

Determination of Roasted Pistachio (*Pistacia vera* L.) Key Odorants by Headspace Solid-Phase Microextraction and Gas Chromatography–Olfactometry

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ABSTRACT: Key odorants in roasted pistachio nuts have been determined for the first time. Two different pistachio varieties (Fandooghi and Kerman) have been analyzed by means of headspace solid-phase microextraction (HS-SPME) and gas chromatography-olfactometry (GCO). The aroma extract dilution analyses (AEDA) applied have revealed 46 and 41 odor-active regions with a flavor dilution (FD) factor ≥ 64 for the Fandooghi and the Kerman varieties, respectively, and 39 of them were related to precisely identified compounds. These included esters, pyrazines, aldehydes, acids, furans, and phenols. The results show that the Fandooghi variety presents, not only more odor-active regions but also higher FD factors than the Kerman variety that can lead to the conclusion that the first variety has a richer aromatic profile than the second one. The descriptive sensory analysis (DSA) showed that the roasted, chocolate/coffee, and nutty attributes were rated significantly higher in the Fandooghi variety, whereas the green attribute was significantly higher in the Kerman one.

KEYWORDS: pistachio aroma, key odorants, GCO, HS-SPME, Fandooghi variety, Kerman variety

INTRODUCTION

Among all the edible nuts, peanuts, almonds, hazelnuts, pecans, pine nuts, macadamias, pistachios, and walnuts are the most popular and commercially valuable. They have been an important source of nutrients^{1,2} since ancient times, and it has been proved that they play a relevant role in the human diet and moreover that they are of great benefit to consumers' health.^{3,4} Nowadays, a minor part of the nut crop is consumed natural or raw, while the majority is roasted and finally used as a snack or as an ingredient in the food industry. Several studies have been published on their chemical^{1,2,5–7} and, for most of them, volatile composition^{8–10} for both raw and roasted nuts, although it must be said that raw nuts have a rather bland aroma. Their characteristic nut odor is mainly due to some compounds generated during the roasting process.^{11–14}

Pistachio (*Pistacia vera* L.) is a member of the Anacardiaceae family, the only pistachio species that provides edible nuts. It is native of the arid zones of Central and West Asia, where it is distributed, as well as throughout the Mediterranean basin,¹⁵ and the Islamic Republic of Iran is the major supplier in the world with an annual production of 230000 t in 2007, followed by Turkey and the United States of America (<http://www.fao.org/corp/statistics/en>).

Some studies have been published on the chemical composition of pistachio^{6,16} and its antifungal,¹⁷ antimicrobial,¹⁸ and antioxidant¹⁹ activities; moreover, different studies have been carried out to determine its geographical origin and the variety effects.^{20–22} Nevertheless, only two previous studies have been published about its volatile composition,^{23,24} although only the latest study considered the contribution of these compounds to aroma. These kinds of studies are very important because, as in any other food product, pistachio quality is closely related to the

aroma detected by consumers, and this attribute will have a great influence on its acceptance or rejection.

The characteristic and unique aroma of every food commodity is attributed to a complex mixture of hundreds of different and very heterogeneous chemicals (alcohols, aldehydes, esters, ketones, pyrazines, acids, terpenes, etc.), present at variable concentrations (ranging from several mg kg⁻¹ to a few ng kg⁻¹), and each one of them with its own chemical properties and a different contribution to the whole aroma.²⁵ Indeed, it is well-known that, although the odorants must be volatile to reach the nose and interact with the appropriate receptors located on the olfactory epithelium,²⁶ only a limited number of volatiles have an actual contribution to the overall aroma.

For assessing the odor-active compounds in complex mixtures such as foods, gas chromatography–olfactometry (GCO) is the most appropriate analytical tool as it allows perceiving the eluted analytes by a conventional detector (FID, MSD) and the human nose, simultaneously. As a result, GCO provides both instrumental and sensory results.^{27,28}

Different techniques have been developed to collect and process the GCO data obtained in order to evaluate the contribution of each odorant to the sample aroma. Among them, the aroma extract dilution analysis (AEDA) is one of the most used.^{29,30} AEDA involves stepwise dilution of the aroma extract followed by an evaluation of each dilution by GCO until no odors are perceived in the GCO effluent; therefore, the last dilution step where an odorant is perceived is its flavor dilution (FD)

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factor, which can be considered a good indicator of the odor potency of that compound. This makes AEDA a very suitable and valuable screening tool for ranking odor-active compounds in a sample according to their relative odor potency.

However, prior to the GCO analysis, a representative aromatic extract has to be obtained to get a reliable aroma characterization. Indeed, the food flavor profile obtained will strongly depend on the isolation procedure performed. Among all the sampling techniques, the headspace solid-phase microextraction (HS-SPME) has proved to be a quick, solvent-free, and quite simple technique that requires very little sample manipulation,³¹ also when applied to the analysis of food aroma compounds.³²

Therefore, the aim of this study is to characterize the roasted pistachio nut aroma by GCO employing the HS-SPME technique, which, in a previous study performed in our laboratory,²⁴ has already demonstrated its suitability for providing representative pistachio aroma extracts. By application of the AEDA, the most potent odorants present in two of the most popular pistachio varieties (Fandooghi and Kerman) have been analyzed and compared.

MATERIALS AND METHODS

Samples. Representative samples of the Fandooghi (Iran) and Kerman (Spain) varieties were supplied from an important nut processing company whose quality control ensures the accomplishment of the specifications of each one of the varieties processed and also that the samples have been collected and transported under the optimal conditions. Moreover, to take into account the sample variability, different batches of pistachios were sampled, and these different aliquots were mixed in order to get a single sample as representative as possible.

To get similar products than those obtained in an industrial roasting treatment, for both varieties, portions of 1 kg of shell-free samples were roasted for 20 min at 160 °C in a laboratory electrical oven. After being roasted, the pistachio samples were vacuum-packed in 100 g portions into nonpermeable polypropylene/aluminum/polyethylene bags and stored at room temperature until used for analysis.

Reagents and Chemicals. The chemical standards of the aroma compounds were supplied by Sigma-Aldrich (Madrid, Spain) and Lancaster (Bischheim, France), and their purity was above 90% in all cases. Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). The oil used to determine the odor thresholds was a deodorized and distillate vacuum pump oil for research applications.

Headspace Solid-Phase Microextraction (HS-SPME). The SPME holder for manual sampling and the StableFlex Divinylbenzene-Carboxen-Polydimethylsiloxane (DVB/CAR/PDMS) fibers with a 50/30 μm film thickness used in this study were purchased from Supelco (Bellefonte, PA, USA). The fibers were conditioned before use and thermally cleaned between analyses by inserting them into the GC injector port at 270 °C.

The volatile compounds were extracted according to the optimum conditions determined in a previous study,²⁴ where the different parameters that influence the HS-SPME were carefully studied by assessing both the chromatographic areas and the number and intensity of the odorants extracted. Thus, 15 g of fresh roasted pistachios finely ground in a coffee mill and passed through a sieve (1.5 mm of diameter) with 15 mL of Milli-Q water (to homogenize the sample and accelerate the extraction^{24,33}) and a magnetic stir bar (as the extraction was carried out under constant magnetic stirring) were placed into a 50 mL glass vial, with the sample/headspace ratio of 1:1. Then, the vial was tightly capped with a silicon septum under nitrogen atmosphere, and it was pre-equilibrated for 15 min at 50 °C in a thermostatic bath. Afterward, the SPME device was manually pushed through the vial septum, and the

fiber was exposed for 2 h (shorter times did not ensure the suitable aromatic perception of all the compounds) at 50 °C (higher temperatures during such a long period could promote changes in sample composition) to the headspace vial. After extraction, the fiber was pulled into the needle assembly, and the SPME device was removed from the vial. It was finally inserted into the injection port of the GC for thermal desorption of the analytes at 270 °C for 1 min in splitless mode.

The precision of the method was assessed in terms of within-day repeatability and between-day repeatability (intermediate precision). These parameters were evaluated not only from the chromatographic response but also from the number, intensity, and quality of the odorants perceived. Both parameters were expressed by means of the percentage of relative standard deviation (% RSD). The first one was calculated by injecting, consecutively, 5 different extracts obtained the same day, while the second one was calculated from the results acquired when injecting 6 different extracts obtained over a month (approximately one extract every week). The results obtained were RSD < 4% for repeatability and RSD < 7% for intermediate precision. These low % RSD values allowed us to confirm that the precision of the optimized HS-SPME technique was very good.

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

The GCO analyses were carried out using a splitting assembly based on Capillary Flow Technology (Agilent Technologies, USA), where the end of the capillary column is connected, which enables the effluent to be split into the FID and the sniffing port. The split ratio for the olfactometric analysis was 1:1 (FID/sniffing port), and it was achieved by employing two deactivated and uncoated fused silica capillaries of the same length and width as a transfer line between the splitting assembly and the detectors. In addition, an olfactory detector control module commercialized by SGE International (Ringwood, Australia), which incorporates a heated transfer section from the GC oven to the glass detection cone, keeps the unit at a suitable temperature to transfer the volatile compounds 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.

Two trained sniffers (replaced at 15 min intervals to avoid fatigue and distractions) recorded the timing and description of the odors perceived during the elution of the sample extract compounds. Every sample was analyzed in triplicate.

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

Aroma Extract Dilution Analysis (AEDA). When working with the SPME, the analytes are retained on the fiber; therefore, no physical extract is obtained. Hence, the usual AEDA cannot be applied. In this study, the most odor-active compounds in roasted pistachios were hierarchically classified by an approach to the AEDA.^{24,34} It consisted of stepwise reduction of the amount of roasted pistachio (1:4) that was placed into the vial before performing the SPME, while a suitable amount of Milli-Q water was added in each dilution to keep constant the headspace/sample ratio. The dilutions were carried out until no odorant was detected by sniffing the highest dilution. Two trained sniffers performed the AEDA experiments, and their response (sensitivity) to the individual compounds did not differ, in any case, by more than 2 FD-factors.

Compound Identification. The identification of the odorants perceived was based on the comparison of three parameters of the unknown odorants with those of the standard compounds analyzed under identical conditions: odor quality perceived at the sniffing port, mass spectra obtained, and retention indices determined 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.

In some cases, due to the coelution of several compounds with similar aromatic qualities, their perception threshold constituted a helpful tool to determine the real contribution to the aromatic region considered. In these cases, because of the important fatty component of the sample, these thresholds were not determined in water but in a deodorized and distillate vacuum pump oil in order to get a suitable matrix.

Descriptive Sensory Analysis. The odor of the two different varieties of fresh roasted pistachios finely ground was evaluated by a panel of 8 trained nonsmoker assessors (5 women and 3 men, between 25 and 42 years old), all of them belonging to the Department of Analytical Chemistry of the Rovira i Virgili University. To get a more specific training on the aroma of roasted nuts, panelists were subjected to the following training: during 10 sessions, each lasting 60 min, they evaluated an array of different commercial roasted edible nuts (hazelnuts, almonds, and pistachios), describing sample odor qualities on the basis of their personal criterion. In that way, multiple odor qualities were achieved, and after an intense discussion, panelists finally agreed on a common list of 8 descriptors for pistachio nuts: green, sweet, roasted, chocolate/coffee, rancid, nutty, fatty, and earthy.

Finally, assessors evaluated the odor of the two different varieties of roasted pistachios analyzed in this study on a discontinuous scale from 0 (not detected) to 5 (maximum detection) for the 8 descriptors selected: green, sweet, roasted, chocolate/coffee, rancid, nutty, fatty, and earthy. Samples (20 g of fresh roasted pistachios finely ground) were singly presented to the panelists in dark glass flasks with random coded numbers (each sample was evaluated in triplicate). Sensory analyses were performed in a sensory panel room at 22 ± 2 °C. The coefficients of variance of each single panelist for every sample replicate of one sample were less than 10%. To evaluate the performance of the panel as a whole, one-way analysis of variance (ANOVA) was carried out.

RESULTS AND DISCUSSION

The sensory analysis of the two roasted pistachio varieties revealed important differences for some of the descriptors evaluated, as can be seen in Figure 1. Thus, although both cultivars gave comparable intensity values ($\alpha = 0.05$) for the sweet, rancid, fatty, and earthy descriptors, the odor profiles of the two varieties differed significantly ($\alpha = 0.05$) with regard to the green, roasted, chocolate/coffee, and nutty descriptors: while the Kerman variety presented a higher intensity for green, the Fandooghi cultivar presented a higher intensity for roasted,

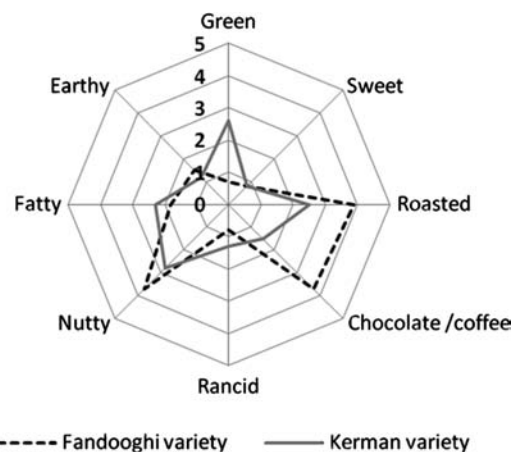


Figure 1. Odor profiles of freshly roasted pistachios.

chocolate/coffee, and nutty, descriptors usually associated with the roasted nut odor. Furthermore, the perception of these three descriptors, especially in this variety, was so high that it disguised the rest of attributes. These results agreed with the ones described in Table 1, where almost all of the different nutty odors detected for the two pistachio varieties presented a higher FD factor for the Fandooghi.

In the same way, when the Fandooghi variety was analyzed by HS-SPME and GC-FID, the chromatographic profile obtained was slightly richer -with more and higher peaks- than the one provided by the Kerman variety. This meant that the Fandooghi variety had a more complex volatile composition than the Kerman cultivar, but both HS-SPME extracts were analyzed by GCO to verify if this higher volatile complexity also implied a greater aromatic response. The results of these analyses confirmed that there was a difference in the number of odor-active regions detected when analyzing the SPME extracts of both varieties by GCO: while 74 different odor-active regions were perceived for the Fandooghi cultivar, only 60 were recorded for the Kerman one. However, nearly all of these latest 60 odor-active areas coincided, both in their retention times and the descriptors employed to define them, with different flavor-active regions detected for the Fandooghi variety. In both cases and as it happens in similar flavor studies when employing a polar column,^{12,14} chemical and fruity notes were perceived at lower retention indices, followed by green and earthy notes. At the end of the analysis appeared the lactic and fatty odors, followed by the burned and caramelized ones.

To get a first hierarchical classification of the odorants in roasted pistachio aroma, the volatiles extracted were analyzed by the new approach to the AEDA developed. The results are summarized in Table 1, where the odor-active areas with higher FD factors (ranging from 64 to 1024) have been arranged following their retention indices in the polar column. Forty-six and 41 flavor-active regions with a $FD \geq 64$ were detected for the Fandooghi and the Kerman varieties, respectively. As can be seen, the actual potent odorants present in pistachio aroma are almost the same, whatever the pistachio variety analyzed. The main difference with regard to the odorants lies in the perception intensity of some of these compounds, as a high FD factor obtained for a variety does not imply a high FD value for the other cultivar. Indeed, only 18 flavor-active regions were detected with the same FD factor for the two varieties: 8 regions with the FD of 1024 and 10 with the FD of 256, while no coincidence

Table 1. Main Odorants Found in Roasted Pistachio Nuts with FD \geq 64 for at Least One of the Varieties Analyzed^a

odor-active regions	RI on			FD factor		compound	identification				previously reported ^d
	CP-WAX	HP-5	odor description	F	K		MSD				
							F	K	RI	odor	
1	a	a	malty, solvent-like	256	256	isobutanal	X	X	X	X	
2	915	a	malty, bitter almonds	1024	1024	2-methylbutanal	X	X	X	X	11,12,14
3	941	nd	fruity, strawberry	64	nd	3-methylbutanal	X	X	X	X	
4	943	b	butter	1024	256	ethyl propanoate			X	X	
5	968	773	strawberry	256	nd	diacetyl	X	X	X	X	12,14
6	983	801	fruity	256	64	2-methylpropyl acetate			X	X	
7	991	702	butter	64	1024	ethylbutyrate			X	X	
8	994	843	fruity, apple	1024	256	2,3-pentanedione	X	X	X	X	11,12,14
9	1020	794	green, grass	1024	256	ethyl 2-methylbutyrate			X	X	11,14
10	1076	753	fruity	64	nd	hexanal	X	X	X	X	10,12,14
11	1183	896	fishy	256	256	(E)-2-pentenal	X	X	X	X	
12	1195		earthy	1024	1024	(Z)-4-heptenal			X	X	12,14
13	1233	1001	citrus, fresh	1024	1024	unknown					
14	1243	975	mushroom	256	256	octanal		X	X	X	12,14
15	1294	920	roasted nuts, corn	1024	1024	1-octen-3-one	X	X	X	X	12,14
16	1314		geranium	256	1024	2-acetyl-1-pyrroline*			X	c	12,14
17	1329	965	sulfur-like	256	256	unknown					
18	1343	997	fruity, sweet	256	64	dimethyltrisulfide	X	X	X	X	12,14
19	1369		rubber, plastic	1024	1024	2-ethyl-5-methylpyrazine	X	X	X	X	11,13
20	1388	nd	mushroom	1024	256	unknown			X	X	10,11
		1077				1-octen-3-ol			X	X	11
21	1402	1085	cooked potato	256	1024	3-ethyl-2,5-dimethylpyrazine	X	X	X	c	11,12,14
		907				2-ethyl-3,5(or 6)-dimethylpyrazine	X	X	X	X	12,14
22	1432		rubber, sulfur-like	1024	1024	methional			X	X	12,14
23	1434		anise-like, fennel	1024	nd	unknown					
24	1449	1150	fatty, green-like	256	256	2,3-diethyl-5-methylpyrazine	X	X	X	X	12,13
		1170				3,5-diethyl-2-methylpyrazine	X	X	X	c	12,14
25	1483	1160	paper-like	256	256	(E)-2-nonenal	X	X	X	X	12,14
26	1510	1232	cheese, disgusting	nd	1024	isobutyric acid			X	X	
27	1519	1026	roasted nuts, popcorn	256	16	2-acetylpyridine	X	X	X	X	12
28	1537		anise-like	1024	1024	unknown					
29	1561	nd	vomit, lactic	1024	4	butyric acid			X	X	12,14
30	1572		green pepper, earthy	256	256	unknown					
31	1591	1043	green roses	1024	256	phenylethanal	X	X	X	X	10,12,14
32	1616	879	blue cheese, sweaty	256	256	2-methylbutyric acid			X	X	12
						3-methylbutyric acid			X	X	12
33	1653	1324	deep-fried	64	256	(E,E)-2,4-decadienal			X	X	12,14
34	1715	1098	roasted nuts	1024	64	2-acetyl-2-thiazoline			X	X	12
35	1760	1520	fatty, flowery	256	256	methyl laurate*			X	X	
36	1776	1383	sweet, peach jam	1024	256	β -damascenone			X	X	14
37	1809	1093	smoky	1024	1024	guaiacol	X		X	X	12,14
38	1828	1039	sweety	256	256	phenylmethanol	X	X	X	X	
39	1865	1119	roses	64	nd	2-phenylethanol	X	X	X	X	10,14
40	1930		roasted, burnt	1024	64	unknown					
41	1951	1387	metallic	256	1024	trans-4,5-epoxy-(E)-2-decenal			X	c	12,14
42	1958	1358	sweet, honey	16	256	γ -nonalactone	X	X	X	X	12
43	2015	1284	disgusting, animal	256	nd	octanoic acid	X	X	X	X	
44	2022	nd	caramel	1024	256	Furaneol	X	X	X	X	12,14
45	2027	nd	urine	256	64	m-cresol	X		X	X	12
46	2116	nd	stall, animal	256	4	4-ethylphenol			X	X	
47	2151	1332	smoky, sweet	256	16	4-vinylguaiacol			X	X	

^a F, Fandooghi variety; K, Kerman variety; RI, retention index on different stationary phases; FD, flavor dilution factor; nd, not detected. *, tentative identification; a, RI not calculated due to solvent interference; b, RI < RI of the first alkane (C6); c, standard not available. ^dThe compound has been previously reported in roasted nuts in the given reference.

between the cultivars was found for the FD of 64. The 8 odor-active areas with a FD factor of 1024 were the regions 2 (malty, bitter almonds), 12 (earthy), 13 (citrus, fresh), 15 (roasted nuts, corn), 19 (rubber, plastic), 22 (rubber, sulfur-like), 28 (anise-like), and 37 (smoky), which were identified by mass spectra, retention indices, and the odor of the reference compounds as 2 and 3-methylbutanal (region 2), octanal (region 13), and guaiacol (region 37). The odorants responsible for the earthy, rubbery, and anise-like notes were not identified. Regarding region 15, we positively identified 2 pyrazines (2,6-dimethylpyrazine and 2-ethylpyrazine) that matched with the aromatic quality of this region in both columns used in this study. However, due to the high odor thresholds that these compounds showed in deodorized and distillate oil ($8000 \mu\text{g L}^{-1}$ and $20000 \mu\text{g L}^{-1}$), we assumed that another odor-active molecule should be responsible for the high FD factor that presented this region. Indeed, Chetschik et al.³⁵ demonstrated, very recently, the unimportance of these pyrazines in the overall aroma of peanut. However, in previous studies related to roasted nut aroma,^{12,14,35} it was pointed out that 2-acetyl-1-pyrroline was responsible for a “roasted, popcorn-like” odor detected at a retention index which coincided with our flavor-active area 15. Since this compound shows a very low detection threshold value ($0.053 \mu\text{g L}^{-1}$),³⁶ even being at very low concentration in roasted nuts,³⁵ it behaves as an important contributor to the overall aroma. This would explain why we could detect it by GCO but not by GC-MS. However, we could not corroborate this identification because its reference standard is not commercially available.

Among the 10 flavor-active regions with a FD factor of 256, 8 of them were chemically identified as shown in Table 1, the regions 1 (malty, solvent-like), 11 (fishy), 14 (mushroom), 17 (sulfur-like), 24 (fatty, green-like), 25 (paper-like), 32 (blue cheese, sweaty), and 38 (sweaty), and only the regions 30 (green pepper, earthy) and 35 (fatty, flowery) were not positively identified. However, with regard to the odor-active region 35, we found the compound methyl laurate as the one possibly responsible for this aromatic region. This hypothesis is due to the fact that the reference standard compound coincided with this region both in its odor and in its RI in both chromatographic columns used in this study. Moreover, although the GC-MS was not able to identify this compound, we found its most abundant m/z ratios at the retention time where this odor eluted when analyzing the spectrum. These facts, together with its low perception threshold ($150 \mu\text{g L}^{-1}$ in deodorized and distillate oil), led us to this assignment.

Although, as can be seen in Table 1, each aroma perceived during the GCO analysis is generally due to a single odorant, we found two aromatic regions which were generated by a mixture of compounds: number 21 (cooked potato) and 24 (fatty, green-like). The first one was positively identified as a mixture of 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5(or 6)-dimethylpyrazine, and methional (region 21), and the second one as a mixture of 2,3-diethyl-5-methylpyrazine, 3,5-diethyl-2-methylpyrazine, and 2-acetylfuran (region 24). Related to region 21, it could be thought that its high FD values were due mainly to methional because of its lower perception threshold in oil ($0.3 \mu\text{g L}^{-1}$ for methional and $5-50 \mu\text{g L}^{-1}$ for the pyrazines identified). However, the greater chromatographic peaks of the pyrazines and also the fact that the “cooked potatoes” note started to be perceived slightly before methional eluted from the column allowed us to ensure that this aromatic region was due to the mixture of the three compounds. However, concerning the aromatic region 24, besides

two pyrazines also 2-acetylfuran was positively identified. Nevertheless, although its odor quality coincided with the one detected in the region considered, its high perception threshold in oil ($500 \mu\text{g L}^{-1}$ when using a distilled and deodorized oil) was a good indicator that this compound was not responsible for such high FD factors. Therefore, we concluded that aromatic region 24 was only due to the mixture of the two pyrazines identified.

When analyzing the differences between varieties, six odor-active regions were detected for the Fandooghi cultivar but not for the Kerman: 3 (fruity, strawberry), 5 (strawberry), 10 (fruity), 23 (anise-like, fennel), 39 (roses), and 43 (disgusting, animal), which correspond to ethyl propanoate (region 3), 2-methylpropyl acetate (region 5), (*E*)-2-pentenal (region 10), 2-phenylethanol (region 39), octanoic acid (region 43), and one unknown odorant (region 23). On the contrary, only flavor-active area number 26 (cheese, disgusting), which was identified as isobutyric acid, was detected for the Kerman but not for the Fandooghi variety. Furthermore, while 22 odor-active regions were perceived in the Fandooghi cultivar with a higher FD factor than in the Kerman cultivar, only 6 odors appeared in this second variety with a higher FD factor: 7 (butter), 16 (geranium), 21 (cooked potato), 33 (deep-fried), 41 (metallic), and 42 (sweet, honey), which were identified as 2,3-pentanedione (region 7), a mixture of 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5(or 6)-dimethylpyrazine, and methional (region 21), and γ -nonalactone (region 42). Region number 16 was not positively identified. With regard to odor-active region 33 (deep-fried), when using the polar column, we found that this odor-active region could be due to (*E,E*)-2,4-decadienal but also to (*E,E*)-2,4-nonadienal because both compounds coeluted. However, although the last one was present among the roasted pistachio volatiles, as well as among the odorants of other roasted nuts,^{12,14} the GCO analyses by using the HP-5 column allowed us to identify them separately, and we could identify the (*E,E*)-2,4-decadienal as the one responsible for this odor-active region. Regarding number 40 (metallic), it has been related to *trans*-4,5-epoxy-(*E*)-2-decenal. Although no reference compound is available, the identification was done on the basis of the odor perceived, the retention indices calculated on the CP-WAX and HP-5 stationary phases, the literature,^{12,14} and the Flavornet database.³⁷

From the results obtained, it can be observed that most of the key odorants of roasted pistachio aroma determined in this study have been previously reported as volatile or aromatic compounds in other roasted nuts.¹⁰⁻¹⁴ This can lead to the conclusion that there is a group of coincident odorants that influences the characteristic roasted nut odor and that they are almost the same, whatever the nut considered. However, we should not forget the role played by those specific compounds present in each nut. Only the global perception of their own characteristic notes, together with the different perception intensity of the coincident compounds, provides the aromatic differences among nuts and among varieties of the same type of nut.

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