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Comparative study of two extraction techniques to obtain representative aroma extracts for being analysed by gas chromatography–olfactometry: Application to roasted pistachio aroma

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ABSTRACT

This research paper presents a comparative study of two different extraction and concentration techniques to obtain representative pistachio aroma extracts: the traditional direct solvent extraction (DSE) followed by high-vacuum transfer (HVT) and the headspace solid-phase microextraction (HS-SPME). The results showed that, although both techniques provide accurate information about the aromatic composition that will be perceived by the consumer, the precision in terms of within-day repeatability and between-days repeatability (intermediate precision) of the chromatographic areas presented better values for HS-SPME than for DSE-HVT. Moreover the solvent-free HS-SPME allows the extraction of more odour-active regions, requires very little sample handling and shorter time for sampling.

1. Introduction

Edible nuts have been widely used since ancient times as an important source of nutrients such as proteins, vitamins, minerals and lipids (mainly mono and polyunsaturated fatty acids) [1,2].

As in any other food product, nuts quality is closely related to the flavour and the aroma detected by consumers will have a great influence on the acceptance or rejection of the nut evaluated. However, it has to be said that raw nuts have a rather bland aroma, being some compounds generated during the roasting process responsible for the characteristic nut odour [3–6]. Due to the importance of nuts on human diet and health [7,8] there are several studies published on their chemical [2,9–12] and volatile composition [13–15] for both raw and roasted nuts. Nevertheless, only a few studies related to their aromatic fraction [3–6,13–15] have been carried out and, for some nuts, such pistachios, there has not been published any study about the compounds responsible for its aroma.

The aroma of any foodstuff is influenced by the action of hundreds of different and very heterogeneous chemicals (alcohols, aldehydes, esters, ketones, pirazines, acids, terpenes, etc.) each of them with a different contribution to the whole aroma [16]. Every odorant must be volatile in order to reach the nose and interact with the appropriate receptors located on the olfactory epithelium [17], but not all the components of the volatile fraction are odour-active.

Gas chromatography–olfactometry (GCO) is a powerful technique in food aroma characterization [18,19] that uses the human nose as a chromatographic detector in parallel with a conventional one, like the Flame Ionic Detector (FID) or the Mass Spectra Detector (MSD). Therefore, this technique allows distinguishing the odour-active compounds within the whole range of volatiles present in a particular product. Several techniques have been developed to collect and process GCO data in order to evaluate the sensory contribution of each odorant to the sample aroma [18]. Aroma extract dilution analysis (AEDA) is one of the most commonly used to determine the relative odour potency of those aromatic compounds present in a sample extract [20,21]. This technique involves stepwise dilution of the aroma extract followed by an evaluation of each dilution by GCO until no odorants are perceived in the GCO effluent. The last dilution step where an odorant is perceived constitutes its flavour dilution (FD) factor, which can be considered a good indicator of the odour potency of that compound (i.e. the higher the FD factor, the higher the sensory contribution of that compound to the sample). In that way, AEDA is a valuable screening tool for ranking odour-active compounds in a sample according to their relative odour potency.

But obtaining reliable data in aroma characterization depends on the representativeness of the extract itself. Indeed, several studies show the large influence of the extraction method employed on

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the results of the olfactometric analyses performed [22], because the extract not only should contain all the sample odorants, but also their relative amounts should remain constant. Nowadays, to obtain a representative extract is still a challenge in most foodstuffs, where their aroma are due to complex mixtures of hundreds of compounds with different chemical properties and, moreover, present at different levels of concentration, ranging from several mg L^{-1} to a few ng L^{-1}. Different sampling techniques, such as liquid–liquid extraction, solid–liquid extraction, distillation, headspace techniques, solid-phase microextraction, demixing, etc. [23–30] have been used to obtain extracts for aroma characterization.

The purpose of this paper was to make a comparative study of two different extraction and concentration techniques to obtain representative pistachio aroma extracts: the direct solvent extraction (DSE) followed by high-vacuum transfer (HVT) [26,31] and the headspace solid-phase microextraction (HS-SPME) [32]. The first technique has been widely used as sample preparation technique on the characterization of many foods but it implies multiple steps in sample handling, it is time-consuming, and, as it employs organic solvents, it is unfriendly with the environment and it can also generate artifacts that will interfere in aroma detection. However, once the extract is obtained it is very easy to proceed with the AEDA analysis. On the other hand, the SPME is a newer solvent-free technique much faster because it carries out the extraction and concentration in a single step, and that requires very little sample manipulation. However, there is an important drawback when dealing with the AEDA because, as the analytes are retained on the fibre and no physical extract is obtained, it is necessary to stepwise dilute each sample before carrying out the extraction for each dilution [27].

2. Materials and methods

2.1. Samples

The samples used in this study were Iranian pistachio nuts of the variety "Fandooghi". Shell-free nuts from the 2008 harvest were roasted at 160 °C for 20 min and immediately vacuum-packed (bags of 250 g) in order to use fresh pistachios batches for every analysis.

2.2. Reagents and chemicals

The chemical standards of the aroma compounds, whose CAS numbers are specified in Table 2, were supplied by Sigma–Aldrich (Madrid, Spain), Fluka (Madrid, Spain) and Lancaster (Bischheim, France) and their purity was above 90%. Dichloromethane, diethyl ether, hexane and sodium chloride were of analytical grade and purchased from Scharlab (Barcelona, Spain), while pure water was obtained from a Milli-Q purification system (Millipore, Bedford, USA).

2.3. SPME

The SPME holder for manual sampling and the polydimethylsiloxane (PDMS) 100 μm, polyacrilate (PA) 85 μm, Carboxen–Polydimethylsiloxane (Carboxen/PDMS) 75 μm and StableFlex Divinylbenzene–Carboxen–Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm fibres used in this study were purchased from Supelco (Bellefonte, USA). All the fibres were conditioned before use and thermally cleaned between analyses by inserting them into the GC injector port at the temperature recommended by the producer.

2.4. Isolation and concentration of the volatile compounds

2.4.1. Direct solvent extraction (DSE) followed by high-vacuum transfer (HVT)

To obtain the extract, 100 g of fresh roasted pistachios finely ground in a coffee mill and passed through a sieve (1.5 mm of diameter) were extracted with 100 mL of dichloromethane for 5 h at 25 °C under constant magnetic stirring and nitrogen atmosphere. Then, the mixture was filtered (paper Whatman® 42) using a water jet filter pump. The liquid phase obtained containing the volatiles and large amounts of lipids, was subjected to a high-vacuum transfer (HVT) [26], in order to isolate the volatile fraction. To proceed with the HVT, the liquid phase was slowly dropped into the distillation flask, which was heated to 36 °C. When the addition was finished, distillation was continued for 30 min. The vacuum used in the apparatus was of ≈5 mPa and the distillate was condensed in the first of the two cooling traps employed. The condensate was finally concentrated to 0.5 mL by means of a Vigreux column and a thermostatic bath at 42 °C. 2 μL of this extract were used for GCO analysis.

2.4.2. Headspace solid-phase microextraction (HS-SPME)

To extract the volatiles, 15 g of fresh roasted pistachios finely ground in a coffee mill and passed through a sieve (1.5 mm of diameter) were placed into a 50-mL glass vial together with 15 mL of Milli-Q water and a magnetic stir bar, being the sample/headspace ratio 1:1. After tightly capping the vial with a silicon septum under nitrogen atmosphere, it was pre-equilibrated for 15 min at 50 °C in a thermostatic bath. Afterwards, the stainless steel needle of the SPME device where the fibre is housed was pushed through the vial septum. Then, the fibre was pushed out of the housing and exposed for 2 h at 50 °C to the vial headspace. After extraction, the fibre was pulled into the needle assembly, the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption of the analytes at 270 °C for 1 min.

2.5. Gas chromatography analysis

2.5.1. GC-FID and GCO

The analyses were performed with a Hewlett-Packard (HP, Palo Alto, USA) 6890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactory detector. The fused silica capillary column employed to carry out the chromatographic separations was a Chrompack (Varian, Middelburg, The Netherlands) CP-WAX 57CB (50 m × 0.25 mm i.d., 0.2 μm film thickness) with helium as a carrier gas at a constant flow-rate of 1 mL min^{-1}. The oven temperature was programmed as follows: 40 °C (2 min), 5 °C min^{-1} to 220 °C (22 min). The fused silica capillary column used to verify the identity of the compounds was a HP-5 (Agilent Technologies, USA) (30 m × 0.32 mm i.d., 0.25 μm film thickness) with helium as a carrier gas at a constant flow-rate of 1 mL min^{-1}. The oven temperature program was: 40 °C (5 min), 3.5 °C min^{-1} to 120 °C, 10 °C min^{-1} to 210 °C (10 min). In both cases, the split–splitless injection port operated in the splitless mode at 270 °C for 1 min and the temperature of the FID was set at 250 °C.

To split the effluent into the FID and the sniffing port, the end of the capillary column was connected to a splitting assembly based on the Capillary Flow Technology (Agilent Technologies, USA). The split ratio for the olfactometric analysis was 1:1 (FID:sniffing port) and it was achieved by using two deactivated and uncoated fused silica capillaries of the same length and width as a transfer line between the splitting assembly and the detectors. Moreover, the use of an olfactory detector control module commercialized by SGE International (Ringwood, Australia) that incorporates a heated transfer section from the GC oven to the glass detection cone, kept the unit at a suitable temperature to transfer the volatile com-

pounds to the detection cone without losses due to condensation. Furthermore, the glass cone is purged with humidified air to prevent nasal mucous membranes from drying out in order to maintain olfactory sensitivity.

Timing and odour descriptions were recorded by two trained sniffers after each sample injection and they were replaced at 15 min intervals to avoid fatigue and distractions. Each sample was analysed in triplicate by each trained researcher.

2.5.2. GC–MS

GC–MS analyses were performed with a Hewlett-Packard (HP, Palo Alto, USA) 6890 gas chromatograph coupled to an HP-5973 mass selective detector. Separation was achieved under the same operating conditions described above and using the same columns as in the GC-FID and GC O analyses. The mass spectrometer operated in the electron impact ionization mode (70 eV). Interface, source and quadrupole temperatures were 200 °C, 230 °C and 150 °C, respectively, and the mass range was from 35 to 300 amu. The split–splitless injection port operated in the splitless mode at 270 °C for 1 min.

2.6. Aroma extract dilution analysis

To get a hierarchically classification of the most odour-active compounds in roasted pistachios we determined the flavour dilution (FD) factors (obtained by two trained sniffers in triplicate) by AEDA, which was carried out in two different ways depending on the extraction technique employed. When dealing with the DSE, the aroma extract obtained was stepwise diluted (1:4) with dichloromethane [33]. But when the sampling was performed with HS-SPME, a new approach to the AEDA was used as no physical extract was obtained. It consists of stepwise reduction of the amount of roasted pistachio that was put into the vial (1:4) before carrying out the SPME by adding a suitable amount of Milli-Q water to keep constant the headspace/sample ratio. In both cases, the dilutions were carried out until no odorant was detected by sniffing the highest dilution. Two experienced sniffers performed the AEDA experiments and their response (sensitivity) to the individual compounds did not differ by more than 2 FD-factors.

2.7. Compounds identification

The odorants detected in the olfactometric study were identified by comparison with reference compounds on the basis of the following criteria: odour quality perceived at the sniffing port, mass spectra obtained and retention indices (RI) on the two stationary phases of different polarity employed (CP-WAX 57CB and HP-5). Retention indices were calculated from the retention times of a series of *n*-alkanes (from 6 to 26 carbon atoms) injected under the same chromatographic conditions.

2.8. Sensory analysis

To determine the similarity between the aroma of the different extracts obtained with the two extraction techniques and the aroma of the fresh roasted pistachios, a panel of 8 trained assessors evaluated the global odour for each particular case. Firstly, panellists were familiarized with the roasted pistachio aroma and were then asked to agree in a common list of 6 descriptors: green, sweet, roasted, chocolate/coffee, rancid and nutty (this last was evaluated as a global impression).

Then, assessors were asked to rate the intensity on a discontinuous scale from 0 (no similarity) to 5 (equal) for the 6 descriptors above specified. The coefficients of variance found for each single panellist for different replicates of one sample were <10%.

3. Results and discussion

3.1. Optimization of DSE parameters

Taking into account that the aim of this study was to extract the odorant compounds of the pistachio samples, when optimizing the parameters that affected the extraction equilibrium we evaluated not only the chromatographic areas of the compounds extracted (FID response), but also the number and intensity of the odorants perceived (GCO response). All the experiments were performed in triplicate.

Among the different solvents usually used to extract food odorants we tested diethyl ether, hexane and dichloromethane. The results showed that the best efficiency was obtained when using dichloromethane because hexane resulted in volatiles losses due to its high boiling point and diethyl ether gave a poorer chromatographic response (nearly 9% smaller than the one obtained when using dichloromethane). Once the solvent was chosen, it was necessary to determine the volume required to get the maximum extraction efficiency. Nevertheless, this parameter is closely related to the sample amount used so, the sample weight/solvent volume ratio was optimized. With this aim, different ratios (from 1:1 to 1:4) were tested by varying both parameters: sample weight ranged from 50 to 200 g and solvent volume ranged from 50 to 200 mL. The best results, both chromatographic and olfactometric, were obtained for the 1:1 ratio. To make easier the sample handling, mainly during the filtration process, we choose as the optimal values 100 g of finely ground roasted pistachios extracted with 100 mL of dichloromethane.

Regarding to extraction time and temperature, both parameters were studied simultaneously as they are strongly influenced one by the other [34]. To determine the optimum sampling conditions, different experiments were carried out for 2.5, 5 and 7.5 h, at 0 °C and 25 °C. Higher temperatures were not evaluated since it could imply losses of some aromatic compounds. The results showed that, whatever the extraction time, the efficiency decreased at lower temperature. With regard to sampling time, the shortest one resulted in a poorer chromatographic profile, while the largest one did not improve significantly (<5%) the extraction yield compared to that of 5 h.

Therefore, the optimum DSE results were obtained when 100 g of finely ground roasted pistachios were extracted for 5 h at 25 °C with 100 mL of dichloromethane.

3.2. Optimization of HS-SPME parameters

Although the reproducibility of fibres has considerably been improved during the last years, we used more than one in order to take into account the variability response among them.

To select the best conditions, we evaluated both chromatographic areas and number and intensity of odorants extracted, in triplicate, as was done in the previous section.

The choice of the SPME fibre coating was the first parameter considered. The coatings checked are listed in Section 2.3. The results showed that, whereas with PDMS, PDA or CAR-PDMS the number of odorant regions was between 40 and 45 for the first two fibres and between 60 and 65 for the third one, when using DVB/CAR/PDMS up to 75 odorants were detected. Thus, this last fibre was selected as the optimum for the extraction.

Another important issue that had to be considered was whether adding a solvent to ground pistachios (solid sample) could improve the SPME extraction. Although different solvents and mixtures were tested, the results showed that the use of organic solvents implied a competence in the extraction process, so we decided to work only with Milli-Q water to homogenize the sample and accelerate the extraction [35].

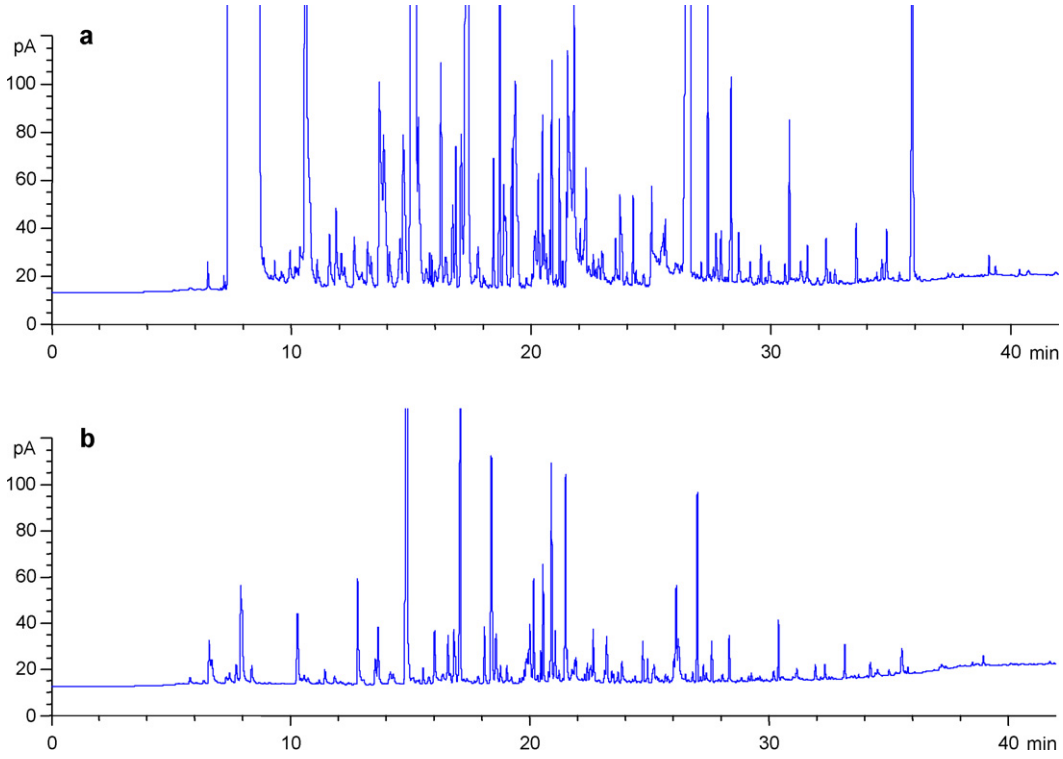

Fig. 1. Chromatograms belonging to the extract of roasted pistachio obtained in the GC-FID (a) with DSE-HVT technique and (b) with HS-SPME technique. The chromatograms were achieved by using a CP-WAX 57CB column and without splitting to the sniffing port.

The sample/headspace ratio was the next parameter studied, because it is well known that the headspace volume affects the extraction efficiency [36]. Thus, we added different water volumes to pistachio samples to reach 5 and 10 mL in 20 mL vials, and 25 mL in 50 mL vials. The best response was obtained when the experiments were carried out with 25 mL of the sample + water mixture in a 50 mL vial.

Related to ionic strength it is known that the higher the ionic strength, the lower the solubility of neutral molecules in the water and the less likely these molecules are to pass from the solid matrix to the water. As a result, the extraction efficiency of these molecules decreases [35]. We checked whether the addition of salt had any influence on the extraction of the odour-active compounds and the results corroborated this negative effect for most of the compounds of interest. Consequently we decided to avoid the salt addition to the samples.

Extraction time and temperature were again simultaneously studied, so different experiments were performed in the range from 2 to 4 h (shorter times did not ensure the suitable aromatic perception of all the compounds) and from 30 to 50 °C. The results showed that the poorest chromatographic and olfactometric responses were obtained when working at the lowest temperature and the shortest sampling time (2 h, 30 °C). However, as the microextraction is an exothermic process, the highest temperatures and the largest times (4 h, 50 °C) did not improved the overall extraction efficiency. Therefore, taking into account that the chromatographic and olfactometric profiles obtained at 2 h, 50 °C were not significantly different (<5%) to the ones obtained at 3 h, 40 °C, we decided to employ the shortest extraction time (2 h, 50 °C) to make faster the sample preparation.

Hence, the optimal conditions for HS-SPME were achieved by mixing 15 g of finely ground pistachios with 15 mL of Milli-Q water (these amounts of sample + water imply a volume of 25 mL) into a 50 mL vial with a little magnetic stir bar and extracting for 2 h at 50 °C.

3.3. Precision of the developed methods

Once the optimum conditions were determined, we evaluated the precision of both methods in terms of within-day repeatability and between-days repeatability (intermediate precision) of the chromatographic areas. Since the chromatogram profiles obtained were very rich but very different depending on the extraction technique employed (Fig. 1), to suitably evaluate these performance parameters, we selected 13 chromatographic peaks which were positively identified by both techniques according to the following criteria: they should present different retention times along the entire chromatogram; they should cover the range of widths and heights of the whole of the chromatographic peaks; they should correspond to aromatic compounds with different contributions to the aroma (i.e. different FD); and they should belong to different chemical families.

Both precision parameters were expressed by means of the percentage of relative standard deviation (%RSD). The repeatability was calculated by injecting, consecutively, 5 different extracts obtained the same day while the intermediate precision was calculated from the results acquired when injecting 6 different extracts obtained over a month (approximately one extract every week). Table 1 shows the results obtained for the compounds selected. The low %RSD values for both parameters and both techniques allowed us to confirm that the accuracy of the optimized methods was very good. However, for almost all the chromatographic peaks, the RSD values (both for repeatability and intermediate precision) obtained when working with HS-SPME were smaller than the ones obtained when working with DSE-HVT. Therefore, although in some cases the %RSD values obtained with each one of the techniques were not statistically different, the fact that the SPME technique always presented lower RSD values allows us to conclude that the SPME technique provided better accuracy.

Table 1
Values of repeatability and intermediate precision for the selected compounds expressed by means of the percentage of relative standard deviation (% RSD).

Retention time (min)	Compound	DSE-HVT			HS-SPME		
		Mean of area	$\%RSD_{rep.}$	$\%RSD_{int.pr.}$	Mean of area	$\%RSD_{rep.}$	$\%RSD_{int.pr.}$
7.8	Isobutanal	69,382	2.4	4.6	793,788	2.4	5.0
9.5	Diacetyl	672,958	2.0	5.2	44,691	2.2	4.3
11	2,3-Pentanedione	699,943	4.9	8.9	126,680	3.4	4.7
11.9	Hexanal	3,278,534	6.5	8.6	563,835	3.1	4.1
13.2	(E)-2-pentenal	452,619	4.7	6.1	75,525	2.1	4.6
15.3	2/3-Methyl-1-butanol[a]	6,190,691	5.1	9.6	469,958	3.9	6.2
18.9	2-Ethylpyrazine	1,827,142	6.9	10.5	934,466	2.6	4.8
19.9	Dimethyltrisulfide	44,293	5.9	9.8	11,267	3.3	6.7
20.3	2-Ethyl-5-methylpyrazine	5,587,016	6.3	7.4	2,647,486	3.0	5.3
23	2-Acetylfuran	383,012	5.1	8.3	177,382	2.5	6.1
31.1	Guaiacol	89,875	4.8	7.7	34,539	3.2	4.9
32.3	2-Phenylethanol	1,808,021	5.2	9.5	876,731	2.8	4.6
35.4	Furaneol	174,012	6.1	10.3	529,962	3.2	6.4

$\%RSD_{rep.}$: relative standard deviation of the repeatability.
$\%RSD_{int.pr.}$: relative standard deviation of the intermediate precision.
[a] Compound with FD 16, so it does not appear in Table 2.

3.4. DSE-HVT versus HS-SPME

As shown in Fig. 1, the GC-FID chromatogram belonging to the extract obtained by application of DSE-HVT presents more and higher peaks than the one obtained by application of HS-SPME. This greater response is due to the fact that, whereas the headspace technique only allows the extraction of those actually volatile compounds, when using DSE, any compound that exhibits affinity to the solvent employed will be extracted.

However, when the extracts obtained with both techniques were analysed by gas chromatography–olfactometry, the results were very similar: 75 and 74 different odour-active regions were detected for the DSE-HVT and the HS-SPME techniques, respectively. Moreover, most of these regions coincided in both cases in their retention times and the descriptors employed to define the odours perceived. In that way, as usually in this kind of flavour studies [5,6,27], when using a polar column, fruity and chemical notes were perceived at lower retention indices, followed by green and earthy notes. At the end of the analysis, lactic and fatty odours, followed by burned and caramelized ones were detected. Therefore, this comparison shows that, although DSE-HVT technique allows the extraction of a higher number of compounds, when we focus only on the extraction of odour-active compounds, these are almost the same ones regardless of the extraction technique employed. The difference lies in the perception intensity of some of these compounds (i.e. different flavour dilution (FD) factors).

By applying the suitable AEDA for each extraction technique, the most odour-active regions were determined: these are the ones with higher FD factors (ranging from 64 to 1024 for at least one of the two extraction techniques). The results are summarized in Table 2, where the different odours perceived have been arranged following their retention indices in the polar column. It should be noted that, although each aroma perceived is mostly due to a single compound, among the different odour-active regions detected in both extracts, we found 3 odours that were originated by a mixture of some compounds. This is the case of the flavour-active regions number **15** (roasted nut, corn), **21** (cooked potato) and **24** (fatty, green-like), that have been positively identified as a mixture of 2,3-dimethylpyrazine, 2,6-dimethylpyrazine and 2-ethylpyrazine (region **15**), a mixture of 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5(or 6)-dimethylpyrazine and methional (region **21**), and a mixture of 2-acetylfuran, 3,5-diethyl-2-methylpyrazine and 2,3-diethyl-5-methylpyrazine (region **24**).

As shown in Table 2, 34 and 45 flavour-active regions with a FD $\geq$ 64 were detected when using DSE-HVT and HS-SPME, respectively. As it can be seen, not always a high FD value obtained when working with an extraction technique implies a high FD value when working with the other one. In fact, only 15 of these regions were perceived with the same FD in both extracts, being 1024 the highest FD factor, which was found for the odorant regions number **2**, **13**, **15**, **19**, **22** and **42**. After these mainly toasted, heavy and rubbery notes, and in descending order of FD, we found other odorant regions with a coincident FD in both extracts: 6 regions with a FD of 256 that mainly provided fruity, earthy and green notes (regions **5**, **11**, **14**, **18**, **25** and **29**) and 3 with a FD of 64 that contribute to the aroma extracts with buttery, fruity and flowery notes (regions **7**, **10** and **38**).

However, there are also some differences between the results obtained from the distillate and the ones from the SPME that give evidence of the different extraction and concentration effectiveness of both techniques. On one hand, 5 odour-active regions were detected on the SPME extract but not on the distillate: **20** (mushroom), **23** (anise-like, fennel), **26** (roasted nuts, popcorn), **28** (vomit, lactic) and **44** (stall, animal), which correspond to two unknown odorants (regions **20** and **23**) plus 2-acetylpyridine (region **26**), butyric acid (region **28**) and 4-ethylphenol (region **44**). On the other hand, while 19 flavour-active regions were perceived in the SPME with a higher FD factor than in the distillate, only 6 odours appeared in the distillate with a higher FD factor: **1** (malty, solvent-like), **3** (fruity, strawberry), **17** (sulphur-like), **21** (cooked potato), **31** (blue cheese, sweaty) and **32** (deep-fried), which have been positively identified as isobutanal (region **1**), ethyl propanoate (region **3**), dimethyltrisulfide (region **17**), a mixture of 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5(or 6)-dimethylpyrazine and methional (region **21**), 2 and 3-methylbutyric acid (region **31**) and (E,E)-2,4-decadienal (region **32**).

From all these results it seems that, although DSE could extract compounds with higher molecular weight and lower volatility than the HS-SPME [37] it is less sensitive to some trace components, which are very important in the flavour perception of foodstuffs. Nevertheless, working with the distillate has some advantages because a liquid extract is obtained so, unlike when working with the SPME, the same sample can be tested many times and it can be concentrated to different degrees to achieve more chromatographic or olfactometric response. On the other hand, the SPME technique shows several advantages: simplicity, rapidity and cleanliness, as it is solvent-free and involves very little sample manipulation.

However, the aroma representativeness of the sample extract is the crucial parameter to decide which extraction technique is the most appropriate. So, to determine the similarity between the aroma of the different extracts obtained and the aroma of the fresh roasted pistachios, a panel of 8 trained assessors evaluated the

Table 2
Main odorants found in roasted pistachio nuts with FD ≥ 64 at least in one of the two extraction techniques.

		RI on			FD factor				Identification			
									MSD			
Odour-active regions	RT (min)	CP-WAX	HP-5	Odour description	DSE-HVT	HS-SPME	Compound	CAS no.	DSE-HVT	HS-SPME	RI	odour
1	7.8	*a*	*a*	Malty, solvent-like	1024	256	Isobutanal	78-84-2	X	X	X	X
2	8.6	936	*a*	Malty, bitter almonds	1024	1024	2/3-methylbutanal	96-17-3	X	X	X	X
					1024	1024		590-86-3	X	X	X	X
3	9.4	958	nd	Fruity, strawberry	256	64	Ethyl propanoate	105-37-3	X		X	X
4	9.5	968	*b*	Butter	256	1024	Diacetyl	431-03-8	X	X	X	X
5	10.2	1005	773	Strawberry	256	256	2-methylpropyl acetate	110-19-0			X	X
6	10.9	1031	801	Fruity	64	256	Ethylbutyrate	105-54-4			X	X
7	11	1041	702	Butter	64	64	2,3-pentanedione	600-14-6	X	X	X	X
8	11.2	1044	843	Fruity, apple	256	1024	Ethyl-2-methylbutyrate	7452-79-1	X		X	X
9	11.9	1071	794	Green, grass	64	1024	Hexanal	66-25-1	X	X	X	X
10	13.2	1122	753	Fruity	64	64	(E)-2-pentenal	1576-87-0	X	X	X	X
11	16	1226	896	Fishy	256	256	(Z)-4-heptenal	6728-31-0			X	X
12	16.3	1233		Earthy	16	1024	*unknown*					
13	17.4	1275	1001	Citrus, fresh	1024	1024	Octanal	124-13-0			X	X
14	17.7	1284	975	Mushroom	256	256	1-octen-3-one	4312-99-6	X	X	X	X
			nd		1024	1024	2,3-dimethylpyrazine	5910-89-4	X	X	X	X
15	18.9	1327	923	Roasted nuts, corn	1024	1024	2,6-dimethylpyrazine	108-50-9	X	X	X	X
			920		1024	1024	2-ethylpyrazine	13925-00-3	X	X	X	X
16	19.5	1350		Geranium	4	256	*unknown*					
17	19.9	1366	965	Sulphur-like	1024	256	Dimethyltrisulfide	3658-80-8	X	X	X	X
18	20.3	1381	997	Fruity, strawberry	256	256	2-ethyl-5-methylpyrazine	13360-64-0	X	X	X	X
19	21	1410		Rubber, plastic	1024	1024	*unknown*					
20	21.2	1429	nd	Mushroom	nd	1024	1-octen-3-ol	3391-86-4		X	X	X
21	21.7	1439	1077	Cooked potato	1024	256	3-ethyl-2,5-dimethylpyrazine	13360-65-1	X	X	X	*c*
			1085		1024	256	2-ethyl-3,5(or 6)-dimethylpyrazine	55031-15-7	X	X	X	X
			907		1024	256	Methional	3268-49-3			X	X
22	22.5	1467		Rubber, sulphur-like	1024	1024	*unknown*					
23	22.7	1469		Anise-like, fennel	nd	1024	*unknown*					
			nd		64	256	2-acetylfuran	1192-62-7	X	X	X	X
24	23	1487	1170	Fatty, green-like	64	256	3,5-diethyl-2-methylpyrazine	18138-05-1	X	X	X	*c*
			1150		64	256	2,3-diethyl-5-methylpyrazine	18138-04-0	X	X	X	X
25	23.7	1521	1160	Paper-like	256	256	(E)-2-nonenal	18829-56-6	X	X	X	X
26	24.4	1573	1026	Roasted nuts, popcorn	nd	256	2-acetylpyridine	1122-62-9		X	X	X
27	25.1	1584		Anise-like	16	1024	*unknown*					
28	25.5	1604	nd	Vomit, lactic	nd	1024	Butyric acid	107-92-6			X	X
29	26	1621		Green pepper, earthy	256	256	*unknown*					
30	26.5	1649	1043	Green roses	16	1024	Phenylethanal	122-78-1		X	X	X
31	26.8	1659	879	Blue cheese, sweaty	1024	256	2/3-methylbutyric acid	116-53-0			X	X
					1024	256		503-74-2			X	X
32	27.7	1694	1324	Deep-fried	256	64	(E,E)-2,4-decadienal	25152-84-5			X	X
33	29.1	1761	1098	Roasted nuts	64	1024	2-acetyl-2-thiazoline	29926-41-8			X	X
34	30	1805	nd	Fatty, flowery	64	256	Methyl laurate	111-82-0			X	X
35	30.4	1824	1383	Sweet, peach jam	256	1024	β-damascenone	23726-93-4			X	X
36	31.1	1861	1093	Smoky	256	1024	Guaiacol	90-05-1	X	X	X	X
37	31.4	1872	1039	Roasted, sweety	64	256	Phenylmethanol	100-51-6	X	X	X	X
38	32.3	1916	1119	Roses	64	64	2-phenylethanol	60-12-8	X	X	X	X
39	33.5	1981		Roasted, burnt	4	1024	*unknown*					
40	33.9	2006	1387	Metallic	64	256	trans-4,5-epoxy-(E)-2-decenal	134454-31-2			X	X
41	35.1	2063	1284	Disgusting, animal	64	1024	Octanoic acid	124-07-2			X	X
42	35.4	2080	nd	Caramel	1024	1024	Furaneol	3658-77-3	X	X	X	X
43	35.5	2085	nd	Pee odour	64	256	m-cresol	108-39-4	X	X	X	X
44	37	2201	nd	Stall, animal	nd	256	4-ethylphenol	123-07-9			X	X
45	37.6	2215	1332	Smoky, sweet	4	256	4-vinylguaiacol	7786-61-0		X	X	X

RI: Retention index on different stationary phases.
FD: Factor of dilution.
nd: not detected.
a: RI not calculated due to the solvent interference.
b: RI < RI of the first alkane (C6).
c: standard not available.

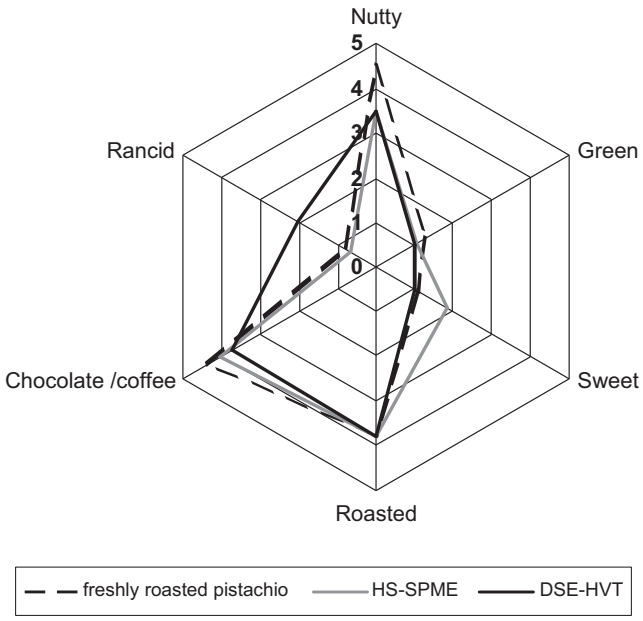

Fig. 2. Odour profiles of freshly roasted pistachio and its aromatic extracts obtained by DSE-HVT and HS-SPME.

global odour for each particular case by using the selected descriptors: green, sweet, roasted, chocolate/coffee, rancid and nutty.

For the DSE-HVT extract, similarity tests were performed by placing a drop of it on a perfume sampling paper and comparing the aroma perceived with the original roasted pistachio odour as a pair.

To check the aroma similarity between the SPME extracts and the roasted pistachio nuts we used the direct gas chromatography–olfactometry technique (D-GCO) [38]. Since a short deactivated capillary column is used, this technique avoids chromatographic separation of flavour volatiles, so the analyst perceives the extract as a global odour. In this case, the aroma detected is also compared with the original roasted pistachio one as a pair.

The results are shown in Fig. 2. As it can be seen, both extracts gave similar intensity values of roasted, green and coffee to the natural roasted pistachio. However, when dealing with DSE-HVT, the descriptor "rancid" was perceived with a higher intensity. This behaviour can be due to the fact that, although the panellists spent some seconds before sniffing the drop of extract placed on the perfume sampling paper to eliminate the solvent by evaporation, this solvent note persisted. Regarding to the HS-SPME technique, all the panellists agreed with the good correlation between the extract aroma and the real sample except on the perception of an intense plastic note on the SPME extract (in Fig. 2 this descriptor does not appear because this is not a descriptor of pistachio aroma). This fact was because of the high affinity of the fibres used for the sulphur compounds which give this kind of aromas [39]. These low differences observed in the spider-web diagram between the real pistachio samples and both kind of extracts were corroborated when the panellists were asked about the degree of similitude of the extracts to the real sample: the values obtained were 75–80% of likeness in both cases.

4. Conclusions

In conclusion, although the DSE followed by HVT has proved to be a good technique to analyse pistachio aroma as well as other food aroma, the results show that the HS-SPME is a good alternative to obtain representative pistachio aroma extracts with a wide range of odorants, suitable for GCO, and that requires shorter time for sampling. Although both extraction techniques provide accurate information about the volatile fraction that will be perceived by the consumer, HS-SPME has demonstrated to be able to extract more odour-active regions that can be detected until higher flavour dilution factors. Moreover, and thanks to the approach to the AEDA employed, it has been possible to establish an initial hierarchy on the contribution of each compound to roasted pistachio aroma and the reconstitution studies would be the next step.

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