JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, *17*(3), 261-265

Journal of Agroalimentary Processes and Technologies

Gas-chromatography from the GC-MS analysis for the walnut grown in Romania

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Received: 30 June 2011; Accepted: 16 August 2011

Abstract

In this paper, the fatty acids composition in saturated and unsaturated of walnut (Juglans regia L) core oil was determined. The oil has been obtained using the Soxhlet method (petrol ether extraction of the core), solvent distillation and anhydrous calcium chloride drying. Walnut samples were collected during the 2010 harvest.

Of unsaturated fatty acids ,the oleic acid content of the oil was 13.62 % of the total fatty acids, while the linoleic acid content was 56.57 % and the linolenic acid content was 12.09 %. It was found the palmitic acid was 9.75 % while stearic acid was 3.48 %. The fatty acid profile was determinated by GC-MS according to AOAC standards.

Keywords: fatty acids, walnut core oil, GC-MS

1. Introduction

The literature data confirm that walnuts are a rich source of a number of important nutrients that appear to have a very positive effect on human health. Further experiments on the effects of feeding walnut diets to humans would be of great interest to understand the mechanisms of all the nutrients in walnuts. Despite the need for further research it is clear that prudent consumption of walnuts has played and can continue to play an important role in a healthy diet [1].

Walnuts have a special dietary food value, given their carbohydrate content (11-14%), protein (14-16%) represented the essential amino acids and lipids (62-65%, of which 44-48% are polyunsaturated fatty acids) [2].

Walnuts have generated considerable interest because they are believed to possess plasma cholesterol-lowering properties.

This property is believed to result from the fatty acid profile present in walnut oil. The major fatty acids (FA) found in walnut oil are oleic (18:1 n-9), linoleic (18:2 n-6) and linolenic (18:3 n-3) acids. The ratios of these FA are considered important for their economic and nutritional value. For example, lower linoleic and linolenic acid contents in the oils may have a longer shelf life while higher levels of polyunsaturated fatty acids are more desirable because of their potential health benefits.

In one study, the supplementation of a background diet with 68 g of walnut/day reduced the total and low-density lipoprotein cholesterol by 5 and 9 % respectively, and it was suggested that these reductions would have some positive effects in reducing the risk of coronary heart disease. This is important as Greve et al. (1992) [9] have shown that the fatty acid profile and chemical composition of walnut oil varies among cultivars [3].

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The proximate compositions of walnut are as follows. Energy, 630 kcal; protein, 18.10–13.60%; total oil, 62.60-70.30%; dietary fiber, 5.20%; ash, 1.80% [1]. The chemical composition of 12 walnut genotypes as follows: Protein, 20.92 –25.95%; ash, 1.68-2.06%; fat, 66.30-74.95%. 141-146 141 from 62.4 to 68.7%, the oleic acid content of the oils ranged from 14.3 to 26.1% of the total fatty acids, while the linoleic acid content ranged from 49.3 to 62.3% and the linolenic contents from 8.0 to 13.8% [4].

As a result, walnuts are a rich source of n-3 and n-6 polyunsaturated fatty acids. The effects of walnut consumption on hyperlipidemia and systolic blood pressure, triglyceride and cholesterol were determined. In conclusion, the composition of the fatty acids consumed in the human diet appears to be more important than its total content. The benefits of walnuts in the human diet against hypercholesterolemia were studied [1].

Walnut kernels (*Juglans regia* L.) generally contain about 60% oil, but this can vary from 50 to 70% depending on the cultivars, location, and irrigation rate. The major fatty acids found in walnut oil are oleic (C18:1), linoleic (C18:2, w-6), and linolenic (C18:3, w-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].

Walnut (*Juglans regia* L.) fruits have been used in human nutrition since ancient times. The walnut seed contains high levels of oil (52–70%). The major constituents of walnut oil (WO) are TG, in which monounsaturated FA (mainly oleic acid) and PUFA (linoleic and a-linolenic acids) are present in high amounts. The proportions of these FA are important to the economic and nutritional value of the nut. Higher linoleic and linolenic acids contents may result in a poorer oxidative stability and a shorter shelf life of the oils. Higher oleic acid levels are desirable because of their potential health benefits [6]. The total oil content of the walnut (*Juglans regia* L.) samples were collected ranged from 62.4 to 68.7%. The oleic acid content of the oils ranged from 14.3 to 26.1% of the total fatty acids, while the linoleic acid content ranged from 49.3 to 62.3% and the linolenic contents from 8.0 to 13.8% [7].

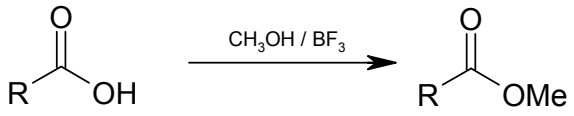
2. Materials and Method

*Soxhlet extraction:*Plant material (walnut core) was collected from the west of Romania, cleaned of impurities, crushing, drying, grinding and Soxhlet extracted with petroleum ether, achieving crude extract.

Materials. Reagents
- Petroleum ether (p.f. = 40-60 °C)
- Soxhlet extraction
- Reflux condenser
- Nest electric thermostat
- Boiling indicator (porous porcelain)

Determination of fatty acids by GC-MS chromatographic technique in oils - Fatty acid derivatization

*General Procedure:*For GC-MS analysis of fatty acid derivatization was necessary to obtain their corresponding methyl esters, more volatile. Derivatization was performed in round-bottomed flask 100 ml, fitted with reflux condenser, which were weighed 100 mg of oleic acid or linolenic • 5 ml methanolic BF_3 (20% BF_3, Lewis acid) to reflux on a water bath for 2 minutes, were introduced 2 ml hexane and continued refluxarea another minute. After cooling esterified mass was treated with 15 ml of saturated NaCl, shake vigorously for 15 seconds, then the flask was filled with the same salt solution to separate the organic layer in the neck of the flask, where it was separated and dried on anhydrous $CaCl_2$.



oleic acid R=$(CH_2)_7$**CH=CH**$(CH_2)_7CH_3$
linoleic acid R=$(CH_2)_7$(**CH=CH**$CH_2)_2(CH_2)_3CH_3$
linolenic acid R=($CH_2)_7$(**CH=CH**$CH_2)_3CH_3$

Figure 1. Fatty acid derivatization (linolenic, linoleic and oleic acid) the system methanol·BF_3.

*Recovery of fatty acids in the complex - General Procedure:*To fatty acids by extraction in hexane, were weighed on analytical balance of samples 100 mg were dissolved in 4 ml of distilled water in a thermostatically controlled extractor provided the mantle, condenser and cooling efficient vigorous magnetic stirring system.

Were brought to 2 ml hexane and stirred vigorously for 20 minutes near the boiling point of hexane, then cool, upper organic layer was separated by pipetting and the aqueous layer was extracted again in a similar manner, yet three times with 2 ml hexane. Combined hexane extracts were then dried on anhydrous CaCl2, derivatized and analyzed by GC-MS.

*GC-MS analysis:*For fatty acid analysis of samples degraded esterified or using a gas chromatographic analysis system coupled with a mass spectrometric detection. We used a Hewlett Packard HP 6890 Series GC coupled with mass spectrometer Hewlett Packard 5973 Mass Selective Detector. For the quantitative determination using a calibration factor of 1.0 (external standard Dodecanese) and the injection was performed using a HP Autosampler GC-MS system attached.

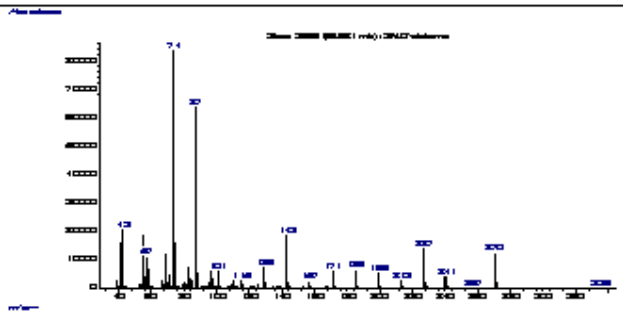
GC analysis conditions were:

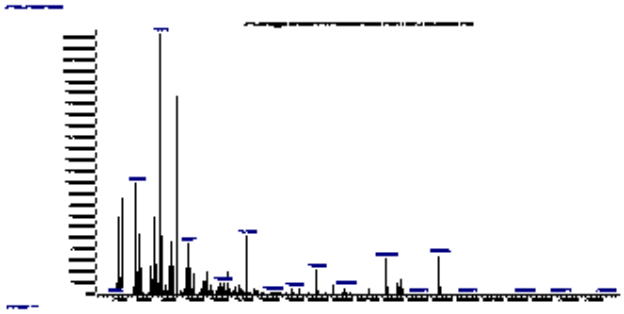
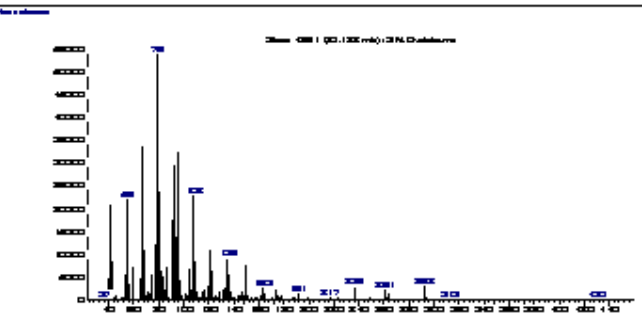
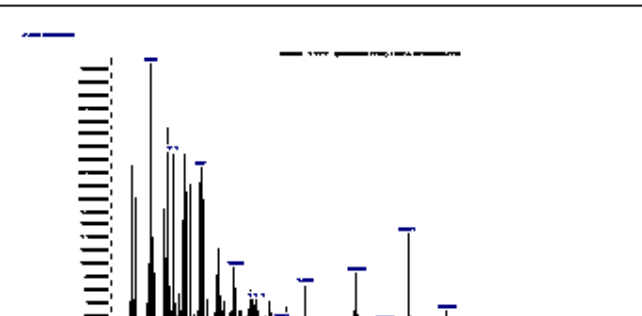
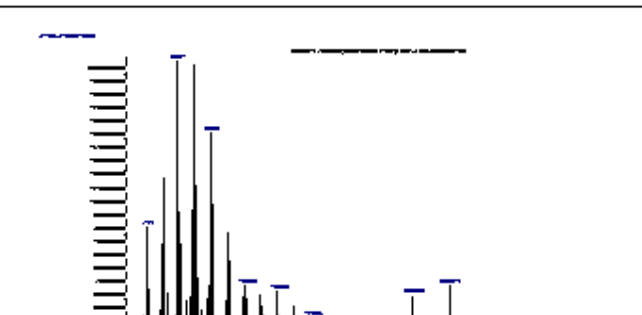
- Column: HP-5 MS 30 m length, inner diameter 0.25 mm, 0.25 mm film thickness;
- Temperature program: 50 °C to 250 °C at a rate of 4 °C/min;
- injector temperature 280 °C;
- detector temperature: 280 °C;
- Injection volume: 2 mL;
- carrier gas: He.

For MS detector has worked with its energy of 70eV, source temperature at 150 ° C, scan range 50-300 AMU, scanning speed 1 s-1 for mass spectrometry and the spectra obtained were compared with database NIST / EPA / NIH Mass Spectral Library 2.0 (2002). Data acquisition was performed using the software package G1701BA Hewlett Packard Enhanced ChemStation ver. B.01.00/1998 and processing of gas chromatography and mass spectrometry was performed using Hewlett Packard Enhanced Data Analysis program of the software package above.The major fatty acids in walnuts, as determined by capillary- column GC (Table 1), were palmitic (16:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). Palmitic acid (9.75%) was the major saturated fatty acid, followed by stearic acid (3.48%). Linoleic acid (56.57%) was the major unsaturated fatty acid followed by oleic acid (13.62%) and linolenic acid (12.09%) [1,3-7].

The differences in individual contents of fatty acids when compared to literature, may be due to the cultivars used and to the cultivation and /or environmental factors.

Table 1. Composition in fatty acids (saturated and unsaturated) for the walnut core oil

Peak nr.	Fatty acids	RT min	%	MS spectra
1	Palmitic acid, methyl ester	26.62	9.75	 Hexadecanoic acid, methyl ester

2	Stearic acid, methyl ester	29.73	3.48	 Octadecanoic acid, methyl ester
3	Linolenic acid, methyl ester	30.12	12.09	 9,12,15 – Octadecatrienoic acid, methyl ester
4	Oleic acid, methyl ester	29.63	13.62	 9 –Octadecenoic acid, methyl ester
5	Linoleic acid, methyl ester	29.80	56.57	 9,12– Octadecadienoic acid, methyl ester

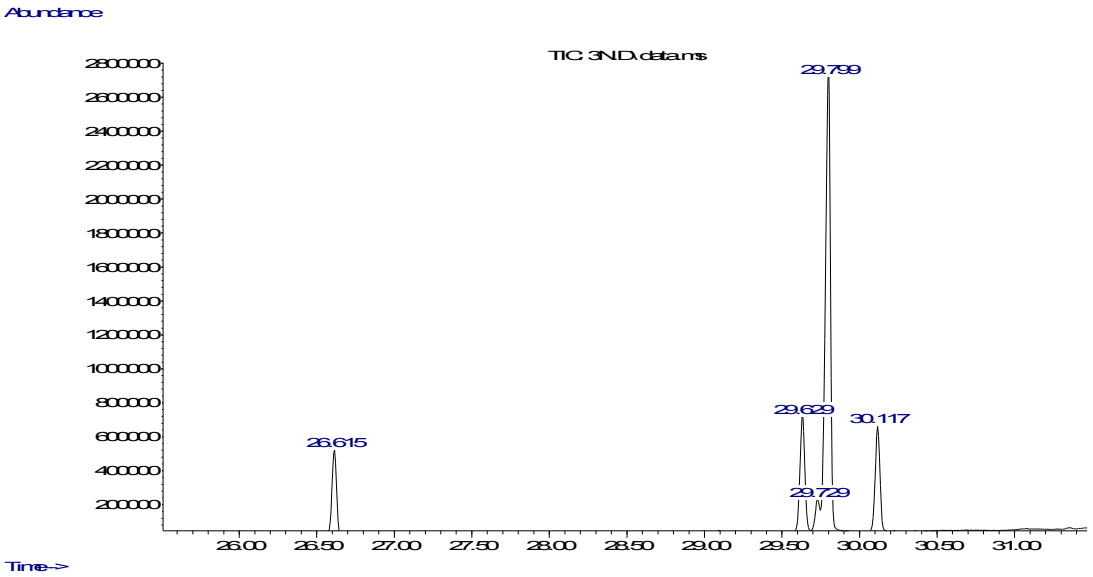


Figure 2. The chromatogram from the GC-MS analysis of the walnut core oil

4. Conclusion

Based on results obtained the fatty acid composition of walnut core oil showed that it falls in the linoleic-oleic acid oils category, therefore the oil be useful for edible purposes and for some industrial applications. The present work confirms previous information from literature that indicates that walnut core are a potential source of edible oil. with high unsaturated fatty acid content and good sources of the essential linoleic fatty acid.

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