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Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds

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Abstract

The composition of fatty acids and phytosterols was investigated in the oil extracted from the pistachio seeds coming from different countries, (Italy, Turkey, Iran and Greece). The oils are characterized by high contents of oleic acid and β-sitosterol, showing a composition almost similar to that of olive oil. The pattern recognition of data for fatty acids sterols, by multivariate analyses, may provide useful criteria for origin authentication of pistachio seeds.

Keywords: Fatty acids; Phytosterols; Pistachio seed oil; *Pistacia vera*

1. Introduction

The pistachio (*Pistacia vera* L.) is a nut having peculiar organoleptic characteristics. It is widely consumed as a raw or toasted ingredient of many desserts, ice cream, cake, pastry and for the production of some sausages. The nuts contain about 50% of oil in which oleic acid is the dominant component of glycerides, followed by linoleic and palmitic acids (Agar, Kaska, & Kafkas, 1995a; Garcia, Agar, & Streif, 1992; Maskan & Karataş, 1998; Satil, Azcan, & Baser, 2003). The main problem of using pistachio in is oxidation of oil which negatively influences the flavour. Moreover, the typical green colour and flavour of Italian pistachio might be reduced or lost during the storage period. Some studies were conducted under modified atmosphere in order to improve the storage stability of pistachio, in particular on the potential protective effect of carbon dioxide (Maskan & Karataş, 1999) and pure nitrogen (Andreini, Piergiovanni, & Beninato, 2000). The modified atmosphere by CO_2 improves the storage stability, especially at low temperature.

In the past few years, many works were conducted to characterize pistachio nuts of different geographic origins. Anderson and Smith (2005) used the profiles of inorganic anions and organic acids to differentiate Turkish, Iranian and Californian products. The fatty acid compositions of pistachio oil were found to be different in the Turkey varieties (Agar et al., 1995a; Agar, Kaska, & Kafkas, 1995b; Garcia et al., 1992; Satil et al., 2003). High temperature seems to reduce the production of saturated fatty acid; in fact, pistachios grown in the hot regions (over 25 °C) have a smaller content than those grown in more temperate regions (about 22 °C) (Satil et al., 2003). Recently the colours of pistachios of different origins were characterized (Bellomo & Fallico, 2007). Results highlighted the presence of cyanidin–β-galactoside on the external skin, and chlorophylls *a* and *b* and lutein inside the seed. Pigment concentration was influenced both by ripening and geographic origin. The Italian samples showed the highest chlorophylls concentration at ripeness.

We have been interested in the lipid fraction of some foods, such as pigmented orange juices (Arena, Campisi, Fallico, & Maccarone, 1998), globe artichoke (Maccarone et al., 1999) and carob seeds (Maccarone, Formica, Rizzo, & Tomaselli, 2004). The aim of the present work was the characterization of the lipid fraction of Italian pistachio

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compared with those from different countries. In particular, the distribution of the sterol fraction was first investigated in order to ascertain the different geographic origins.

2. Materials and methods

2.1. Samples

Pistachio samples, From Italy, Greece, Iran and Turkey, were picked at ripeness. Each sampling was performed from different pistachio producers: 13 Italian samples (Agrigento 2, Bronte 11), 2 Greek, 2 Iranian, 7 Turkish. The Italian samples were representative of 1000 kg of pistachio kernels, from which 10 kg of intermediate samples was obtained, from which, in turn, 1 kg of pistachio was taken. The other samples were 1 kg each, coming from lots between 1000 and 40 000 kg.

2.2. Reagents and standards

All chemicals and solvents used were of analytical grade (Merck and Baker). Thin-layer chromatography (TLC) pre-coated silica-gel 60 plates (20 × 20 cm, 0.25 mm layer thickness) were purchased from Merck (Darmstadt, Germany). Fatty acid methyl esters (FAME) and sterol standards were purchased from Sigma (St. Louis, MO, USA).

2.3. Oil extraction and chemical analyses

Moisture content was determined on pistachio kernels according to a standard method (Balestrieri & Marini, 1996). Oil was extracted on ground pistachio by a Soxhlet extractor for 6 h using petroleum ether as solvent. The solvent was evaporated under reduced pressure, using a rotary evaporator at 40 °C. Acidity (%), peroxide number (meq/kg), UV absorbance at 232, 262, 268, 270, 274 nm and ΔK-value were determined (Balestrieri & Marini, 1996). All the analyses were conducted in triplicate.

2.4. FAME preparation

FAMEs were prepared by refluxing 0.05 g of oil samples with 5 ml of 1 N sodium hydroxide solution in methanol. Five minutes after boiling, 5 ml of BF_3–methanol were added, and, after a further 5 min, 5 ml of heptane. After cooling the mixture, 5 ml of saturated NaCl solution were added and the organic layer with the FAMEs was recovered and dried over anhydrous Na_2SO_4 and then analized by GC/FID and GC/MS.

2.5. Sterols extraction and TMSE derivatization

The unsaponifiable fraction was extracted after alkaline hydrolysis of the oil, and sterols were isolated from the other constituents by thin-layer chromatography according to Balestrieri and Marini (1996). Trimethylsilyl ether derivatives (TMSE) were prepared by adding a mixture of a anhydrous pyridine/trimethylchlorosilane/hexamethyldisilazane (9/3/1 v/v) to each dried sample. The derivatized samples were immediately analyzed by GC/MS.

2.6. FAME and TMSE sterols GC analysis

A gas chromatograph, SHIMADZU 17-A, equipped with a flame ionization detector (FID) was used to analyze both FAME and TMSE sterols. The operating conditions for FAME analysis were the following: a CP-Wax 52 CB capillary column (Chrompack, 50 m × 0.25 mm i.d., 0.2 μm d.f.). Helium was used as carrier gas; split ratio was 56:1; the oven temperature was kept at 140 °C for 3 min, from 140 °C to 230 °C at 4 °C/min, then held 20 min at 230 °C. The injector and detector temperatures were 230 °C and 260 °C, respectively.

TMSE sterols were separated and identified by a capillary column, SE-54 (Supelco, 30 m × 0.25 mm i.d., 0.25 μm d.f). Helium was used as carrier gas; split ratio was 13; the oven temperature was kept at 260 °C (isothermal) for 60 min. Injector was kept at 260 °C.

FAME and TMS-sterol peaks were identified by comparing retention times and mass spectra to those of authentic standards with a GC/MS SHIMADZU QP 5050A under the same conditions as above described. FAMEs were quantified using heptadecanoic acid as internal standard.

2.7. Statistical analysis

Multiple range test and multivariate analysis of experimental data principal component analysis (PCA) and linear discriminant analysis (LDA) were performed using

Table 1
Moisture and oil contents of pistachio nut, and acidity and peroxide number of the extracted oil

Samples	Moisture (%)	Oil[a] (%)	Acidity (%)	Peroxide number (meq/kg)
Italy (Bronte)	6.0	50.4	0.63	5.5
Italy (Agrigento)	6.9	57.6	0.51	2.9
Turkey	4.9	55.3	0.71	6.4
Greece	5.2	55.4	0.81	5.4
Iran	6.3	58.0	0.65	6.8
Mean value	5.7	53.7	0.66	5.7

Each value was the average of three determinations. Standard error of mean value does not exceed ±6%.
[a] Calculated on dry weight of pistachio nut.

Table 2
Spectrophotometric analysis of pistacchio oil

Samples	K_{232}	K_{262}	K_{268}	K_{270}	K_{274}	ΔK
Italy (Bronte)	1.648	0.187	0.199	0.203	0.205	0.004
Italy (Agrigento)	1.645	0.165	0.181	0.185	0.186	0.005
Turkey	1.777	0.285	0.317	0.325	0.331	0.010
Greece	1.909	0.251	0.265	0.268	0.270	0.005
Iran	2.221	0.353	0.373	0.376	0.371	0.011

Each value was the average of three determinations. Standard error of mean value does not exceed ±4%.

Table 3
Fatty acid composition (%) of pistachio oils of different geographic origins

Samples	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Vaccenic (C18:1ω11)	Linoleic (C18:2)	Linolenic (C18:3)
Italy (Bronte)	9.8b	0.86b	1.9c	72.0d	1.6a	13.3a	0.45a
Italy (Agrigento)	9.6a	0.86bc	1.4b	70.3c	1.8b	15.6b	0.46a
Turkey	9.5a	0.67a	2.6e	70.5c	1.5a	14.7b	0.47a
Greece	10.8d	0.95d	2.1d	68.3b	1.9b	15.4b	0.49ab
Iran	10.8c	0.94cd	1.1a	55.1a	2.5c	28.9c	0.60b
Mean value	9.9	0.81	2.1	69.6	1.7	15.4	0.48

Means in the same column followed by common letter are not significantly different ($P < 0.05$).

the Statgraphic Plus software (Manugistic Inc. Rockville, MD, USA).

3. Results and discussion

Table 1 shows the oil yield and the moisture content of pistachio nuts, the acidity and the peroxide number of the oil. The oil content varied from 58% for the Iran samples, to about 50% for the Bronte samples. These results are in agreement with the literature data (Garcia et al., 1992; Satil et al., 2003). The mean value of moisture was 5.7% with a lowest value of 4.2% for the Turkey sample and the highest value 6.9% for the Agrigento (Italy) sample. The very low level of acidity in all samples (<1%) indicates no chemical or enzymatic hydrolysis of glycerides. The peroxide number changed from 2.9 for the Agrigento sample to 6.8 for the Iran sample and indicates the freshness of the samples and the good quality of the oil. The spectrophotometric data and the very small ΔK-values indicate the absence of degradation in unsaturated fatty acids (Table 2).

Table 3 shows the fatty acid composition. The principal fatty acid were oleic (55.1–71%), linoleic (13.3–28.9%), palmitic (9.5–10.8%), stearic (1.1–2.6%), vaccenic (*Z*-(7)-octadecenoic acid) (1.5–2.5%), palmitoleic (0.67–0.95%) and linolenic (0.45–0.60%). Myristic acid, pentadecenoic acid, *Z*-(7)-hexadecenoic acid and arachidic acid were present in all samples in trace amounts. According to Satil et al. (2003), these minor acids do not exceed 0.5%.

All samples, except the Iranians, had about 70% of oleic acid and 15% of linoleic acid, whereas the Iranian samples showed 55% of oleic acid and about 28% of linoleic acid. The fatty acid distribution in Turkish samples appears to be more similar to those in Italian samples, than data reported for Turkish pistachios (Agar et al., 1995a, 1995b; Satil et al., 2003).

The ratio between unsaturated and saturated fatty acids was about 6.5 and the ratio between oleic and linoleic acid was about 4.5. These data, as well as the distribution of fatty acids, show that pistachio oil is very similar to olive oil (Belitz & Grosch, 1999; Parcerisa, Richardson, Rafecas, Codony, & Boatella, 1998).

The low values of acidity and peroxide number, the ΔK near to zero, the absence of erucic acid and the fatty acids distribution (similar to olive oil), indicate a high oil quality both from nutritional and stability aspects.

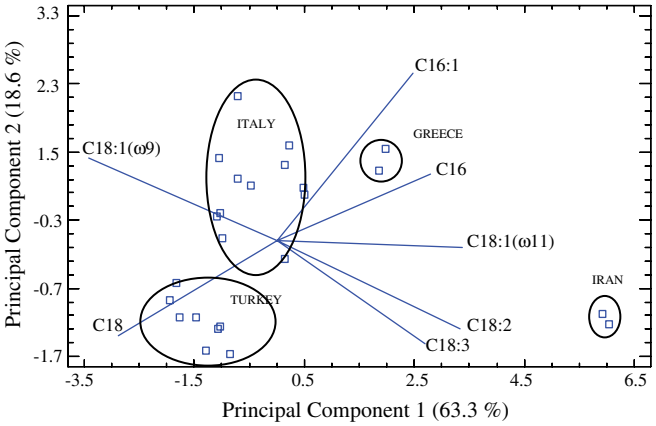

Fig. 1. Plot of principal components.

Principal component analysis of the seven major fatty acids percentage gives two linear combinations which explain, overall, 94.8% of the variance, in particular 82% for the first component and 12.8% for the second. Fig. 1 shows the vectors of each variable and the distribution of the oil samples in the plane defined by the values of the two principal components. Italian (Bronte, Agrigento) and Greek pistachio samples were similar, and differ from the other samples by the high value of oleic and palmitoleic acid. Turkish samples were located in the negative quadrant of the plane, along the vector of stearic acid,

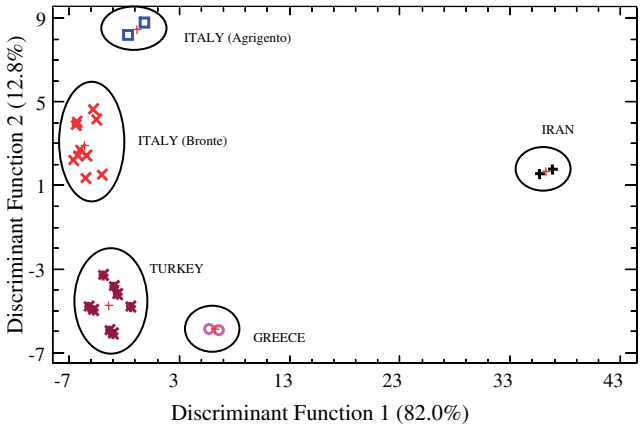

Fig. 2. Plot of the first two discriminant functions for the acids distribution.

which is the characterizing variable. In a completely different zone of the plane were the Iranian samples, characterized by high levels of linoleic and linolenic acids. The linear discriminant analysis confirms the diversity among the samples of different geographic origin (Fig. 2). Table 5 lists the statistical parameters and the classification of results.

Nine sterols were identified by GC/MS and quantified for the first time in this work. Table 4 shows the distribution of the four main phytosterols of pistachio seed oil. β-Sitosterol was the predominant component in all samples, varying from about 85% in the Italian samples to 88% in Iranian samples; Δ^5-avenasterol was present in about 9% of Agrigento samples and in 5.7% of Iranian samples; campesterol was at about 3% in all samples except the Iranian samples (4.55%). Among the minor sterols, clerosterol was about 0.94% in Turkish samples while, in the other samples, it changed from 0.51% to 0.72%. Stigmasterol varied from 0.64 in the Iranian sample to 0.95% and 0.92% in Italian and Turkish samples, respectively; $\Delta^{5,24}$-stigmastadienol was similar in all samples (0.46–0.59%); Δ^7-stigmastenol was absent in Iranian samples but, in the other samples, changed from 0.16 to 0.52%. Δ^7-Avenasterol was detected only in Agrigento and Turkish samples while cholesterol was detected only in the Turkish samples. Cumulatively, the minor sterols varied from 1.07% for the Iranian samples to 2.37% for Turkish ones.

With respect to other seed oils, the pistachio oil had a typical distribution of sterols, characterized by a high content of β-sitosterol, (cf. hazelnut and olive oil), and by a low content of campesterol and stigmasterol as in olive oil. Δ^5-Avenasterol had a distribution intermediate between hazelnut and olive oil (Cercaci, Rodriguez-Estrada, & Lercker, 2003; Parcerisa et al., 1998).

A linear discriminant analysis was performed, using the percentage of the four principal sterols (campesterol, stigmasterol, β-sitosterol and Δ^5-avenasterol) as variables in

Table 4
Distribution (%) of sterols of pistachio oils of different geographic origins

Samples	β-Sitosterol	Δ5-Avenasterol	Campesterol	Stigmasterol	Others
Italy (Bronte)	85.5ab	9.24cd	3.04ab	0.95abc	1.71
Italy (Agrigento)	85.5a	8.82d	3.56a	0.84c	1.29
Turkey	86.2ab	7.24b	3.56b	0.92bc	2.37
Greece	87.2ab	7.41bc	3.25ab	0.71ab	1.49
Iran	88.0b	5.69a	4.55c	0.64a	1.07

Means in the same column followed by common letter are not significantly different ($P < 0.05$).

Table 5
Linear discriminant analysis of fatty acids and sterols: statistics and classification of results

FD	Eigenvalue	Variance (%)	Canonical correl.	*p*-Value
Fatty acids				
1	163.6	81.96	0.997	0.0000
2	25.5	12.75	0.981	0.0000
Sterols				
1	8.14	87.30	0.944	0.0000
2	0.81	8.70	0.669	0.0500

Classification of results

Groups	Cases	Cases correctly classified	Cases not correctly classified	Total cases correctly classified (%)
Fatty acids				
Italy (Bronte)	10	10	–	100
Italy (Agrigento)	2	2	–	100
Greece	2	2	–	100
Iran	2	2	–	100
Turkey	8	8	–	100
Total	24	24	–	100
Sterols				
Italy (Bronte)	11	11	–	100
Italy (Agrigento)	2	1	1[a]	50
Greece	2	2	–	100
Iran	2	2	–	100
Turkey	7	7	–	100
Total	24	23	1	95.83

[a] Classified as Italy (Bronte).

Table 6
Performances of linear discriminant analyses

Samples	Group size	Predicted Groups					
		Agrigento	Bronte	Greece	Iran	Turkey	Unknown
Fatty acids							
Italy (Agrigento)	2	2	–	–	–	–	–
Italy (Bronte)	9	–	9	–	–	–	–
Greece	1	–	–	1	–	–	–
Iran	1	–	–	–	1	–	–
Turkey	7	–	–	–	–	7	–
Unknown	4	–	–	1	1	1	1
Sterols							
Italy (Agrigento)	2	1	1	–	–	–	–
Italy (Bronte)	10	–	10	–	–	–	–
Greece	1	–	–	1	–	–	–
Iran	1	–	–	–	1	–	–
Turkey	6	–	–	–	–	6	–
Unknown	4	–	1	1	1	1	–

24 samples. Two discriminant functions of the variables were used to distinguish samples of different origins (Table 5). These discriminant functions together explain 96% of the variance and show multiple correlation coefficients and *p*-values with a confidence level higher than 95%. The high values of the standardized coefficients of the functions indicate that all the variables were important for differentiating the oil. The classification of samples was very good: 23 cases out of 24 were correctly classified (95.8%); only 1 case, a sample coming from Agrigento was classified as Bronte. All Italian samples were separated from those from Turkey, Greece and Iran. Agrigento samples were collocated near the Bronte ones, while Greece samples were near to Turkish samples. Iranian samples were positioned in a different region of the plot.

The robustness of statistical models was evaluated as follows: four samples were randomly left out from the data set (one for each group of origin) and the model was calibrated on the remaining data (20 samples). The statistical results were very similar to those of the previous model (24 samples). The four samples left out from the model were then introduced into the data set as an unknown group. As concerns sterols, all four samples were correctly classified; for fatty acids, instead, three samples were correctly classified and 1 as unknown (Table 6).

Both PCA for fatty acids and LDA for fatty acids and sterols had highlighted the possibility of distinguishing the geographic origin of pistachios. Bronte and Agrigento sample results were similar to each other, both for fatty acids and sterols distribution; Greek samples similar to Bronte and Agrigento samples for fatty acid distributions, but similar to Turkish samples in sterol distributions. Turkish and Iranisn samples were completely different from the other samples and from each other in both the distribution of fatty acids and sterols. In conclusion, despite the limited number of samples used in this work, in particular as concerns those from Greece and Iran, the composition of pistachio oil might be used as a marker to distinguish the geographic origin of seed.

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