Ripening Attributes of New Passion Fruit Line Featuring Seasonal Non-climacteric Behavior

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Ⓢ Supporting Information

ABSTRACT: The passion fruit hybrid cultivar 'Passion Dream' (PD) produces two cycles of fruiting per year, in the summer and winter. Self-hybridization of PD created various lines, including 'Ripens during Summer' (RS), which lacks the ability to abscise during winter, suggesting a seasonal nonclimacteric behavior. The two lines were characterized by several quality traits: PD produced high ethylene levels in both seasons; RS produced significantly less ethylene during summer and almost none during winter. The ratio of total soluble solids to titratable acidity (TSS/TA), and aroma volatiles production, as determined by solid-phase microextraction/gas chromatograph−mass spectrometer (SPME/GC-MS) techniques, and taste indices were highest in PD summer fruits and lowest in RS winter fruits. Peel color in PD was affected by environmental and storage temperatures, whereas RS fruits always showed a strong purple color. The present findings suggest that ethylene production levels in passion fruit greatly influence various ripening processes, including acid degradation, increased TSS/TA ratio, accumulation of aroma volatiles, and tastiness.

KEYWORDS: *Passiflora edulis, ethylene, postharvest storage, heat map, odor activity value (OAV)*

■ INTRODUCTION

Passion fruit (*Passiflora edulis*), native to South America, has been grown worldwide during the past few decades for its edible fruit,[1,2] which emits an exotic and flavorful aroma.[2,3] Of the 400 species in the genus *Passiflora*, only 50 are edible, and of those, only the purple passion fruit, *Passiflora edulis* Sims, and the yellow passion fruit, *Passiflora edulis* Sims f. *flavicarpa* Degener, are grown commercially.[4,5] In Israel and most subtropical countries, passion fruit vines yield in both winter and summer. Hybrids of the two passion fruits are reported to be superior to those of either subspecies, because of integration of their desirable agricultural and organoleptic qualities.[1,4,6] The dark-purple peel color is an important quality for passion fruit consumers and, consequently, for growers; however, hybridization of purple and yellow passion fruits often results in a light-purple peel.[1,4]

Like every climacteric fruit, passion fruit produces elevated ethylene levels during its ripening, accompanied by accelerated respiration, both on and off the vine.[7] In comparison with other climacteric fruits, passion fruit produces extremely high ethylene levels, mainly in the arils.[8] But significant ethylene production also takes place in the abscission zone and ultimately causes the fruit to abscise and fall to the ground.[9] In purple passion fruit, for instance, Mita et al.[8] have demonstrated high accumulation of ethylene receptors in both arils and abscission zone during the turning stage of the fruit. A nonclimacteric fruit, such as grape or certain lines of melon, maintains a steady level of carbon dioxide production during its ripening and emits only basal ethylene levels.[10−12] Exposure of a climacteric fruit to exogenous ethylene will accelerate its endogenous ethylene production, whereas ethylene production in a nonclimacteric fruit will not be influenced.[12,13]

Various studies investigated the volatiles composition of purple and yellow passion fruits, and over 200 compounds were identified to date.[1−5] Some of these volatiles contribute to the exotic and distinct flavor of passion fruit, especially unique components such as the apocarotenoid edulans,[14] mostly identified in the purple subspecies, and sulfur-containing molecules[15,16] that are present mainly in the yellow subspecies. However, esters form the largest group of volatiles in passion fruit aroma and constitute more than half of all volatile compounds in the fruits.[3,17] Despite intensive analysis of the volatiles of fresh fruit and industrial juice, only a few studies have reported postharvest changes in passion fruit volatile compounds.[2] Moreover, to date, no research has examined the aroma differences between winter and summer fruits. Temperature, water availability, and sunlight intensity or duration were reported to affect fruit flavor and volatile contents.[18] For instance, in 'Delicious' apples Miller et al.[19] found that ester volatiles concentrations increased with increasing light intensity. In addition to volatiles concentration, odor thresholds of the volatiles form another parameter to be taken into account in estimating their contribution to the total fruit aroma; for example, the apocarotenoid β-ionone exhibits a very low odor threshold of merely 0.007 $\mu g\ L^{-1}$ and, therefore, can be sensed at very low concentrations, whereas the ester ethyl acetate has an odor threshold of ∼5 000 $\mu g\ L^{-1}$, so that only high levels can be detected by the olfactory system.[20]

Whereas fruit aroma is undisputedly significant, acidity level and total soluble solids (TSS) are often more crucial elements

of the overall flavor.[18] In fact, in many fruits such as peach, melon, and citrus, the TSS/TA ratio has great impact on flavor quality and acceptability.[21−23] In climacteric fruits, ripening processes proceed continuously after the fruit has been detached from the plant.[24] Thus, during storage of passion fruit, acid degradation processes elevate the TSS/TA ratio and thereby improve the generally acidic taste.[25,26]

The 'Passion Dream' (PD), a hybrid of purple and yellow passion fruits, is the commercial line in Israel;[6] it blooms and yields fruit twice yearly, during winter and summer, for a total of 5 months. Progeny from self-fertilized PD was screened under field conditions,[6] and one line showing unique behavior was named 'Ripens during Summer' (RS). The most prominent characteristic of RS is that its fruits do not abscise during the winter, which suggests a seasonal nonclimacteric behavior. The aim of the present study was to investigate the influence of changes in climacteric behavior on fruit quality and aroma in the RS and PD lines, at harvest and after storage, in both winter and summer seasons.

■ MATERIALS AND METHODS

Plant Material and Storage. Passion fruits (*Passiflora edulis* Sims) of lines PD and RS (the RS variety is registered as the cultivar 'Dena') were obtained from an experimental orchard of the Faculty of Agriculture of the Hebrew University, Rehovot, Israel. The average temperatures in the orchard were 20/8 °C (day/night) in the winter, and 32/24 °C in the summer, provided by the Wunderground weather station, Nes Tziona, Israel, 2009 (http://www.wunderground.com/weatherstation/WXDailyHistory.asp). Fruits were allowed to abscise and fall on the ground or, in the case of RS winter (RSw) fruits, harvested. Abscised fruits were collected from the ground within 24 h after abscission, whereas RSw fruits were picked after reaching full color and size, using only slight manual force, to confirm fruit maturity.[27] Fruits were packed in 1 L plastic boxes, 6 fruits per box (230−300 g of fruit in each box), and overwrapped with a perforated 22 μm thick polypropylene (PP) stretchable shrink film (Amir Marketing, Emek Hefer, Israel) to prevent peel shriveling, according to previous work on passion fruit.[28] The shrink film enables sufficient gas transition; therefore carbon dioxide accumulation in the boxes did not surpass 1.6 kPa. Also Matta et al.[28] found minor accumulation of CO_2 during storage in their wrapped passion fruit using similar film. The boxes were stored at 12 °C for 3 weeks and then transferred to shelf life at 20 °C for an additional 5 days, after the PP wrapping was removed.

Fruit Quality and Peel Color. Total soluble solids (TSS) and titratable acidity (TA) were assayed in fruit juice extracted from six individual fruits from each line and season. Juice samples were taken on the day of harvest, after 3 weeks at 12 °C, and after a further 5 days at 20 °C. Every fruit was cut into halves and the juice was squeezed through two-layered cotton gauze, to remove seeds. Percentage TSS was determined with a PR-1 digital refractometer (Atago, Tokyo, Japan), and TA (calculated as citric acid equivalent) was determined with a TIM 850 automatic titration system (Radiometer, Copenhagen, Denmark).

Peel color indices were recorded at two diametrically opposite sides of each fruit with a Chroma Meter CR-310 (Minolta, Tokyo, Japan). Results were expressed as hue angle (H°), in which 90° represents a full yellow color, 0°/360° represents a full red color, and 270° represents full blue. Accordingly, full purple is between 270 and 360°.

Ethylene Measurements. Box headspaces were sampled for ethylene concentration every 3−4 days during cold storage at 12 °C. A silicone septum was glued onto each PP bag, and headspace samples were taken with a 10 mL syringe, as previously reported for mango fruits.[29] Ethylene levels were analyzed by gas chromatograph with a model 3300 flame ionization detector (GC-FID) (Varian, Walnut Creek, CA, USA). Ethylene levels were calculated as μL L^{-1}

present in the headspace of the PP bags,[29] and average production was calculated from all data through the course of the storage period at 12 ± SE °C.

Volatiles Analysis. Fresh passion fruit juice was extracted as described above, at the same three time points. Volatile analysis was conducted according to the method of Pesis et al.,[30] with small modifications. Samples were placed in 20 mL amber vials; each sample comprised 1 mL of juice, 1 mL of 200 g L^{-1} NaCl solution, and an additional 0.6 g of NaCl. The high salt concentration was intended to prevent enzymatic activity associated with volatiles biosynthesis and to increase partitioning of volatiles, as was also reported in passion fruit juice.[17] To each vial was added 5 μL of toluene (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:1000 (v/v), which was previously suited to the chromatograms as an internal standard. The vials were sealed with screw caps and stored at −20 °C pending analysis.

Volatile compounds were detected by gas chromatograph−mass spectrometer (GC-MS). Prior to analysis, samples were incubated for 1 h at 30 °C to attain equilibrium. Volatiles were then absorbed from the vial headspace for 10 min by solid-phase microextraction (SPME) onto a fiber (1 cm long, 100 μm thick) coated with polydimethylsiloxane (PDMS) (Sigma-Aldrich/Supelco, Bellefonte, PA, USA). The fiber was desorbed for 5 min at 250 °C in the splitless inlet of a model 7890A GC (Agilent, Santa Clara, CA, USA) equipped with an HP-5 capillary column (30 m × 0.25 mm inner diameter (i.d.), 0.25 μm film thickness; Agilent), coupled to a model 5975C MS detector (Agilent). Helium was used as the carrier gas at a flow rate of 0.8 mL min^{-1}. The oven temperature was initially 40 °C for 1 min, ramped up to 150 at 10 °C min^{-1}, and then up to 220 at 15 °C min^{-1}. Detection was performed by the mass spectrometer in the electronic impact mode (EI at 70 eV) with a mass acquisition range of 40−206 *m/z* and a scanning rate of 7.72 spectra s^{-1}. Volatile compounds were identified by two methods: (i) comparing their mass spectra with the National Institute of Standards and Technology (NIST) 2006 mass spectra library and (ii) calculating the volatiles' linear retention indices (RIs) by using a series of *n*-alkanes (C5−C20) and comparing their values with published RI values, obtained by comparison to authentic purchased or synthesized compounds or published by Adams.[31] Peaks of interest were quantified by using calibration curves of external standards (Sigma-Aldrich, St. Louis, MO, USA) of each structural group— esters were quantified as ethyl butanoate equivalents, alcohols as octanol equivalents, ketones as hepten-6-methyl-5-hepten-2-one equivalents, linalool as terpene equivalents, and limonene as cyclic terpene equivalents—and adjusting values according to the amount of internal standard (toluene) present.[32]

Sensory Evaluation. Taste indices were tested on the day of harvest for both the PD and RS lines, in both winter and summer seasons. Fruits were cut into halves, and the content (pulp plus seeds) was emptied into 50 mL plastic cups, with each sample comprising a mixture prepared from six different fruits. Sensory evaluation was performed by 12 panelists, 6 men and 6 women, aged from 22 to 50. The panelists were members of the department of postharvest science, with previous experience in sensory assessment of tropical fruzits. Each panelist was asked to evaluate the taste of the samples according to four criteria—sourness, sweetness, off-flavor, and tastiness—on intensity scales of 1−9, and to rinse their mouth with water between samples.[23]

Mathematical and Statistical Analysis. To estimate the difference in volatiles production between summer and winter seasons, we created the summer/winter ratio (SWR) index. It was calculated for each volatile compound in both PD and RS lines, as follows:

$$\frac{\text{summer volatile concentration}}{\text{winter volatile concentration}} = \text{SWR}$$

Thus, a difference between seasons in favor of summer results in SWR > 1; the larger the difference between summer and winter, the higher the SWR value will be, reaching a ratio of tens, hundreds, or thousands, whereas a difference in favor of winter will lead to values of 0 < SWR < 1.

The odor activity value (OAV) represents the contribution of a specific volatile to the odor of a sample and is calculated as follows:

$$\frac{\text{volatile concentration}}{\text{volatile odor threshold}} = \text{OAV}$$

Volatiles with log OAV > 0 contribute to sample odor.[33] Because volatiles quantification was based on equivalents of external standards according to their functional group, the OAVs are calculated from approximate data for comparison purposes.

Fruit-quality indices were determined as means of six samples, whereas volatile analysis was performed on three samples. Ethylene headspace measurements are means of three boxes with least significant difference (LSD) at $P < 0.05$. One-way analysis of variance (ANOVA) was used to identify volatiles whose levels differed significantly among treatments (line/season). Data were analyzed with JMP 5.0 software (SAS Institute, Cary, NC, USA). Hierarchical clustering and heat map analysis were performed with the Multi-experiment Viewer (MeV) software,[34] with data being standardized (mean = 0 and variance = 1 for each volatile) prior to analysis and visualization.[23]

■ RESULTS AND DISCUSSION

Changes in TSS and TA Contents during Storage. Results of measurements of TSS and acid contents as well as TSS/TA ratios of lines PD and RS during storage are presented in Figure 1. Summer fruits of line PD showed mild decreases in
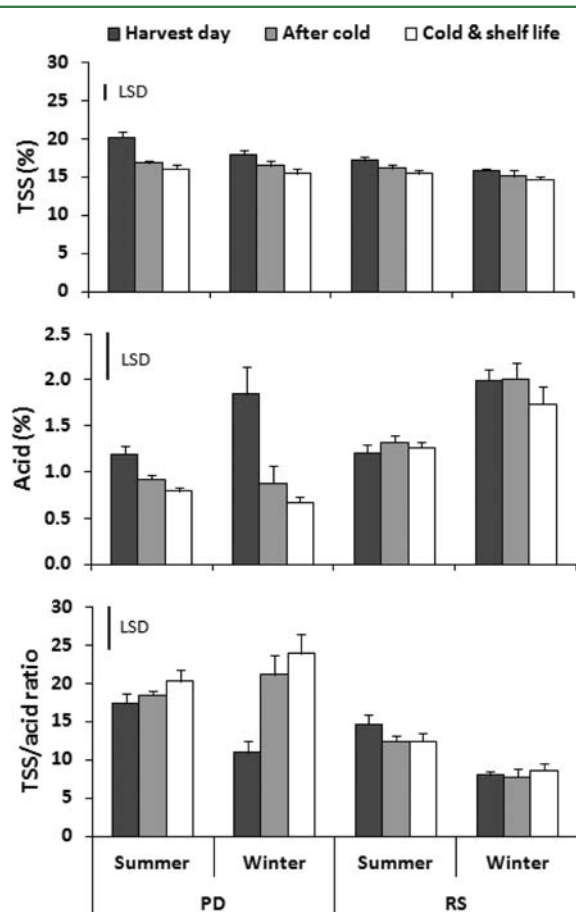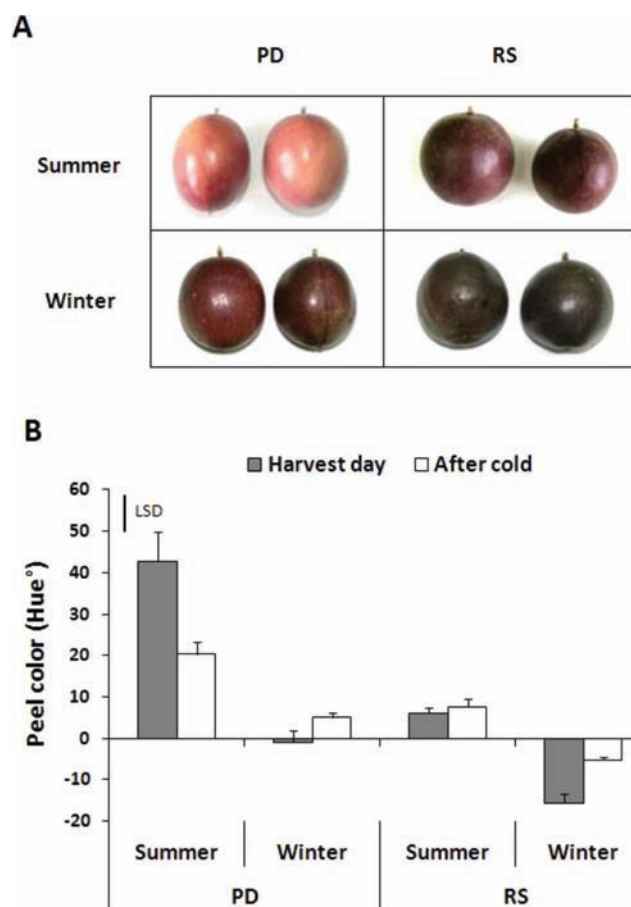

Figure 2. Peel color of lines PD and RS in summer and winter seasons on the day of harvest or collection (A), and hue angle on the day of harvest and after cold storage of 3 weeks at 12 °C (B). Data are means of 10 measurements ± SE. LSD = 8.72 at the $P < 0.05$ level.

Figure 1. TSS and acid contents as well as TSS/acid ratios of lines PD and RS in summer and winter seasons on the day of harvest or collection, after cold storage (3 weeks at 12 °C), and after cold storage plus shelf life (3 weeks at 12 °C plus 5 days at 20 °C). Data are means of six measurements ± SE. LSDs at the $P < 0.05$ level are 2.05, 0.51, and 5.44 for TSS, acid, and TSS/acid ratio, respectively.

TSS and acid contents after cold storage and shelf life, which led to moderate increases in their TSS/TA ratio. In contrast, PD winter (PDw) fruits had notably higher acid contents than the summer ones on the day of harvest, but they decreased significantly during storage, causing a dramatic increase in the poststorage TSS/TA ratio (Figure 1). The reductions in TSS during cold storage are in agreement with those accepted for yellow passion fruit after storage at 10 or 15 °C.[28] Recent studies have shown a decrease in acid content accompanied by an increase in TSS/TA ratio in passion fruit during storage at 10 and 20 °C.[35,36] In addition, our results agree with previous findings on passion fruit grown under controlled-temperature conditions: under a high-temperature regime of 30/30 °C, day/night, fruits had the lowest acid contents, whereas under a 24/17 °C regime they exhibited the highest acidity level.[37] In passion fruit, acid degradation begins on the vine, prior to natural abscission,[7] and during summer the acid degradation process in PD fruits was accelerated, probably while they still were on the vine, because of the higher temperatures.[38] However, in the winter, when acidity of PD fruits on the vine remains high, a storage period is needed, to enhance acid degradation which elevates the TSS/TA ratio and thereby improves the fruit flavor quality. In addition to growth temperature, other environment conditions such as growth altitude were reported to influence passion fruit acidity.[35] In RS fruits there were small differences in TSS values between summer and winter. On the other hand, acid contents in RS summer
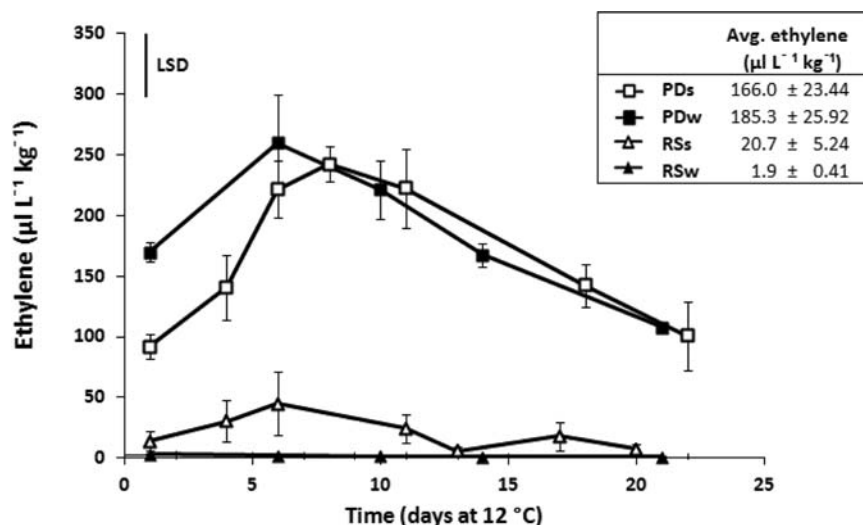
Figure 3. Ethylene accumulation during cold storage at 12 °C: of PD summer fruits (PDs), PD winter fruits (PDw), RS summer fruits (RSs), and RS winter fruits (RSw). Values are means ± SE of headspace measurements in 3 boxes at each time point. Each box contained 6 fruits wrapped in PP film (M&M). Average ethylene accumulation throughout the storage period appear in the inset table as mean ± SE. LSD = 50.42 at the $P < 0.05$ level.
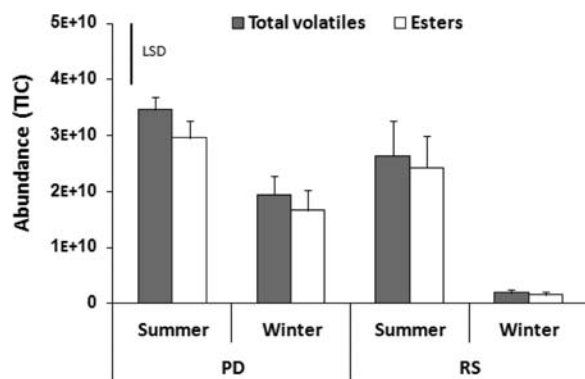

Figure 4. Abundance of total volatiles and total esters (total ion current) in PD and RS summer and winter fruits on the day of harvest or collection. Values are means ± SE of 3 juice headspace measurements. LSD = 1.11×10^{10} at the $P < 0.05$ level.

(RSs) fruits were significantly lower than those in RSw fruits, so that the TSS/TA ratio was higher in the summer than in the winter (Figure 1). However, the lack of changes in TSS and acidity in RS fruits during storage leads to constant TSS/TA ratios after cold storage and shelf life, in both summer and winter (Figure 1). The cessation of acid degradation in RS fruits during storage in both seasons is indicative of reduced ripening processes, as well as repressed fruit respiration and metabolism.[39] Slow metabolism is common in nonclimacteric fruits such as citrus, grapes, and various berries, in contrast to the climacteric tropical passion fruit.[25,26] Our results are in accordance with those of several studies which found that whether various melon lines exhibited climacteric or nonclimacteric behavior depended on the speed of the ripening processes.[12,40]

Peel Color Development under Cold Conditions. Peel color was lightest in PDs fruits on the day of harvest, when it attained the highest hue value of more than 40 °H, manifested as a pinkish peel color (Figure 2). Likewise, in passion fruits that were grown under controlled temperatures, peel color development was poor under a high-temperature regime (30/30 °C)

whereas under a low-temperature regime (24/17 °C) fruits developed an attractive dark-purple peel color.[37] PDs fruits became more purple, with hue angle below 20 °H, after removal from cold storage, probably because of induction of anthocyanins during storage at 12 °C. Our findings are with accordance to those of Matta et al.,[28] who showed that purple passion fruit, picked as green fruit, becomes more purple in cold storage at 10 °C. Pruthi et al.[41] had previously shown that anthocyanins were the pigments mainly responsible for the purple peel color of passion fruit. Biosynthesis of anthocyanins is known to be induced by exposure to low temperatures.[42] Thus, PD fruits were subjected to low temperatures in the field during winter and during storage at 12 °C in the summer, and biosynthesis of anthocyanins probably was stimulated by both situations, resulting in a darker peel color. RSs fruits were significantly darker than PDs ones, as indicated by their lower hue angle (Figure 2), which remained the same after 3 weeks at 12 °C. The fruits with the darkest purple peel color were RSw fruits (350 °H), whose color changed a little after cold storage (Figure 2B).

Differences in Ethylene Measurements during Cold Storage. PD fruits produced ~70 times more ethylene than RS fruits in both seasons (Figure 3); they emitted considerable amounts of ethylene immediately after they were collected from the ground, whereas RS fruits emitted negligible amounts at this point in time (Figure 3). RSs fruits produced at least 10 times as much ethylene as RSw fruits, but nevertheless, the average ethylene production by RSs fruits (5.2 μL L^{-1}) was very low compared with that by PD in the present study (50.4 μL L^{-1}), as well as very low compared with those found in other studies, in both yellow and purple passion fruits.[7,26,43] Nevertheless, summer ethylene production levels in line RS seem to be sufficient to induce fruit abscission; all summer fruits were abscised and collected from the ground. Ethylene is an extremely potent plant hormone that can be effective in minimal concentrations.[9,11] In the present study ethylene levels measured in the headspace of RSw fruits (averaged ~0.4 μL L^{-1}) were negligible compared with those of all other fruits (Figure 3). Furthermore, it should be mentioned that detached winter fruits of line RS that were treated with exogenous
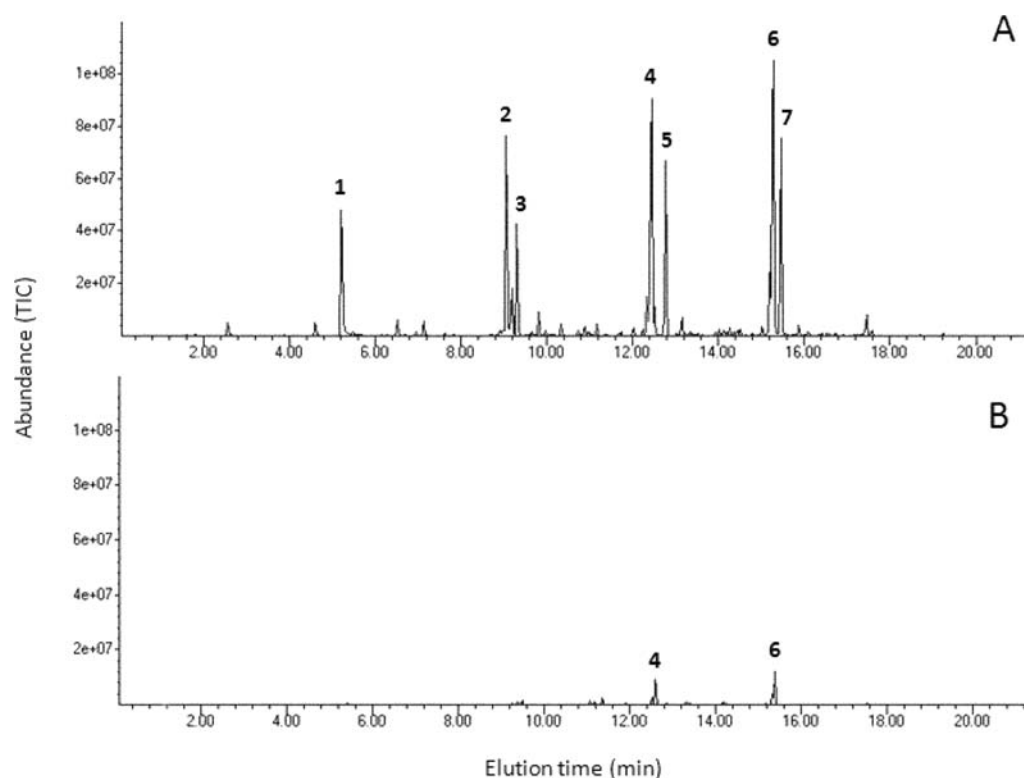
Figure 5. GC-MS chromatograms of passion fruit juice volatiles for (A) RS summer fruit and (B) RS winter fruits, sampled with PDMS SPME fiber on the day of harvest or collection. Major peaks: (1) ethyl butanoate, (2) ethyl hexanoate, (3) hexyl acetate, (4) hexyl butanoate, (5) methylhexyl butanoate, (6) hexyl hexanoate, and (7) methylhexyl hexanoate.

ethylene at 20 °C showed no rise in ethylene during storage (Supporting Information, Figure S1) or carbon dioxide production, a further confirmation of their nonclimacteric behavior.[12,13]

Volatiles Production on the Day of Harvest or Collection. PDs fruits produced the largest amount of volatiles, followed by RSs fruits (Figure 4). During winter, aroma volatiles production in PDw fruits was fairly low compared with that in PD and RS summer fruits. However, it was significantly higher than that in RSw fruits, which emitted the lowest level of volatiles, around one-tenth of that in all other fruits (Figure 4). Because volatiles are secondary metabolites, their production is influenced by changes in the metabolic rate,[12] such as the deceleration of respiratory and ripening processes at low temperatures during winter.[38] In addition, the substantially smaller ethylene production of RSw fruits than of RSs ones probably led to reduction of volatiles synthesis, as ethylene plays a key role in aroma development in climacteric fruits.[11,12,24]

Analysis of the aroma volatile compounds showed that esters comprised 78−92% of the total aroma volatiles in all examined fruits (Figure 4). In parallel with the total volatiles, total ester contents were found to be highest in PD and RS summer fruits, and significantly higher than those in RSw fruits (Figure 4). In apples and melons, biosynthesis of esters was reportedly induced by ethylene production,[12,44,45] and treatment of apples with 1-methyl cyclopropene (1-MCP), a known ethylene-action inhibitor, reduced biosynthesis of esters in three different apple cultivars.[45] In the present study, the low ester production in RSw fruits was similar to that found in other nonclimacteric fruits such as citrus[32] or nonclimacteric melons.[12] Comparison

between the summer and winter GC-MS chromatograms of RS illustrates the wide difference between the RS volatile production levels in the two seasons (Figure 5). The seven main peaks in the RSs fruit chromatogram belong to various ethyl, hexyl, and methylhexyl esters, all assumed to be derived from fatty acids.[5,24] In the RSw fruit chromatogram only two of these peaks are evident, at extremely low levels (Figure 5). The large percentage of esters among the total volatiles in both lines, and the association of decreased production of esters in RSw fruits with reduced ethylene emission, all suggest that ethylene is involved in control of biosynthesis of esters in PD and RS passion fruit lines. The involvement of ethylene in ester production was demonstrated in apples by using ethylene-suppressed transgenic apples.[44]

Identification and quantification of some of the main volatiles identified in the juice headspace by the SPME technique are presented in Tables 1 and 2. The major compounds in both PD and RS lines were the hexyl esters—hexyl butanoate and hexyl hexanoate—which contributed >45% of the total volatile concentrations in the winter (Table 2). In the summer, in addition to these two hexyl esters, two branched-chain esters, methylhexyl butanoate (MHB) and methylhexyl hexanoate (MHH), were significantly induced and, together with the hexyl esters, contributed >62% of the total volatiles in both lines. It should be noted that the hexyl esters and the methylhexyl eaters are probably not composed of the same alcohol. The hexyl group is donated by hexanol,[46] and the methylhexyl group is donated by the secondary alcohol, 2 heptanol.[5] Both of these alcohols react with butanoic and hexanoic acids, derived from fatty acids,[5,46,47] to create the four main compounds in the juice during summer. The induction of MHB and MHH esters

Table 1. Identification and Odor Quality of Major Headspace Volatile Compounds, Determined by SPME/GC-MS Analysis of Passion Fruit Lines PD and RS Summer and Winter Fruits

compound	calculated RI[a]	literature RI	reference	odor quality[c]
ethyl acetate	651	664	Jordan et al., 2002[3]	sweet, etherial, grape, rum
methyl butanoate	754	726	Jordan et al., 2002[3]	pungent, perfume, fermented
ethyl butanoate	812	781	Jordan et al., 2002[3]	strawberry, apple, banana
butyl acetate	825	811	Shalit et al., 2001[53]	sweet, banana, pear
hexanol	879	863	Jordan et al., 2002[3]	grass, fruity, floral
2-heptanone	901	895	Pino et al., 2003[54]	fruity, ketonic, green, cream
methyl hexanoate	936	923	Pino et al., 2003[54]	pineapple, ether
heptanol	981	970	Flamini et al., 2003[55]	musty, pungent, green
6-methyl-5-hepten-2-one	999	988	Flamini et al., 2003[55]	green, fruity, pear
β-myrcene	1003	982	Jordan et al., 2002[3]	herbaceous, woody, peppery
butyl butanoate	1007	947	Azodanlou et al., 2003[56]	sweet, fresh, fatty
ethyl hexanoate	1011	995	Jordan et al., 2002[3]	green, anise, brandy, waxy
3-hexenyl acetate	1019	1002	Jordan et al., 2002[3]	fresh, green, sweet, fruity
hexyl acetate	1025	1010	Jordan et al., 2002[3]	sweet, fatty, fresh, pear
limonene	1044	1022	Jordan et al., 2002[3]	orange peel-like, fresh
methylhexyl acetate	1054	1047	Pino et al., 2003[57]	fenugreek, fruity
cis β-ocimene	1062	1051	Pino et al., 2003[54]	warm, floral, herb flower, sweet
octanol	1083	1077	Jordan et al., 2002[3]	waxy, green, citrus, rose
linalool	1114	1103	Jordan et al., 2002[3]	citrus, floral, waxy, rose
hexyl propanoate	1121	1108	Ruther at al., 2000[58]	pear, green
rose oxide	1127	1112	Guth et al., 1997[59]	green, spicy, geranium
ethyl 3-hydroxy hexanoate	1142	1136	Jordan et al., 2002[3]	green
benzyl acetate	1182	1161	Jordan et al., 2002[3]	sweet, fruity, floral, jasmin
3-hexenyl butyrate	1202	1187	Ruther at al., 2000[58]	fresh, apple, fruity, vegetative, grape
hexyl butanoate	1211	1192	Jordan et al., 2002[3]	green, sweet, waxy
methylhexyl butanoate[b]	1231	1194	Adams, 2001[31]	fruity, green, vegetable
hexyl 2-methyl butanoate	1253	1236	Flamini et al., 2003[55]	green, waxy, spicy, tropical
3-mercaptohexyl acetate	1269	1244	Lin et al., 2002[60]	floral, fruity, pear, tropical, passion fruit
geraniol	1285	1257	Jordan et al., 2002[3]	floral, sweet, fruity, citrus
edulan II[b]	1287			strong floral, rose-like aroma[1]
dihydro edulan I	1325	1292	Campeol et al., 2001[61]	camphoraceous aroma[1]
edulan I[b]	1343	1309	Pino et al., 2003[54]	strong floral, rose-like aroma[1]
benzyl butanoate	1370	1337	Jordan et al., 2002[3]	sweet, fresh, pineapple
3-hexenyl hexanoate	1402	1380	Ruther at al., 2000[58]	fruity, waxy, green, tropical, balsamic
hexyl hexanoate	1409	1387	Ruther at al., 2000[58]	green, sweet, waxy, berry
methylhexyl hexanoate	1424	1390	Adams, 2001[31]	sweet, fruity, ester
3-mercaptohexyl butanoate[b]	1445			sulfurous, passion fruit, fruity, tropical
geranyl acetone	1478	1463	Shalit et al., 2001[53]	fresh, magnolia, aldehydic
β-ionone	1522	1493	Shalit et al., 2001[53]	floral, woody, sweet, berry
3-mercaptohexyl hexanoate[b]	1684			fruity, sulfurous, tropical, passion fruit

[a]Calculated retention indices using a series of *n*-alkanes on HP-5 column. [b]Compounds were tentatively identified by comparison to mass spectral database and/or retention index was taken from Adams.[31] [c]Odor descriptions for each compound were taken from: http://www.thegoodscentscompany.com.

was most prominent in the RS line, where the SWRs for MHB and MHH were 185 and 100, respectively (Table 2). Strohalm et al.[5] previously found that these esters were more prevalent in the purple passion fruit than in the yellow one, but to date, we did not find any reports on seasonal differences. We assume that the enzymes responsible for synthesis of the esters MHB and MHH are induced during summer because of the high temperatures, which causes a dramatic elevation of their concentration in the juice. This assumption is further supported by the fact that these esters are derived from fatty acids,[5] and at higher temperatures the natural process of fatty acid breakdown is probably induced during fruit ripening and senescence.[48] In our present study the induction of volatiles during summer was associated with the higher levels of ethylene than in winter,

in both lines (Figure 3), which led to further acid breakdown (Figure 1), as also shown by Yonemoto et al.[37] Two other principal components in the aromas of lines PD and RS, in both summer and winter, were the ethyl esters ethyl butanoate and ethyl hexanoate (Table 2, Figure 5). Both of these esters are presumably synthesized by esterification of the corresponding acids with ethanol, a process reported to be induced in high temperatures of between 30 and 40 °C,[49] the average day temperatures during summer. However, the concentrations of hexyl esters were at least twice as great as those of the ethyl esters, especially in RS winter fruits, in which they were 10 times as great (Table 2). Greater amounts of hexyl esters than of ethyl esters were previously found in various lines of apples.[50]

Table 2. Major Headspace Volatile Compounds Concentrations, Determined by SPME/GC-MS Analysis of Passion Fruit Lines PD and RS Summer and Winter Fruits, on the Day of Harvest or Collection and Their Summer/Winter Ratio[a]

| | concentration (nL L^{-1}) | | | | summer/winter ratio (SWR) | |
| | line PD | | line RS | | | |
compound	summer	winter	summer	winter	PD	RS
ethyl acetate	809a	157a	432a	51a	5.14	8.39
methyl butanoate	42a	34a	47a	nd	1.24	473.89
ethyl butanoate	8328a	4253ab	6010a	145b	1.96	41.54
butyl acetate	475a	187ab	173ab	20b	2.55	8.65
hexanol	52a	24b	62a	8b	2.16	7.94
2-heptanone	208a	30b	28b	0.28b	7.02	101.43
methyl hexanoate	26a	18a	116a	nd	1.40	1161.11
heptanol	4a	2a	3a	1a	1.88	2.40
6-methyl-5-hepten-2-one	49b	137a	7c	12c	0.36	0.57
β-myrcene	42a	47a	36a	6a	0.89	6.39
butyl butanoate	1128a	650a	167a	5a	1.74	31.34
ethyl hexanoate	6791a	3454a	9467a	101a	1.97	93.48
3-hexenyl acetate	893a	805a	944a	107b	1.11	8.85
hexyl acetate	1399ab	953ab	2140a	181b	1.47	11.81
limonene	200a	134a	211a	40a	1.49	5.26
methylhexyl acetate	563a	19b	486ab	4b	30.20	111.28
cis β-ocimene	215a	37b	30b	2b	5.82	13.41
octanol	28ab	11b	59a	4b	2.57	15.97
linalool	71a	47ab	17ab	8b	1.51	2.20
hexyl propanoate	113a	160a	67a	31a	0.70	2.18
rose oxide	28a	5b	3b	nd	5.33	26.96
ethyl 3-hydroxy hexanoate	5a	23a	41a	nd	0.21	413.48
benzyl acetate	147a	97ab	192a	15b	1.52	13.10
3-hexenyl butyrate	3978a	2971a	1732a	229a	1.34	7.57
hexyl butanoate	22657a	14567ab	15081ab	1139b	1.56	13.24
methylhexyl butanoate	9999a	1263bc	5529ab	30c	7.92	185.42
hexyl 2-methyl butanoate	181b	80b	520a	81b	2.25	6.43
3-mercaptohexyl acetate	10b	8b	47a	3b	1.15	14.12
geraniol	15a	8a	9a	1a	2.02	11.26
edulan II	51a	12b	7b	0.14b	4.20	50.06
dihydro edulan I	137a	6ab	20b	1ab	22.20	29.18
edulan I	345a	122ab	85b	2ab	2.83	34.83
benzyl butanoate	365a	108ab	189b	12ab	3.37	15.33
3-hexenyl hexanoate	2865a	2486a	2356a	341a	1.15	6.90
hexyl hexanoate	15594a	12902ab	16738a	1730b	1.21	9.67
methylhexyl hexanoate	6634a	1097b	6040a	60b	6.05	100.05
3-mercaptohexyl butanoate	19a	76a	68a	6a	0.25	11.86
geranyl acetone	49ab	91a	31ab	4b	0.53	7.31
β-ionone	44a	12b	11b	3b	3.56	3.24
3-mercaptohexyl hexanoate	nd	8a	8a	1a	0.01	7.59

[a]Values followed by the same letter within a row are not significantly different, $P < 0.05$, $n = 3$ (Tukey test). SWR - summer/winter ratio; see Materials and Methods. nd = not detected. For SWR calculation, an arbitrary value of 0.1 nL L^{-1} was used.

In addition to the strong prevalence of the ethyl and hexyl esters, there are various, even unique, volatiles that constitute an integral part of the passion fruit aroma, despite their relatively low concentrations. Among the esters there is a small group of ester thiols derived from cysteine,[16] another group containing terpenes and cyclic terpenes generated by methylerythritol phosphate (MEP) and mevalonate pathways,[24] and a group of the benzo-pyrans edulans, the breakdown products of carotenoids.[14] All these groups of volatiles are generally found at much higher levels in summer than in winter, in both lines (Table 2).

In general, the seasonal differences in volatiles production, as indicated by the SWR parameter, were much smaller in line PD

than in line RS (Table 2). Nevertheless, higher SWR values of 30.2, 22.2, 7.9, and 6.1 were found for four volatile compounds in line PD: methylhexyl acetate, dihydro edulan I, methylhexyl butanoate, and methylhexyl hexanoate, respectively. These findings suggest that the biosynthesis of these four volatiles, three of which are methylhexyl esters, might be influenced by the higher temperatures and higher ethylene production during summer (Figure 3). On the other hand, the production of volatiles with SWR values of hundreds in RS fruits but not in PD fruits was probably due to elevated ethylene production at higher temperatures. The most significant inductions in RS fruits in summer included many methyl esters—methyl

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Table 3. Log Odor Activity Values (OAVs) of Certain Volatile Compounds in Passion Fruit Lines PD and RS in Summer and Winter Seasons on Day of Harvest or Collection; log OAV = log (Volatile Conc./Volatile Threshold)[a]

| | | | log OAVs | | | |
| | | | line PD | | line RS | |
compound	odor threshold (μg L^{-1})	reference	summer	winter	summer	winter
ethyl acetate	5000	Pino et al., 2006[20]	−0.79	−1.50	−1.06	−1.99
methyl butanoate	59	Pino et al., 2006[20]	−0.15	−0.24	−0.10	−2.77
ethyl butanoate	1	Pino et al., 2006[20]	**3.92**	**3.63**	**3.78**	**2.16**
butyl acetate	66	Pino et al., 2006[20]	**0.86**	**0.45**	**0.42**	−0.52
hexanol	500	Pino et al., 2006[20]	−0.98	−1.31	−0.91	−1.81
2-heptanone	140	Pino et al., 2006[20]	**0.17**	−0.68	−0.70	−2.70
methyl hexanoate	70	Pino et al., 2006[20]	−0.43	−0.58	**0.22**	−2.85
heptanol	425	Pino et al., 2006[20]	−2.08	−2.36	−2.18	−2.56
6-methyl-5-hepten 2-one	50	Buttery et al., 1990[62]	−0.01	**0.44**	−0.87	−0.62
β-myrcene	15	Pino et al., 2006[20]	**0.44**	**0.49**	**0.38**	−0.42
butyl butanoate	400	Pino et al., 2006[20]	**0.45**	**0.21**	−0.38	−1.87
ethyl hexanoate	1	Pino et al., 2006[20]	**3.83**	**3.54**	**3.98**	**2.01**
3-hexenyl acetate	8	Buttery et al., 1990[62]	**2.05**	**2.00**	**2.07**	**1.12**
hexyl acetate	2	Takeoka et al., 1990[63]	**2.84**	**2.68**	**3.03**	**1.96**
limonene	10	Fazzalari, 1978[64]	**1.30**	**1.13**	**1.32**	**0.60**
octanol	190	Pino et al., 2006[20]	−0.82	−1.24	−0.51	−1.71
linalool	6	Pino et al., 2006[20]	**1.08**	**0.90**	**0.46**	**0.12**
hexyl propanoate	8	Takeoka et al., 1990[63]	**1.15**	**1.30**	**0.92**	**0.58**
rose oxide	0.5	Guth, 1997[65]	**1.76**	**1.03**	**0.73**	−0.70
ethyl-3-hydroxy hexanoate	270	Plotto et al., 2008[66]	−1.75	−1.07	−0.81	−3.43
benzyl acetate	364	Pino et al., 2006[20]	−0.39	−0.57	−0.28	−1.40
hexyl butanoate	203	Pino et al., 2006[20]	**2.05**	**1.86**	**1.87**	**0.75**
hexyl-2-methyl butanoate	22	Takeoka et al., 1990[63]	**0.91**	**0.56**	**1.37**	**0.56**
3-mercaptohexyl acetate	0.02	Steinhaus et al., 2009[51]	**2.69**	**2.63**	**3.37**	**2.22**
geraniol	40	Buttery et al., 1990[62]	−0.42	−0.72	−0.66	−1.71
benzyl butanoate	376	Pino et al., 2006[20]	−0.01	−0.54	−0.30	−1.48
hexyl hexanoate	500	Zea et al., 2001[67]	**1.49**	**1.41**	**1.52**	**0.54**
geranyl acetone	60	Pino et al., 2006[20]	−0.09	**0.18**	−0.29	−1.15
β-ionone	0.007	Pino et al., 2006[20]	**3.80**	**3.25**	**3.18**	**2.67**
No. of contributing volatiles			**17**	**18**	**17**	**12**
no. of noncontributing volatiles			12	11	12	17

[a]OAVs = odor activity values; see Materials and Methods. Specific volatile contribute to the fruit aroma at OAV > 0. Values of volatiles contributing to the juice aroma appear in **bold**.

butanoate, methyl hexanoate, methylhexyl acetate, methyl 3-hydroxy hexanoate, methylhexyl butanoate, and methylhexyl hexanoate—all of which exhibited SWR values of hundreds of units (Table 2).

Odor Activity Values of the Various Volatiles on the Day of Harvest or Collection. The contributions of volatiles to the aroma and flavor of each line and season are presented in Table 3. Although not all volatiles have positive OAV values, their presence in the juice is important and even desirable, because they are perceived as background odors.[33] Volatiles with high concentrations and low odor thresholds, such as ethyl butanoate, ethyl hexanoate, and hexyl acetate, contributed to the aromas of both lines in both seasons (Table 3). It should be noted that, despite the higher concentrations of the hexyl esters, their influence on fruit aroma was lower than that of the ethyl esters, which have odor thresholds hundreds of times smaller than those of the hexyl esters.[31] In addition, volatiles that were present in very low concentrations in both lines—3-mercaptohexyl acetate and β-ionone—(Table 2) contributed greatly to the fruit aroma, as indicated by their high OAVs (Table 3). These high OAVs (2.2−3.8) resulted from the very low odor thresholds of both compounds (0.02 and 0.007 μg L^{-1},

respectively), which enable us to sense them in minimal concentrations (Table 3). It was shown that thiols can serve as novel odorants because of their typically low odor thresholds,[16,51] as in the case of PD and RS lines (compare Table 1 versus Table 2). 3-Mercaptohexyl acetate, which possesses a pleasant passion fruit/black currant scent, was found in the aromas of yellow passion fruit and guava.[15,51] In blackberry, β-ionone is a significant contributor to the aroma, despite its relatively low concentration, and is an important fragrance chemical, used in perfumery.[24,33,52] β-Ionone is derived from degradation of carotenoids, which are present at high levels in the arils of passion fruit[1,4] and previously were shown to be part of the passion fruit aroma.[3,4]

Generally, there is little difference among the OAVs of PD fruits in both seasons and among those of RSs fruits: out of 29 compounds tested, 17 or 18 had positive AOVs (Table 3), meaning that these compounds contributed to the fruit aroma. However, in RSw fruits only 12 of the 29 compounds had positive OAVs, and 17 of them had negative OAVs (Table 3). The absence of some of the fruity and herbaceous volatiles from the aroma of RSw fruits, combined with the high acidity of
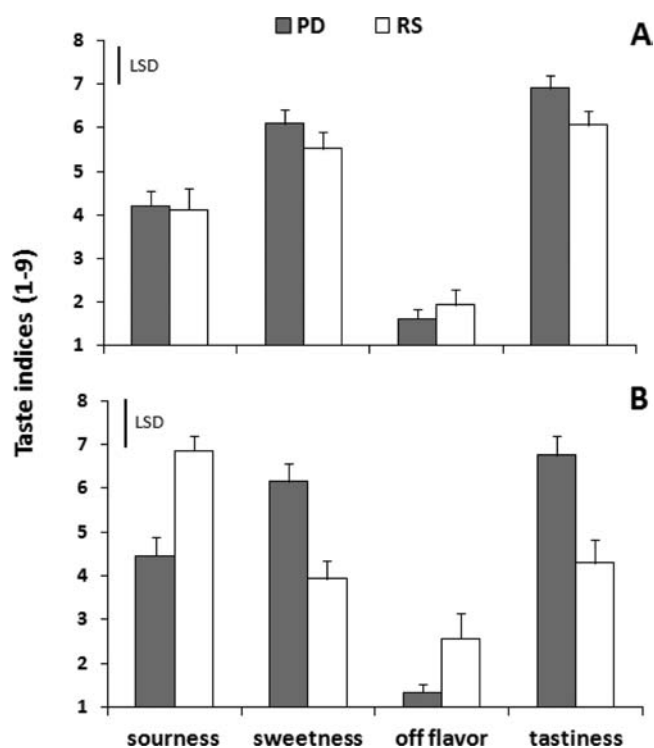
Figure 6. Taste indices of PD and RS lines on the day of harvest or collection in summer (A) and winter (B) seasons. Data are means ± SE of grades given by 12 panelists. LSDs at the $P < 0.05$ level are 0.88 and 1.12 for summer and winter, respectively.

these fruits (Figure 1), might diminish their total flavor and aroma.

Sensory Evaluation of the Two Passion Fruit Lines on the Day of Harvest or Collection. Both PD and RS fruits were examined for several parameters on the day of harvest or collection in the summer and winter. The parameters were sourness, sweetness, off-flavor, and tastiness representing the overall flavor of the sample. The PD fruits received similar grades in summer and winter, whereas the grading of RS fruits differed considerably (Figure 6). In the summer, RS fruits resembled PD fruits in the sourness, sweetness, and off-flavor indices, so that their total grades for tastiness were similar (Figure 6). However, in the winter, RS fruits were graded significantly more acidic and less sweet than PD ones, which correlated with their low TSS/TA ratio (Figure 1). In addition the former fruits received higher off-flavor grading, so that, in total, they were graded as less tasty (Figure 6). In many fruits, inferior sweetness and presence of an off-flavor often reduces the fruit acceptability,[18,23,40] as was also found in passion fruit.[25] The lower tastiness of RSw fruits resulted from a combination of low TSS/TA ratio (Figure 1) and relatively poor volatiles production (Figures 4 and 5). Examination of various passion fruit lines in our lab have shown high correlation between TSS, acid, and TSS/TA ratio and taste indices (Supporting Information, Figure S2). However, the additional explanation for fruit sensory acceptability is the crucial effect of volatile compounds on flavor was previously shown in various fruits such as citrus,[23] blackberry,[52] tomato,[33] and others. Among volatiles, esters possess a sweet fruity taste (Table 1), which contributes most of the aroma in our present passion fruit lines (Figure 4). In feijoa, fruits containing higher ester

concentrations were rated as having sweeter taste, irrespective of their acid or TSS contents.[27] The low levels of naturally occurring esters in RSw fruits probably further compromised their overall taste.

Aroma Volatiles Production During Cold Storage and Shelf Life. In general, the levels of aroma volatiles production, as illustrated in the heat map (Figure 7, parts A and B), throughout the storage period (T0, collection; T1, cold storage; T2, shelf life) were quite similar in PDs and PDw fruits. In the summer and winter seasons, some of the PD volatiles placed in the lower part of the hierarchical clustering showed low levels of volatiles on the day of collection (blue color), especially in PDs fruits, and these levels rose during cold storage and shelf life (Figure 7A). However, more than half of the volatiles in the upper part of the heat map showed a distinct pattern of high levels on the day of collection (red color), followed by a reduction in these volatiles after removal from cold storage and after shelf life (Figure 7, parts A and B). The high levels of volatiles on the day of collection, immediately after the fruit had abscised naturally, might have occurred because the fruit had been within or just after its climacteric peak (Figure 3)—a period when ethylene production and ripening processes are accelerated and at their maximum.[11]

In contrast, the heat map and hierarchical clustering of RS summer and winter fruits revealed significantly different behavior patterns of volatiles in the two seasons and during storage. In RSs fruits there were high levels of many volatiles on the day of collection, similarly to the findings for PDs fruits (Figure 7, parts C vs A). In contrast, RSw fruits, which were picked and did not abscise, emitted negligible amounts of ethylene (Figure 3), which led to production of extremely low volatiles levels on the day of harvest (Figures 4, 5 and 7D). According to the heat map, most of the volatiles in RSw fruits rose during storage (Figure 7D). However, it should be noted that, even after cold storage and shelf life, the level of total volatiles in RSw fruit was lower than in all the other fruits (data not shown).

Generally in PDs, PDw, and RSs fruits there were reductions in volatiles levels during storage, probably because of evaporation and the decline in ethylene production during storage at 12 °C (Figure 3). Moreover, the fruits had expended most of their resources in respiration and had reached the senescence stage, characterized by reduced amounts of volatiles. However, some of the volatiles in PD and RS fruits increased during storage, e.g., the ester thiols 3-mercaptohexyl acetate and 3-mercaptohexyl butanoate (Figure 7). Recently, a thioesterase responsible for the release of these thiols was isolated from passion fruit mesocarp.[16] We speculate that cell-wall degradation during ripening and senescence facilitates contact between the thiosterase enzyme and its substrates in the juice, which results in elevation of ester thiol levels during storage.

In conclusion, our findings suggest that ethylene production levels in the two passion fruit lines greatly influence various ripening processes. In the PD line, which produced considerable amounts of ethylene on the vine in both seasons, there was significant accumulation of aroma volatiles as early as the collection day, which led to high taste scores. In contrast, in RSs fruits, which produced ethylene and were abscised naturally, the accumulation of volatiles was higher and fruit taste indices were superior in comparison with RSw fruits, which exhibited nonclimacteric behavior, remained on the vine, and were very sour.
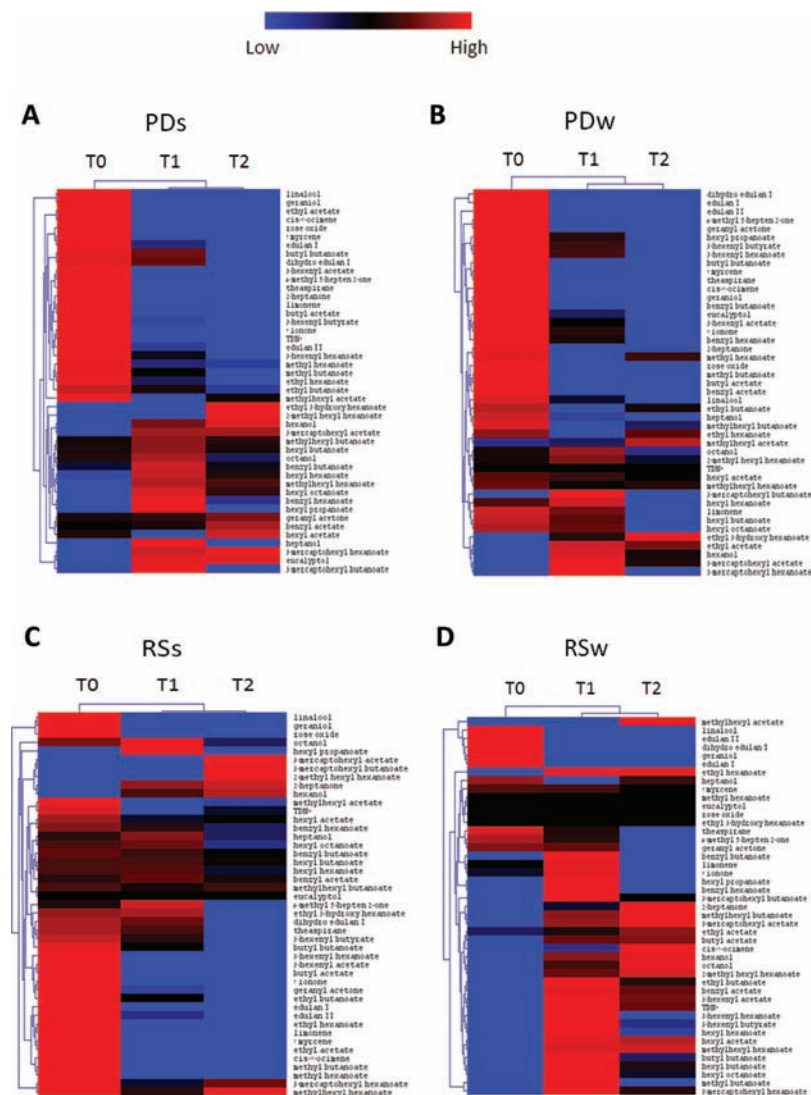
Figure 7. Heat map and hierarchical clustering analysis representing levels of volatile compounds in juice headspace of PD and RS summer and winter passion fruits on the day of harvest or collection (T0), after 3 weeks at 12 °C (T1), and after additional 5 days at 20 °C (T2). Red color represents high levels, and blue color represents low levels. Data are means of three measurements.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Contains Figure S1, ethylene production in RSw fruits treated with ethylene at 12 °C, and Figure S2, correlations between TSS, acid, and TSS/TA ratio and taste indices in various passion fruit lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

GC-MS, gas chromatograph–mass spectrometer; OAV, odor activity value; PD, passion dream; RS, ripens during summer; SPME, solid-phase microextraction; SWR, summer/winter ratio; TA, titratable acid; TSS, total soluble solids.

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