

## CHEMICAL COMPOSITION OF WALNUT OIL FROM FRUITS ON DIFFERENT YEARS OLD BRANCHES

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### Abstract

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The influence of the year of branches on the physico-chemical composition of walnut oil was investigated. There was an increasing of oil content with increasing the year of the branch found. The latter did not exhibit significant effect on fatty acid composition. All samples content predominantly unsaturated fatty acids - mainly oleic and linoleic acids. Linolenic was in the range of 9.66–10.77%. The highest content of polyunsaturated linoleic and linolenic acids was 64.14% and 10.77% respectively in fruits grown on four year old branch. The amount of tocopherols was the highest in the oil isolated from one year old branch - 567 mg kg<sup>-1</sup>. In tocopherol fraction of all tested oils  $\gamma$ -tocopherol dominates, followed by  $\delta$ -tocopherol and  $\alpha$ -tocopherol. The highest content  $\delta$ -tocopherol was observed in the oil of one-year old branch - 9.7%.  $\beta$ -tocopherol was not found in studied walnut oils. The amount of the sterols was in the range of 0.5–0.7%.  $\beta$ -sitosterol dominates in sterol fraction - 86.2–89.8%.

*Key words:* walnut oil, fatty acid, tocopherol, sterol

### Introduction

Walnuts (*Juglans regia* L.) contain kernels that have high content of glyceride oil. It varies widely (52-75%), depending on the variety, cultivation, place of growing and irrigation of walnut trees. Regarding the fatty acid composition unsaturated fatty acids are dominating - oleic, linoleic and linolenic. Ratio of these acids determines the nutritional value of walnut oil. Monounsaturated fatty acids have a beneficial effect on human health (Ali et al., 2010; Greve et al., 1992, Oliveira et al., 2002; Tsamouris et al., 2002; Yaerlikaya et al., 2012).

There are data about fatty acid composition of walnut oil, derived from nuts, growing in different places. The content of fatty acids varies widely: oleic (12.7-34%), linoleic (49.7-72%) and linolenic acid (9-25%) from the saturated palmitic (5.24-8.2%) and stearic (1.4-2.5%) (Ali, 2010; Amaral et al., 2003; Dogan and Akgul, 2005; Greve et al., 1992; Özcan, 2009; Özcan et al., 2010; Rabrenovic et al., 2011; Savage et al., 1999; Yerlikaya et al., 2012).

According to literature data the amount of tocopherol in the walnut oil varies within the range 100-436 mg.kg<sup>-1</sup>. In

tocopherol fraction the predominant is  $\gamma$ -tocopherol - 88% of total tocopherols (Rabrenovic et al., 2011; Savage et al., 1999). Lavedrine et al. (1997) does not establish the presence of  $\beta$ -tocopherol in studied walnut oils. The presence of large amounts of  $\gamma$ -tocopherol provides some level of protection against oxidation (Dolde et al., 1999).

Sterols are physiologically active substances, which constitute the main part of the unsaponifiables matters. In references (Amaral et al., 2003; Oliveira et al., 2002) the sterol fraction of total sterols of tested walnut oils contains  $\beta$ -sitosterol (85%),  $\Delta$ 5-avenasterol (7%) and campesterol (4%). There is an identified presence of cholesterol, stigmasterol,  $\Delta$ 7-stigmasterol and  $\Delta$ 7-avenasterol. Verlehen et al. (2002) found out only campesterol and  $\beta$ -sitosterol in investigated walnut oil. The amount of total sterols varies widely - 120-200 mg.100g<sup>-1</sup> oil.

Walnut oil is used as salad oil, because the oil phase emulsifies sauces. It is not used for cooking or heat treatment of products due to the presence of unsaturated fatty acids - 70%. Their oxidation leads to the appearance of unwanted taste and odor.

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The influence of different climatic and soil factors on chemical composition of walnut oil obtained from different varieties of nuts has been investigated (Özcan, 2009; Özcan et al., 2010).

No data on the influence of the location of the walnut fruit on the vines branches, backlight and the year old of branches were found in the literature.

The aim of this study was to determine the effect of the year of branches at which the walnut fruit were grown on the physic-chemical composition of walnut oil.

## Materials and Methods

Nuts of varieties Izvor 10 and walnut oil from them were investigated. Studied kernels are single fruit grew on a one year (№ 1), two years (№ 2), three (№ 3) and four years old (№ 4) branches. The oil content of nuts was determined by the extraction method with Soxlet apparatus (Hadzhiyski and Perifanova – Nemska, 1994).

Fatty acid composition of the oil was determined by gas-liquid chromatography (ISO 5508, ISO 5509). Tocopherols were determined by liquid chromatography. They were identified by retention times compared with those of the witnesses from the individual pure tocopherols. Total tocopherols were calculated as the sum of individual tocopherols based on the absolute values of the peak areas of the standard solution and the tested oil (Ivanov and Aitzmueller, 1995).

The total content of sterols was determined spectrophotometrically (Ivanov et al., 1972). The individual qualitative and quantitative composition of sterols is set at gas chromatography (ISO5509, Homberg and Bielefeld, 1989). The standard deviation of repeatability is 0.05%. The sterols were identified by retention times compared with those of the witnesses' individually pure sterols. The total amount of sterols was calculated as the sum of individual sterols based on the absolute values of the peak areas of the standard solution and the test oil (in %).

## Results and Discussion

The oil content of the investigated nuts was determined. Data are present in Table 1. The oil content increases with an increasing of the year of the branch.

Data on the fatty acid composition of the derived oils are presented in Table 2. The year of the branch had no significant effect on fatty acid composition. The results indicated that in all samples the predominant unsaturated fatty acids were oleic and linoleic acids. Linolenic was in the range of 9.66-10.77%. The highest is the content of polyunsaturated - linoleic and linolenic acids - 64.14% and 10.77% respectively, in nuts grown on four years old branch. Data for the amount of saturated - palmitic and stearic acids do not differ from those cited in the literature for different varieties and climat-

**Table 1**  
**Characteristics of walnuts**

Sample	1. Single fruit grown on a year old branch	2. Single fruit grown on a two-years old branch	3. Single fruit grown on a three-years old branch	4. Single fruit grown on a four-years old branch
Oil content, %	63.02	69.27	70.36	73.86

**Table 2**  
**Fatty acid composition of walnut oils**

Sample, %	1. Single fruit grown on a year old branch	2. Single fruit grown on a two-years old branch	3. Single fruit grown on a three-years old branch	4. Single fruit grown on a four-years old branch
Palmitic (C16:0)	6.02	6.31	6.57	6.46
Palmitoleic (C16:1)	0.11	0.11	0.12	0.1
Stearic (C18:0)	2.92	2.89	2.75	2.65
Oleic (C18:1)	15.82	16.63	17.12	15.61
Linoleic (C18:2)	64.69	63.57	63.52	64.14
Linolenic (C18:3)	10.13	10.53	9.66	10.77
Aracidic (C20:0)	0.11	0.10	0.10	0.10
Eicosonic (C20:1)	0.19	0.16	0.16	0.17
Ratio Saturated: Unsaturated fatty acids	9.05:90.95	9.30:90.70	9.42:90.58	9.21:90.79

ic conditions. The content of oleic acid - 16.53 to 17.12% is lower than the data reported for Turkish varieties - 20-26%. The content of linoleic acid was higher - 63.52-64.69% than that cited by some authors (49.7-55.5%) and the content of linolenic acid - 9.66-10.77 were lower than that quoted by these authors - 14.3-14.8%.

There are not significant differences in the established fatty acids composition compared with the data cited by other authors for different varieties and climatic conditions.

The content of biologically active components in the investigated oils is presented in Table 3.

The amount of total tocopherols is the highest in the oil sample number 1-567 mg.kg<sup>-1</sup>, which is higher than the cited data in the literature (1-100 mg.kg<sup>-1</sup>).  $\gamma$ -tocopherol dominates in tocopherol fraction for all tested oils followed by  $\delta$ -tocopherol and  $\alpha$ -tocopherol. The highest content of  $\delta$ -tocopherol was found again in oil extracted from one one-year old branch fruits. This has an impact on the increase in oxidative stability of the oils extracted from sample 1. The highest content of  $\gamma$ -tocopherol 9.7% in sample 4 provides some level of protection against oxidation. In the same sam-

ple a high content of linoleic and linolenic fatty acids was also observed.

$\beta$ -tocopherol was not found in the tested nut oils, which is confirmed by other authors for different varieties of nuts.

The amount of the sterols was in the range 0.5-0.7%, which is higher than data cited in the literature.

$\beta$ -sitosterol, campesterol and stigmasterol are identified in a larger amount (Table 4). The stigmasterol was not identified or it was at a minimum amount of most nut oils discussed in the literature. In sterol fraction  $\beta$ -sitosterol - 86.2-89.8% dominates, which is higher than the data in the literature - 85%. The content of campesterol is also compliant to data cited in literature - 4.1-5.4%.

## Conclusion

It was found that the synthesis of lipids was highest in single fruit, grown on multi year old branches and lowest in those grown on one-year old. The content of tocopherols is inversely proportional to the amount of oil content. There was no significant difference found in the synthesis of unsat-

**Table 3**  
Biologically active components in walnut oils

Sample №	Sterols in oil, %	Total tocopherols, mg.kg <sup>-1</sup>	$\alpha$ - tocopherol, %	$\beta$ - tocopherol, %	$\gamma$ -tocopherol, %	$\delta$ - tocopherol, %
1.Singel fruit grown on a year old branch	0.5	567	4.4	—	86.3	9.7
2.Singel fruit grown on a two -years old branch	0.7	529	4.9	—	88.2	6.1
3.Singel fruit grown on a three -years old branch	0.7	551	5.7	0.2	86.8	7.1
4.Singel fruit grown on a four -years old branch	0.6	449	5	—	85.1	9.1

**Table 4**  
Individual sterol composition of walnut oils, %

Sample, %	1. Single fruit grown on a year old branch	2. Single fruit grown on a two-years old branch	3. Single fruit grown on a three-years old branch	4. Single fruit grown on a four-years old branch
Holesterol	trace	trace	trace	trace
Campesterol	5.4	4.7	4.6	4.1
Stigmasterol	2.6	2.9	2	2
$\beta$ - Sitosterol	89.2	89.5	86.2	89.8
$\Delta^5$ Avenasterol	0.5	0.7	2.4	0.8
$\Delta^{7,25}$ Stigmaenol	1	0.9	0.9	1
$\Delta^7$ Stigmasterol	0.5	0.5	3	0.6
$\Delta^7$ Avenasterol	0.8	0.9	0.9	1.7

urated fatty acids in one-year and four-year old branches. The content of these acids was lower in the oils extracted from two and three year old branches fruits. The highest quantity of essential linoleic acid was observed in the oil extracted from four year-old branch fruit. It was not found any correlation between the quantity of unsaturated linolenic acid and content of tocopherols and their fractional composition in the derived oils. There is no significant difference in the sterol composition of the oils derived from the fruit of the different year old branches.

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