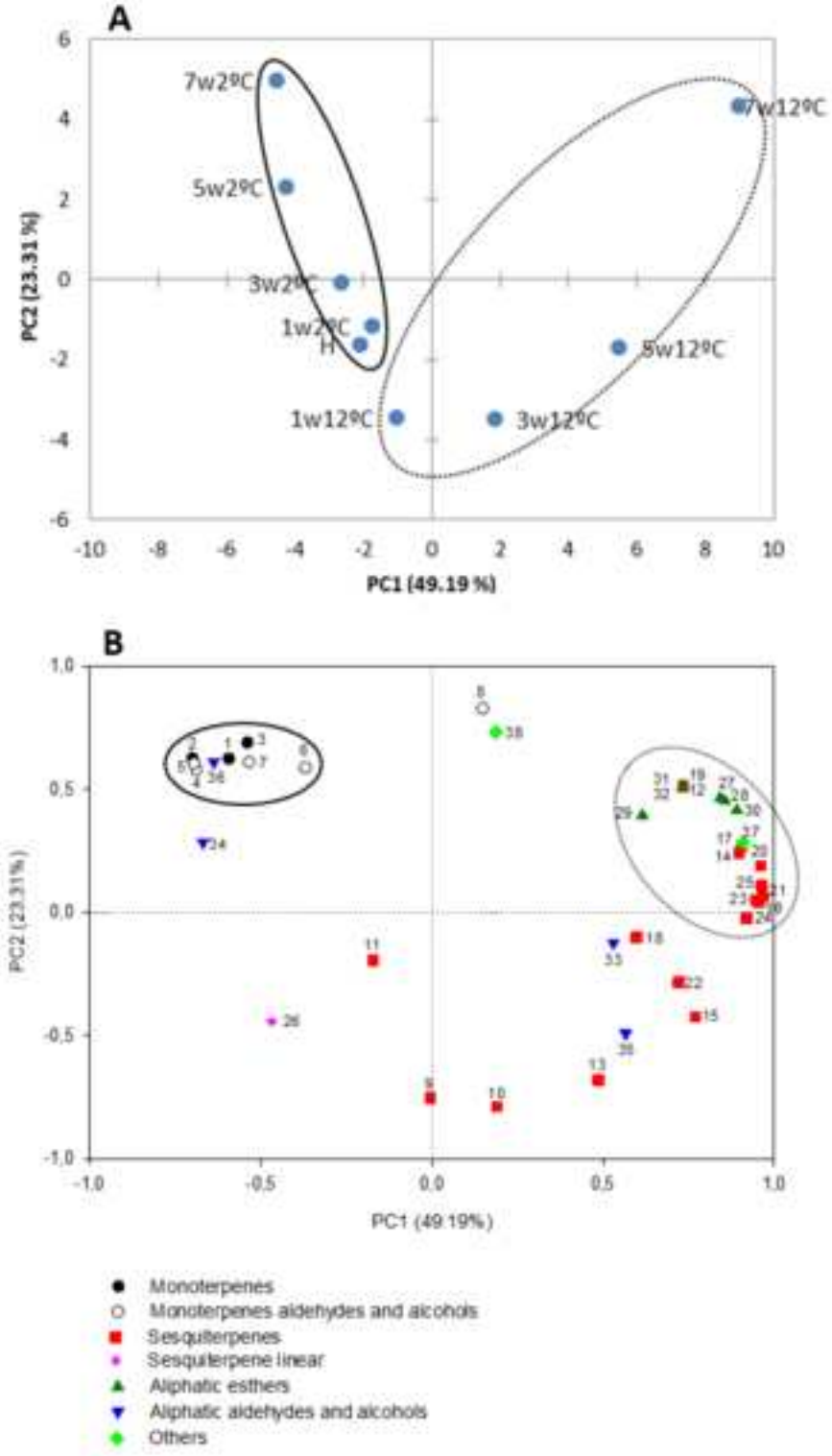
Figure 4. Lado et al.



***Declaration of Interest Statement**

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☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: