Chemistry of Natural Compounds, Vol. 44, No. 2, 2008

PHYSICOCHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF *Juglans regia* CULTIVARS GROWN IN SERBIA

B. Rabrenovic,[1*] K. Picuric-Jovanovic,[1] S. Sobajic[2]

UDC 547.916

*Tree cultivars (Jupiter, Sejnov, and Elit) of walnut (*Juglans regia *L.) were collected during the 2004 harvest from Cacak, Central Serbia. The chemical composition, including moisture, total oil content, crude protein, ash, and carbohydrates, was determined. Afterwards, two techniques of oil extraction were implemented: cold pressing extraction and organic solvent extraction. Iodine value, saponification value, acid value, and peroxide value of obtained walnut oils were analyzed. The fatty acid composition of the walnut oils was determined using gas chromatography with flame ionization detector. The oleic acid content of the oils ranged from 15.9-23.7% of the total acids, while linoleic acid content ranged from 57.2-65.1% and the linolenic acid from 9.1-13.6%. The process of oil extraction had no significant effect on content and composition of fatty acids.*

Key words: walnut oil, cold pressing extraction, solvent extraction, physical and chemical constants, fatty acid composition.

Nuts have been part of the human diet for a long time and 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 [1].

Walnut kernels (*Juglans regia* L.) generally contain about 60% oil [2], but this can vary from 50 to 70% depending on the cultivars, location, and irrigation rate [3, 4].

The major fatty acids found in walnut oil are oleic (C18:1), linoleic (C18:2, ω-6), and linolenic (C18:3, ω-3) acids. The good proportion of these fatty acids is important to the walnut nutritional value. Higher levels of these polyunsaturated fatty acids (PUFAs) are more desirable because of their health benefits, although lower linoleic and linolenic content may provide longer shelf life [5, 6].

Walnuts have generated considerable interest in the last decade because several studies suggest that their intake decrease total plasma cholesterol and low-density-lipoprotein cholesterol [7, 8]. 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 their favorable ratio.

Epidemiological and clinical trials suggest that ω-3 PUFAs may have a significant role in the prevention of coronary heart disease. Several mechanisms were suggested for the action, including antiarrhythmic, hypolipidemic, and antithrombotic roles [9]. Foods of plant origin rarely have a high content of ω-3 fatty acids (FA), and walnuts and walnut oil together with linseed oil, canola oil, and soy oil make an important contribution to ω-3 FA daily intake.

The data in Table 1 show the results of the chemical composition obtained for the three analyzed walnut cultivars. Oil was the predominant component, ranging from 67.6% to 71.7%, followed by carbohydrates, 13.3-15.4%, and protein, 10.9-12.6%. The moisture content of the samples was in the range from 4.2 to 4.8% and total ash from 1.9-2.1%.

The determined values were in agreement with those published previously [10, 11].

The physical and chemical characteristics of the oils, extracted in two different ways, are summarized in Table 2.

1) Department of Food Technology and Biochemistry, Faculty of Agriculture, 11000 Belgrade, Nemanjina 6, Serbia and Montenegro; 2) Department of Bromatology, Faculty of Pharmacy, 11000 Belgrade, 450 Vojvode Stepe, Serbia and Montenegro. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 118-120, March-April, 2008. Original article submitted November 30, 2006.

0009-3130/08/4402-0151

TABLE 1. Chemical Composition (g/100 g of Fresh Weight) of Tree Walnut Cultivars Grown in Serbia

Cultivars	Moisture	Total fat	Ash	Crude protein	Carbohydrates
JUPITER	4.8	67.6	1.9	12.6	15.4
SEJNOVO	4.5	69.6	2.1	12.1	13.4
ELIT	4.2	71.7	1.9	10.9	13.3

TABLE 2. Physical and Chemical Characteristics of Oil Samples Extracted by Cold Pressing (A) and Solvent Extraction (B)

Cultivars	Free fatty acid content		Iodine value, g I_2/100 g oil		Saponification number, mg KOH/g oil	
	A	B	A	B	A	B
JUPITER	0.26	0.27	145.4	149.1	187.9	187.5
SEJNOVO	0.23	0.23	145.5	154.5	191.0	190.0
ELIT	0.18	0.19	144.1	149.5	189.0	189.0

_____

Refractive index at 25°C: 1.473-1.476.

TABLE 3. Fatty Acids Composition of Examined Walnut Oils

Cultivars	$C_{16:0}$		$C_{16:1}$		$C_{18:0}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		$C_{20:0}$	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
JUPITER	6.9	7.0	0.2	0.1	1.7	1.8	23.7	22.9	58.3	58.1	9.1	9.9	-	-
SEJNOVO	6.3	6.7	-	-	1.7	1.7	15.9	16.2	65.1	63.3	10.6	11.2	0.3	0.8
ELIT	7.1	7.1	0.1	0.3	1.7	2.2	21.2	21.6	60.3	58.8	9.6	9.9	-	-

_____

Cold pressing (A) and solvent extraction (B).

Acid value was calculated as the free fatty acid content, and the obtained values were low, ranging from 0.18-0.27%. This means that the analyzed walnut samples were fresh and stored under good conditions prior the analyses.

The results presented in Table 2 clearly show that the iodine values and refractive indexes of the oils were relatively high. This indicates that the analyzed oils belong to oils rich in PUFAs. The iodine value was in the range from 144.1 to 154.5 g/100 g. There was a slight difference between the iodine values of samples obtained by solvent extraction and by pressing. The iodine values of extracted oils were higher than that of pressed oils. Shijie et al. noticed the same in their study [12].

There were no significant differences in saponification values and refractive indexes among extraction methods. Saponification values were in the range from 187.5 to 191.0 mg/g. The refractive index values at 20°C were in the range from 1.473 to 1.476.

The results for fatty acid composition of the investigated walnut oils are presented in Table 3. No significant differences in fatty acid composition were found between the oils extracted by petrol-ether in Soxhlet or by cold pressing.

The major fatty acids in the samples were linoleic (C18:2, 58.1-65.1%), oleic (C18:1, 15.9-23.7%), α-linolenic (C18:3, 9.1-11.2%), and palmitic (C16:0, 6.3-7.1%). The stearic acid accounted for 1.7-2.2%, and palmitoleic acid (C16:1) accounted for 0.1-0.4%.

Arachidic acid was detected only in samples of Sejnovo cultivar and in a small amount, while palmitoleic fatty acid was not detected in this cultivar.

The profile of fatty acids found in this study is comparable with data previously reported in the literature [13-15]. This study is a preliminary investigation of the fat, physical and chemical constants, and fatty acid compositions of cultivars grown in Central Serbia. These data should help in selecting cultivars that are suitable for commercial production of walnut oil.

EXPERIMENTAL

Plant Material. Tree walnut (*Juglans regia* L.) cultivars, Jupiter, Sejnovo, and Elit, 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 the crop year 2004, and a final sample of 2 kg was taken. The walnuts were stored in the shell and closed in paper bags in a dark room at approximately 12°C for 2 months.

Extraction. Before chemical analysis the walnuts were manually cracked and shelled and then chopped in a coffee mill (Braun, Germany). Two techniques of oil extraction were implemented: cold pressing extraction and organic solvent extraction.

Cold pressing extraction was performed by a hand-held cold press. After pressing, the oil samples were filtered. To obtain oil samples by solvent extraction, chopped walnuts (20 g) were extracted with light petroleum ether (Merck, 40-60°C) in a Soxhlet apparatus and the remaining solvent was removed by vacuum distillation.

Both extracted oil samples had a light yellow color and a very characteristic nutty flavor.

All samples were stored in polypropylene tubes at -20°C prior to analysis.

Chemical Analysis of Walnuts. Moisture was determined by oven drying at 105°C for 3 hours. Ash, crude protein (% N×5.3), and total fat contents were determined according to AOAC Official Methods [16]. Carbohydrate content was estimated by difference using the following formula: carbohydrate content = 100% - (% moisture + % protein + % fat + % ash) [17]. All analyses were carried out in duplicate.

Physicochemical Characteristics of Oil Samples. The ordinary oil constants, e.g., acid value, iodine, saponification, and peroxide number, and refractive index, were estimated according to the AOAC Official Methods [18].

Preparation of Fatty Acid Methyl Esters. The total fatty acid composition of the kernel oils was determined by GC in the methyl ester form. Fatty acid methyl esters were prepared using 14% BF3–MeOH solution and extracted with hexane [19].

Gas Chromatography (GC). GC analysis was performed on a VARIAN chromatograph, Model 1400, equipped with a flame ionization detector and a 30 m × 0.32 cm steel column, packed with LAC-3R-728 (20%) on Chromosorb W/AW (80-100 mesh). Nitrogen was used as a carrier gas (flow rate 24 mL/min). The GC oven temperature was kept at 180°C. The detector and injector temperature was 200°C. GC analysis was performed according to the International Standards [20]. Fatty acids were identified by comparison with retention times (Rt) of standards (SupelcoTM FAME Mix). The relative content of each fatty acid, in %, was calculated from the ratio of the relevant peak area to the total peak area for fatty acids.

REFERENCES

1. K. Laskowski and A. Kulikowska, *Akad. Med*., Lublin, Pol. Roczniki Pastwowego Zakladu Higieny, **18,** 483 (1967).
2. R. B. N. Prasad: Walnuts and pecans, In: *Encyclopaedia of Food Science, Food Technology and Nutrition,* Academic Press, London (1994), 4828.
3. C. Greve, G. McGranahan, J. Hasey, R. Snyder, K. Kelly, D. Goldhamerer, and J. Labavitch, *J. Soc. Hort. Sci.,* **117,** 518 (1992).
4. J. M. Garcia, I. T. Agar, and J. Streif, *Turk. J. Agric. Forestry,* **18,** 195 (1994).
5. C. Crews, P. Hough, J. Godward, P. Brereton, M. Lees, S. Guiet, and W. Winkelmann, *J. Agric. Food Chem.,* **53**, 4835 (2005).
6. G. P. Savage, P. C. Dutta, and D. L. McNeil, *J. Am. Oil Chem. Soc.,* **76**, 1059 (1999).
7. J. Sabate, G. E. Fraser, K. Burke, S. F. M. Knutsen, H. Bennett, and K. D. Lindsted, *N. Engl. J. Med.*, **328,** 603 (1993).
8. M. Abbey, M. Noaks, G. B. Belling, and P. J. Nestel, *Am. J. Clin. Nutr.,* **59,** 995 (1994).
9. C. R. Harper and T. A. Jacobson, *Arch. Intern. Med.,* **161,** 2185 (2001).
10. G. P. Savage, *Plant Foods Hum. Nutr.,* **56,** 75 (2001).
11. J. S. Amaral, S. Casal, J. A. Pereira, R. M. Seabra, and B. P. P. Oliveira, *J. Agric. Food Chem.,* **51,** 7698 (2003).
12. Y. Shijie, W. Caire, K. Xiaohong, D. Junmin, and H. Juping, *Shipin Gongye Keji.,* **23,** 33 (2002).
13. S. Ruggeri, M. Cappelloni, L. Gambelli, S. Nicoli, and E. Carnovale, *Ital. J. Food Sci.,* **10,** 243 (1998).
14. L. Zwarts, G. P. Savage, and D. L. McNeil, *Int. J. Food Sci. Nutr.*, **50,** 189 (1999).

15. M. A. Koyuncu, T. Yarilgac, A. Kazankaya, F. Koyuncu, M. Kucuk, and S. M. Sen, *Acta Hortic.,* **544,** 557 (2001).
16. *Official Methods of Analysis*, AOAC (17th ed.), Arlington, Virginia, 2000, Vol II, Ch. 40.
17. N. R. Grosso, V. Nepote, and C. A. Guzman, *J. Agric. Food Chem.,* **48,** 806 (2000).
18. Official Methods of Analysis, AOAC (16th ed.), Arlington, Virginia, 1995, Vol II, Ch. 41.
19. L. D. Metcalfe and A. A. Schmitz, *Annal. Chem.,* **33**, 363 (1961).
20. International standard ISO 5508-1978 (E), ISO 5508-1979 (E).