

# The Fatty Acids Composition and Antioxidant Activity of Walnut Cold Press Oil

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*The fatty acids composition and antioxidant activity have been determinate for cold press walnut oil. It has been found that the total saturated fatty acids have been 8.8 % while poly unsaturated fatty acids have been 72.84 %. The ratio between omega-6 and omega-3 has been determined as 5.06 which could help in human healthy diet. On the same side, the antioxidant activity of the oil is very high at a level of 3.65 mmol L<sup>-1</sup> and a ratio between lipophilic and hydrophilic fractions of 9.45.*

**Keywords:** fatty acids, antioxidant activity, walnut cold press oil

The Persian walnut (*Juglans regia* L.) is one of the most cultivated species throughout Northern Africa, Eastern Asia and Southern Europe. The world production of walnut has been in 2013 3.458 million tones with China having the highest production (46%) followed by Iran, USA, Turkey and Ukraine (www.fao.org). In Romania, the productions of walnuts with shell have been more than 30.000 tones, in 2013. The walnuts oil is used in food preparation, mostly in cold dishes. Cold press walnuts oil is one of the most expensive culinary oils and is produced mostly in East Europe. Walnuts oil is dietary sources of essential fatty acids which could not be synthesized in the body and must be supplied by food [1]. Recently, has been demonstrated that walnut consumption could protect against colon cancer by changes of the gut microbiome [2]. As well, cold press oil obtained from walnuts could decrease the sugar concentrations in patients with diabetes mellitus type 2 [3]. Even more recent studies have been shown that walnuts oil could be used in cosmetics due to their exceptionally lipid quality [4].

Triacylglycerol is the major component of different nut oils which contain in high amounts monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Most of the nuts oils have high content of monounsaturated fatty acids [5]. For example, monounsaturated fatty acids percentage in almond oil vary between 66% and 78%, depending on cultivars [6, 7], while hazelnuts oil content of monounsaturated fatty acids is around 80% [8]. In contrast, walnuts oil has been shown to have high percentages of polyunsaturated fatty acids as linoleic (PUFA) acid and linolenic acid [9, 10, 11]. Indeed, the concentration of total PUFA in different cultivar of walnuts oil has been shown to be more than 60 % [9]. For example, Martinez and Maestri [12] has been found in different genotypes grown in Argentina a variation in oleic acid between 16.1 and 25.4%, linoleic acid between 52.5 and 58.9% and linolenic acid between 11.4 and 16.5%. The same type of values have been determined by Amaral et al. [13] for six walnut cultivars grown in Portugal. They have found that total PUFA vary between 71 and 75 % while total MUFA vary between 16 and 20 % (with the oleic acid main component). In the case of Romanian cultivars there are not any information regarding to fatty

acids composition, the only information came for total phenolic coming from six different cultivars [14].

## Experimental part

### Determination of fatty acid concentration

The fatty acid contained in walnut oils were transmethylated into corresponded fatty acid methyl esters by treating 0.2 mL of oil samples with 1.5 mL of methanol/toluene/sulphuric acid (88/10/2 v/v/v) for 1 h, at 80° C. The resulted methyl esters were then extracted twice with 1 mL of heptane and analyzed by GC-MS in a Shimadzu 2010 Plus gas chromatography apparatus (Shimadzu, Kyoto, Japan). The column used was a capillary column DB 1 (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<sup>-1</sup>. The injector temperature and MS source were maintained at a temperature of 250 and 200° C, respectively. Identification of different fatty acid methyl esters have been done based on their MS spectra using NIST 14 library and Willy 09 library.

### Radical scavenging activity

The in vitro antioxidant activity of walnut oil was determined by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, a spectrophotometric method presented by Tuberoso et al. [15]. The data were shown as Trolox equivalent antioxidant capacity (TEAC, mmol/L), by using a Trolox calibration curve. The antioxidant activities of walnut oil and as well its lipophilic and hydrophilic fractions were determined. In order to separate the lipophilic and hydrophilic fractions we mixed 500 µL of walnut oil with 500 µL of methanol in an Eppendorf microtest tube, then we shook in a vibration mixer for 10 s, putted in a rotary shaker for 30 min and then shook in the vibration mixer for 10 s. After 10 min of centrifugation at 800 rpm, the solution was allowed to separate and the methanolic phase that contain the polar compounds was separated from the lipophilic compounds. The hydrophilic fraction was tested using 20 µL of extract added to 3 mL of methanolic DPPH (0.04 mM). The walnut oil and the lipophilic fraction were tested in similar way but using DPPH dissolved in heptane. The spectrophotometric readings were carried out after one hour period of incubation, in dark and at room temperature,

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with a ScanDrop 200 Nano-Volume spectrophotometer (Analytic Jena, Germany) at 517 nm using a 10 mm cuvette. All the determinations were performed two times. The Trolox calibration curve in methanol, in the range 0.02–4.00 mM was prepared, and data were calculated in Trolox equivalent antioxidant capacity (TEAC, mmol/L). The Flash Soft Pro software were used to perform the analysis.

All solvents are gas-chromatography purity and have been purchase from Sigma-Aldrich.

All measurements have been done in triplicates.

## Results and discussions

### Fatty acids profile

The fatty acid profile of both walnut oils have been determined as corresponding methyl esters, using gas chromatography. A typical chromatogram is presented in figure 1.

It could be seen than eight different saturated and unsaturated fatty acids could be separated. The fatty acids composition is presented in table 1. From all poly unsaturated fatty acids we found in the samples only linoleic acid ( $\omega$ -6,  $C_{18:2n,9,12}$ ) and  $\alpha$ -linolenic acid ( $\omega$ -3,  $C_{18:3n,9,12,15}$ ) which is quite one of the feature of the walnut

oils which have been determined in other studies as well (table 1).

The amount of the fatty acid with or without double bonds varies between different studies with less than 5%, even if the nut varieties, growing time, and growth conditions are different (table 2). Usually, the compositions of active principle from plants and their parts (root, stem, leaves, flowers, fruits) vary depending on climate, soil composition, and meteorological conditions [16-19].

The ratio between omega 6 and omega 3 for our determination is around 5, in good agreement with other authors (table 2). Simopoulos [20] showed that a ratio of 5 to 1 had a valuable effect on patients with asthma. Even more, in Greece varieties those ratio is around 3 which is the most valuable ratio for human health [21].

### DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by using a spectrophotometric method detailed by Tuberoso et al. [15]. The DPPH $\cdot$  radical is a stable organic free radical which presents an absorption maximum band at 515-528 nm and it is widely used to assess the scavenging ability of an antioxidant. During DPPH and

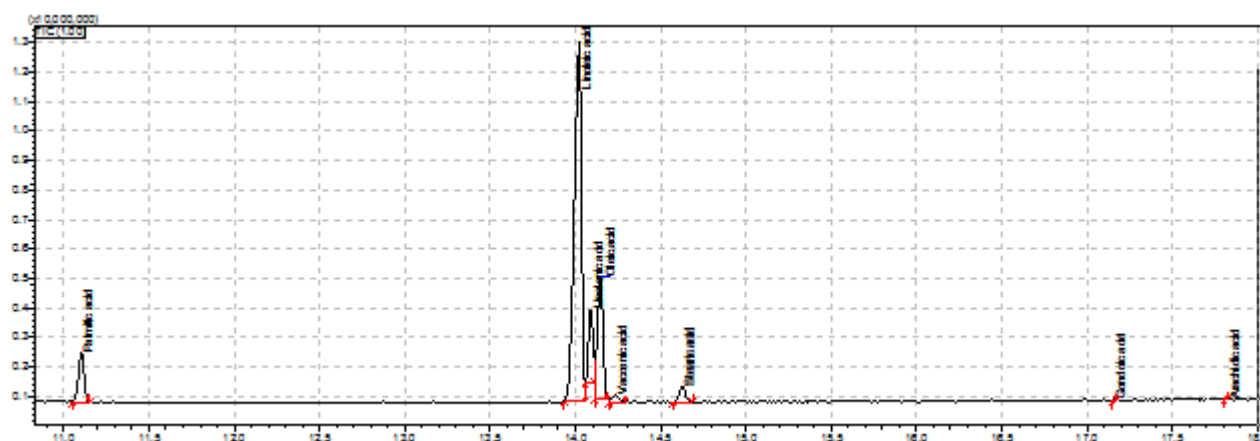


Fig. 1. The separation of fatty acids using gas-chromatography

Table 1

FATTY ACIDS PROFILE FOR WALNUTS OILS COMPARED WITH THE LITERATURE DATA

Fatty acid/%	Present study Romania	(Christopoulos and Tsantili 2015 ) Greece <sup>1</sup>	(Arranz et al. 2008) Spain	(Martinez and Maestri 2008) Argentina <sup>2</sup>	(Bialek et al. 2016 ) Poland	(Amaral et al. 2003 ) Portugal <sup>3</sup>	(Bujdosó et al. 2016) Hungary <sup>4</sup>
Palmitic acid $C_{16:0}$	6.58	7.47	7.04	7.12	7.40	6.98	6.82
Stearic acid $C_{18:0}$	2.16	3.05	2.27	2.07	2.60	2.51	4.60
Arachidic acid $C_{20:0}$	0.06	0.09	0.08	-	0.10	0.07	0.09
$\Sigma$ SFA (%)	8.80	10.61	9.39	9.18	10.10	9.56	11.50
Oleic acid $C_{18:1n-7}$	17.04	18.07	13.45	20.86	15.20	16.30	21.19
Vaccenic acid $C_{18:1n-11}$	1.23	0.75	0.97	-	-	1.27	-
Gondoic $C_{20:1n-11}$	0.09	0.21	11.85	-	-	0.19	-
$\Sigma$ MUFA (%)	18.36	19.04	26.27	20.86	15.20	17.76	21.19
linoleic acid ( $\omega$ -6, $C_{18:2n,9,12}$ )	60.82	53.70	63.19	56.65	59.50	60.31	57.06
$\alpha$ -linolenic acid ( $\omega$ -3, $C_{18:3n,9,12,15}$ )	12.02	14.39	0.05	13.88	9.60	11.95	11.06
$\Sigma$ PUFA (%) <sub>I</sub>	72.84	68.09	63.24	70.53	69.10	72.26	68.12
$\omega$ -6/ $\omega$ -3	5.06	3.73	-	4.08	6.20	5.05	5.16

<sup>1</sup>Average data for oils from 5 different varieties of *Juglans regia* L. grown in Greece; <sup>2</sup>Average data for oils from 8 different varieties of *Juglans regia* L. grown in Argentina;

<sup>3</sup>Average data for oils from 6 different varieties of *Juglans regia* L. grown in Portugal;

<sup>4</sup>Average data for oils from 11 different varieties of *Juglans regia* L. grown in Hungary

**Table 2**  
THE SUM OF DIFFERENT FATTY ACIDS CLASSES AS FOLLOWING: SATURATED, MONOSATURATED AND POLYSATURATED

Fatty acid/%	Present study Romania	(Christopoulos and Tsantili 2015) Greece <sup>1</sup>	(Arranz et al. 2008) Spain	(Martinez and Maestri 2008) Argentina <sup>2</sup>	(Bialek et al. 2016) Poland	(Amaral et al. 2003) Portugal <sup>3</sup>	(Bujdoso et al. 2016) Hungary <sup>4</sup>
Σ SFA (%)	8.80	10.61	9.39	9.18	10.10	9.56	11.50
Σ MUFA (%)	18.36	19.04	26.27	20.86	15.20	17.76	21.19
Σ PUFA (%)	72.84	68.09	63.24	70.53	69.10	72.26	68.12
ω-6/ω-3	5.06	3.73	-	4.08	6.20	5.05	5.16

antioxidants reactions, the blue colour of the DPPH solution may reach a yellow colour, in accordance with the hydrogen donating ability of the antioxidants. The DPPH-free radical scavenging activities of (i) walnut oil, (ii) lipophilic fraction (insoluble in methanol), and (iii) hydrophilic fraction (soluble in methanol) were evaluated. TEAC values determined are the following: (i) walnut oil:  $3.6566 \pm 0.035 \text{ mmol L}^{-1}$ , (ii) lipophilic fraction (insoluble in methanol):  $3.6527 \pm 0.0272 \text{ mmol L}^{-1}$  and (iii) hydrophilic fraction (extracted in methanol):  $0.3864 \pm 0.104 \text{ mmol L}^{-1}$ . The lipophilic fraction has higher antioxidant activity compared with that of hydrophilic fraction, result that is in good agreement with the earlier published data for diverse seed oils [15]. The ratio between lipophilic and hydrophilic fractions is 9.45, demonstrating the high contribution of the lipophilic fraction for the antioxidant activity of the samples.

### Conclusions

Determination of fatty acid composition reveals that eight different saturated and unsaturated fatty acids could be separated and the total saturated fatty acids have been 8.8 %, while poly unsaturated fatty acids have been 72.84 %. The ratio between omega-6 and omega-3 has been determined as being 5.06, value that could help in human healthy diet. The antioxidant activity of the oil is very high, at a level of  $3.65 \text{ mmol L}^{-1}$ , and the ratio between lipophilic and hydrophilic fractions was 9.45.

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