Determination of Fatty Acid and Tocopherol Compositions and the Oxidative Stability of Walnut (*Juglans regia* L.) Cultivars Grown in Serbia

Biljana RABRENOVIC[1], Etelka DIMIC[2], Milan MAKSIMOVIC[3], Sladjana SOBAJIC[4] and Ljiljana GAJIC-KRSTAJIC[5]

[1]Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Zemun, Serbia; [2]Faculty of Technology, University of Novi Sad, Novi Sad, Serbia; [3]Institute of Hygiene, Military Medical Academy, Belgrade, Serbia; [4]Department of Bromatology, Faculty of Pharmacy, Belgrade University, Belgrade, Serbia; [5]Faculty of Agronomy, University of Kragujevac, Cacak, Serbia

Abstract

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Five cultivars (Sampion, Jupiter, Sejnovo, Elit, and Geisenheim 139) of walnuts (*Juglans regia* L.) were collected during the 2008 harvest, from Cacak, Central Serbia. Two techniques of oil extraction were implemented – cold pressing and organic solvent extraction. The influence of the implemented methods on the fatty acid composition, tocopherol content as well as oxidative stability was examined. Predominant fatty acids were palmitic, oleic, linoleic, and linolenic acids. The oleic acid content of the oils ranged from 15.9–23.7% of the total fatty acids, while the linoleic acid content ranged from 57.2–65.1% and that of linolenic acid from 9.1–13.6%. The process of oil extraction had no significant effect on the content and composition of fatty acids in the oil. The total content of tocopherols ranged from 28.40 mg/100 g to 42.40 mg/100 g of the extracted oil. The most common tocopherol in all samples was γ-tocopherol. The oil extracted using the Soxhlet method contained higher amounts of total tocopherols while the stability of the oil samples, expresed as induction period, ranged from 5.0 h to 7.1 hours. Reduced stability of the oil samples as measured by the Rancimat method was negatively correlated with the level of linolenic acid and total content of tocopherols.

Keywords: walnut oil; solvent extraction; cold pressing; fatty acid composition; tocopherol content; oxidative stability

Nuts have been part of the human diet for a long time and their remains have been found in archaeological sites dating back to before 10 000 BC. They are a good source of macronutrients and micronutrients, as well as other bioactive constituents (Laskowski & Kulikowska 1967).

Walnut kernels (*Juglans regia* L.) generally contain about 60% oil (Prasad 1994), but this can vary from 50% to 70% depending on the cultivar, location, and irrigation rate (Greve 1992; Garcia *et al.* 1994).

The major fatty acids found in walnut oil are oleic (C18:1), linoleic (C18:2, ω-6) and linolenic (C18:3, ω-3) acids. High levels of these polyunsaturated fatty acids (PUFAs) are more desirable because of their health benefits, although lower linoleic and linolenic acids contents may provide longer shelf life (Cunnane *et al.* 1993).

Walnuts have generated considerable interest in the last decade because several studies suggested that their intake decreases total plasma cholesterol

and low-density-lipoprotein cholesterol (Abbey *et al.* 1993; Sabate *et al.* 1993). These properties may be attributed to the fatty acid profile found in walnut oil, in particular the presence of ω-3 and ω-6 PUFAs which are essential dietary fatty acids, and to their favourable ratio in walnut oil.

Epidemiological and clinical trials suggest that ω-3 PUFAs may have a significant role in the prevention of coronary heart disease. Several mechanisms have been suggested for their action, including antiarrhythmic, hypolipidemic, and antithrombotic roles. The foods of plant origin rarely have a high content of ω-3 fatty acids (FA), and walnuts and walnut oil together with lineseed oil, canola oil, and soy oil make important contribution to ω-3 FA daily intake (Harper & Jacobson 2001).

This study is a preliminary investigation into the fatty acid compositions and tocopherol content and their influence on the oxidative stability of oil from five walnut cultivars grown in the Central Serbia. These data should help in selecting the cultivars that are suitable for the commercial production of walnut oil.

MATERIALS AND METHODS

Walnut cultivars. Five walnut (*Juglans regia* L.) cultivars Sampion, Jupiter, Sejnovo, Elit, and Geisenheim 139 (G-139) were studied. The walnut fruits were taken from the orchard of the Institute for Fruit and Vegetables, Cacak, Central Serbia.

The walnut fruits were harvested in autumn 2008, and a final representative samples of 2 kg were collected for analysis. The walnuts were stored in shell in paper bags, in a dark room at approximately 12°C for 2 months.

Walnut oil. Prior to chemical analysis, the walnuts were manually cracked and shelled and then milled into a fine powder in a coffee mill (Braun, Kronberg, Germany). Oil was extracted using two different techniques of oil extraction, cold pressing using a hand press and organic solvent extraction. To obtain oil samples by solvent extraction, chopped walnuts (20 g) were extracted with light petroleum ether (b.p. 40–60°C) in a Soxhlet apparatus following the AOCS method Aa 4–38 (AOCS 1993), and the remaining solvent was removed by vacuum distillation. Both extracted oil samples had a light yellow colour and very characteristic nutty flavour. All samples were stored in polypropylene tubes at –20°C prior to analysis.

All solvents and reagents used in the analytical determinations were from Merck (Darmstadt, Germany) and Sigma (Poole, UK), p. a. type.

Fatty acid composition. Fatty acids were analysed as fatty acid methyl esters using GLC method. Fatty acids were transformed to fatty acid methyl esters by direct transesterification for neutral samples (ISO 5509:2000, Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids). Fatty acid composition was determined by gas chromatography (GC; VARIAN chromatograph, model 1400; Varian Associates, Walnut Creek, CA), equipped with a flame ionisation detector and a 3.0 m × 0.32 cm steel column, packed with LAC-3R-728 (20%; Cambridge Ind. Co., Cambridge, UK) on ChromosorbW/AW(80-100 mesh; Merck, Darmstadt, Germany). Nitrogen was used as a carrier gas (flow rate, 24 ml/min) (ISO 5508:1990, Analysis by gas chromatography of methyl esters of fatty acids).

Fatty acids were identified by comparison of their retention times (Rt) with those of standards (Supelco^{TM} FAME Mix). All determinations were carried out in triplicates.

Composition of tocopherols. Quantification of tocopherols was carried out using high performance liquid chromatography (Waters M600E, Milfold, USA) on a reversed phase column Nucleosil 50-5 C18 with fluorescence detection. The *n*-hexan extraction was applied, with the extract vaporisation and reconstitution in methanol using membrane filtration. Mobile phase was 95% ethanol with the flow rate of 1.2 ml/minute. The detection conditions for tocopherol were 295 nm wavelength for excitation and 330 nm wavelength for emission.

The relative retention value and maximum values of absorption at the given relative retention time were used to identifiy and confirm the presence of tocopherol in the extracted oil samples. The data was processed by using Class-VP software V. 6.2.

Oxidative stability. The oxidative stability was measured using a Rancimat apparatus 617 (Metrohm AG, Herisau, Switzerland). The extracted oil samples (2.5 g) were weighed into test tubes and heated at 100°C while 20 l/h of air flowed through the tubes. The volatile compounds released during oxidation were collected in a cell containing distilled water, and the increasing water conductivity was continually measured. The time taken to reach the conductivity inflection point was recorded as the induction time. All determinations were carried out in triplicates.

Statistical analysis. The correlation analysis was performed using Statistica softver Version 5.0. (Stat Soft Co., USA).

RESULTS AND DISCUSSION

The results obtained for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the walnut oils analysed are presented in Table 1. No significant differences in the fatty acid composition were found between the oils extracted with petroleum ether in Soxhlet apparatus or by cold pressing.

The major fatty acids in the samples were C18:2 (57.2–65.1%), C18:1 (15.9–23.7%), C18:3 (9.1–13.6%), and palmitic acid (C16:0, 6.3–7.7%). Stearic acid (C18:0) accounted for 1.6–2.2% and palmitoleic acid (C16:1) for 0.1–0.4%, respectively.

Out of these fatty acids, α-linolenic acid (C18:3), which has been associated with health benefits, was present in a significant amount especially in the cultivar G-139 (13.6%).

Arachidic acid (C20:3) was detected in very small amounts in the cultivar Sejnovo while, in contrast, palmitoleic acid was not detected in this cultivar.

The profile of fatty acids found in this study is comparable with the data previously reported in literature (Colombini *et al.* 1979; Ebrahem *et al.* 1994; Ruggeri *et al.* 1998; Zwartz *et al*. 1999; Savage 2001; Shijie *et al.* 2002; Amaral *et al.* 2003). Some differences in the amounts of C16:0 (8–10%) and C18:0 (0.9–2.0%) were noticed in comparison with Turkish walnut cultivars (Koyuncu *et al*. 2001). Caglarirmak (2003) also found different ranges for C18:0 (1.9–15.0%), C18:2 (42.1–49.0%), C18:3 (4.7–7.4%), and C20:0 (3.1–16.0%) in the oils from four Turkish walnuts. In the cold-pressed oils from six Californian cultivars, the levels of C16:0 (2.3–4.4%) and C18:0 (0.6–0.8%) were lower as well as the levels of C18:1 (in one sample 11.8%, in five other samples 5.5–7.7%) but the levels of C18:3 were higher (16.5–25%) (Greve *et al.* 1992).

PUFAs were the main group of fatty acids in walnut oil obtained from the cultivars studied, ranging from 67.4% to 75.7%, while MUFAs ranged from 15.9 to 23.9%, SFAs were the minor group,

Table 1. Fatty acid composition (%) of analysed walnut oil samples (mean ± SD)

Fatty acid		Sampion	Jupiter	Sejnovo	Elit	G-139
C16:0	cold pressing	7.5 ± 0.05	6.9 ± 0.01	6.3 ± 0.23	7.1 ± 0.03	7.7 ± 0.13
	solvent extraction	7.1 ± 0.05	7.0 ± 0.04	6.7 ± 0.01	7.1 ± 0.06	7.4 ± 0.18
C16:1	cold pressing	0.4 ± 0.19	0.2 ± 0.15	–	0.1 ± 0.05	0.4 ± 0.02
	solvent extraction	0.4 ± 0.01	0.1 ± 0.03	–	0.3 ± 0.10	0.4 ± 0.17
C18:0	cold pressing	1.7 ± 0.01	1.7 ± 0.11	1.7 ± 0.17	1.7 ± 0.13	1.7 ± 0.06
	solvent extraction	1.6 ± 0.12	1.8 ± 0.01	1.7 ± 0.07	2.2 ± 0.09	1.6 ± 0.06
C18:1	cold pressing	20.7 ± 0.20	23.7 ± 0.09	15.9 ± 0.11	21.2 ± 0.07	18.6 ± 0.17
	solvent extraction	19.0 ± 0.13	22.9 ± 0.02	16.2 ± 0.15	21.6 ± 0.07	19.8 ± 0.23
C18:2	cold pressing	59.8 ± 0.09	58.3 ± 0.21	65.1 ± 0.01	60.3 ± 0.30	58.0 ± 0.05
	solvent extraction	60.9 ± 0.11	58.1 ± 0.04	63.3 ± 0.11	58.8 ± 0.18	57.2 ± 0.02
C18:3	cold pressing	9.8 ± 0.17	9.1 ± 0.09	10.6 ± 0.30	9.6 ± 0.10	13.1 ± 0.15
	solvent extraction	11.0 ± 0.22	9.9 ± 0.19	11.2 ± 0.06	9.9 ± 0.07	13.6 ± 0.01
C20:0	cold pressing	–	–	0.3 ± 0.01	–	–
	solvent extraction	–		0.8 ± 0.14	–	–
ΣSFA	cold pressing	9.2 ± 0.06	8.6 ± 0.12	8.3 ± 0.41	8.8 ± 0.16	9.4 ± 0.19
	solvent extraction	8.7 ± 0.17	8.8 ± 0.05	9.2 ± 0.22	9.3 ± 0.15	9.0 ± 0.24
ΣMUFA	cold pressing	21.1 ± 0.39	23.9 ± 0.24	15.9 ± 0.11	21.3 ± 0.12	19.0 ± 0.19
	solvent extraction	19.04 ± 0.14	23.0 ± 0.05	16.2 ± 0.15	21.9 ± 0.17	20.2 ± 0.40
ΣPUFA	cold pressing	69.6 ± 0.26	67.4 ± 0.30	75.7 ± 0.31	69.9 ± 0.40	71.1 ± 0.20
	solvent extraction	71.9 ± 0.33	68.0 ± 0.23	74.5 ± 0.17	68.7 ± 0.25	70.8 ± 0.03

Table 2. Tocopherol contents (mg/100 g oil) of analysed walnut oil samples (mean ± SD)

		Sampion	Jupiter	Sejnovo	Elit	G-139
α-Tocopherol	cold pressing	1.9 ± 0.11	2.0 ± 0.47	2.6 ± 0.31	1.8 ± 0.09	2.0 ± 0.23
	solvent extraction	1.5 ± 0.02	1.6 ± 0.13	2.1 ± 0.09	1.6 ± 0.15	2.0 ± 0.01
γ + β-Tocopherol	cold pressing	33.2 ± 0.21	25.4 ± 0.18	30.9 ± 1.07	27.5 ± 0.22	33.2 ± 0.09
	solvent extraction	34.5 ± 0.29	29.6 ± 0.03	32.1 ± 0.03	32.1 ± 1.06	38.4 ± 0.30
δ-Tocopherol	cold pressing	1.4 ± 0.21	1.0 ± 0.01	2.6 ± 0.23	1.5 ± 0.13	2.4 ± 1.12
	solvent extraction	2.0 ± 0.11	2.0 ± 0.09	3.4 ± 0.30	3.1 ± 0.08	2.0 ± 0.49
Total content	cold pressing	36.5 ± 0.53	28.4 ± 0.66	36.1 ± 0.61	30.8 ± 0.44	37.6 ± 0.44
	solvent extraction	37.9 ± 0.42	33.2 ± 0.25	37.6 ± 0.42	36.8 ± 0.29	42.4 ± 0.80

ranging from 8.3% to 9.4%. The cultivars with lower PUFA contents (Jupiter, Sampion and Elit) should be more stable and it is also possible that these more stable cultivars may have some commercial advantages, for example, they could be probably stored much better over a long period of time.

As Table 2 shows, the total content of tocopherols in the analysed samples ranged between 28.4 and 42.4 mg/100 g oil which is comparable with other data published (Demir & Cetin 1999; Savage *et al.* 1999; Crews *et al.* 2005). The highest content of total tocopherols was found in G-139 cultivar oil extracted by the Soxhlet method, while the lowest content was found in the Jupiter cultivar oil, obtained by cold pressing.

This research verified the dominant presence of γ-tocopherol in walnut oil. The content of γ-tocopherol ranged between 25.4 and 38.4 mg/100 g oil. β-Tocopherol was present in a significantly smaller percentage and was difficult to separate from γ-tocopherol.

The results of the oxidative stability measured by Rancimat test and expressed by the induction period (IP) are presented in Table 3. The IP represents the time needed for the decomposition of hydroperoxides produced by oil oxidation (IUPAC 1987).

The IP of the extracted oils ranged from 5.0 h to 7.1 hours. The results reported by Amaral *et al.* (2003) for six French cultivars are much lower, ranging from 2.7 h to 3.4 hours. In all cases was the oxidative stability of the oil extracted using the Soxhlet method higher than that of the same oil extracted by cold pressing. The Soxhlet method was more effecient in extracting tocopherols from each of the walnut oils (Table 2) and this may explain the increased IP values of these samples.

In addition, the reduced stability of the walnut oil appears to be correlated with the high level of PUFA, especially C18:3, in the oils. The linear regression analysis shows that the IP value has a strong but negative correlation with the C18:3 content (r = –0.82 and r = –0.76) regardless of the method of extraction. The oil samples obtained from the cultivar G-139 had the lowest IP and a higher amount of C18:3. The relative rate of oxidation of C18:3 is much faster than those of C18:2 and C18:1 and that could explain the correlation observed.

Also, the linear regression analysis of IP and tocopherol contents show a strong but negative correlation with the solvent extraction method (r = –0.78). That could be explained by the fact that the antioxidative potenial of tocopherols becomes lower with time and with the decrease of the oxidation level.

Owing to the high commercial and high nutritive values of the cold-pressed walnut oil, extreme care needs to be taken to prevent oxidation of the unstable PUFAs in the oil. The susceptibility to oxidation of the walnut oils from the cultivars used in this study as mesaured by the Rancimat test was similar or even better than in almost all the data which we could find in literature.

These data should help in selecting cultivars that are suitable for commercial production of walnut oil in Serbia.

Table 3. Oxidative stability of walnut oil samples expressed as a induction period – IP (h)

		Sampion	Jupiter	Sejnovo	Elit	G-139
IP	cold pressing	5.8	5.7	5.8	5.5	5.0
	solvent extraction	7.1	7.0	6.8	6.5	5.8

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Corresponding author:

Biljana Rabrenovic, MSc., University of Belgrade, Faculty of Agriculture, Department of Food Technology and Biochemistry, Nemanjina 6, 11080 Zemun, Serbia
tel.: + 381 112 615 269, e-mail: biljanar@agrif.bg.ac.rs