

## FATTY ACIDS PROFILE AND ANTIOXIDANT ACTIVITY OF ALMOND OILS OBTAINED FROM SIX ROMANIAN VARIETIES

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### Abstract

The almond nuts are an important source of unsaturated fats that may contribute to a significant decreasing of cholesterol level in blood. Six patented almond varieties grown in North-Western part of Romania, Bihor County (Sort A, Sort B, Sort C, Sort D, Sort E, and Sort F) were selected in order to determine the fatty acid composition and the antioxidant capacity of the oils obtained from almonds. Four fatty acids (stearic, palmitic, oleic and linoleic) were determined through their methylated derivatives by using gas-chromatography coupled with mass spectrometry. The major fatty acid was found to be the oleic acid, with the percentage varying from 61% (Sort C) to 77% (Sort D), followed by linoleic acid (19% for Sort A variety and 28% for Sort F). Linoleic acid content was negatively correlated with oleic acid content ( $r = -0.862$ ) and low correlations have been found between the oleic acid and stearic acid. The antioxidant capacity determined by using DPPH method ranged values between 73% (Sort F) and 91% (Sort A) for the investigated samples. The study is important as it investigates the fatty acid profile and the antioxidant activity of almond nuts that are recommended in the human diet.

### Rezumat

Migdalele sunt o sursă importantă de grăsimi nesaturate care pot contribui la scăderea semnificativă a nivelului colesterolului în sânge. Șase soiuri de migdali brevetate, cultivate în partea de nord-vest a României, (Soiul A, Soiul B, Soiul C, Soiul D, Soiul E și Soiul F) au fost selectate pentru a determina compoziția de acizi grași și capacitatea antioxidantă a uleiurilor obținute din migdale. Patru acizi grași (stearic, palmitic, oleic și linoleic) au fost determinați prin derivații metilici, utilizând cromatografia de gaze cuplată cu spectrometria de masă. Acidul gras predominant a fost acidul oleic, procentul variind de la 61% (Soiul C) la 77% (Soiul D), urmat de acidul linoleic (19% pentru Soiul A și 28% pentru Soiul F). Conținutul de acid linoleic a fost corelat negativ cu conținutul de acid oleic ( $r = -0,862$ ) și au fost găsite corelații scăzute între acidul oleic și acidul stearic. Capacitatea antioxidantă determinată prin utilizarea metodei DPPH are valori între 73% (Soiul F) și 91% (Soiul A), pentru probele investigate. Studiul este important, deoarece investighează profilul de acizi grași și activitatea antioxidantă a migdalelor care sunt recomandate în dieta umană.

**Keywords:** fatty acids, almonds, antioxidant activity

### Introduction

Almond (*Prunus dulcis* syn. *amygdalus*) has an important economic value being one of the most cultivated nut trees worldwide. The total production of almond with shell exceeds 3.2 million tons in 2016 from which United States provided 63% of the total. In Europe, almost all almonds are produced in Spain [22]. In Romania, *Prunus dulcis* is less cultivated due to its low resistance to extreme temperatures. There are only few Romanian varieties, most of them cultivated in Eastern and Western parts of the country where the climate conditions and soil

properties [24, 25] are optimal for them. The cold pressed almond oil is one of the most expensive oils used for pastry, cosmetics and pharmaceutical products. The impact of the almond seeds on human health has been presented in many papers. Due to their high content in monosaturated fatty acid, particularly oleic acid and low content of saturated fats [16, 26], almond seeds could contribute to lowering the cholesterol level [12] and decrease the risk of cardiovascular diseases [1, 8, 10]. It has been shown that almond consumption would alter the serum fatty acids profile for hyperlipidaemia individuals when they use a diet that incorporates almond [20]. Furthermore,

consumption of nuts is essential to decrease aortic atherosclerosis in female apoE-deficient mice compared with consumption of a palm oil diet [28]. Moreover, it has been shown that almond oil consumption could decrease triglyceride level and improve lipid profiles in type 2 diabetic patients [14]. In contrast, dietary supplementation with a functional almond-based beverage did not improve performance neither for senior nor for young athletes [5].

Regarding the chemical composition, almond seeds contain different polyphenols (para-coumaric acid, caffeic acid, vanillic acid, quercetin, kaempferol, isorhamnetin) - with important antioxidant properties [8, 10, 18, 19, 21-23], and vitamin B2 and B3 [2]. In general, fatty acids profiles of almond oil have shown high percentages of monounsaturated fatty acids and low content of unsaturated fatty acids [35]. Anyway, the composition of oils depends on origin, country and climatic conditions and even vary between different genotypes [13, 18, 19, 21, 22, 34]. Sathe *et al.* have shown that the most abundant fatty acids in almonds grown in California are oleic and linoleic acids, but the quantity depends on the location and the crop year [26]. The same results have been found for different almond varieties grown in Spain [16]. Furthermore, significant differences have been determined for the fatty acid profile depending on the pollination treatment [16].

The aim of the present study is to examine the fatty acids composition of six different almond oils varieties from Romania and to show their unique antioxidant capacities.

## Materials and Methods

### Chemicals

The commercial chemicals and solvents used in the study are reagent grade and were used without further purification. Acetone was purchased from Merck, Germany. All solvents used for chromatographic determination were of gas-chromatography purity and have been purchased from Sigma-Aldrich, Germany.

### Sample collection

Six almond varieties (patented by Gîtea M. A. *et al.*), noted as Sort A [29], Sort B [9], Sort C [30], Sort D [31], Sort E [32] and Sort F [33] were grown in an orchard, located in North-Western Romania [10].

### Preparation of almond seed oils

The almond seeds were collected during August-September period, 2017, dehulled and sorted to remove all mesocarp. The seeds were sundried for several days and then finely ground into flour using a blending machine. 100 g of the prepared almond sample flour and 300 mL petroleum ether were subjected to a Soxhlet extraction for 4 hours.

### Determination of fatty acid concentration

The fatty acids were chromatographed as methyl esters according to a modified protocol of the method

described by Copolovici *et al.* [8]. Briefly, 0.3 mL of oil samples and 0.6 mL of methanol/toluene/sulphuric acid (88/10/2, v/v/v) were pipetted into Eppendorf tubes, vortexed and then kept at 80°C for 1 h. The resulting methyl esters were extracted twice with 0.5 mL of heptane and analysed by GC-MS in a Shimadzu 2010 Plus gas chromatography equipment (Shimadzu, Kyoto, Japan). A DB 1 capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 0.93 L/min have been used. The injector temperature and MS source were maintained at a temperature of 250°C and 200°C, respectively. In order to calculate the retention indexes (Kovats's indexes) we used a standard mixture of alkanes (C7-C40) Supelco, USA. Identification of different fatty acid methyl esters has been done based on their MS spectra using NIST 14 library and Willy 09 library.

### Antioxidant activity

The *in vitro* antioxidant activity of almond oils obtained from the six genotypes investigated was determined by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, a spectrophotometric method depicted by Tuberoso *et al.* (2007) [36]. The data are expressed as Trolox equivalent antioxidant capacity (TEAC, mg/L), by using a Trolox calibration curve. 20 µL of sample was added to 3 mL of methanolic DPPH (0.2 mM). The spectrophotometric measurements were performed after a 1-hour period of incubation, in the dark, at room temperature, with a ScanDrop 200 nano-volume spectrophotometer (Analytic Jena, Germany) at 517 nm, using a 10 mm cuvette. Positive controls containing 0.2 mM DPPH solution were used as reference. Inhibition of the DPPH stable free radical was calculated with eq. (1):

$$\%Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (1)$$

where  $Abs_{control}$  is the absorbance of 0.2 mM DPPH in ethanol and  $Abs_{sample}$  is the absorbance of 0.2 mM DPPH in extract.

The Trolox calibration curve in methanol, in the range 0.02 - 4.00 mM was also attained, and data were calculated in Trolox equivalent antioxidant capacity (TEAC, mg/L). The Flash Soft Pro software was used to make the analysis. All measurements have been performed in triplicates.

## Results and Discussion

### Fatty acids profile

Diverse oils present different quantities of common fatty acids. Therefore, the chromatographic determination of the fatty acids contained in oils is crucial to determine the quality and possible alteration of the oil sample. In the gas chromatographic analysis of the almond oils investigated in the present study, palmitic acid (16:0), stearic acid (18:0), oleic acid

(18:1), and linoleic acid (18:2) were determined. They have been shown the average concentrations as follows: palmitic acid 7.04%, stearic acid 2.28%,

oleic acid 67.34%, and linoleic acid 23.87%, as it is depicted in Table I.

**Table I**

Fatty acids profile (in %) and retention indexes for almond oils obtained from six Romanian varieties

Almond variety	Palmitic ac. C <sub>16:0</sub>	Stearic ac. C <sub>18:0</sub>	∑ SFA (%)	Oleic ac. C <sub>18:1 n=9</sub>	Linoleic ac. ω-6, C <sub>18:2 n=9,12</sub>	Ratio of oleic ac. to linoleic ac.
RI*	2006	2217	-	2192	2183	-
Sort A [29]	6.50 ± 0.79	2.91 ± 0.69	11.59 ± 1.96	69.02 ± 1.68	19.39 ± 2.34	3.56
Sort B [9]	5.65 ± 0.62	2.52 ± 0.31	8.17 ± 0.93	70.82 ± 3.97	21.02 ± 3.66	3.37
Sort C [30]	10.78 ± 4.33	2.83 ± 0.07	13.61 ± 4.41	70.82 ± 3.97	26.47 ± 4.35	2.32
Sort D [31]	4.44 ± 0.51	ND	4.44 ± 0.51	77.27 ± 4.19	19.77 ± 1.87	3.91
Sort E [32]	6.8 ± 0.62	2.06 ± 0.07	8.86 ± 0.69	64.26 ± 1.08	27.89 ± 1.03	2.30
Sort F [33]	8.22 ± 1.85	3.36 ± 0.36	11.57 ± 2.21	61.34 ± 1.60	28.66 ± 1.63	2.14
General average	7.06	2.28	9.70	67.34	23.87	2.93

\*RI: retention index (Kovats's index); ND: Not determined

In Sort D [31], stearic acid was not determined. In the analysed samples, saturated fatty acids (SFA) 9.70%, unsaturated fatty acids (USFA) 91.21% and a rate of USFA/SFA of 9.40, monounsaturated fatty acids (MUFA) 67.34%, polyunsaturated fatty acids (PUFA) 23.87% and a MUFA/PUFA ratio of 2.82 were found. As it can be observed, the highest

concentration in any fatty acid in almond oil is oleic acid, a MUFA important in human consumption.

In order to compare the fatty acid composition of almond oils obtained from different almond varieties grown in diverse parts of the world we used the data obtained in this study and that was reported in some papers [4, 6, 13, 15, 17, 26, 27, 34]. These data are shown in Table II.

**Table II**

The average sum values of different fatty acids classes: SFA, MUFA and PUFA, in %, determined in the present study and some data reported in literature

Country of study	Reference No.	Fatty acids classes		
		∑ SFA	∑ MUFA	∑ PUFA
Present study, Romania	-	9.70	67.34	23.87
China	27	8.89	66.06	24.40
Turkey	3	9.33	70.84	18.48
Turkey	10	6.19	74.46	17.89
Spain	24	8.5	62.5	29.0
Spain	5	8.38	66.06	25.62
Spain	14	8.31	70.93	19.65
Spain	13	8.15	71.85	18.64
USA	23	6.69	65.51	27.27

Tian *et al.* determined 18 fatty acids, from (14:0) till (23:0), in 13 varieties of almond oils, from which 13 fatty acids presented a concentration smaller than 1% [35]. The major percentage was determined as oleic acid (64.78%), followed by linoleic acid (22.71%). The total amounts of fatty 8.89%, as it is acids were MUFA 66.06%, PUFA, 24.40% and SFA, depicted in Table II.

Askin *et al.* have selected 26 genotypes from almond population grown in Turkey, with the kernel weight of the genotypes ranging from 0.50 to 1.34 g [4]. For the almond oils investigated the fatty acids content varied as follows: oleic acid: 50.41% and 81.2%, linoleic acid: 6.21% to 37.13%, palmitic acid: 5.46% and 15.78%, stearic acid: 0.80% and 3.83%, palmitoleic acid: 0.36% and 2.52%. The study revealed that the almond genotypes with high kernel weight

presented higher contents of oleic, stearic, and palmitic acids, but lower contents of linoleic acid, meanwhile the shell thickness was positively correlated with the oleic acid content and negatively correlated with SFA content.

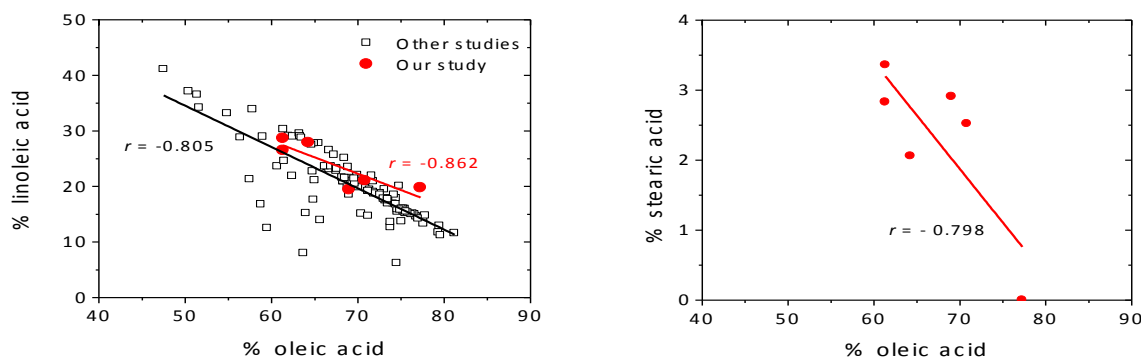
Soler *et al.* determined the fatty acid composition of Pons almond variety seeds depending on harvesting in three different development stages, and these contained an average of: 6.5% palmitic acid, 1.5% stearic acid, 0.5% palmitoleic acid, 62.5% oleic acid and 29.0% linoleic acid [27].

Data obtained from the investigation of fatty acid profile of 19 almond varieties [5] were also reported. In this study was also revealed that two unsaturated acids (18:1 and 18:2) represent more than 90% of the total fatty acids.

Kodad *et al.* reported detailed analyses of fatty acid composition and tocopherol concentration for 44 local varieties of almonds from Spain in three different years of harvesting [16]. The study showed values between 64% and 80% of the concentration of oleic acid (the main acid) in the samples, and oleic acid/linoleic acid ratio varying from 2.8 to 5.5. The results demonstrated that the chemical composition of almond oils depends on the phenotypic variability, even grown

in similar climate conditions and the diversity of germplasm collections must be kept.

Figure 1a presents the correlation between the percentage of linoleic and oleic acids content in almond oils determined in our study and in articles mentioned in Table II, and Figure 1b shows the correlation between stearic and oleic acids in almond oils investigated in the present study.



**Figure 1.**

Correlation between the percentage of **a.** linoleic and oleic acids content in almond oils and **b.** stearic and oleic acids in almond oils

Furthermore, we evaluated the correlation analyses for the Romanian almond varieties analysed in our study. We determined that linoleic acid content was negatively correlated with oleic acid content, the highest correlation coefficient being  $r = -0.862$ . The meta-correlation analyses for 108 different almond oils from different countries and varieties discussed above indicate the same trend, with a correlation coefficient of  $r = -0.805$ . On the other hand, low correlations have been found between the oleic acid and stearic acid ( $r = -0.798$ ). Similarly, a negative correlation has been found for almond genotypes from Anatolia, Turkey [4].

The ratio of oleic acid to linoleic acid is an important parameter to evaluate kernel quality, and the values obtained for our samples are between 2.14 (Sort F [33]) and 3.91 (Sort D [31]), with an average of 2.39 (Table I). The high content of oleic acid is assuring a good resistance of almond nuts for

diverse processes as industrial processing, storage, transportation [16].

*DPPH radical scavenging activity*

The DPPH radical scavenging activity was determined by using a spectrophotometric method [36]. The DPPH· radical, which is a stable organic free radical, presents an absorption maximum band at  $\lambda = 515 - 528$  nm and it is widely used to assess the scavenging ability of an antioxidant. During the reactions of the DPPH with antioxidant molecules the blue colour of the DPPH solution may reach to a yellow-coloured solution, in accordance with the hydrogen donating ability of the antioxidants. The DPPH· free radical scavenging activities of almond seeds oil of the six Romanian varieties were evaluated. Inhibition values determined for the studied oils varied from 73.13% for sort F till 91.21% for Sort A [29].

Similarly, in Table III are presented the values of TEAC, mg/L, for the investigated samples, Sort A [29] exhibiting the highest antioxidant activity.

**Table III**

Antioxidant activity of the oils obtained from six new almond varieties grown in the North-Western part of Romania

Almond variety	Inhibition %	TEAC (mg/L)
Sort A [29]	91.2 ± 2.3	1.54 ± 0.02
Sort B [9]	85.4 ± 1.6	1.48 ± 0.02
Sort C [30]	89.2 ± 1.7	1.52 ± 0.02
Sort D [31]	76.2 ± 1.6	1.38 ± 0.01
Sort E [32]	79.5 ± 5.8	1.42 ± 0.06
Sort F [33]	73.1 ± 1.4	1.35 ± 0.02

Our study presents similar results as that reported by Keser *et al.* which determined that almond extract

scavenged 89.50% of the ABTS radical, 66.77% of the hydroxyl radical, and 87.30% of the DPPH

radical [15]. In their study, the major fatty acids were oleic (76.23%) and linoleic acid (15.43%) with a ratio of them around 3.5. It could be speculated that such ratio of oleic acid to linoleic acid is responsible for high antioxidant activities and lower rancidity. Indeed, it has been shown that white apricot almond oil presented a higher scavenging capacity compared with that of ascorbic acid in the DPPH system [35].

Arranz *et al.* determined the antioxidant activity of several nut oils and obtained the following ranking: peanut oil < almond oil < walnut oil < hazelnut oil < pistachio oil (with EC<sub>50</sub> 712.2 ± 36 g oil/g DPPH for almond oil) [3]. These results are well corroborated with our earlier published data related to antioxidant activity of walnut cold-pressed oil [8], That contained 8.80% SFA, 18.36% MUFA and 72.84% PUFA.

### Conclusions

The fatty acid composition and antioxidant capacity were determined for six patented almond varieties grown in the North-Western part of Romania, Bihor County. The main fatty acid is oleic acid, with the percentage varying from 61% to 77%, followed by linoleic acid (19 - 28%). Linoleic acid content was negatively correlated with oleic acid content, the highest correlation coefficient being  $r = -0.862$  and low correlations have been found between oleic and stearic acids ( $r = -0.798$ ). The inhibition percentage determined by using DPPH system varied between 73% and 91% for the investigated samples. This study indicates, once again, the importance of nuts in the human diet, including almond kernels that are rich in MUFA and present a significant antioxidant activity.

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