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Solid Phase Microextraction (SPME) of Orange Juice Flavor: Odor Representativeness by Direct Gas Chromatography Olfactometry (D-GC-O)

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The sensorial quality of solid phase microextraction (SPME) flavor extracts from orange juice was measured by direct gas chromatogrphy−0lfactometry (D-GC-O), a novel instrumental tool for evaluating odors from headspace extracts. In general, odor impressions emerging from SPME extracts poorly resembled that of the original orange juice. In an attempt to improve the sensorial quality of extracts, sample equilibration and exposure times were varied on Carboxen/polydimethylsiloxane (CAR/PDMS) and divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibers. Best sensorial results were obtained with the DVB/CAR/PDMS fiber exposed for the shortest time; a trained panel of eight assessors judged its odor as the most representative of the reference orange juice. The analysis of odor active compounds by classical GC-O accounted for odor characteristics revealed by D-GC-O. A principal component analysis (PCA) was applied on SPME and headspace extracts using flavor recoveries as variables. Interestingly, PCA discriminated samples according to their odor representations described by D-GC-O analysis. This paper provides the first comprehensive methodology to "smell" SPME extracts and "evaluate" their sensorial quality. This method will enable future investigations to further improve SPME performance.

KEYWORDS: Citrus; freshly squeezed orange juice; SPME; headspace; olfactometry; direct GC−olfactometry (D-GC-O); similarity; sensorial; principal component analysis (PCA)

INTRODUCTION

Orange juice is the most appreciated juice beverage worldwide. Its high quality, which is key for the consumer demand, is greatly dependent on the characteristic "fresh orange juice" flavor. Orange juice flavor has been extensively investigated and reviewed (*1*). The qualitative and quantitative makeup of aromatic constituents in freshly squeezed orange juice have been reported in many studies (*2*, *3*). Maccarone et al. (*3*) investigated aroma compounds from 72 orange juices derived from the most widespread blond and blood cultivars. Hinterholzer and Schieberle (*4*) identified the most odor-active volatiles in fresh Valencia Late juice by odor dilution techniques.

Solid phase microextraction (SPME) is extensively applied to the study of orange juice aroma. This technique offers the advantages of being rapid, solventless, and relatively inexpensive. Kataoka et al. (*5*) recently reviewed its applications to food science. However, SPME analysis shows sensitivity to experimental conditions such as heating temperature and time, sample volume, stirring, and concentration. As such, SPME requires careful optimization of these experimental parameters, which strictly depend on the type of food sample and matrix characteristics (*6*).

Investigations on orange juice flavor analysis using SPME methods have mainly dealt with selection of fibers or optimization of both extraction and desorption parameters. Steffen and Pawliszyn (*7*) developed a SPME method based on a polyacrylate (PA)-coated fiber. Their results pointed out that such a fiber extracts more of the target compounds than the classical polydimethylsiloxane (PDMS)-coated fiber.

Jia et al. (*8*) optimized SPME sampling and gas chromatography (GC) conditions for qualitative and quantitative analysis of volatile compounds in the headspace of orange juice. Those authors used a PDMS-coated fiber and studied the effects of temperature, time, and sample agitation on the amount of aromas in the headspace at equilibrium. Miller and Stuart (*9*) detected a drastic improvement in the extraction abilities of SPME fibers over the traditional static headspace method in fresh and aged orange juice—the PDMS/divinylbenzene (DVB) fiber was the most able to recover flavor volatiles of different chemical classes.

Thus, many efforts are being made to enhance the performance of SPME. Notwithstanding, the odor quality of the SPME extract—a crucial aspect for flavor analysis—remains virtually uncovered by actual investigations. Basically, global odor obtained from flavor extraction techniques including SPME have

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to come close to the original sample odor, yet the methods classically used to assess odor representativeness cannot be applied to SPME extracts (*10*).

To solve this methodological problem, we developed a simple and rapid technique for evaluating the sensory quality of orange juice extracts using direct gas chromatography−olfactometry (D-GC-O). This novel technique permits one to assess the global sensory quality of the solventless extracts (*11*). Practically, solventless samples are injected into a short capillary column, the phase of which is deactivated and which is directly connected to the GC-sniffing port. This method avoids chromatographic separation of flavor volatiles. Sniffers thus perceive the sample as a global odor. Using this method, we previously tested the representativeness of orange juice extracts obtained by static syringe-sampled headspace (SSHS), vacuum distillation, and three SPME fibers commonly used in orange aroma analysis (Carboxen/polydimethylsiloxane, CAR/PDMS; polydimethylsiloxane/divinylbenzene, PDMS/DVB; divinylbenzene/Carboxen/polydimethylsiloxane, DVB/CAR/PDMS). We found that static headspace and aqueous distillate produced odor closely resembling that of the original juice. On the contrary, SPME produced odors with lower similarity to the reference. The three fibers, however, showed differential odor representativeness, DVB/CAR/PDMS being the best rated (*12*). Why persevere in utilizing SPME despite its low sensorial quality? It is beyond controversy that SPME is a rapid and very sensitive extraction method compared to other methods such as static headspace and vacuum distillation: the former method leads to low-concentration aroma extracts, whereas the latter is time-consuming and not reproducible enough. Therefore, if optimization methods succeed in improving SPME sensorial quality, the SPME method would be among the most attractive extraction procedures for flavor analysis.

The aim of this work was to optimize SPME conditions for orange juice flavor analysis and to evaluate the sensorial quality of SPME extracts using a novel, instrumental tool, that is, D-GC-O, which is dedicated to assessing global odor from solventless extracts. On the basis of our previous results, we restricted the analysis to those fiber types showing the best sensorial results, namely, CAR/PDMS and DVB/CAR/PDMS. In that preceding study, we also showed that best odor representativeness with respect to the reference juice was perceived from static headspace extract (*12*). As such, our optimization strategy consisted of varying SPME equilibrium and fiber exposure times in order to approach the chromatographic profile of the orange juice static headspace.

Remarkably, the odor active compounds recovered in SPME extracts by means of classical GC-O accounted well for the odor impression emerging from D-GC-O. Thus, this paper provides the first comprehensive methodology to "smell" SPME extracts and "evaluate" their sensorial quality.

MATERIALS AND METHODS

Orange Juice. Fresh orange juice (Naveline, Spain) was obtained by a "Santos" extractor in the CIRAD laboratories (Montpelier, France) and stored at −30 °C under nitrogen atmosphere in glass bottles. All analyses took place 3 months after sample preparation. Just before analysis, juice was rapidly thawed (10 min at 25 °C) to minimize eventual degradations. This orange juice was submitted to different flavor extraction methods.

SSHS. Five milliliter aliquots of fresh juice were poured into 20 mL vials sealed with PTFE-lined caps (Supelco, Bellefonte, PA). Samples were kept for 1 h under agitation in a 40 °C water bath.

Two milliliters of headspace was injected in the GC equipped with a TCT injector (Chrompack) in order to cryofocus volatile compounds in the cold capillary trap. The sampled headspace was injected onto a glass tube heated at 240 °C to prevent component condensation. Volatiles were thus stripped by the carrier gas (desorption flow = 10 mL min^{-1}) and cryofocused on capillary silica tubing kept at −130 °C with liquid nitrogen. Once the sample was collected, the cold trap was flash heated to 250 °C to inject the sample onto the GC column.

Table 1. SPME Experimental Conditions for Aroma Extraction from Orange Juice Headspace (All Conditions Are Performed at 40 °C under Agitation)

sample	SPME fiber type	equilibrium time (min)	fiber exposure time (min)
G20	Stableflex 50/30 mm DVB/CAR/PDMS	5	15
G6	Stableflex 50/30 mm DVB/CAR/PDMS	5	1
G31	Stableflex 50/30 mm DVB/CAR/PDMS	30	1
N20	75 mm CAR/PDMS	5	15
N6	75 mm CAR/PDMS	5	1

SPME. Volatiles from orange juice headspace were extracted using two SPME fibers: 75 μm CAR/PDMS and Stableflex 50/30 μm DVB/CAR/PDMS SPME (Supelco). The fibers were conditioned in a splitless/split GC injector port (2 h at 300 °C for CAR/PDMS; 4 h at 270 °C for DVB/CAR/PDMS SPME). Before each extraction they were held at 260 °C for 5 min and then at room temperature for 2 min. SPME extraction was performed on 1 mL of stirred juice (40 °C) contained in a 4 mL vial sealed with a PTFE-lined screw cap. The five sampling conditions applied to the same orange juice are reported in **Table 1**. They were chosen after preliminary GC-FID analyses giving chromatographic profiles similar to that of a SSHS on the same orange juice.

D-GC-O. An HP 5890 equipped with a sniffing port and a 0.75 mm injector liner was supplied with a short capillary of untreated silica (80 cm × 0.32 mm i.d.). The flow rate of the carrier gas (H_2) was 25 mL min^{-1}, and the oven temperature was kept at 50 °C. The five SPME extracts were introduced in successive sequences into the GC port (splitless mode, T = 240 °C). Because no chromatographic separation was carried out by the short silica capillary, aroma compounds arrived simultaneously at the sniffing port. Here, for each SPME extract, a trained panel of eight assessors perceived and evaluated the resulting global odor. Fibers were kept in the GC inlet until the end of the sensorial stimulus.

Sensory analysis sessions were performed only after a suitable training: panelists were first familiarized with five commercial orange juices and asked to agree on a common list of 15 descriptors. After that they were familiarized with the D-GC-O device.

A similarity test was performed in triplicate on the five SPME odors issued from the same fresh orange juice. Extracts were presented in Latin square. Sniffers were asked to smell the reference juice (5 mL) contained in a plastic cup sealed with a pierced cap (T = 20 °C). They had to memorize the odor and then describe it using the descriptors list. Then they evaluated with the D-GC-O device the different extracts, rating their similarity to the reference using a 10 cm scale ranging from 0 (close to the reference) to 10 (far from the reference). They were also asked to give descriptors. SPME extracts were injected every 4 min. Between two sample evaluations panelists had to smell the reference again.

GC-O. The odor active compounds of G20 and G31 SPME extracts were analyzed by high-resolution GC-O on a 5890 HP equipped with a flame ionization detector (FID, 250 °C) and a sniffing port. After sampling, the SPME fibers were placed into the injection port of the GC equipped with a 0.75 mm i.d. liner (Supelco) for 5 min at 240 °C; for the first 3 min the purge was off and then for the remaining 2 min the purge was on to further clean the fiber. Operating conditions were as follows: DB-Wax column (J&W Science, i.d. = 0.32 mm, 30 m, film thickness = 0.5 μm) held at 35 °C for 5 min, then increased at 5 °C min^{-1} to 240 °C, and then held for 5 min. Hydrogen was used as carrier gas with a linear velocity of 37 cm s^{-1}. The GC effluent was

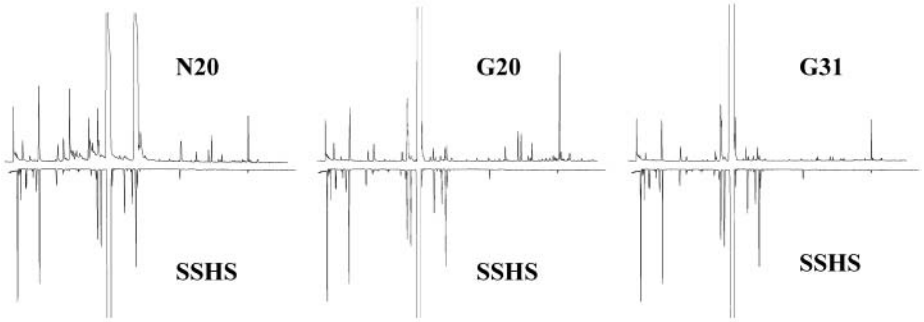

Figure 1. Chromatographic profiles of three SPME orange juice extracts (N20, G20, and G31) with respect to the syringe-sampled static headspace (SSHS) profile.

split 1:1 between the FID and the sniffing port (250 °C). A panel of eight assessors (same as for D-GC-O experiments) evaluated the effluents enriched with purified, humidified air (100 mL min^{-1}). For each odor stimulus, panelists recorded the detection time and gave an odor description. GC-O frequency analysis was performed following the methodology described by Charles et al. (*13*).

Linear retention indices (RI) of the compounds were calculated using a series of *n*-alkanes (C10−C30) injected in the same chromatographic conditions.

Identification and Quantification of Volatile Compounds by GC-MS. Volatile compounds were identified by an HP 6890 GC equipped with an MSD 5973 mass detector (Agilent Technologies, Palo Alto, CA). Chromatographic conditions were the same as those applied for GC-FID-O, but helium was used as carrier gas in constant flow mode with a linear velocity of 40 cm s^{-1}. The source was kept at 200 °C, and the transfer line and the detector were kept at 250 °C. Mass spectra in the electron impact (EI) mode were generated at 70 eV; they were collected from *m*/*z* 29 to 450, at 3.45 scans s^{-1}. Mass spectral matches were made by comparison of NIST (NIST, Gaithersburg, MD) and INRAMASS mass spectra libraries. Linear Kovats indices of authentic compounds were used to confirm identification.

For quantification, relative recovery of volatile compounds was calculated. Among the 66 volatile compounds identified, 19 key compounds were selected on the basis of their abundance in the headspace, their impact on orange flavor, and their chemical class. Relative recovery of the *x*th marker compound was obtained by the formula ($TIC_x/TIC_{sum} \times 100$) considering the sum of the markers' TIC as 100% of the overall odor compound response.

Statistical Analysis. Statistical analyses were done with the Statistic Analytical System (SAS). A three-way ANOVA was performed on similarity rates considering the sampling method, the judge, and the repetition effects. The Newman−Keuls test ($p < 0.1$) and the Student's *t* test for pairs of variables were also done. Principal component analysis (PCA) was performed with the relative recovery of 19 aroma compounds and the 6 extracts (G6, G20, G31, N6, N20, and SSHS) using Statbox software (Grimmer, France) in order to determine relationships among variables and different extraction methods.

RESULTS AND DISCUSSION

Representativeness of SPME Extracts. Five SPME extraction procedures were applied to the same orange juice using two fiber types and three sampling conditions (**Table 1**). G20 and N20 procedures were already applied by Rega et al. (*12*); the G31 SPME procedure was chosen because it gave a GC profile most resembling that obtained by SSHS (**Figure 1**).

The G6 method, with very short equilibrium and fiber exposure times, showed minor differences in its chromatographic profile relative to the G31 (data not shown). Representativeness of the resulting five SPME extracts was tested by a similitude test and an odor profile obtained by means of D-GC-O, a rapid tool (three sessions of 20 min for each assessor) that has the advantage of not tiring the assessors.

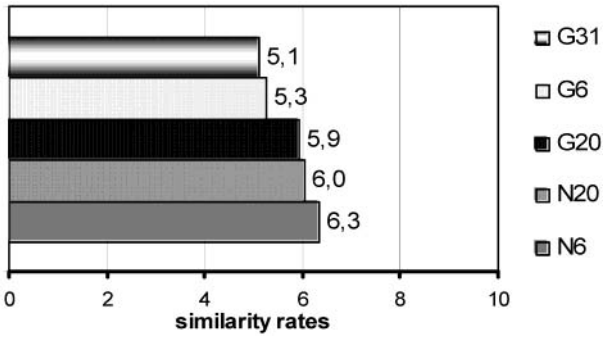

Figure 2. Similarity rates obtained for SPME samples by the sensorial panel of 13 assessors; the scale ranges from 0 (close to the reference) to 10 (far from the reference).

Table 2. Three-Way ANOVA of the Similarity Rates Obtained by D-GC-O for SPME Extracts G31, G20, G6, N6, and N20

factor	degrees of freedom	ANOVA sum square	mean square	F value	*P* value
sampling method	4	25.61	6.40	2.10	0.09
subject	7	217.34	31.00	10.17	<0.0001
repetition	2	4.25	2.12	0.70	0.50
method × subject interaction	28	106.42	3.80	1.25	0.22

The three-way ANOVA on similarity rates showed a significant "sampling method" effect (p value = 0.09) and also a strong "subject" effect (p value 0 < 0.001). Conversely, the "repetition" factor did not significantly influence the variance (**Table 2**).

Figure 2 shows the results of the similarity scaling obtained for the five SPME global odors with respect to the reference juice. Rates range from 5.1 to 6.3, the same range found previously. This shows that in general the odor emerging from SPME extracts poorly resembled that of the original orange juice. Among samples, G31 generated the most representative odor (score of 5.1). The G31 extract is obtained by a SPME procedure applying the longest equilibrium time (30 min) and the shortest fiber exposure time (1 min). It is worth noting that in GC-FID analysis, the G31 chromatographic profile most resembled the SSHS profile. This is coherent with previous observations (*6*). No significant sensorial differences were found between G31 and G6 extracts that were obtained using the same short fiber exposure time. Conversely, a significant ($\alpha < 0.09$) decrease in similarity rates was found when the fiber exposure time of the DVB/CAR/PDMS SPME fiber was decreased from 15 to 1 min, namely, passing from G20 to G31, due to a better similarity with respect to the reference. As a general trend, the Stableflex 50/30 μm DVB/CAR/PDMS SPME fiber gives global odors more resembling that of the reference juice than 75 μm

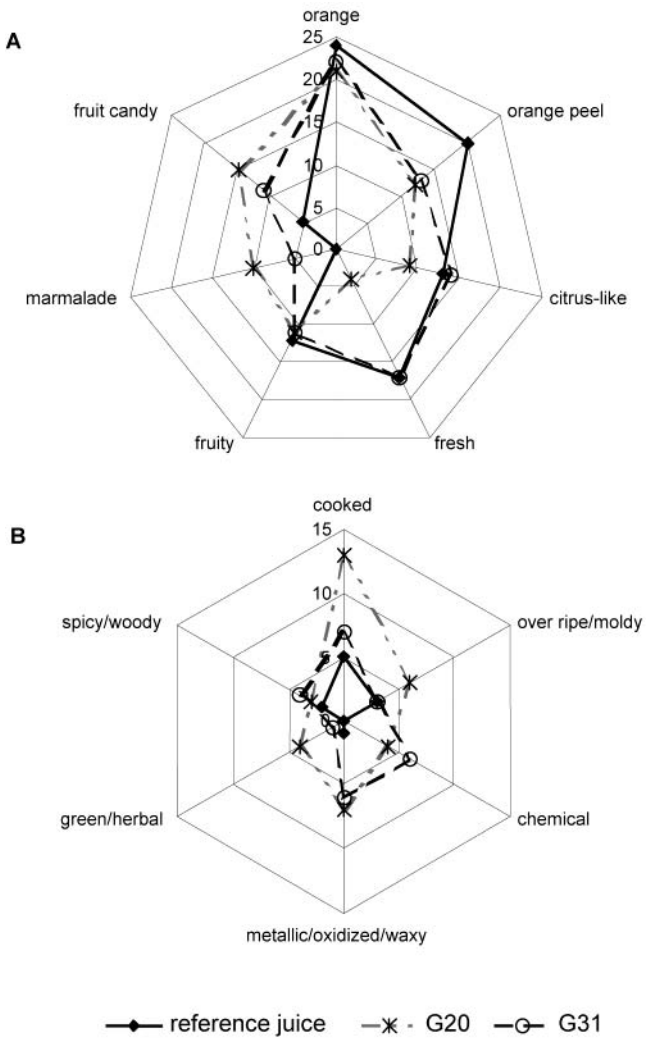

Figure 3. Spider-web representation of odor descriptors for G20 and G31 SPME extracts: (**A**) pleasant descriptors; (**3**) unpleasant descriptors.

CAR/PDMS (Student's *t* test, $\alpha < 0.03$). In fact, extracts N20 and N6 are obtained using a 75 μm CAR/PDMS fiber and their global odor is perceived by assessors as the farthest from the reference juice. This allowed us to make two main observations: the sensorial quality of SPME extracts of orange juice depends not only on the type of SPME absorbent but also on the time during which the fiber is in contact with the headspace of the orange juice. An increase in representativeness of SPME extracts was thus obtained using a DVB/CAR/PDMS SPME fiber and simply decreasing its exposure time to the headspace. Nevertheless, the improvement of SPME sensorial quality is limited, and further investigations are needed to reach better results. The best representativeness obtained for the G31 extract could also be explained by D-GC-O descriptive analysis. **Figure 3** shows pleasant and unpleasant attributes (a and b, respectively) characterizing the G20 and G31 global odors. Spider-web graphics also represent reference orange juice odor profile (black bold line).

Passing from the G20 to the G31 extract, "fresh" and "citrus-like" attributes became very similar to that of the reference juice. Moreover, the intensity of unpleasant attributes, such as "herbal", "cooked", and "over ripe−moldy", decreases. However, it should be noted that some differences from the reference juice odor profile persist. SPME extracts, in fact, are perceived as having a less "orange-peel" character than the genuine juice; furthermore, unpleasant notes such as "chemical" and "metallic-oxidized−waxy" persist. This could explain why similarity scores are only slightly improved.

Identification and Quantification of Odor Compounds Responsible for the Global Perception. Classical GC-O was applied to G31 and G20 extracts to find discriminant odorant zones in their olfactograms and then identify the aroma compounds responsible for these odors. **Table 3** shows the odor detected by panelists in G20 and G31 SPME extracts and the corresponding identified molecular components. Thirty-two and 25 molecules were detected in these extracts as contributing to the overall orange juice aroma. Among them ethyl butanoate, limonene, and β-myrcene were the best perceived aroma compounds (they were detected by all eight panelists) in both SPME extracts.

Ethyl butanoate is responsible for a fruity-orange note, and it is known to be one of the most potent odorants as judged by its high FD factor in aroma extract dilution analysis (AEDA) (*14*). Limonene is the most abundant aroma compound in orange juice (on the order of milligrams per kilograms); thus, even if its odor thresholds in air and in water (orthonasal) are very high [424 ng L^{-1} and 200 μg kg^{-1} (*3*, *15*)], it is potently detected. Regarding the differences in the perception of key aroma compounds between G20 and G31, we were able to identify 14 discriminant odorant zones.

The boldface zones in **Table 3** correspond to descriptors that present significant differences in frequency of detection between G20 and G 31 ($|G20 - G31| \geq 3$). For example, δ-carene gives a pleasant floral note in G20, but it is not detected in G31. Similarly, ethyl hexanoate (fruity-orange note) is mostly detected in G20. On the other hand, *n*-octyl acetate (RI 1481), responsible for an unpleasant dusty note in G20, is not perceived in G31 at all. This compound was recently identified in yellow passion fruit juice by Jordàn et al. (*15*) as responsible for a woody, tar, burnt plastic odor by GC-O. At a retention index (RI) of 1807 we found, in the G20 sample, an unidentified compound responsible for a strong unpleasant note. This note significantly decreases in G30 (frequency of detection decreases from 4 to 1). **Table 3** shows some general trends: oxygenated aroma compounds are better perceived in sample G20 than in G31. This is the case for alcohols such as linalool and 1-octanol, which give, respectively, very potent floral and herbal notes to G20. These data are confirmed by GC quantitative analysis (**Figure 4**), showing that these compounds are present in G20 in higher amounts than in G31.

Looking at the balance between pleasant and unpleasant descriptors (**Table 3**), we can deduce that G31 presents a lower number of odorant zones but it has been preferred by panelists mainly due to a lower abundance of unpleasant descriptors (23% instead of 44% for G20).

The relative recovery of 19 aroma compounds from SPME and SSHS extracts is reported in **Figure 4**. These compounds were chosen with regard to their impact on orange flavor, their chemical class, and their abundance in the headspace. Considering *n*-octyl acetate, this compound is totally absent in the SSHS. SPME greatly increases the concentration of this aroma compound, leading to an unpleasant note in the extract. Nevertheless, relative recovery of *n*-octyl acetate by G31 is significantly lower than in G20. This could explain the higher detection of the unpleasant note in the G20 extract by both classical GC-O and D-CG-O (higher over-ripe/moldy global note).

Linalool and 1-octanol are responsible for fruity/floral and herbal notes, respectively. They are known as important

Table 3. Orange Juice Odor Active Components Identified in SPME G20 and G31 Extracts by GC–Olfactometry (Eight Panelists) (Boldface Zones Correspond to Discriminant Odorant Zones)

						G20 descriptors		G31 descriptors	
RI	component[a]	descriptors (intensity)	frequency of detection G20	frequency pf detection G31	diff in frequency of detection (G20 – G31)	pleasant	unpleasant	pleasant	unpleasant
677[b]	acetaldehyde	fruity	2	3	–1	2	0	3	0
814[b]	2-propanone	fruity	2	0	2	2	0	0	0
893[b]	ethyl acetate	orange	2	0	2	2	0	0	0
993[b]	methyl butanoate	fruity	1	3	–2	0	1	3	0
1020	α-pinene	citrus, chemical, spicy, woody (+)	7	6	1	0	7	6	0
1041	ethyl butanoate	fruity, orange (++)	8	8	0	8	0	8	0
1083	hexanal	fruity, orange, floral (+)	7	6	1	7	0	6	0
1100	β-pinene	citrus, terpene-like	0	2	–2	0	0	2	0
1149	δ-carene	**floral (+)**	3	0	**3**	3	0	0	0
1168	β-myrcene	peel, unpleasant, geranium, medicine	8	7	1	0	8	0	7
1211	limonene	fruity, lemon, anise (+)	8	8	0	8	0	8	0
1241	ethyl hexanoate	**fruity, orange**	8	4	**4**	8	0	4	0
1257	terpene	**fruity, orange, floral**	2	2	0	2	0	2	0
1287/1293	α-terpinolene/ octanal	unpleasant, citrusy, chemical, orange (+)	6	7	–1	0	6	7	0
1312	4,8-dimethyl-1,3,7-nonatriene	mushroom (+)	3	4	–1	0	3	0	4
1362	1-hexanol	**floral**	0	3	**–3**	0	0	1.5	1.5
1382	3-hexen-1-ol	**woody, benzene-like**	3	0	**3**	0	3	0	0
1398	nonanal	floral, medicine	2	0	2	0	2	0	0
1420/1414	carvacrol/*trans*-2-hexenol	fruity, plastic, India rubber	3	4	–1	0	3	4	0
1441	ethyl octanoate + 3-octen-1-ol[c]	spicy, floral, mushroom	4	4	0	0	4	4	0
1481	*n*-octyl acetate	**unpleasant, dusty, green**	4	0	**4**	0	4	0	0
1503	decanal	chemical fruity, lemon	4	4	0	4	0	4	0
1545	β-cubebene	floral, terpene-like, lemon (+)	6	4	2	6	0	4	0
1554	linalool	**floral, fruity, lemon (++)**	5	2	**3**	5	0	2	0
1566	1-octanol	grapefruit, herbal	2	0	2	1	1	0	0
1668	1-nonanol	fruity, floral	2	1	1	2	0	1	0
1807	*c*	**aldehyde-like, unpleasant, naphthalene**	4	1	**3**	0	4	0	1
1828	*p*-menth-1-en-9 yl acetate	**citrusy, fruity, floral**	5	2	**3**	5	0	2	0
1855	geraniol	**rose, floral**	4	0	**4**	4	0	0	0
1873	hexanoic acid	**fruity, fatty, sweet**	0	4	**–4**	0	0	2	2
1940	β-ionone	**rose, lilac (+)**	5	0	**5**	5	0	0	0
1976	*c*	unpleasant, floral	2	0	2	1	1	0	0
2012	*c*	**soapy, caramel-like**	0	3	**–3**	0	0	3	0
2091	octanoic acid	**moldy, unpleasant, bouillon (+)**	3	0	**3**	0	3	0	0
2182	*c*	solvent, plastic, India rubber	5	5	0	0	5	0	5
2237	*c*	smoky, phenol-like, plastic	3	2	1	0	3	0	2
		sum	133	99	34	75	58	76.5	22.5
		%				56	44	77	23

[a] Identified by comparing it with the reference substance on the basis of retention index (RI) on DB-Wax, mass spectra, and odor quality. [b] From the literature. [c] Not positively identified.

contributors to the fresh orange juice odor (*16*) even if their amount in orange juice is strictly dependent on orange variety (*3*).

These compounds are better extracted by the G20 than the G31 SPME method, so this explains higher frequencies of citation and also a higher perception of fruit candy and green/herbal descriptors during D-GC-O sessions for the G20 extract. Nevertheless, these two odorant molecules are present in very low amounts in SSHS. This could explain why fruit candy and green/herbal odors are less perceived for reference orange juice during D-GC-O (**Figure 3A**).

In the 1287−1293 RI range (**Table 3**) assessors perceived either pleasant or unpleasant notes due to the proximity of α-terpinolene and octanal. GC-MS quantification (**Figure 4A**) showed that α-terpinolene is mostly present in G31, whereas octanal is more prevalent in G20. α-Terpinolene is also present in very high amount in SSHS. Shorter SPME exposure times (1 min for G31 and G6) permits increased α-terpinolene relative recovery consistent with an increase in the G20 sample. Moreover, the G31 procedure led to a significant decrease in the octanal selective extraction and, thus, to a result closer to the static headspace profile.

Roberts et al. (*6*) showed that although short sampling times with SPME have reduced sensitivity as compared to exhaustive SPME (long fiber exposure times), it better approaches static headspace. Moreover, they used short sampling times to reduce the possibility of fiber overloading and resulting biases. Competitive effects can affect the results when two samples are being compared for target compounds. In our case only one orange juice was sampled, so TIC area measurements reflect changes in impact odorant recoveries under different extraction conditions.

Miller and Stuart (*9*) compared gas- versus SPME-sampled static headspace in orange juice. They found that SPME had an 1800-fold sensitivity for nonpolar compounds relative to gas-sampled headspace. In this case a long exposure time was used

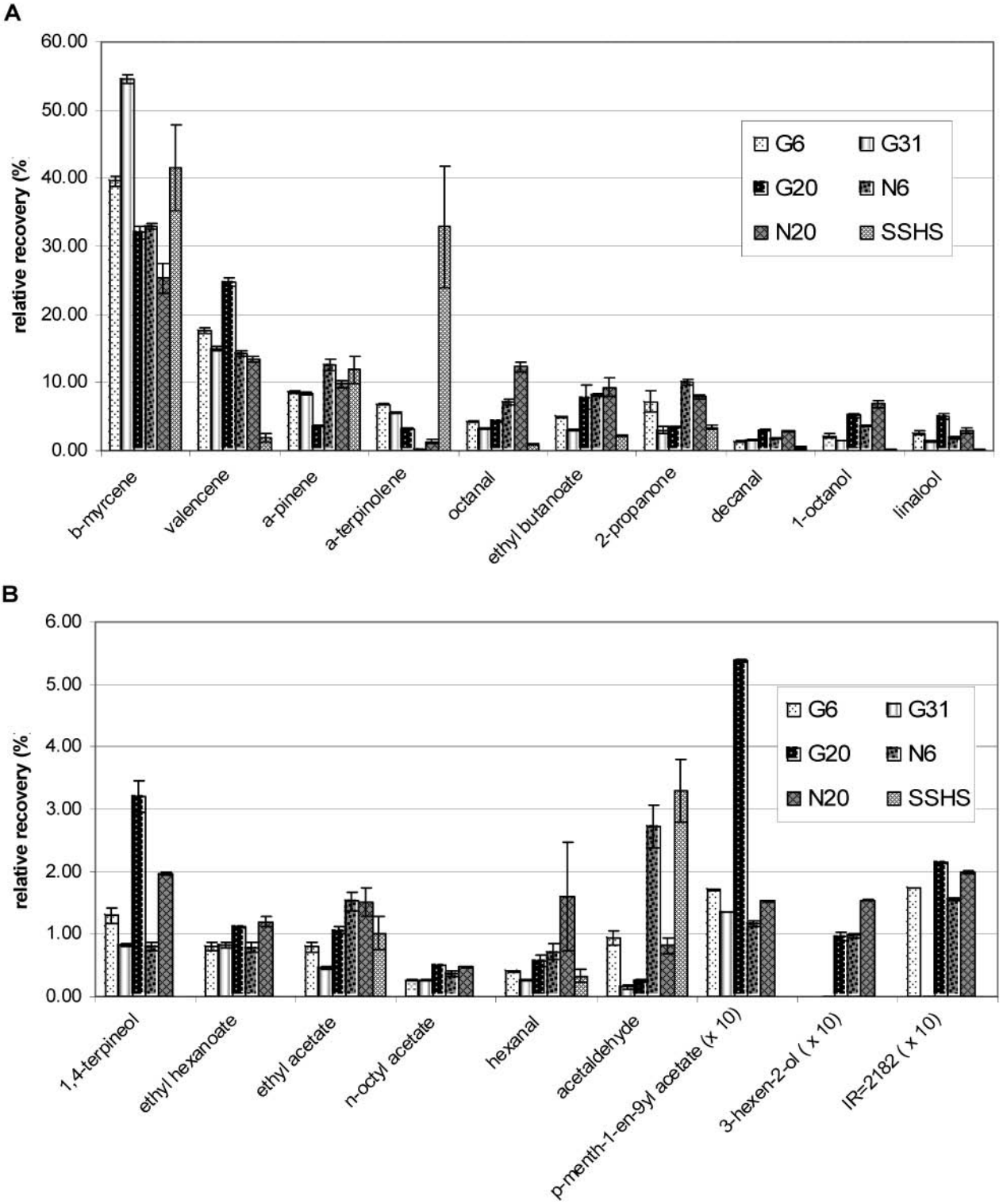

Figure 4. Relative recovery of aroma compounds in SPME (G6, G20, G31, N6, and N20) and syringe-sampled headspace (SSHS) samples.

(60 min). When using a shorter sampling time, the profiles of conventional and SPME headspace analysis are more similar, although the less volatile compounds are detected better with SPME and highly volatile compounds are detected better with static headspace (*6*).

Recovery of limonene is reported in **Table 4**. This molecule represents up to 93% of all aroma compounds (*17*). Concerning the DVB/CAR/PDMS SPME fiber, short fiber exposure times (1 min for G6 and G31) led to a relative limonene amount of 93%. Short equilibrium time (5 min) applied to G6 leads to a lower absolute limonene amount (TIC area) due to a lower limonene concentration in the headspace (no exhaustive extraction). A longer fiber exposure time (15 min for G20) leads to a decrease in limonene relative to the amount corresponding to less volatile compounds (lower $K_{air-water}$) higher recovery. Concerning the CAR/PDMS fiber, a different absorption process explains the lower affinity for limonene (lower TIC areas).

Figure 3 shows that the CAR/PDMS fiber is more efficient in extracting very early eluting compounds such as acetaldehyde and 2-propanone responsible for fruity notes. Moreover, these two compounds are better extracted at short equilibrium and fiber exposure times (N6), which is in agreement with Bazemore

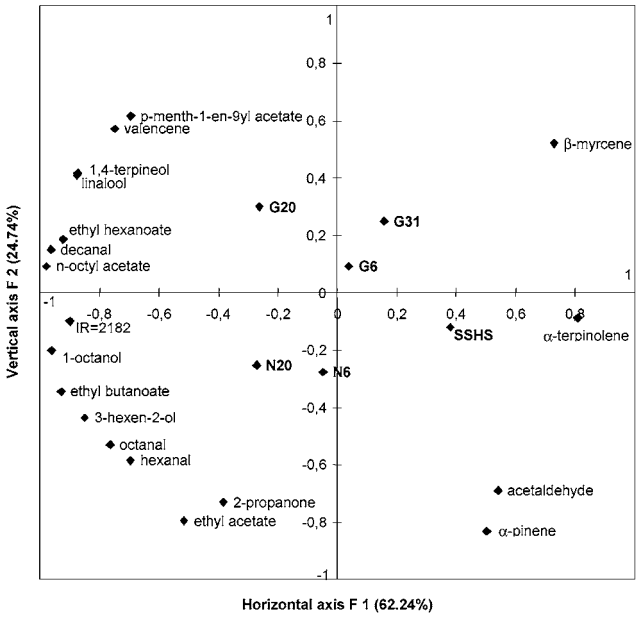

Figure 5. PCA representation of samples (G6, G31, G20, N6, N20, and SSHS) and variables (relative amount of aroma compounds) on the first principal plane.

Table 4. Recovery of Limonene from SPME Samples

sample	TIC[a]	CV %	% of all detected compounds
G6	6371123560	7	93
G31	11116360428	2	93
G20	10730681861	4	88
N6	3576893984	6	94
N20	1192298028	6	51

[a] Total ion current by GC-MS.

et al. (*18*). However, longer equilibration times are needed to increase the recovery of less volatile compounds such as 1,4-terpineol. This selectivity could cause an imbalance in the global odor of N6 and N20 extracts, explaining similarity results. Limonene is responsible for the orange peel note in GC-O and is perceived with the same frequency in both G20 and G31 SPME extracts. Its high supraliminal concentration in headspace explains why the "orange peel" character of the global odors is the same not only for G20 and G31 (**Figure 2**) but also for N6 and N20 extracts (data not shown).

It is worth noting that some high molecular weight components, very late eluting, are completely absent in the SSHS, among them sesquiterpenes and oxygenated compounds, which are responsible for unpleasant notes. Some examples are 3-hexen-1-ol (woody, benzene-like), fatty acids (moldy, fatty), the unidentified compound at RI 2182 (plastic, solvent-like, rubber note), and the sesquiterpene at RI 2237 (smoky, phenol-like, plastic). Most probably this is why unpleasant notes are detected in the global odors of SPME extracts.

PCA. Figure 5 shows the representation of samples and variables on the first principal plane. As variables we used relative aroma amounts from SPME and SSHS extracts. The first principal axis (horizontal) explains 62.24% of the variance; on this axis extracts are separated according to the sampling method: first, SSHS at equilibrium with the highest positive value; second, long equilibrium time and short fiber exposure time SPME (G31), then short equilibrium and fiber exposure time SPME (G6 and N6), and finally long fiber exposure time SPME (G20 and N20) with negative scores. The second axis (24.74%) discriminates the two fiber types: CAR/PDMS and DVB/CAR/PDMS (N and G codes) on the negative and positive sides, respectively. On the second axis esters and aldehydes are oriented as a function of their carbon chain length. This is coherent with the observation that the CAR/PDMS fiber is more selective for very early eluting compounds.

Finally, on the principal plane G20 and N20 extracts are placed farthest from the SSHS sample, which confirms their lower representativeness with respect to the reference juice; the other extracts are located in intermediate positions. This also means that the flavor compounds chosen for this representation are closely related to the perceived global aroma. It is worth noting that all aroma compounds which are responsible for negative impressions are located on the same side of the plane as the SPME methods with the worst representativeness notations. Conversely, β-myrcene, α-terpinolene, α-pinene, and acetaldehyde—which give important contribution to the fresh orange aroma (*19*)—are located on the side of the plane that is better represented by SSHS, G31, and G6 extracts.

Conclusion. It is beyond controversy that SPME is a rapid and very sensitive extraction method. In particular, it allows higher flavor recovery and, thus, easier odor identification if compared to other flavor extraction methods such as static headspace and vacuum distillation. Notwithstanding, the odor quality of the SPME extract—a crucial aspect for flavor analysis—remains virtually uncovered by actual investigations. This paper provides the first comprehensive methodology to "smell" SPME extracts and "evaluate" their sensorial quality on the basis of D-GC-O. Using this novel methodology on fresh orange juice, we showed that odor impressions emerging from SPME extracts poorly resembled that of the original orange juice. An optimization of SPME parameters, that is, fiber type and time of exposure and sample equilibration time, permitted slight improvement of SPME representativeness with respect to the reference juice: the best sensorial results were obtained for DVB/CAR/PDMS fiber exposed for the shortest time (5 min). In this way the selective extraction of unpleasant odors is minimized as shown by classical GC-O and aroma compound quantification. Interestingly, PCA discriminated SPME and SSHS extracts according to their odor representations described by D-GC-O analysis.

In conclusion, D-GC-O will enable future investigations to further improve SPME performance.

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