Research Article

Effects of ripening degree and sample preparation on peach aroma profile characterization by headspace solid-phase microextraction

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Abstract: Peaches are consumed worldwide and have great market demand. Compared to apricots, the volatiles defining the typical peach aroma are still poorly analytically characterized. The aim of our study was to determine the impact of the stage of ripening, sample storage conditions, and type of fiber coating and extraction glassware on peach aroma compounds. The peach aroma components were extracted by headspace solid-phase microextraction (HS-SPME) and with the application of a specific fiber (DVB/CAR/PDMS fiber) that retained the main group of volatiles present in peaches. Artisan-made glassware that enabled bigger sample loads was used. It was found that its application provided a 3-fold higher extraction of aromatic compounds on average. Significant differences were also found when the same peach pulp was analyzed fresh or frozen (in liquid nitrogen or in the freezer at –16 °C). In fresh peaches, a higher amount of some alcohols and important esters was determined. Liquid nitrogen had a positive impact on hexanal and (*E*)-2-hexenal, whereas the storage of samples in the freezer had a major impact on most other aldehydes, as well as limonene. The study revealed the importance of sample preparation and storage on the overall aromatic profile of peaches.

Key words: Aromatic profile, artisan-made glassware, fruit volatiles, HS-SPME, DVB/CAR/PDMS fiber, peach aroma

1. Introduction

The peach belongs to the subfamily Prunoideae of the family Rosaceae. There are 3 major groups of cultivars: nectarines, freestone peaches, and clingstone peaches. The peach is grown on all continents except Antarctica, and world peach production has increased steadily in recent years (Hui, 2010). Peaches and nectarines (*Prunus persica* (L.) Batsch) are the second-most important fruit after apples in the EU (Konopacka et al., 2010).

In the peach fruit, there is a close link between on-tree physiological maturity and the development of key traits responsible for its quality (Visai and Vanoli, 1997). A delayed harvest could improve fruit organoleptic characteristics, but melting flesh peaches and nectarines undergo rapid ripening and soften quickly after harvest, leading to losses in the supply chain. Therefore, peaches are commonly picked at an early stage of ripening to withstand handling better (Ziosi et al., 2008).

The influence of flavor compounds in peaches is not strictly proportional to their absolute quantity or volatility, but also depends on the sensorial impact and their interaction with other compounds (Agozzino et al., 2007). The main groups of components that define peach aroma, according to their perception in relation to concentration, are alcohols (e.g., (*E*)-2-hexenol) and aldehydes (e.g., (*E*)-2-hexenal) as the representatives of the "green aroma" and esters (e.g., hexyl acetate) and lactones (e.g., γ-decalactone) as the representatives of the fruity, ripe aroma (Kakiuchi and Ohmiya, 1991). These volatile substances are continuously synthesized and accumulated during fruit growth and maturation. Thus, the form of qualitative and quantitative volatile constituents varies greatly during fruit development (Agozzino et al., 2007). During peach ripening, the amount of C6 compounds (aldehydes and alcohols) decreases, whereas the amount of lactones increases (Visai and Vanoli, 1997). Technologically, the most important group of peach volatiles is the latter, since β- and γ-lactones are commonly pointers of peach quality status in cool chambers (Benedetti et al., 2008).

The extraction of peach volatiles, until now, has been poorly studied. Several techniques have been employed and it is difficult to compare them. The content of aromatic

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compounds in the gas media above the peach juice headspace solid-phase microextraction (HS-SPME) (Riu-Aumatell et al., 2004) differs from the aromatic profile gained after liquid–liquid microextraction (Aubert and Milhet, 2007). Previously, the peach and the nectarine were analyzed employing several classical extraction techniques, such as vacuum distillation–extraction (Takeoka et al., 1988), ether liquid–liquid extraction (Engel et al., 1988a, 1988b; Kakiuchi and Ohmiya, 1991), continuous steam distillation–hexane extraction (Horvat and Chapman, 1990; Horvat et al., 1990), ethanol extraction (Chapman et al., 1991), dichloromethane liquid–liquid extraction (Aubert et al., 2003), or pentane liquid–liquid extraction (Jia et al., 2005). Most of these methods are very time-consuming and require many steps.

Solid-phase extraction (SPE) of peach volatiles was first tested in 1997, and later again in 2009 (Visai and Vanoli, 1997; Yang et al., 2009). Analysis of the peach vapor phase by applying a dynamic headspace was first done in 2002 (Lavilla et al., 2002). Recently, solid-phase microextraction (SPME), which is based on the partitioning of compounds between a sample and a coated fiber immersed in it, was proposed as a sample preparation technique for the analysis of volatile and semivolatile compounds in various matrices (Stashenko and Martínez, 2007). The comparison between the extraction techniques on apricot cultivars (Versari et al., 2002) showed that all the aforementioned techniques do not exclude each other, but, in fact, complement each other. SPE and LLE enable the extraction of glycosidically bound aromatic compounds, whereas SPME is more appropriate for the isolation of free aromatic compounds, especially esters, aldehydes, and alcohols (Solís-Solís et al., 2007).

Peaches are soft fruits that ripen and overmature rapidly at ambient temperatures. Low-temperature storage represents the primary technology for minimizing deterioration after harvest; however, despite their importance or fruit quality, not much information can be found in the literature about the influence of low-temperature storage on the volatile constituents of the peach (Aubert and Milhet, 2007; Raffo et al., 2008; Wang et al., 2009). Although peaches are climacteric, they will continue ripening after being picked from the tree. The taste of the peaches can decrease if they are not properly stored or if they are contaminated by microorganisms (Guohua et al., 2012).

There are many variables in HS-SPME analysis that affect the final analysis, e.g., agitation conditions, sampling time, temperature, sample volume, headspace volume, vial shape, condition and geometry of the fiber coating, sample matrix, and injector setup (Pawliszyn, 1999). Thus, it is important to monitor the formation of volatiles not only in order to fulfil the consumers' expectations, but also to

catch the optimal technological maturity of fruits.

The present study aimed to determine the impact of stage of ripening, sample storage condition, and type of fiber coating and extraction glassware on peach aroma compounds. For this purpose, HS-SPME was applied to determine the volatile constituents of fruits from 2 yellow-fleshed peach cultivars.

2. Materials and methods

2.1. Chemicals
1-Octanol, the internal standard, was purchased from Sigma Aldrich (Germany). Lactone components (γ-hexalactone, γ-heptalactone, γ-octalactone, γ-nonalactone, γ-decalactone, and δ-decalactone) and terpene components (α-terpineol, β-citronellol, geraniol, and nerol) were also obtained from Sigma Aldrich. Furthermore, 1-hexanol, hexanal, 1-heptanol, linalool, octanal, (*E*)-2-hexenyl acetate, and 2-nonenol were purchased from Alfa Aesar (Germany). All the chemicals used in this study were of analytical grade quality or at least 95% purity.

2.2. Samples
For extraction efficiency testing among different SPME fibers, a commercial peach juice was chosen as a medium with uniform content of the main constituent of peach aroma compounds. The commercial peach juice was obtained from the company Fructal (Slovenia). The juice contained, in 100 mL, 213 kJ, 0.3 g protein, 11.9 g carbohydrates, and 0.0 g fat. A 10-mL aliquot of the commercial peach juice, spiked with the internal standard 1-octanol (0.45 mg/L final concentration in juice) and peach volatiles standards, was placed in a standard 20-mL Supelco headspace vial. Afterwards, it was put in the magnetic stirrer water bath (Ecorototherm; Dinkelberg Analytics GmbH, Germany) at 40 °C for 10 min for thermal equilibration. After thermal equilibration, different fibers were tested over a 20-min extraction period.

2.3. Plant material
Two yellow-fleshed peach cultivars, Royal Glory and Redhaven, were used in our study. Cultivars were freestone types, mainly used for eating fresh or for processing. Approximately 30 undamaged peaches (approximately 4–5 kg) from 5–7 trees of each cultivar were harvested at 3 stages of ripening: pretechnological maturity, commercially ripe, and tree-ripe. The whole period from pretechnological maturity to tree-ripeness period ranged from 7 to 10 days in seasons 2009 and 2010. From each fruit, 3 longitudinal slices (from stem end to calyx end) were taken. The slices were put in a blender equipped with a tube through which a gentle stream of nitrogen was passed in order to prevent oxidation during mixing. The peach pulp was then immediately analyzed, while a part of

the pulp was immersed into liquid nitrogen and the other part was transferred into inert plastic boxes (25 mL) in the freezer at –16 °C.

2.4. Preparation of plant material for analysis

Ten grams (in a vial) or 20 g (in a flask) of peach pulp (fresh, frozen in liquid nitrogen, and frozen in a freezer at –16 °C) were spiked with internal standard 1-octanol (0.23 mg/kg final concentration in pulp) and placed into a vial or in an artisan-made glassware single-neck round-bottom flask. The concentration of internal standard in 20 g of peach pulp was around 50% lower (0.23 mg/kg) than the concentration of internal standard in 10 mL of commercial peach juice (0.45 mg/L). The levels of volatile compounds were expressed as 1-octanol equivalents assuming that all of the response factors were 1. The internal standard addition procedure followed literature data (Aubert and Milhet, 2007; Wang et al., 2009). The flask was then put in a magnetic stirrer water bath at 40 °C for 10 min for thermal equilibration, and left for 20 min for extraction.

2.5. Glassware

A standard headspace vial (Supelco, 75 mm height × 23 mm opening) with a volume of 20 mL has some important drawbacks. For instance, with the increasing amount of the sample, the headspace above the sample is critically reduced, and consequently a homogeneous stirring is questionable. With the intention to solve this problem, we designed an artisan-made single-necked round-bottom flask artisan made glassware with a final volume of 50 mL (75 mm height × 23 mm opening), a size approximate to the standard septa seal vial. Figure 1 shows the comparison between the standard headspace vial and the artisan-made glassware.

Figure 1. A standard headspace vial (Supelco, 20 mL) on the left and an artisan-made single-neck round-bottom flask (50 mL) on the right.

2.6. Analytical procedure

A SPME device (Supelco) and fibers (Sigma Aldrich) with 5 different coatings (100 μm polydimethylsiloxane (PDMS), 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS), and 85 μm polyacrylate (PA)) were used for extraction. After the extraction, the SPME device was removed into a gas chromatograph with a mass-selective detector (GC-MS-Agilent 6890 Series GC System with Agilent 5973 Mass Selective Detector) in the splitless injector at 270 °C for 10 min. Prior to daily analysis, the fiber was conditioned and activated by inserting it into the GC injector at 270 °C for 30 min. The volatiles were separated on an Rtx-20 column (60 m, 0.25 mm ID, 1 μm df, Restek, USA). The temperature program was as follows: initial temperature 50 °C (2 min), 10 °C min^{-1}, 150 °C (for 3 min), 10 °C min^{-1}, and 250 °C (for 5 min) (Aubert and Milhet, 2007; Wang et al., 2009). Total run time was 30 min. The mass spectrometer was operated in the electron ionization mode at a voltage of 70 eV, the temperature of the MS Quad was set to 150 °C, and the ion source was set to 230 °C. The compounds were identified on the basis of their retention times (compared with standards) and spectra using the searchable EI-MS spectra library (NIST02). The peak area for quantification was measured either in a TIC chromatogram or in an extracted ion chromatogram in the case of coelution with other compounds. The average relative standard deviation of the method applied was 13%.

2.7. Isolation of volatiles

For headspace sampling, the SPME fibers presented in Section 2.6 were used, and headspace sampling was done by a method carried out in the previous works of Aubert and Milhet (2007) and Wang et al. (2009). The fibers were activated according to manufacturer's instructions. HS-SPME was used for the isolation and concentration of volatiles. The process of preparing peach pulp for analysis of volatiles was previously described in Section 2.4. The flask with the peach sample was put in the magnetic stirrer water bath at 40 *°C for 10 min for thermal equilibration, and then the SPME fiber was exposed to the headspace of the sample for 20 min for extraction of the* analytes. The fibers were then introduced into the heated chromatograph injector port for desorption at 270 °C for 10 min in the splitless mode.

2.8. Data treatment

Descriptive statistics, such as arithmetic means, were used to describe the main features (comparison) of round-bottom and vial flasks. The differences among individual stages of ripening and among different methods of storage were determined by ANOVA. Tukey's multiple comparison tests were performed to determine the differences between

group means. All statistical calculations were performed with SPSS 18.0 and Statistica for Windows.

3. Results

3.1. Comparison of fibers

The components representing immature peach aromas consisted of 2 groups: a group of alcohols that were collected and are presented in Figure 2 (standard addition of alcohols in spiked commercial peach juice was as follows: 166 µg/L 3-pentanol, 175 µg g/L 1-heptanol, 189 µg g/L linalool, and 180 µg g/L 2-nonenol), and a group of aldehydes and alcohols (named as C6 components) that were collected and are shown in Figure 3 (standard addition of C6 components in spiked commercial peach juice was as follows: 186 µg/L hexanal, 220 µg/L (Z)-3-hexenal, 166 µg/L (Z)-3-hexen-1-ol, 168 µg/L (E)-2-hexenol, and 174 µg/L (E)-2-hexenal).

Figure 4 shows the lactone components that represent mature, ripened peach aroma (standard addition of lactones in spiked commercial peach juice was as follows:
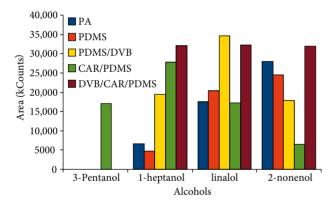

Figure 2. Extraction of alcohols on 5 microextraction fibers (PA, PDMS, PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS).

196 µg/L γ-hexalactone, 194 µg/L γ-heptalactone, 212 µg/L γ-octalactone, 190 µg/L γ-nonalactone, 194 µg/L γ-decalactone, and 222 µg/L δ-decalactone).

All 3 groups of peach aroma components were extracted on 5 commercially available fibers as previously described. The concentration of all spiked standards ranged from 166 µg/L 3-pentanol to 222 µg/L δ-decalactone. For the group of alcohols, 3 fibers (PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS) exhibited high efficiency, with an exception in the case of 3-pentanol, which was only retained with the CAR/PDMS fiber. With the PDMS/DVB fiber, a stronger retention was proportional to higher molecular masses. In addition, the DVB/CAR/PDMS fiber retained 1-heptanol, 2-nonenol, and linalool similarly. We determined that the efficient retention of the so-called C6 group was achieved by employing only 2 fibers, CAR/PDMS and DVB/CAR/PDMS (Figure 2). Additionally, compounds that were efficiently extracted with CAR/PDMS fiber were less efficiently extracted with DVB/CAR/PDMS fiber, and vice versa.

Moreover, in the case of lactones (Figure 3), the best results were achieved by employing the DVB/CAR/PDMS fiber, although PA and PDMS were sufficiently potent for lactone extraction. The extraction of δ-lactones was less efficient in comparison to γ-lactones. The extraction of γ-lactones increased with the increasing molecular mass with all 3 fibers.

The same concentration of individual volatiles was tested on 5 different fibers. The response was evaluated as the average area of an individual volatile. The statistical analysis of the 5 fibers showed no statistically significant difference between fibers in retention of analyzed volatiles. However, different responses could be seen from the graphical representation (Figures 2–4). Since the PA, PDMS, and PDMS/DVB fibers were not efficient in the
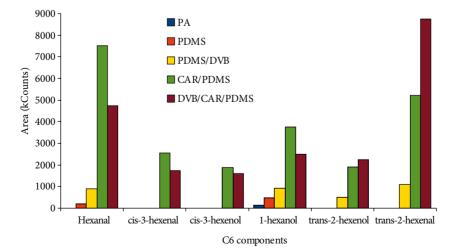

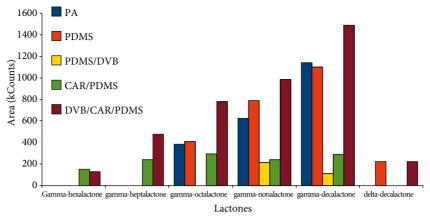**Figure 3.** Extraction of C6 components on 5 microextraction fibers.

Figure 4. Extraction of lactones on 5 microextraction fibers.

retention of several volatiles (low sensitivity), only CAR/PDMS and DVB/CAR/PDMS could be proposed for this purpose, and in particular the latter (DVB/CAR/PDMS). The DVB/CAR/PDMS fiber represents a good compromise for the extraction of aromatic compounds in peaches belonging to different series of compounds, and consequently it was selected for further work.

3.2. The impact of glassware
It was observed that when a 20-mL standard headspace vial was filled with 10 g of samples, it was half full, and a homogeneous mixing of fruit pulp was quite difficult, because the stirrer was mixing only the bottom layer. On the other hand, the 50-mL artisan-made round-bottom flask offered a bigger sample load, enabling a homogeneous stirring (Figure 1). The relative response (peak area) of identified compounds increased in the same way as the sample load.

Table 1 lists 34 volatiles identified in immature Royal Glory peaches. In only 2 cases of alcohols (2, 3) was there a higher peak area with 10 g of the sample load, whereas in all other 32 compounds, a significant increase in peak area was measured using higher loads. The increase was not general for all compounds but was rather highly specific, ranging from 1.08 for hexanal (7) to 7.73 in the case of limonene (25) when a 20-g load was compared to a 10-g load. In cases of a 30-g load, even higher areas were measured, but a rather worse sample stirring was noticed during the extraction. The sum of areas of all volatiles analyzed under higher loads (30 g and 20 g) showed a similar total area in case of 30-g and 20-g samples, whereas in the case of 10-g loads, a 50% lower response was obtained (Table 1). The obtained results indicated that the use of 20 g of the sample load in the artisan-made round-bottom flask was more appropriate.

3.3. The impact of sample storage on volatiles
The effect of ripening and maturity on the volatile profile of peaches (Redhaven) was examined on fresh fruit (FF),

fruit frozen in liquid nitrogen (LN), and fruit frozen in a freezer (FZ).

Tables 2–7 show the results of arithmetic means of concentration of volatiles, expressed as the concentration of internal standard 1-octanol (mg/kg).

Fruits were picked at 3 stages of ripening. The comparison between maturity stages and volatile profile according to sample storage (FR, LN, and FZ) was made using 2-factor ANOVA with Tukey's post hoc test.

Even with careful sample preparation, we determined a significant difference in the amount of volatiles in the same peach samples. This could be explained by the fact that it is difficult to completely avoid the presence of oxygen in shorter versus longer freezing times.

4. Discussion
The most exploited fiber in volatile analysis is based on a PDMS stationary phase (Jia et al., 2005) or its upgraded phase using divinyl-benzene, known as PDMS/DVB fiber (Wang et al., 2009). In a study of compounds characterizing the aroma of oblate-peach fruit during storage by GC-MS (Cheng et al., 2012), 3 fibers were tested and compared: PDMS at 100 µm, CAR/PDMS at 65 µm, and DVB/CAR/PDMS at 50/30 µm. The results showed that the last of these fibers was the most efficient fiber to trap the volatile compounds. In our research, the extraction of volatiles was also efficient with the DVB/CAR/PDMS 50/30 µm fiber.

LN was significantly different from the other 2 methods of storage (Tables 2, 3, and 5) in the case of the following compounds: 2 alcohols (4-penten-1-ol (35) and (*E*)-2-hexen-1-ol (3)), 3 aldehydes (hexanal (7), (*E*)-2-hexenal (8), and (*E,E*)-2,4-hexadienal (42)), and 1 ester ((*Z*)-2-hexenyl butyrate (22)). Furthermore, a higher abundance of volatiles was determined for all 3 aldehydes. In fact, aldehyde concentrations (7, 8, 22) were up to 4-fold higher than in FZ or FR. Moreover, these compounds are one of the key marker compounds that are generally used

Table 1. Comparison of round-bottom flask (20-g and 30-g load) versus vial (10-g load)[a].

Compounds		Sample ratio		
		30 g / 10 g	20 g / 10 g	30 g / 20 g
Alcohols	(1) ethanol	2.5	2.2	1.1
	(2) 1-hexanol	0.7	0.5	1.2
	(3) (E)-2-hexen-1-ol	0.8	0.6	1.2
	(4) 1-octanol	2.2	3.0	0.7
	(5) 2-octen-1-ol	2.9	2.8	1.0
	(6) maltol	30.8	12.2	2.5
Aldehydes	(7) hexanal	1.1	1.1	1.0
	(8) (E)-2-hexenal	1.2	1.2	1.0
	(9) furfural	7.1	4.3	1.6
	(10) heptanal	3.3	2.3	1.4
	(11) benzaldehyde	2.2	1.3	1.6
	(12) butanedial	4.1	3.4	1.2
	(13) nonanal	2.9	2.3	1.3
	(14) 2,5-furandicarboxaldehyde	3.4	2.3	1.5
	(15) 5-(hydroxymethyl)-2-furancarboxaldehyde	10.7	5.3	2.0
Esters	(16) hexyl acetate	2.1	2.0	1.0
	(17) 3-hexenyl acetate	0.0	0.0	1.1
	(18) (E)-2-hexenyl acetate	2.3	2.3	1.0
	(19) (E)-2-hexenyl butyrate	1.8	2.1	0.8
	(20) hexyl butyrate	0.0	0.0	0.3
	(21) ethyl octanoate	0.2	2.1	0.1
	(22) (Z)-2-hexenyl butyrate	2.8	4.4	0.6
	(23) γ-decalactone	2.3	1.3	1.8
Terpenic com.	(24) α-pinene	1.6	1.3	1.2
	(25) (-)-limonene	10.8	7.7	1.4
	(26) 3,7-dimethyl-1,6-octadien-3-ol, linalool	1.1	0.9	1.2
Others	(27) acetic acid	7.7	5.6	1.4
	(28) 2,5-furandione	0.0	0.0	1.1
	(29) 1,3-dihydroxy-2-propanone	0.0	0.0	2.1
	(30) 1,2-cyclopentanedione	0.0	0.0	1.8
	(31) 2-pentyl-furan	5.5	6.0	0.9
	(32) 5-methyl-2-furancarboxaldehyde	2.8	3.2	0.9
	(33) 2,5-dimethyl-4-hydroxy-3(2H)-furanone	5.0	3.4	1.4
	(34) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	11.2	5.0	2.2

[a]Area of the individual compound extracted from headspace above peach matrix in the round-bottom flask divided by area of the same individual compound extracted from headspace above the same peach matrix in the standard headspace vial (cultivar Royal Glory).

Table 2. Minimum–maximum range and arithmetic means of volatiles (alcohols).

Compounds		R	Sample storage					
			LN		FZ		FR	
Alcohols	(1) ethanol	n.s.	0.0136 0–0.0403	A	0.0000 n.d.	A	0.0000 n.d.	A
	(35) 4-penten-1-ol	n.s.	0.0141 0.0059–0.0297	A	0.0000 n.d.	B	0.0000 n.d.	B
	(36) (Z)-2-penten-1-ol	n.s.	0.0000 n.d.	A	0.0024 0.0021–0.0027	B	0.0000 n.d.	A
	(37) (Z)-3-hexen-1-ol	***	0.0043 0.0027–0.0064	A	0,0061 0.0037–0.0086	B	0.0086 0.0062–0.0108	C
	(2) 1-hexanol	*	0.4646 0.3785–0.5285	A	0,5368 0.4533–0.6471	A	0.5499 0.4037–0.6258	A
	(3) (E)-2-hexen-1-ol	***	0.3151 0.2372–0.4238	B	0.6710 0.4106–0.9090	A	0.6113 0.4264–0.7042	A
	(38) 2,4-hexadien-1-ol	n.s.	0.0000 n.d.	A	0.0018 0.0000–0.0057	A	0.0000 n.d.	A
	(39) (Z)-2-hexen-1-ol	n.s.	0.0000 n.d.	A	0.0011 0.000–0.0036	A	0.0065 0.0053–0.0084	B
	(40) 1-nonanol	*	0.0012 0.000–0.0038	A	0.0020 0.0019–0.0021	A	0.0050 0.0029–0.0081	B

Legend: Ripening (R), liquid nitrogen (LN), freezer (FZ), fresh (FR).
In Tables 2–7, means followed by the same letter in the same row are not significantly different at P > 0.05 according to Tukey's test; n.s.: not significant at P > 0.05, *: 0.01 < P < 0.05, **: 0.001 < P < 0.01,*** : P < 0.001 (Redhaven); n.d.: not detected.

Table 3. Minimum–maximum range and arithmetic means of volatiles (aldehydes).

Compounds		R	Sample storage					
			LN		FZ		FR	
Aldehydes	(41) pentanal	n.s.	0.0000 n.d.	A	0.0075 0.0050–0.0090	B	0.0000 n.d.	A
	(7) hexanal	n.s.	3.2913 2.8927–3.8605	B	0.6303 0.4198–0.8748	A	0.2843 0.1082–0.4024	A
	(8) (E)-2-hexenal	**	3.0789 2.5159–3.4567	B	0.5005 0.2980–0.7549	A	0.7346 0.3181–0.9464	A
	(10) heptanal	n.s.	0.0071 0.0057–0.0105	A	0.0157 0.0105–0.0199	B	0.0000 n.d.	C
	(42) (E,E)-2,4-hexadienal	**	0.3995 0.2950–0.4386	B	0.0529 0.0287–0.0809	A	0.0845 0.0300–0.1161	A
	(43) 2-(E)-nonenal	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0055 0.0043–0.0063	B
	(44) 2-heptenal	n.s.	0.0000 n.d.	A	0.0087 0.0000–0.0126	B	0.0000 n.d.	A
	(11) benzaldehyde	n.s.	0.2978 0.2559–0.3818	A	0.5426 0.3317–0.8870	B	0.0896 0.0659–0.1017	A

Table 4. Minimum–maximum range and arithmetic means of volatiles (aldehydes and acids).

Compounds		R	Sample storage					
			LN		FZ		FR	
Aldehydes	(45) (E,E)-2,4-heptadienal	n.s.	0.0000 n.d.	A	0.0047 0.0000–0.0088	B	0.0000 n.d.	A
	(46) (E)-2-octenal	n.s.	0.0000 n.d.	A	0.0170 0.0088–0.0280	B	0.0000 n.d.	A
	(13) nonanal	*	0.0222 0.1988–0.0241	A	0,0185 0.0118–0.0298	A	0.0076 0.0045–0.0109	B
	(47) benzeneacetaldehyde	n.s.	0.0000 n.d.	A	0.0005 0.0000–0.0028	A	0.0000 n.d.	A
	(48) decanal	n.s.	0.0056 0.0034–0.0089	A	0.0034 0.0020–0.0048	A	0.0051 0.0030–0.0090	A
	(49) 3-ethylbenzaldehyde	***	0.0022 0.0011–0.0031	A	0.0008 0.0000–0.0027	B	0.0016 0.0000–0.0030	AB
	(50) 2,6-dimethylbenzaldehyde	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0022 0.0000–0.0037	B
Acids	(51) acetic acid	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0327 0.0243–0.0603	B
	(52) hexanoic acid	n.s.	0.0210 0.0136–0.0239	B	0.0030 0.0000–0.0053	A	0.0000 n.d.	A

Table 5. Minimum–maximum ranges and arithmetic means of volatiles (esters).

Compounds		R	Sample storage					
			LN		FZ		FR	
Esters	(53) methyl acetate	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0007 0.000–0.0021	A
	(54) ethyl butyrate	n.s.	0.0000 n.d.	A	0.0015 0.0000–0.0054	A	0.0000 n.d.	A
	(55) ethyl-2-methylbutyrate	n.s.	0.0000 n.d.	A	0.0005 0.0000–0.0022	A	0.0000 n.d.	A
	(56) pentyl acetate	*	0.0012 0.0000–0.0037	A	0.0000 n.d.	A	0.0024 0.000–0.0072	A
	(57) ethyl hexanoate	n.s.	0.0000 n.d.	A	0.0041 0.000–0.0185	A	0.0000 n.d.	A
	(16) hexyl acetate	**	1.4046 0.4719–2.6795	A	2.6349 1.2732–3.4401	B	4.8040 4.6272–5.0611	C
	(17) 3-hexenyl acetate	n.s.	0.3976 0.3133–0.4850	A	0.2521 0.1691–0.3171	A	1.0791 0.9337–1.2897	B
	(18) (E)-2-hexenyl acetate	***	1.0760 0.3114–2.0266	A	1.8079 0.6018–2.4696	B	3.5005 3.2697–3.7113	C
	(58) (Z)-3-methylpenta-1,3-diene-5-yl acetate	n.s.	0.0000 n.d.	A	0.0032 0.0000– 0.0048	B	0.0109 0.0095–0.0120	C

Table 5. (Continued).

Compounds		R	Sample storage					
			LN		FZ		FR	
Esters	(59) (*E*)-2-hepten-1-yl acetate	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0021 0.0000–0.0034	B
	(60) methyl octanoate	n.s.	0.0050 0.0000–0.0160	A	0.0000 n.d.	A	0.0000 n.d.	A
	(20) hexyl butyrate	n.s.	0.0000 n.d.	A	0.0063 0.0057–0.0068	B	0.0024 0.0012–0.0039	C
	(21) ethyl octanoate	**	0.0024 0.0000–0.0078	A	0.0028 0.0014–0.0048	A	0.0070 0.0013–0.0170	A
	(22) (*Z*)-2-hexenyl butyrate	***	0.0061 0.0015–0.0126	B	0.0189 0.0030–0.0274	A	0.0198 0.0077-0.0326	A
	(61) octyl acetate	n.s.	0.0144 0.0020–0.0320	A	0.0127 0.0000–0.0394	A	0.0869 0.0433–0.1575	B
	(62) (*E*) -hex-2-enylpentanoate	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0033 0.0000–0.0058	B
	(63) (*E*)-hex-2-enylhexanoate	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0025 0.0000–0.0051	A
	(64) hexyl hexanoate	n.s.	0.0000 n.d.	A	0.0005 0.0000–0.0018	A	0.0000 n.d.	A

Table 6. Minimum–maximum ranges and arithmetic means of volatiles (lactones).

Compounds		R	Sample storage					
			LN		FZ		FR	
Lactones	(65) 5-ethyloxolan-2-one, γ-hexalactone	***	0.0105 0.0077–0.0161	A	0.0114 0.0084–0.0150	A	0.0118 0.0074–0.01870	A
	(66) 5-butyloxolan-2-one, γ-octalactone	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0007 0.0000–0.0021	A
	(23) 5-hexyl dihydro-2(3H)-furanone, γ-decalactone	***	0.0053 0.0021–0.0110	A	0.0057 0.0000–0.0151	A	0.0079 0.0017–0.0184	A
	(67) 6-pentyloxan-2-one, δ-decalactone	*	0.0000 n.d.	A	0.0012 0.0000–0.0043	A	0.0013 0.0000–0.0038	A

as common descriptors of peach maturity (Horvat et al., 1990). Liquid nitrogen had, on the other hand, a positive impact on 4-penten-1-ol (35) (the effect of ripening was not confirmed), since this compound was found only in LN. On the other hand, LN had a negative impact in the cases of (E)-2-hexen-1-ol (3) and (Z)-2-hexenyl butyrate (22). The last 2 compounds (3, 22) could be markers of ripeness, since both expressed highly significant differences (P < 0.001). In fact, their concentrations decreased with maturation. Storage in the freezer had a significant impact on the formation of some volatiles. They were not identified in FR or LN samples: (Z)-2-penten-ol (36), pentanal (41), 2-heptenal (44), (E,E)-2,4-heptadienal (45), and (E)-2-octenal (46) (Tables 2–4).

Concentrations of benzaldehyde (11) (with a pleasant almond-like odor), on the other hand, were significantly higher in FZ compared to FF and LN (Table 3). In our research, a slight increase of benzaldehyde concentration was detected with ripeness, but the statistical difference was not confirmed. The same pattern was shown when peach samples were stored at −40 °C (Wang et al., 2010). Besides benzaldehyde (11) and limonene (25) (Table 7), cyclic terpene (strong smell of oranges) was found in a significantly higher concentration in FZ than in FR and LN. In contrast, the concentration of p-cymene (69) (herbaceous odor) was significantly higher in FR (Table 7). All the other terpenic compounds were not affected by the applied method of storage and/or during the ripening process, except linalool (26) (Table 7). Linalool (26) and limonene (25) are known as the most abundant terpenic

compounds found in many fruits (Wang et al., 2009) and their concentrations significantly increased with ripeness. A statistical difference in the concentration of volatiles in FR was determined for (Z)-2-hexen-1-ol (39) (green leafy odor) and 1-nonanol (40) (citrus odor similar to citronella oil). In the group of aldehydes, 2-(E)-nonenal (43) (strong tallow odor) and 2,6-dimethylbenzaldehyde (50) were identified only in FR, whereas nonanal (13) (fruity or floral odor) expressed a statistically lower value in FR compared to samples FZ and LN (Tables 2–4).

Acetic acid (51) and 4 esters (3-hexenyl acetate (17) (fruity-green, green banana-like), (E)-2-hepten-1-yl acetate (59) (sweet fruity fatty), octyl acetate (61) (orange-like), and (E)-hex-2-enylpentanoate (62) (fruity, apple, pear)) (Brechbill, 2007) were found in statistically higher concentrations in FR, whereas (59) and (62) were found only in FR samples (Tables 4 and 5). As seen in Tables 2, 3, and 5, the effect of storage had the most noticeable effects on 6 compounds: (Z)-3-hexen-1-ol (37), heptanal (10), and 4 esters (hexyl acetate (16), (E)-2-hexenyl acetate (18), (Z)-3-methylpenta-1,3-diene-5-yl acetate (58), and hexyl butyrate (20)). Compounds 16 and 18 are volatiles that commonly define the typical peach odor and aroma (Sevenants and Jennings, 1966; Spencer et al., 1978; Rizzolo et al., 1993; Sumitani et al., 1994).

The peach aroma extracted by HS-SPME was characterized by 3 groups of compounds: groups of alcohols and C6 components, both representing immature peach aroma, and lactones, the representatives of ripened peach aroma. Based on our findings, this is the first study

Table 7. Minimum–maximum ranges and arithmetic means of volatiles (terpenic compounds).

Compounds	R	Sample storage					
		LN		FZ		FR	
(68) 7-methyl-3-methylideneocta-1,6-diene	n.s.	0.0000 n.d.	A	0.0009 0.0000–0.0034	A	0.0034 0.0000–0.0101	A
(25) (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene, limonene	*	0.0020 0.0000–0.0123	A	0.0310 0.0241–0.0443	B	0.0022 0.0000–0.0128	A
(69) 1-methyl-4-propan-2-ylbenzene, p-cymene	***	0.0033 0.0000–0.0113	A	0.0032 0.0000–0.0100	A	0.0133 0.0111–0.0149	B
(70) 2,2,4-trimethyl-3-oxabicyclo[2.2.2]octane, eucalyptol	n.s.	0.0000 n.d.	A	0.0028 0.0000–0.0095	A	0.0000 n.d.	A
(71) 2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol, carveol	n.s.	0.0000 n.d.	A	0.0051 0.0000–0.0160	A	0.0067 0.0056–0.0072	A
(26) 3,7-dimethyl-1,6-octadien-3-ol, linalool	***	0.0471 0.0301–0.0694	A	0.0478 0.0339–0.0677	A	0.0685 0,0353–0.1225	A
(72) 2-methyl-6-methylideneoct-7-en-2-ol, myrcenol	n.s.	0.0000 n.d.	A	0.0000 n.d.	A	0.0020 0.0000–0.0060	A

Terpenic compounds (row group labels along left margin)

to integrate the influence of different parameters on peach aroma characterization. Comparing the 5 fibers indicated that the DVB/CAR/PDMS fiber allowed the best extraction of multiple series of aromatic compounds present in peaches. In order to extract as many compounds as possible, a new style of artisan-made glassware was proposed, offering on average a 3-fold higher extraction of aromatic compounds. Significant differences were found when the same peach pulp was analyzed fresh or frozen, either in liquid nitrogen or in the freezer at –16 °C. In FR, higher amounts of (Z)-3-hexen-1-ol, (Z)-2-hexenol, 1-nonanol, 2-(E)-nonenal, 2,6-dimethyl-benzaldehyde, acetic acid, hexyl acetate, 3-hexenyl acetate, (E)-2-hexenyl acetate, (Z)-3-methylpenta-1,3-diene-5-yl acetate, (E)-2-heptenyl acetate, octyl acetate, (E)-2-hexenylpentanoate, and p-cymene were found. Furthermore, LN storage had a positive impact on 4-penten-1-ol, hexanal, (E)-2-hexenal,

(E,E)-2,4-hexadienal, and hexanoic acid. LN had a negative impact on (E)-2-hexen-1-ol and (Z)-2-hexenyl butyrate. FZ storage had a major positive impact on aldehydes, resulting in higher amounts of pentanal, 2-heptenal, benzaldehyde, (E,E)-2,4-heptadienal, (E)-2-octenal, and hexyl butyrate, and also limonene, an important representative of the terpenic compounds. This study reveals the importance of sample storage on the overall aromatic profile of peaches. The same pattern could be expected in other fruit and food when the impact of a naturally present oxidizing atmosphere cannot be completely avoided.

Acknowledgments

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