Matching Changes in Sensory Evaluation with Physical and Chemical Parameters

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ORIGINAL PAPER

Matching Changes in Sensory Evaluation with Physical and Chemical Parameters

A Case Study: Argentinean Pistachio Nuts (*Pistachia vera* L. cv Kerman)

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Abstract Changes in pistachio nuts induced by roasting and salting processes, evaluated by both sensory and instrumental analyses are reported, looking for a match in both methods. Dried (DP), roasted (RP), and salted–roasted pistachios (SRP) were studied, including four groups of sensory attributes, six basic chemical parameters, complemented by volatile organic compounds (VOCs) and breaking force. Multivariate statistics helped with data interpretation, enabling correlations between two groups of variables. Key results show that the perception of roasting is associated with increased amounts of α-pinene and 3-carene in RP and SRP, while DP are associated with higher amounts of limonene, moisture, and bitterness. Sensory and

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puncture test measurements (texturometer) showed opposite results, which could be explained considering the loss of plasticity in RP and SRP. Current results present a novel approach for the evaluation of changes in the perception of pistachio quality by consumers, supported by both sensory and instrumental analyses using multivariate statistics for data evaluation.

Keywords Pistachio nut · Sensory analysis · Volatile compounds · Multivariate statistics

Introduction

Nuts are globally popular and valued for its nutritional and sensory attributes. Fresh natural kernels are consumed mainly as whole nuts or used in various confectioneries. Since ancient times, it has been proved that they play an important role in human diet, bringing benefits to health of consumers. Additionally, kernels are a rich source of oil (50–60 % of their composition), containing essential fatty acids (linolenic and linoleic) as well as oleic acid (USDA 2010; Emadzadeh et al. 2012).

Pistachio nuts share previously mentioned characteristics but also contains proteins, carbohydrates, fibers, minerals, vitamins, and other hydrosoluble compounds like polyphenols and anthocianes (Chen et al. 2006; Amoo et al. 2012). These nuts are highly nutritious (Dreher 2012). Furthermore, different Pistachio varieties are reported as medicinal plants (Flamini et al. 2004), while its essential oils are reported having antioxidant, antibacterial, antifungal, and neuroprotective potential (Alma et al. 2004; Orhan et al. 2012).

Pistachio (*Pistacia vera* L.) is a member of the Anacardiaceae family, which is a species native from Central and Western Asia but currently distributed throughout the Mediterranean basin (Gentile et al. 2007). In the 1980s, the first seeds of pistachio were introduced to Argentina, being the Province of San Juan the main producer, mainly because of environmental factors and calcium-rich soils, allowing the production of a wide open healthy pistachio nut, with nice aromatic taste. All of these characteristics are highly appreciated by consumers at national and international markets (Pisté 2012).

The quality of pistachio nut is usually related to its moisture content and biochemical composition (e.g., carbohydrate, fat, and protein contents) (Crane 1978). In spite of its nutritional characteristics, the aroma detected by consumers will determine its acceptance or rejection (Aceña et al. 2010; Aceña et al. 2011). Moreover, there are reports indicating that some biochemical characteristics can be affected by rootstock, ecological conditions, growing areas, and horticultural practices (Kader et al. 1982; Seferoglu et al. 2006).

Consumer perception (flavor and texture) is an important aspect defining the quality of food products, determining their acceptability (Muñoz et al. 1992; Vincent 2004). Nowadays, pistachio is rarely consumed raw (natural) but roasted and salted to be used as a snack. Moreover, pistachio is used as flavoring in cookery and confectionery and, because of the deep green color of its kernel, also employed in ice creams and pastry industries.

During the drying process (40 °C), nuts can undergo undesirable oxidative reactions, mainly associated almost with unsaturated fatty acid (linolenic, linoleic, and oleic acid), which cause a decrease of its quality, producing odd colors and flavors (Fennema 1985). Nuts dried to low moisture content (0.04 gg^{-1} dry solids) present higher crispness and sweetness but lower bitterness and rancidity in comparison to nuts dried to a higher moisture content (0.06 to 0.11 gg^{-1} dry solids). Consequently, nuts dried to 0.06 gg^{-1} moisture also present higher sweetness but lower bitterness and rancidity than those dried to 0.11 gg^{-1} moisture (Kader et al. 1982). Normally, after the drying process, pistachio is roasted to increase its overall palatability (Nikzadeh and Sedaghat 2008). The roasting process (90–150 °C) modifies the original constituents of pistachio (fatty acids, sugars, and amino acids) more than the drying process (Luh et al. 1981). Simultaneously, the flavor, color, texture, and appearance are also modified during heating (Vincent 2004; Nikzadeh and Sedaghat 2008; Raei et al. 2009; Shakerardekani et al. 2011). Therefore, analyses of volatile organic compounds (VOCs) coupled to sensory analysis could be useful in predicting changes in VOC profile, leading to variations in consumers' response to pistachio (Aceña et al. 2010; Aceña et al. 2011). Moreover, the loss of moisture during roasting also results in a more rigid product, which can be perceived as crispy by consumers. This last attribute could be associated with the rupture force measured by physical methods (texturometer).

There are scarce published data describing how roasted and salted–roasted processes affect the color, the texture, and the appearance of pistachio (Kader et al. 1982; Luh et al. 1981; Kashani-Nejad et al. 2003; Nikzadeh and Sedaghat 2008; Tsantili et al. 2010; Shakerardekani et al. 2011). Aceña et al. (2010) studied the contribution of volatile compounds to roasted pistachio (RP) aroma, although these authors did not report enough information on the relationship between descriptive sensory analysis and VOC patterns. Also, the VOC profile reported by Aceña et al. (2010) did not agree with other reports on VOCs in the plant, fruits, and essential oil of pistachio. This difference in VOC profile could be attributed to the use of diverse analytical methods (Flamini et al. 2004; Aceña et al. 2010; Orhan et al. 2012). Moreover, the VOC profile and other characteristics for Argentinean pistachios have been not yet reported.

So far, the main goal of this study was to evaluate changes in sensory properties, breaking force (texturometer), and chemical parameters (basic composition and VOC profile) among dried pistachios (DP), RP, and salted–roasted pistachios (SRP), thus, searching for evidences on the correlation among descriptive sensory analysis and physical–chemical changes. This correlation should enable matching changes in instrumental measurable parameters (breaking force, basic chemical parameters, and VOC profile) with the consumer perception, bringing a useful tool for the study of process-induced changes affecting both the chemical composition and the consumer perception of food quality.

Materials and Methods

Pistachio nuts (*P. vera* L. cv Kerman) were obtained from an industrial factory located in the province of San Juan, Argentina. This factory has its own plantation, from where raw pistachios nuts were obtained. The sampling area is located at both riverbanks of San Juan River (latitude 31° S, longitude 69° W). The altitude varies from 650 to 750 m asl.

Pure VOCs, bovine albumin, citric acid, and caffeine were purchased from Sigma-Aldrich Argentina (Buenos Aires, Argentina). Sucrose and NaCl were purchased from Merck Química Argentina (Buenos Aires, Argentina). Ultrapure water (conductivity≤0.05 μS cm^{-1}; TOC≤5 μg L^{-1}) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). All reagents were of analytical grade.

Sample Treatment

After collection, raw pistachio nuts were dried at 40 °C (4 h) up to 3 % moisture content in a grain drier (Mega S.A., Argentina). DP were roasted (RP) using a rotating bakery oven (Argental, Argentina) at 120 °C for 90 min. SRP were prepared by immersion of DP in a brine solution (NaCl 10 % w v^{-1}) during 1 min, slurred and then toasted in according to the experimental procedure for RP. All samples were collected immediately after drying or roasting and stored under vacuum in individual bags (500 g each) at 5 °C until further use. Seven independent samples from each treatment were stored (seven DP, seven RP, and seven SRP).

Pistachio Nut Elemental Composition

Samples were analyzed determining total oil, protein, moisture, and ash content in accordance to standard AACC methods (AACC 2000). Oil extraction was performed at 20 °C using a Komet screw press (Model CA 59 G, IBG Monforts, Germany). The peroxide value for oil samples was determined using Cd 8b-90 standard AOCS method. This method determines all substances, in terms of milliequivalent peroxide per 1,000 g of test sample, which oxidize potassium iodide under the conditions of the test (AOCS 2009).

Sensory Descriptive Analysis

A total of 12 trained panelists (ten females and two males) participated during the descriptive analysis of pistachio nuts. The number of panelists was decided considering previous reports (Meilgaard et al. 1991; Anzaldua-Morales 1994). All panelists were selected considering the following criteria: (1) people without food allergies, (2) nonsmokers, (3) people with complete natural dentition, (4) between 18 and 64 years old, (5) nut's consumers, (6) availability for sensory sessions, (7) interest in participating, and (8) capability for verbally communicating observations (Plemmons and Resurreccion 1998). All panelists were trained and calibrated during 24 sessions (2 h each) over 3 months.

Descriptive analysis was performed in accordance to Nepote et al. (2009). A 10 cm unstructured line scale (0–10) was used for sample evaluation. A list of attribute definitions, a sheet with warm-up (nonsalty medium-roasted commercial pistachio nut from EEUU) as well as intensity ratings and references (a material that clearly represents each attribute intensity) were developed during training sessions (Table 1). Attributes and their definitions were established by panelists, resulting in nine descriptors for pistachio nuts: roasted, oxidized, bitterness, salty, sour, sweetness, hardness, crunchiness, and glossy. The definition of these attributes was based on previously reports on descriptive sensory analysis of nuts (Kader et al. 1982; Nepote et al. 2009; Martínez et al. 2011). All samples were evaluated using partitioned booths, under fluorescent light at room temperature. Thirty nuts from each treatment (DP, RP, and SRP) were placed in plastic cups, coded using three-digit random numbers. Sample temperature was kept constant (28±2 °C). Panelists were instructed to take a sample of three nuts in the mouth, chewing (12 times) to swallow the sample, followed by the evaluation of aromatic and basic taste attributes. Odorless water and green apple were used for cleansing the mouth between samples. Each panelist evaluated three samples per day, in addition to a warm-up sample, until completing the evaluation of the entire sample set (n=21). Before starting the sensory evaluation, all panelists retested both references and warm-up samples. The final lists of warm-up and reference ratings as well as established definitions were posted in the booths during all test sessions. Samples were tested using a randomized complete block design. Results were registered on paper ballots, which were recorded in a personal computer for data processing.

Analysis of VOCs in Pistachio Nut Samples

Head-Space Solid Phase Extraction

The evaluation of VOC profiles was performed by head-space solid phase extraction (HS-SPME), followed by gas chromatography coupled to mass spectrometry (GC–MS). A

Table 1 Definition of attributes and standard reference intensity ratings used during the descriptive analysis of pistachio nuts

Attribute	Definition[a]	References[b]	Intensity[e]	Warm-up intensity[e,f]
Aromatics				
Roasted	The aromatic associated with medium-roasted pistachio	Sunflower kernels (Grandiet, Cordoba, Argentina)	3	6.4
		Toasts (Criollitas, Bagley, Cordoba, Argentina)	8	
Oxidized	The aromatic associated with rancid fats and oils	Mixture of 10 % rancid sunflower oil[c] in oil[d]	2	0.3
		Mixture of 20 % rancid sunflower oil[c] in oil[c]	5.5	
Basic tastes				
Sweetness	Taste on the tongue associated with sucrose solutions	0.5 % sucrose solution	0.5	3.5
		2 % sucrose solution	3	
Bitterness	Taste on the tongue associated with bitter solutions such as caffeine	0.05 % caffeine solution	1.2	0.2
		0.08 % caffeine solution	4.5	
Salty	Taste on the tongue associated with sodium chloride solutions	0.2 % NaCl solution	1	0.3
		0.35 % NaCl solution	3	
		0.5 % NaCl solution	7	
Sour	Taste on the tongue associated with acid agents such as citric acid solutions	0.05 % citric acid solution	1.5	0.2
		0.08 % citric acid solution	5	
Texture				
Hardness	Force needed to compress a sample (a unit) between molar teeth	Walnut (Grandiet, Cordoba, Argentina)	2.5	4.6
		Almond (Grandiet, Cordoba, Argentina)	6	
Crunchiness	Force needed and amount of sound generated from chewing a sample (a unit) with molar teeth	Roasted peanut (Grandiet, Cordoba, Argentina)	4.5	3.7
		Corn flakes (Granix, Buenos Aires, Argentina)	8	
Appearance				
Glossy	The appearance associated with the amount of light reflected by the product surface	Peeled crude peanut (Grandiet, Cordoba, Argentina)	2.5	3.9
		Peanut coated with chocolate (ARCOR, Colonia Caroya, Córdoba, Argentina)	8	

[a] Attribute definitions are based on the literature (Kader et al. 1982; Nepote et al. 2009; Martínez et al. 2011)

[b] Reference: a material that clearly represents the intensity for each attribute

[c] Rancid sunflower oil obtained from Rancimat test (air flux 20 Lh^{-1} , 110 °C, exposition time 10 h)

[d] Commercial sunflower oil ("Natura," General Deheza, Córdoba, Argentina)

[e] Intensity ratings are based on 10 cm unstructured line scale (0–10)

[f] Warm up: nonsalty medium-roasted commercial PN (EEUU)

50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellafonte, PA) 1-cm-long SPME fiber was used for the fractionation of volatile and semivolatile compounds from the headspace (HS) of samples, conditioned in the extraction unit of a Combi PAL autosampler (Varian, Inc., USA, CA). The fiber was first conditioned for 1 h at 270 °C in the injection port of the GC using helium (5.0 grade). The absence of carry over effect was checked by extracting and injecting blanks every six samples. All samples were analyzed in triplicate.

Gas Chromatography–Mass Spectrometry

Analyses were performed on a GC (Varian Saturn 3800) equipped with a Combi PAL autosampler (Varian, Inc., USA, CA). Samples were grinded using ceramic mortar and pestle, introducing 1 g each into glass vials (10 mL) having magnetic screw caps (CTC Combi PAL, PN 20091405; Supelco, USA) and ultraclean, thin premarked septum (CTC Combi PAL, PN 18032063; Supelco, USA). Attempts to use different septa resulted in bending and further destruction of the needle containing the SPME fiber during the extraction. Prior to SPME extraction, samples were conditioned adding 1 mL ultrapure water containing 100 mg NaCl. SPME extraction was performed in the extraction unit of the Combi PAL autosampler during 50 min at 50 °C, shaking at 250 rpm. The GC was equipped with a Factor Four (Varian, Inc., CA, USA) capillary column VF-5; 30 m x 0.25 mm ID; DF=0.25 μm, a 1079 injector equipped with Merlin seals working in splitless mode. Mass detector was an ion trap (Varian 4000, CA, USA), operated in the electron ionization mode at 10 mV, scanning the range *m/z* 40–650. The SPME fiber was desorbed at 250 °C during 3 min, with further heating during 5 min at 250 °C in the purge unit of the Combi PAL autosampler using nitrogen (5.0 grade) to flush the purge chamber. The oven temperature was programmed starting at 40 °C, held at this temperature during 4 min, increased to 220 °C at a rate of 5 °C min^{-1}, followed by a final increase to 250 °C at a rate of 20 °C min^{-1}; the total run time was 40 min. The carrier gas was helium (ultrapure, grade 5.0) at a constant flow of 1 mL min^{-1}.

Identification and Quantification of VOCs

The initial identification of VOCs (qualitative) was performed during preliminary runs with pistachio nuts by comparison of the GC retention times and mass spectra with both pure compound and match with spectral library (NIST MS search 2.0). The method and compound identity were further verified by the addition of pure compounds to pistachio samples (spiked samples). A relative quantitation was performed using pure compounds, dissolved in ultrapure water. Thus, a calibration plot was constructed by linear regression using the peak area resulting from variable amounts of each pure compound. Considering that the calibration solutions and samples have different analytical matrices, VOC results reported here are only comparative for different pistachio nuts treatments. Sample analyses were carried out in triplicate. The limit of detection (LOD) was taken at a signal-to-noise ratio (S/N) of 5, while the limit of quantification (LOQ) was taken at a S/N of 15.

Puncture Test (Texturometer)

DP, RP, and SRP samples were measured using an Instron Universal Testing Machine (Model 3342, Instron Inc., USA). Pistachios were first divided into two halves using a thin knife, placing each half on an aluminum plate (flat surface side down). A 1.5 mm diameter probe punctured completely through each sample. The probe was set to move down at a speed of 0.5 mm s^{-1} until the texturometer reached a force of 0.1 N, at which point the probe continued moving down into and through the pistachio until fracturing it. The maximum force (*N*), from the force-deformation curve, was used to express the breaking force of the sample. Seven replicates (two halves each) were measured for each set of samples (DP, RP, and SRP; $n=7$). Data were analyzed using the Blue Hill 2 software (Instron Inc., USA).

Statistical Analysis

Analytical determinations were averaged over triplicate measurements from independent samples. Statistical differences were estimated by ANOVA test at the 95 % level ($p<0.05$) of significance. Whenever ANOVA indicated a significant difference, a pairwise comparison of means by least significant difference (LSD) was carried out. Considering previous experiences (Baroni et al. 2006; Di Paola-Naranjo et al. 2011), multivariate statistics was used to point out differences between treatments as well as matches or discrepancies between sensory and instrumental analyses (VOCs and puncture test). Thus, cluster analysis (CA), principal components (PC), factor analysis (FA), and stepwise linear discriminant analysis (LDA) were applied to the dataset looking to verify differences among DP, RP, and SRP.

The statistical package STATISTICA 7 from StatSoft (2005) was used for statistical calculations.

Results and Discussion

Pistachio Nuts Elemental Composition

Changes in pistachio nut basic composition, induced by different treatments (DP, RP, and SRP) are shown in the Table 2. Pistachio nuts (DP, RP, and SRP) had lower moisture content (0.013–0.033 g g^{-1} dry solids) than values reported by Kader et al.(1982) and Kashani-Nejad et al. (2003) (0.04 to 0.06 g g^{-1} dry solids). The moisture content is important for food safety (e.g., not supporting fungal grow) and also for the flavor of the product (Kader et al. 1982). The significant decrease of moisture content in pistachio kernels upon roasting at 120 °C (Table 2, DP and RP) has been also reported by Nikzadeh and Sedaghat (2008). Additionally; it was observed that moisture levels of kernels were gently increased after salting. This may be explained by the immersion of nuts in the brine solution (10 % w v^{-1} NaCl), increasing the initial moisture content. Current results also indicate that the industrial process of pistachio nuts to obtain SRP increased the amount of ashes in pistachio kernels, presenting significant differences with DP and RP (Table 2).

No significant effects of drying and roasting processes on protein and fat contents were observed. The crude protein, ranging from 21 to 22 g 100 g^{-1} dry solids, was in agreement with values reported by Tsantili et al. (2010) but lower than those found in pistachios from California (Kader et al. 1982).On the other hand, Luh et al. (1981) described that drying, blanching, and roasting produce the loss of sugars present in the kernel.

Also, drying and roasting processes resulted in changes in the peroxide value of pistachio nut oils, showing a significant increase in the peroxide index between DP, RP, and SRP (Table 2). These results are in agreement with those reported by Swern (1964), indicating an increase in the lipid peroxidation, mainly during roasting.

Table 2 Raw composition and peroxide values of studied pistachio nuts

Parameter	Dried pistachio (DP)	Roasted pistachio (RP)	Salted–roasted pistachio (SRP)
Moisture content ($g g^{-1}$ dry solids)	0.033^{c}±0.001	0.0135^{a}±0.002	0.021^{b}±0.001
Total protein ($g g^{-1}$ dry solids)	0.216^{a}±0.003	0.223^{a}±0.004	0.215^{a}±0.006
Ashes ($g g^{-1}$ seed)	0.029^{a}±0.001	0.030^{a}±0.001	0.047^{b}±0.001
Carbohydrates ($g g^{-1}$ seed)	0.232^{b}±0.005	0.243^{b}±0.022	0.172^{a}±0.003
Oil content ($g g^{-1}$ seed)	0.498^{a}±0.001	0.495^{a}±0.016	0.508^{a}±0.003
Peroxide index (meq peroxide kg^{-1} oil)	1.56^{a}±0.04	2.02^{b}±0.25	4.29^{c}±0.15

Reported values are means ± standard deviation (n=7). Different superscript letters at each column indicate significant differences between pistachio nut products ($p \leq 0.05$)

Sensory Evaluation: Descriptive Analysis in Pistachio Nuts Samples

Nine sensory attributes with their respective intensity (determined by panelists) were used during the descriptive analysis of pistachio nuts. Results from sensory analysis are presented in Table 1. Diverse effects of roasting and salting treatments were detected by trained panelists. The roasted flavor was higher in SRP and RP than in DP (Table 3), showing significantly different scores between three treatments. Probably, the salt present in SRP produced a stimulation of the taste buds, intensifying its perception; a similar behavior was observed for the oxidized flavor. As it is almost obvious, the salty perception was much higher in SRP with respect to DP and RP. It is important to highlight that all samples presented an oxidized perception close to zero, which is important for the quality of the product considering that this is a negative attribute for consumers (Table 3).

Panelists detected few significant differences in sweetness scores, with DP and RP presenting a little higher sweetness than SRP (Table 3). On the other hand, intensities for basic tastes (bitterness and sourness) were not significantly different (p>0.05) between DP, RP, and SRP.

Conversely, there were significant differences between DP, RP, and SRP with respect to the texture and appearance attributes. Hardness is widely used as a texture attribute in nuts. During roasting, the texture became more crumbly and fragile (less hard) (Vincent 2004). Our current results show that RP and SRP had higher values for hardness, while crunchiness was significantly higher in SRP in comparison with DP and RP (Table 3).

Physical and Chemical Analyses

Puncture Test

Texture influences the mouth feel of a product during eating (Jha et al. 2011). During this test, the evaluation of the rupture force of a pistachio sample, using a texturometer, was evaluated. Results are shown in Fig. 1a. SRP showed the lower breaking force, while DP and RP presented similar values among them. This result is opposite to that obtained during sensory analysis (Table 3), where trained panelists assigned the higher hardness to RP and SRP. These contradictory results could be explained considering differences in the sample plasticity between DP and RP-SRP. Apparently,

Table 3 Attribute values for descriptive analysis of pistachio nuts

Attribute	Dried pistachio (DP)	Roasted pistachio (RP)	Salted–roasted pistachio (SRP)
Aromatics			
Roasted	5.34^{a}±0.97	6.24^{b}±0.23	6.90^{c}±0.20
Oxidized	0.31^{a}±0.07	0.33^{a}±0.05	0.43^{b}±0.10
Basic tastes			
Sweetness	3.51^{b}±0.40	3.35^{b}±0.15	2.12^{a}±0.43
Bitterness	0.26^{a}±0.02	0.24^{a}±0.02	0.24^{a}±0.02
Salty	0.29^{a}±0.04	0.32^{a}±0.12	6.25^{b}±1.34
Sour	0.18^{a}±0.01	0.18^{a}±0.01	0.20^{a}±0.03
Texture			
Hardness	3.98^{a}±0.29	4.10^{b}±0.45	4.45^{b}±0.38
Crunchiness	3.80^{a}±0.27	3.65^{a}±0.53	4.28^{b}±0.57
Appearance			
Glossy	4.33^{b}±0.52	4.32^{b}±0.56	3.62^{a}±0.56

Different superscript letters in each column indicate significant differences between pistachio nut products ($p \leq 0.05$)

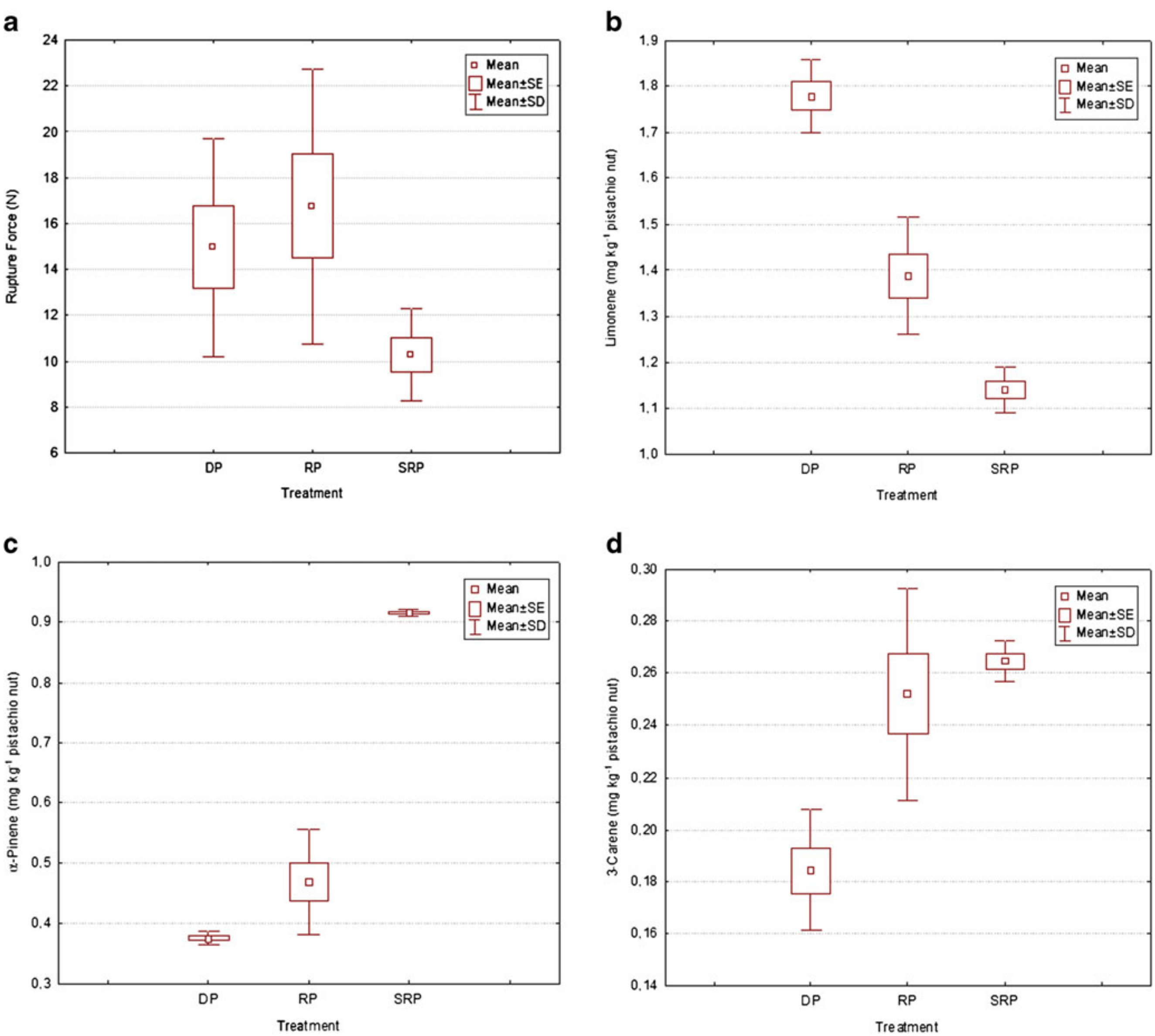

Fig. 1 **a** Rupture force (puncture test) pointing out differences between three treatments (DP, RP, and SRP) in pistachio nut products. *DP* dried pistachio, *RP* roasted pistachio, *SRP* salted–roasted pistachio. **b** Limonene content pointing out differences between three treatments (DP, RP, and SRP) in pistachio nut products. *DP* dried pistachio, *RP* roasted pistachio, *SRP* salted–roasted pistachio. **c** α-Pinene content pointing out differences between three treatments (DP, RP, and SRP) in pistachio nut products. *DP* dried pistachio, *RP* roasted pistachio, *SRP* salted–roasted pistachio. **d** 3-Carene content pointing out differences between three treatments (DP, RP, and SRP) in pistachio nut products. *DP* dried pistachio, *RP* roasted pistachio, *SRP* salted–roasted pistachio. **b**, **c**, **d** LOD ($mg L^{-1}$) calculated for pure compounds dissolved in water: α-pinene (0.021), 3-carene (0.008), and limonene (0.010). LOQ ($mg L^{-1}$) calculated for pure compounds dissolved in water: α-pinene (0.063), 3-carene (0.025), and limonene (0.030)

when measuring the breaking force, DP were more compressible (plastic), needing a higher energy to be broken than RP and SRP. Conversely, RP and SRP were detected as harder by panelists because of their lack of plasticity.

VOC Analysis

Based on previous experiences (Baroni et al. 2006), qualitative and semiquantitative analyses of VOCs using pistachio samples were done (two samples from each treatment) to establish the best chromatographic conditions, retention times as well as the approximate analytical range to be used during the effective quantitation of selected VOCs. This first exploratory analysis allowed the detection of more than 30 compounds, which were tentatively identified by comparison with NIST MS library. After background (blank) subtraction and removal of compounds arising from plastic bags used (phthalic acid esters), 22 compounds were tentatively identified (Table 4). Considering relative amounts and occurrence in three studied treatments (DP, RP, and SRP), only major and minor compounds (α-pinene, 3-carene, and limonene) were further used for analysis (confirmation with pure compounds and quantitation), excluding compounds found in trace amounts.

VOC analysis shows that Argentinean pistachio nuts are rich in monoterpenes, mainly limonene (citrus, mint), α-pinene (pine, turpine), and 3-carene (lemon, resin) (Table 4, Fig. 1b–d). These results coincide with those reported by Parfitt et al. (2005) for pistachios dried at commercial temperatures from California. Our current results also agree with several reports on the presence of monoterpenes in pistachio essential oils (Flamini et al. 2004; Orhan et al. 2012) but are, to some point, contradictory with compounds reported by Aceña et al. (2011), who did not inform on the presence of terpenes within VOCs identified by SPME–GC–MS/olfactometry. Moreover, European cultivars have different flavor characteristics. For instance, Tsantili et al. (2010) evaluated the variety Kerman from Greece, Iran, and Syria with the highest scores for overall flavor. Also, Aceña et al. (2011) reported differences between Kerman and Fandooghi varieties, the last one being richer in flavors than the first.

Comparing Fig. 1b, c, and d, we can see that limonene is the major component in DP. However, a significant change in the relative amount of monoterpenes using different treatments (DP, RP, and SRP) is also observed. So, the amount of limonene decreases from DP to SRP (Fig. 1b) but α-pinene and 3-carene contents were increased during roasting and salting procedures (Fig. 1c and d). These results suggest that roasted and salted process influence the content of monoterpenes, being possible to define a VOC (limonene) as marker for unroasted pistachio, while α-pinene and 3-carene seem to be associated with RPs. Alsavar et al. (2003) observed large differences between natural and roasted hazelnut, where concentrations of several classes of compounds, like 3-carene, were increased upon roasting. Also, Gogus et al. (2011) reported changes in VOCs of

Table 4 List of compounds in studied pistachio nuts, tentatively identified by GC–MS

Retention time (min)	Compound (MS-id)	Relative amount	DP	RP	SRP	Previously reported
8.95	α-Pinene[a]	Main cpd.	XX	XX	XX	[b]
10.71	β-Pinene	Traces	XX	ND	X	[b]
10.99	*n*-Decane	Traces	XX	ND	XX	[c]
11.32	3-Carene[a]	Min. cpd.	XX	XX	XX	[d]
11.96	Limonene[a]	Main cpd.	XX	XX	XX	[b]
12.64	2-Propyl-1-heptanol	Traces	XX	ND	XX	
13.30	7-Hexadecene	Traces	ND	ND	X	
17.05	3-Methyldecane	Traces	XX	ND	X	
17.42	Methyl salicylate	Traces	XX	ND	ND	
19.83	1-Dodecanol, 3,7,11-trimethyl	Traces	XX	ND	ND	
20.06	7-Tetradecene	Traces	XX	ND	ND	
20.28	2-Hexyl-1-decanol	Traces	XX	ND	ND	
21.55	*o*-Bromophenyl methyl ketone	Traces	XX	ND	ND	
22.52	Myristic acid	Traces	ND	XX	X	[e]
22.53	2,3-Dimethyldecane	Traces	XX	ND	ND	
22.95	Dodecanal/pentadecanal	Traces	XX	XX	X	
25.7	2,4-di-*tert*-Butylphenol	Traces	XX	ND	ND	
26.72	Ethylparaben	Traces	XX	ND	ND	
27.13	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	Traces	XX	XX	XX	
28.39	2′,5′-Dichloroacetophenone	Traces	XX	XX	XX	
32.33	Isopropyl myristate	Traces	XX	ND	X	
37.67	1-Propylpentyl laurate	Traces	XX	XX	XX	

Identification based on ≥75 % matching with NIST 2005 spectral library

ND not detected, *X* identified at least in three samples from this treatment, *XX* identified in all samples from this treatment

[a] Identity confirmed using pure compound and spiked samples

[b] Flamini et al. (2004), Orhan et al. (2012), Gogus et al. (2011)

[c] Gogus et al. (2011)

[d] Alma et al. (2004), Tsokou et al. (2007), Orhan et al. (2012), Gogus et al. (2011)

[e] Tsantili et al. (2010)

roasted and unroasted *Pistacia terebinthus*. These authors observed a drop of limonene during first 5–10 min roasting, but a return to initial values after 25-min roasting, which means that the amount of limonene in RPs could depend on the roasting time. Moreover, Gogus et al. (2011) found a drop in the amount of α-pinene, 2-carene, and 3-carene upon 20-min roasting. So far, our current results are contradictory with those reported by Gogus et al. (2011). It is worthy to mention that we used a unique higher time (90 min) in an industrial oven, reaching 120 °C instead of 250 °C. So, we used much more time but less temperature for roasting than those reported by Gogus et al. (2011), which could explain these differences, in addition to different pistachio varieties studied. Furthermore, the report of Gogus et al. (2011) did not simulate the salting procedure, which strongly influenced the drop of limonene and the increase of α-pinene and 3-carene. To our knowledge, this is the first report on VOC profile corresponding to DP, RP, and SRP nuts from Argentinean pistachios (*P. vera* L. cv Kerman).

There is still an open question to explain the drop of limonene and the rise of α-pinene and 3-carene during roasting or salting–roasting procedures. There are many scientific reports on the thermal isomerization of α-pinene to limonene (Stolle et al. 2008), but any pointing out that the inverse reaction is possible. So far, we should discard a thermal isomerization of limonene to α-pinene and 3-carene as the main cause of changes in the quantitative profile of monoterpenes observed during this work. Thus, we could suggest that the drop of limonene could be due to hydrodistillation from the pistachio kernel during roasting (together with removed moisture), while the augmented α-pinene and 3-carene could be the consequence of further biosynthesis of these compounds from their natural precursors as far as enzymatic systems remain active (Mikkelsen and Poll 2002). However, we do not have objective evidences for these last points and need further research to answer this open question.

Pattern Recognition Methods (Multivariate Statistics)

There are several methods used to evaluate changes in pistachio composition and sensory perception upon three treatments (DP, RP, and SRP). Looking to associate results from different methodologies, multivariate statistics, namely, pattern recognition methods, were used, looking for a match between sensorial descriptors and objective measurements (either physical or chemical). Methods used were based on previous experiences (Baroni et al. 2009, 2011; Di Paola-Naranjo et al. 2011; Wunderlin et al. 2001).

A first exploratory method, carried out using CA (Ward's method), showed that SRP can be completely separated from DP and RP (Fig. 2a). Further PC and FA were allowed to evidence the association between sensory descriptors and instrumental analyses. Figure 2b shows results from FA, evidencing at least three groups of variables explaining differences observed between three studied treatments (DP, RP, and SRP; Fig. 2c).

From Fig. 2b, we can observe that the perception of RP is closely linked to the amount of 3-carene but opposite to the amount of moisture, limonene, and bitterness, which is in good agreement with results presented in Tables 1 and 3 and Fig. 1d. It is remarkable that the perception of bitterness is not different for diverse treatments when evaluating results from Table 3. However, it looks evidently associated with unroasted pistachio when analyzing this parameter in a multivariate context (Fig. 2b and c).

Also from Fig. 2b, it is possible to evidence an association between the oxidized flavor perception and the peroxide index, which is the chemical measurement of such perception. In this last case, trained panelists were able to differentiate only two groups (DP and RP from SRP; Table 3), while chemical evaluation (peroxide index) allowed differentiation of three groups (Table 2).

All in all, from Fig. 2b, a clear separation of three groups along Factor 1 (*x*-axis) was observed, with SRP strongly associated with oxidized, sour, hard, crunchy, salty, and roasted perceptions in addition to the amount of ashes, 3-carene, α-pinene, proteins, and the value of peroxide index. Conversely, DP and RP are more associated with bitterness, sweetness, and glossy perceptions, plus the amount of limonene, moisture, and carbohydrates. Considering Factor 2, a further separation between DP and RP is achieved (Fig. 2c). Thus, DP is more strongly associated with higher amounts of limonene, moisture, and a higher perception of bitterness than RP.

Further discriminant analysis (DA) was performed on the entire dataset (sensory, physical, and chemical variables). Stepwise DA allowed 100 % right classification between three groups (DP, SP, and SRP) (Fig. 2d), pointing out eight parameters necessary to discriminate between three groups (ashes, moisture, α-pinene, 3-carene, carbohydrates, limonene, oxidized, and salty) (data not shown). Thus, DA pointed out the need of using both sensory and chemical analyses to verify changes occurring during roasting and salting.

Finally, it is interesting to evaluate if sensory perception could be linked to the analysis of VOCs using a formal statistical method. Thus, canonical correlation (CC) was carried out. To do that, two datasets was defined: the first set includes values from sensory attributes (roasted, oxidized, sweet, bitter, salty, and sour), while the second set includes relative amounts of VOCs (α-pinene, limonene, and 3-carene), plus a new variable that accounts for the treatment. Significant correlation (r: 0.97; P 0.0002) was observed between these two sets of variables, indicating that sensory attributes are reflected in VOCs with an overall correlation of 97 %. It is worthy to remark that the correlation calculated from canonical analysis

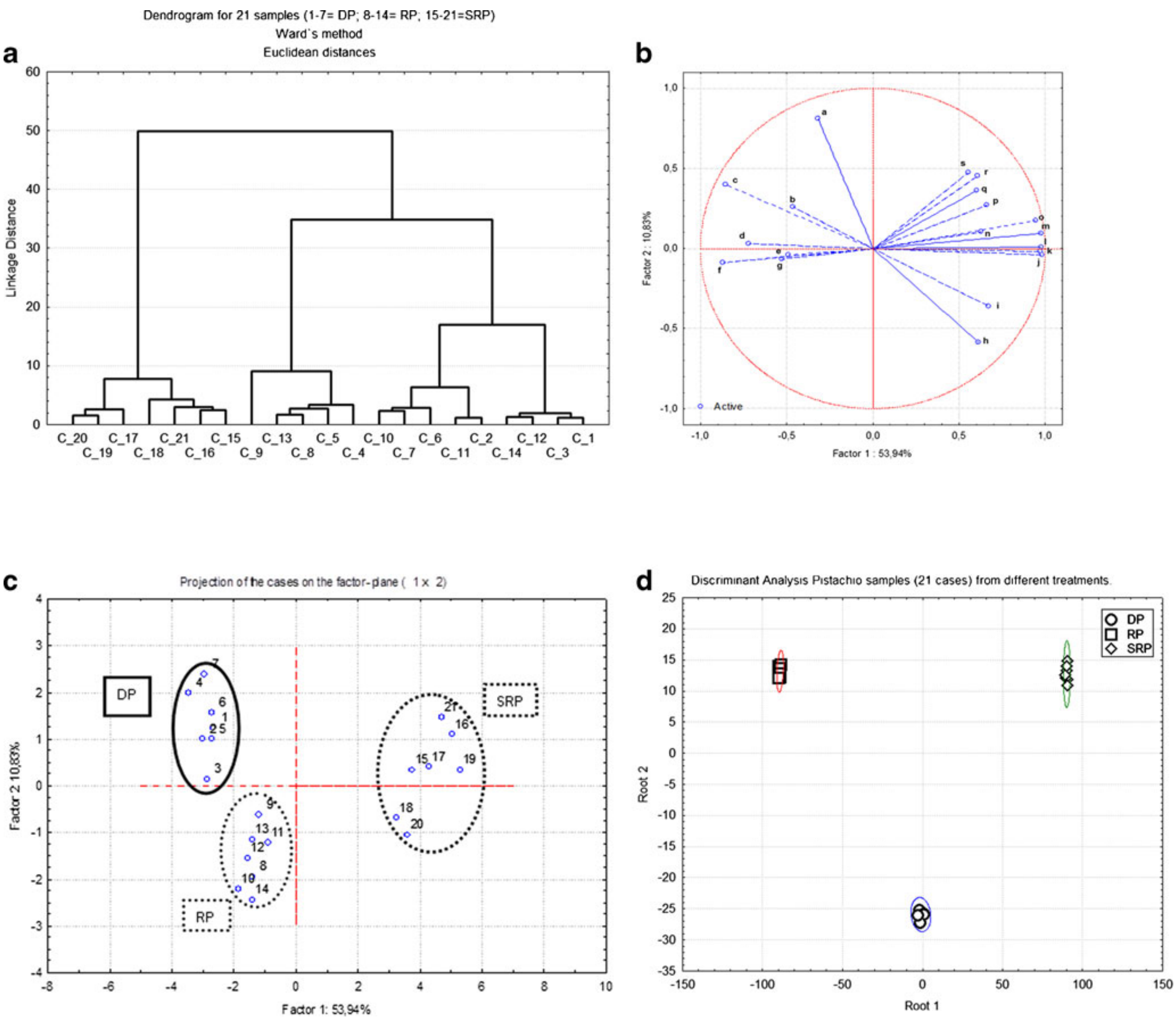

Fig. 2 **a** Cluster analysis of pistachio nut products (*DP* dried pistachio, *RP* roasted pistachio, *SRP* salted–roasted pistachio). **b** Projection of variables on the factor plane (1×2) corresponding to factor analysis (FA) of pistachio nut products. *a* Moisture, *b* bitterness, *c* limonene, *d* carbohydrates, *e* rupture force, *f* sweetness, *g* glossy, *h* 3-carene, *i* roasted, *j* proteins, *k* α-pinene, *l* peroxide index, *m* ashes, *n* crunchiness, *o* salty, *p* hardness, *q* sour, *r* fat, *s* oxidized. **c** Projection of the cases (samples) on the factor plane (1×2) corresponding to factor analysis (FA) of pistachio nut products. **d** Discriminant analysis of pistachio nut products. (*DP* dried pistachio, *RP* roasted pistachio, *SRP* salted–roasted pistachio)

is coincident with the correct classification obtained by DA. Thus, two independent multivariate statistical methods afforded similar predictions. Considering the study case, 97 % of aromatic and basic tastes in pistachio nuts could be explained by three VOCs, namely, limonene, α-pinene, and 3-carene.

Conclusion

Different analytical and statistical methods allowed associating physical and chemical parameters measured in DP, RP, and SRP samples. Key results show that the perception of roasting is associated with increased amounts of α-pinene and 3-carene in RP and SRP, while DP is associated with higher amounts of limonene, moisture, and bitterness. Sensory and physical measurement of hardness and breaking force, respectively, showed opposite results, which could be explained considering the loss of plasticity in RP and SRP. Current results present a novel approach for the evaluation of changes in the perception of nuts quality by consumers.

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