

Pistachio (*Pistacia vera*) seed oil composition: geographic situation and variety effects

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RESUMEN

Composición del aceite de la semilla del pistachio (*Pistacia vera*): efectos de la situación geográfica y de la variedad.

Este estudio tiene como objetivo caracterizar cuatro cultivos de pistachio (*Pistacia vera*) de Túnez, de las regiones de Mateur (Norte), Nabeul (Nordeste), Kairouan (Centro) y Sfax (Centro-Este) y dos variedades, *Mateur* and *Ohadi*, considerando la composición en ácidos grasos y de lípidos mayoritarios (lípidos polares, diglicéridos, triglicéridos y ácidos grasos libres) de semillas maduras. Los lípidos se separaron mediante cromatografía en capa fina, y los ésteres metílicos de los ácidos grasos (FAMES) se analizaron por cromatografía de gases. El ácido oleico (C18:1 Δ^9) fue el mayoritario en todas las muestras; las de la región de Mateur (Norte) fueron significativamente distintas, presentando el contenido más bajo de C18:1 (54.2 % del total de ácidos grasos) y más alto de C18:2 (24.1 %). La cantidad total de ácidos grasos (TFA) en las semillas de la variedad *Ohadi* fue significativamente más bajo que el de la variedad *Mateur* de la región de Sfax. Los triglicéridos fueron el componente más importante en la semillas de pistachio de Nabeul (98.5% del total de glicerolípidos).

PALABRAS-CLAVE: Ácidos grasos – Glicerolípidos – Pistachio – *Pistacia vera* – Variedad Mateur – Variedad Ohadi .

SUMMARY

Pistachio (*Pistacia vera*) seed oil composition: geographic situation and variety effects.

This study was aimed at characterizing four Tunisian pistachio cultivations (*Pistacia vera*) from the Mateur (North), Nabeul (North-East), Kairouan (Middle) and Sfax (Middle-East) regions and two varieties, *Mateur* and *Ohadi*, considering fatty acid composition and main lipid class contents (polar lipids, diacylglycerols, triacylglycerols and free fatty acids) in ripe seeds. Lipid classes were separated using thin layer chromatography, and fatty acid methyl esters (FAMES) were analyzed by gas chromatography. Oleic acid (C18:1 Δ^9) was the major fatty acid for all samples; those of the Mateur region (the northern region) differed significantly with the lowest content in C18:1 (54.2 % of total fatty acids) and the highest in C18:2 (24.1 %). The total amount of fatty acids (TFA) in the seeds of *Ohadi* variety was significantly lower than that of the *Mateur* variety from the Sfax region. Triacylglycerols were most abundant in pistachio seeds from Nabeul (98.5% of total glycerolipids).

KEY-WORDS: Fatty acids – Glycerolipids – Mateur variety – Ohadi variety – Pistachio – *Pistacia vera*.

1. INTRODUCTION

Pistacia vera (*Anacardiaceae*) is the only pistachio species providing edible nuts. Furthermore, pistachio seeds present a high nutritive value due to their oil richness which varies from 40 to 63% on a dry weight basis (Garcia *et al.*, 1992; Agar *et al.*, 1995; Yildiz *et al.*, 1998; Küçüköner and Yurt, 2003; Satil *et al.*, 2003). Their oil composition depends on environmental factors such as climate, geography, soil type... as well as intrinsic factors such as variety. In Tunisia, since about fifty years ago, special efforts were made to improve pistachio cultivations as this species can thrive in dry regions (Hadj-Hassan and Kardouch, 1995) and poor soils like calcareous and saline soils (Joley, 1969).

In 2005, Pistachio production reached 2500 tons (Ministère de l'Agriculture et des Ressources Hydrauliques, 2006). The main varieties found in Tunisia are: *Mateur*, also called *AEgine*, which is originated from Greece and is the most commonly cultivated in Tunisia (Ghrab *et al.*, 2004); the *Sfax* variety occupies second place in terms of production, followed by the *El Guettar* variety (Jacquy, 1973).

The present work was carried out to assess the quality of pistachio seeds through their fatty acid (FA) composition. Total fatty acids (TFA), non-polar and polar glycerolipids classes were analyzed for pistachio seeds of the *Mateur* variety taken from four Tunisian regions: Mateur (North), Grombalia (North-East), Kairouan (Middle) and Sfax (Middle-East). Furthermore, in order to study the variety effect between *Mateur* and *Ohadi* varieties, the same analyses were made for these varieties which were both cultivated in the Sfax region.

2. EXPERIMENTAL

2.1. Material

Seed samples were taken from four Tunisian stations (Figure 1): Ghézala, Grombalia, Hendi Ezzitouna and Taouss, located in the following regions, respectively: Mateur in the north (sub humid climate), Nabeul in the north-east (semi-arid climate), Kairouan in the middle region (arid



Fig. 1

Location of sampling stations Ghz, Ghézala; Grb: Grombalia; HZ: Hendi Ezzitouna. Pistachio fruits were sampled in four stations, belonging to four geographically different regions: Ghézala (Mateur, north), Grombalia (Nabeul, north-east), Hendi Ezzitouna (Kairouan, middle region) and Taouss (Sfax, middle-east).

climate), Sfax in the middle-east (arid climate). Two varieties were considered: the *Mateur* variety, found in the four studied regions, and *Ohadi* variety, only present in the region of Sfax.

2.2. Moisture content

Portions of thirty fresh seeds were used in triplicate to assess moisture content. They were placed in an oven at 105 °C until dry weight was constant.

2.3. Lipid extraction

Freshly harvested seeds were fixed in boiling water for two minutes in order to inactivate phospholipases (Douce, 1964). The extraction of TFA was performed according to the method described by Bligh and Dyer (1959), grinding a known amount of pistachio nuts in the presence of a methanol/chloroform (1:2 v/v) mixture at 30ml/g of fresh weight. Three replicates of samples were considered.

2.4. Fatty acids methylation

Fatty acids were transformed into their methyl esters according to the method described by Cecchi *et al.* (1985) using sodium methylate, in order to be analyzed by gas chromatography. A known quantity of methyl heptadecanoate used as internal standard was added to the sample for quantification purposes.

2.5. Glycerolipid class separation by thin layer chromatography

Glycerolipid classes were separated by thin layer chromatography according to the method of

Mangold (1964) using plates (20 cm x 20 cm x 0.2 mm) covered with silica gel (G60, Merck, Darmstadt, Germany) and petroleum ether/ethyl ether/acetic acid (70:30:0.4, v/v/v) as developing mixture. Spots were revealed by placing the plates into a hermetic vat containing iodine vapours. Spots were scraped off and transmethylated by the previous method.

2.6. Fatty acid analysis

The gas chromatograph (GC) was an HP 6890 apparatus (Agilent, Palo Alto, CA) equipped with a flame ionization detector (FID) and an Innowax capillary column (30 m, 250 µm i.d., 0.25 µm film thickness) with a stationary phase made of polyethylene glycol (PEG). Analyses were conducted in split mode (60:1). The oven temperature program was: isotherm at 150 °C for 1 min., 150 to 200 °C at a rate of 15 °C/min., 200 to 242 °C at a rate of 2 °C/min., isotherm at 242 °C for 2 min. Total flow was 1.6 ml/min. Injector and detector temperatures were held to 250 and 275 °C, respectively.

An HP Chemstation (Rev.A.0401) software connected to the GC allowed peak areas to be determined.

Results were obtained from the mean of three replicates of samples with their corresponding standard deviations to the threshold of 5%.

2.7. Statistical analysis

The results were compared by one-way analysis of variance (one-way ANOVA) using Statistica software (StatSoft, France, 1999). Means were judged significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Moisture content

Moisture contents are presented in Figures 2 and 3.

Values ranged from 25% (seeds from Sfax, *Ohadi* variety) to 38.4% (seeds from Kairouan,

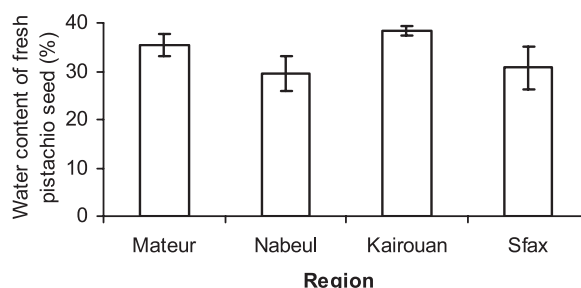


Fig. 2

Moisture content in fresh pistachio seeds of the *Mateur* variety from different regions. Moisture content was determined for fresh newly harvested pistachio seeds.

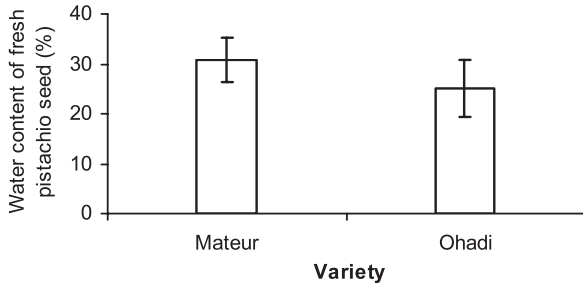


Fig. 3
Moisture content in fresh pistachio seeds of the *Mateur* and *Ohadi* varieties from the Sfax region. Moisture content was determined for fresh newly harvested pistachio seeds.

Mateur variety) (Fig. 2). According to Kashaninejad *et al.* (2004), pistachio seeds present a water content varying from 37 to 40%. It is worth noting that water content is decreased to about 4% when seeds are sold, in order to inhibit enzyme and micro-organism activities. For comparison, Macadamia nut water content was found to be 30% (Kaijser *et al.*, 2000).

3.2. Fatty acid and glycerolipids contents

3.2.1. Fatty acid composition

Seed fatty acid composition presents saturated (C16:0, C18:0), monounsaturated (C16:1, C18:1) and polyunsaturated (C18:2, C18:3) fatty acids.

Oleic acid (C18:1) was the major fatty acid, accounting for more than 50% of TFA for all samples (Tables 1 and 2). This result is in accordance with many others (Shokraii, 1977; Garcia *et al.*, 1992; Agar *et al.*, 1995; Maskan and Karataş, 1998; Yildiz *et al.*, 1998; Küçüköner and Yurt, 2003; Satil *et al.*, 2003).

For comparison, the Uzun, Kırmızı, Siirt and Halebi varieties from Turkey studied by Küçüköner and Yurt (2003) presented similarities with our samples (except those from the *Mateur* region) concerning oleic and linolenic acid contents (Tables 1 and 2).

Oleic, palmitic and linoleic acids were the most abundant fatty acids. This corresponds to the result of

the *de novo* fatty acid biosynthesis where palmitic acid is in general the first FA to be formed after a thioesterase activation on palmitoyl-ACP. In most cases, palmitoyl-ACP is transformed by a 3-Ketoacyl-ACP Synthase II (KAS II) to stearyl-ACP (Ohlrogge and Browse, 1995) which is generally converted to oleic acid (C18:1^{Δ⁹}) thanks to a stearyl-ACP Δ^9 -desaturase and a thioesterase releasing ACP moiety.

This FA composition resembles that of olive oil where oleic acid is the major FA (Mancha, 1974) and whose content varies from 55 to 83% of TFA (CODEX STAN, 1989).

It appears that pistachio oil is characterized by the predominance of monounsaturated fatty acids (56.6% for the *Mateur* region and more than 70% for the Nabeul, Kairouan and Sfax regions); thus, pistachio consumption could constitute a protection against cardiovascular diseases (Fraser, 1999; Kris-Etherton *et al.*, 2001) and oxidization.

3.2.2. Glycerolipids content

Glycerolipids can be classified as polar and neutral lipids. The latter are formed by neutral classes i.e. monoacylglycerols (MAG), diacylglycerols (DAG) and triacylglycerols (TAG), and by free fatty acids (FFA). Tables 3 and 4 give the proportions of these lipid categories.

The major class is represented by TAG, accounting for more than 90% of total glycerolipids (Tables 3 and 4). They constitute the storage lipid form of pistachio seeds.

The remaining lipids i.e. polar lipids (PL), DAG and FFA are marginally represented in fully ripe seeds. PL are mainly found in the biomembranes and constitute the minor components of seeds. DAG and FFA serve as metabolic intermediates, explaining their low contents in the seeds.

3.3. Regional effect

Table 1 gives the fatty acid composition of *Mateur* seed variety sampled from four different geographical regions.

The oleic acid content ranged from 73 and 77% except for samples from the *Mateur* region where it was significantly lower (54.2%).

Table 1
Fatty acid composition (%)
of *Mateur* variety pistachio seeds from different regions.

Region	Mateur	Nabeul	Kairouan	Sfax
C16:0	15.7 ± 1.2 ^a	12.5 ± 1.4 ^b	11.8 ± 0.6 ^b	11.2 ± 0.9 ^b
C16:1	2.5 ± 1.2 ^a	1.5 ± 0.1 ^a	1.6 ± 0.2 ^a	1.9 ± 0.1 ^a
C18:0	2.7 ± 2.1 ^a	2.6 ± 0.3 ^a	2.5 ± 0.3 ^a	2.3 ± 0.3 ^a
C18:1	54.2 ± 2.0 ^b	75.1 ± 2.9 ^a	73.0 ± 4.6 ^a	76.8 ± 1.3 ^a
C18:2	24.1 ± 3.0 ^a	7.8 ± 1.1 ^b	10.3 ± 3.4 ^b	7.6 ± 1.1 ^b
C18:3	0.9 ± 1.6 ^a	0.6 ± 0.4 ^a	0.9 ± 0.6 ^a	0.2 ± 0.1 ^a

Results are given as means ± SD from triplicate estimations.
Means in the same line followed by different letters are significantly different.

Table 2
Fatty acid composition (%)
of pistachio seeds from the Sfax region.

Variety	<i>Mateur</i> variety	<i>Ohadi</i> variety
C16:0	11.2 ± 0.9 ^a	11.5 ± 1.1 ^a
C16:1	1.9 ± 0.1 ^a	2.1 ± 0.4 ^a
C18:0	2.3 ± 0.3 ^a	1.4 ± 1.1 ^a
C18:1	76.8 ± 1.3 ^a	70.0 ± 1.7 ^b
C18:2	7.6 ± 1.1 ^b	14.1 ± 1.9 ^a
C18:3	0.2 ± 0.1 ^a	0.9 ± 0.5 ^a

Results are given as means ± SD from triplicate estimations. Means in the same line followed by different letters are significantly different.

Palmitic (C16:0) and linoleic (C18:2) acids were present in comparable proportions for Nabeul, Kairouan and Sfax samples with mean values of 11.7 and 9.9%, respectively. On the contrary, samples from the *Mateur* region had higher linoleic and palmitic acid contents (24.1 and 15.7%, respectively) (Table 1).

Table 3
Main Glycerolipid class contents (%)
of *Mateur* variety pistachio seeds from the different regions.

Region	<i>Mateur</i>	Nabeul	Kairouan	Sfax
PL	0.9 ± 0.0 ^b	0.7 ± 0.2 ^b	2.7 ± 1.3 ^a	2.3 ± 0.2 ^a
DAG	3.9 ± 1.4 ^a	0.5 ± 0.2 ^b	3.5 ± 1.8 ^{ac}	1.5 ± 0.6 ^{bc}
FFA	2.8 ± 1.3 ^a	0.3 ± 0.1 ^{bc}	2.7 ± 1.0 ^a	1.2 ± 0.7 ^{ac}
TAG	92.4 ± 2.8 ^{bc}	98.5 ± 0.1 ^a	91.1 ± 2.7 ^b	95.0 ± 1.5 ^{ac}
NL	99.1 ± 0.0 ^a	99.3 ± 0.2 ^a	97.3 ± 1.3 ^b	97.7 ± 0.2 ^b

Results are given as means ± SD from triplicate estimations. Means in the same line followed by different letters are significantly different. PL, polar lipids; DAG, diacylglycerols; FFA, free fatty acids; TAG, triacylglycerols; NL, neutral lipids.

The particular fatty acid composition of pistachio seeds from the *Mateur* region could be explained by desaturases regulation by temperature, i.e. a higher desaturase activity in the seeds from the *Mateur* region, certainly due to the cooler climate of this region (Mc Carthy and Stumpf, 1980; Mazliak, 1988). Indeed, temperatures were the lowest from April to June (before storage lipid biosynthesis) in the *Mateur* region compared to the other regions. Similarly, Satil *et al.* (2003), studying nuts from Turkey, reported that the C16:0 content was higher in cooler climates whereas that of C18:1 was lower for the same variety (*Uzun* variety).

This result is in contradiction with many works on Tunisian and Italian olive fruits. For instance, Montefredine and Laporta (1963) suggested that low temperatures resulted in higher contents of oleic acid in disfavour of linoleic and palmitic acids. Moreover, Fedeli (1977) pointed out that linoleic acid content increased with higher temperatures.

As for the *Ohadi* variety (Table 2), oleic acid content was higher in our samples with 70.0 % against 56.6% in Turkish pistachio nuts (Küçüköner

Total fatty acids amounts for the *Mateur* variety are given in Table 5. They decreased in the southern regions, dropping under 400 mg/gDM for the Kairouan and Sfax stations. This could be due to the drought affecting the primary metabolic pathways by slowing down fatty acid biosynthesis. Nevertheless, no significant differences were detected.

Considerable amounts of oleic acid were found for all samples (more than 200 mg/gDM). Furthermore, essential fatty acids, represented by linoleic acid and linolenic acid, account for 107.62 mg/gDM in seeds from the *Mateur* region and 35.59 mg/gDM for seeds of the *Mateur* variety from the Nabeul, Kairouan and Sfax regions (mean value) (Table 5).

Samples from Nabeul showed the highest capacity for TAG biosynthesis (98.5% of total lipids), as well as seeds of the *Mateur* variety from the Sfax region (95 %) (Table 3). As these regions are not comparable, no relationship seems to exist between TAG biosynthesis and geographical effect.

and Yurt, 2003). On the contrary, linoleic acid content was found to be higher in Turkish pistachio nuts (31.0%) while our sample showed a mean value of 14.1% TFA for the *Ohadi* variety. Their geographical location may explain these differences.

Table 4
Main Glycerolipid class contents (%)
of pistachio seeds from the Sfax region.

Variety	<i>Mateur</i> variety	<i>Ohadi</i> variety
PL	2.3 ± 0.2 ^a	0.6 ± 0.6 ^b
DAG	1.5 ± 0.6 ^b	6.5 ± 0.8 ^a
FFA	1.2 ± 0.7 ^a	1.6 ± 1.1 ^a
TAG	95.0 ± 1.5 ^a	91.3 ± 0.8 ^b
NL	97.7 ± 0.2 ^b	99.4 ± 0.6 ^a

Results are given as means ± SD from triplicate estimations. Means in the same line followed by different letters are significantly different. PL, polar lipids; DAG, diacylglycerols; FFA, free fatty acids; TAG, triacylglycerols; NL, neutral lipids.

Table 5
Fatty acid amounts (mg/g DM)
of *Mateur* variety pistachio seeds from different regions.

Region	Mateur	Nabeul	Kairouan	Sfax
C16:0	67.65 ± 0.48	55.60 ± 1.07	43.52 ± 10.22	40.98 ± 5.53
C16:1	10.64 ± 0.15	6.79 ± 0.27	5.71 ± 0.98	6.92 ± 0.61
C18:0	11.59 ± 0.25	11.37 ± 2.81	9.07 ± 2.60	8.25 ± 1.68
C18:1	233.47 ± 0.70	334.65 ± 56.93	268.71 ± 36.00	279.74 ± 22.33
C18:2	103.84 ± 0.48	34.76 ± 0.40	37.74 ± 10.79	27.64 ± 6.73
C18:3	3.78 ± 0.16	2.64 ± 1.60	3.25 ± 2.20	0.75 ± 0.47
Total (mg/gDM)	430.98^a ± 1.65	445.81^a ± 59.09	367.99^a ± 52.42	364.28^a ± 49.41

Results are given as means ± SD from triplicate estimations.

Means in the same line followed by different letters are significantly different.

DM: dry matter.

3.4. Variety effect

A comparison of fatty acid composition between *Mateur* and *Ohadi* varieties in the Sfax region is reported in Table 2.

Oleic acid content was significantly higher in seeds of the *Mateur* variety whereas the *Ohadi* variety seeds were significantly richer in linoleic acid, suggesting a genetic control for the biosynthesis of these fatty acids. Küçüköner and Yurt (2003) found the same result when comparing the *Ohadi* variety to other varieties grown under the same environmental conditions, i.e. *Ohadi* variety seeds were significantly richer in linoleic acid whereas their oleic acid content was significantly lower.

As for total fatty acids, significant differences were noted between both varieties of the Sfax region, with a higher value in the *Mateur* variety seeds (Table 6).

Seeds from the *Ohadi* variety exhibited a slightly higher amount of essential fatty acids (C18:2 + C18:3) with 43.93 mg/gDM (Table 6).

Considering lipid classes, Table 4 clearly illustrates the variety effect due to higher TAG content in the seeds of the *Mateur* variety.

4. CONCLUSIONS

Pistachio nuts are oleaginous seeds characterized by the predominance of oleic acid (C18:1 Δ^9) for all

samples from the Mateur, Nabeul, Kairouan and Sfax regions (> 50 %) and for both varieties, *Mateur* and *Ohadi*. However, seeds from the Mateur region (*Mateur* variety) had an oleic acid content significantly lower than the other samples of the same variety, and a higher content in linoleic acid (C18:2), indicating a probable effect of geographical location with an increasing desaturase activity in the northern regions.

Total fatty acid amount was the lowest in seeds from the Sfax region, suggesting a lower biosynthetic activity partly due to drought.

Triacylglycerols, the predominating class (> 90%), constitute the main storage form of glycerolipids in the seeds.

A variety effect was determined, however, since TFA amounts in seeds of the *Mateur* and *Ohadi* varieties from the Sfax region were not comparable to the higher value for the *Mateur* variety. Furthermore, seeds of the *Mateur* variety were significantly richer in oleic acid and in TAG. Hence, this variety could represent better nutritional value than the *Ohadi* variety.

In regard to the nutritional value of pistachio seeds (richness in monounsaturated fatty acids such as C18:1 and presence of essential fatty acids such as C18:2) and considering the rusticity of pistachio trees, Tunisia has been striving to increase its production for the past thirty years, leading to a production increase of about 400 fold (43000 ha) (Ghrab *et al.*, 2004). The characterization of varieties and cultivars could be a useful criterion for the pistachio market.

Table 6
Fatty acids amounts (mg/g DM)
of pistachio seeds from the Sfax region.

Variety	<i>Mateur</i> variety	<i>Ohadi</i> variety
C16:0	40.98 ± 5.53	33.93 ± 3.90
C16:1	6.92 ± 0.61	6.14 ± 0.32
C18:0	8.25 ± 1.68	4.23 ± 0.87
C18:1	279.74 ± 22.33	206.06 ± 13.52
C18:2	27.64 ± 6.73	41.35 ± 5.84
C18:3	0.75 ± 0.47	2.58 ± 0.37
Total (mg/gDM)	364.28^a ± 49.41	294.29^b ± 32.34

DM: dry matter. Means in the same line followed by different letters are significantly different.

Results are given as means ± SD from triplicate estimations.

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