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Fatty acid profiles and antioxidant properties of raw and dried walnuts

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Abstract

Background: *Walnuts consumption produces beneficial effects on human health. Health-promoting benefits are dedicated to its desirable fatty acid profile and high content of antioxidants. Heat treatment of walnuts may alter their fatty acid composition and antioxidant capacity.* ***Aim:*** *In general the aim of this work was to compare fatty acids profiles and antioxidative properties of raw and dried walnuts at 60 °C for 12 hours.* ***Methodology and results:*** *FA profiles were analyzed using gas chromatography. Antioxidative capacities of walnut samples were determined by DPPH and ABTS tests. There were no significant differences in fatty acid profiles comparing dried and raw walnuts. The most abundant fatty acid was linoleic with mean content of 61.38 ± 1.11% in raw and 62.40 ± 0.99% in dried walnuts. Walnuts oil contained 10.64 ± 0.46% and 10.49 ± 0.81% of α-linolenic acid (ALA) in raw and dried walnuts, respectively. Antioxidative capacity of methanolic extracts showed no difference comparing raw and dried walnut by DPPH and ABTS test. Heat treatment at 60 °C for 12h induced no change in fatty acid profiles of walnuts and led to minor decrease in antioxidative capacity measured only by ABTS test.* ***Conclusion:*** *We suggest that drying process in our experiment did not decreased nutritional capacity which is mostly mediated by conservation of fatty acids content in walnuts.*

Key words: *walnuts, fatty acids, linolenic acid, antioxidant capacity.*

INTRODUCTION

Walnuts (Jugulans regia L.) represent nutrient dense food containing macro and micronutrients, soluble fiber, high amounts of unsaturated fatty acids, polyphenols, vitamin E, as well as other bioactive constituents which separately or together may produce beneficial effects on human health [1]. Prospective, observational and large clinical trial suggests that walnuts consumption may reduce risk of cardiovascular disease, shows cholesterol-lowering effect [2] and has beneficial effect on oxidative stress, inflammation, vascular reactivity [3]. Accumulating evidence from numerous studies also show that dietary intake of walnuts may lower blood pressure and have positive health effects in visceral adiposity [4], metabolic syndrome and type II diabetes [5]. Moreover, walnuts consumption effects are found in studies of prevention and treatment of certain cancers, as well as the reducing of symptoms attributed to age related and other neurological disorders [5].

Walnuts contain from 50–70% of oil [6]. Numerous health–promoting benefits of walnut oil are dedicated to its desirable fatty acid profile which is rich in unsaturated fatty acids [7,8]. The fatty acids present in high amount in walnut kernels are: oleic (C18:1, n-9), linoleic (C18:2, n-6) and α-linolenic acid (ALA, C18:3, n-3). Among all tree nuts walnuts have the highest n-3: n-6 ratio as ALA content in walnuts is about 10% [9–11]. Monosaturated fatty acids (MUFA) are present in substantial amounts with 15.9-23.9 % of total fatty acids in five walnut cultivar grown in Central Serbia [9]. Saturated fatty acids (SFA) is the minor group of fatty acids containing less than 10% of total fatty acids in walnut oil [9–11].

Studies related to antioxidant potential of nuts demonstrated that walnuts represent polyphenol rich food [12]. It is well known that polyphenols are large group of phenolic compounds widely distributed in plant food that posses antioxidant and anti-inflammatory bioactivity. The main polyphenol in walnuts is pedunculagin, an ellagitanin that is found only in limited numbers of fruits and nuts species [13,14]. Several studies demonstrate walnut polyphenols beneficial

effect in health maintenance and disease prevention [13,15].

Heat treatment of walnuts may alter their fatty acid composition and content of naturally occurring antioxidants leading to changes in their beneficial effects to health. In general our aim was to compare fatty acids profiles and antioxidative properties in raw and dried walnuts.

MATERIAL AND METHODS

Walnuts samples

Walnuts were collected from four representative large markets in ten biggest cities in Serbia. A composite sample was defined as a mix of equal portion (100 g) of 10 primary samples. Primary sample was generated by mixing an equal portion of four samples taken from markets in representative parts of Serbia. Raw and dry walnuts samples were analyzed. Dry walnuts were prepared by heating in oven about 10 g of raw samples on 60 °C for 12 hours. Weights before and after heating were measured.

Determination of antioxidant capacity of samples

Milled samples powders (raw and dry walnut) were defatted with n-hexane [16]. Methanolic extract of defatted raw and dried walnuts were prepared as described by Barthet et al. [17]. The final volumes of methanolic extracts prepared from 0.5 g of defatted milled powder were adjusted to 10 ml. Antioxidant capacity of methanolic extract were analyzed by 2,2 diphenyl-1-picrilhydrazyl test (DPPH) [18] (Espin) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) test (ABTS) [19] (van den Berg).

Analysis of fatty acid compositions of samples

Total lipids were extracted using Folch method [20]. Then, fatty acid methyl esters were prepared by transmethylation with sodium hydroxide (2 M) in methanol (heated at 85 °C for 1 h) followed by heating in sulfuric acid (1 M) in methanol (heated 85 °C for 2 h) [21]. Fatty acid methyl esters were extracted into hexane before analysis. Samples were centrifuged and upper phase (n-hexane phase) of samples were put into tubes and evaporated with technical nitrogen. Separations of the methyl esters were carried out using a gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a split/splitless injector and a flame ionization detector. The methyl ester separation was carried out on capillary column RTX 2330 column (60 m X 0.25 mm with a 0.20 ìm film) from RESTEK, Bellefonte, PA using helium as the carrier gas. The injector and detector temperature was set at 220 °C and 260 °C, respectively. The injection was performed in split mode with a 1:20 split-ratio. The temperature of the column was initially set at 140 °C for 5 min., and then increased to 210 °C at the rate of 3 °C/min, and held at final temperature for 20 min. Each fatty acid was identified with reference to the retention time of that in a PUFA-2 standard mixture (Sigma-Aldrich, St. Louis, MO). The content of FA was expressed as percentage of total fatty acids.

Statistical analysis

Data are presented as the mean ± standard deviation. The comparison between dried and raw walnuts was performed using Student's *t* test. The level of statistical significance was set to $p<0.05$.

RESULTS

Samples of walnuts (n=6) were weight before and after heating at 60 °C for 12 h (**Table 1**). The mean weight loss of samples was 2.55 ± 0.26%.

Table 1. Weight of walnut samples before and after 12 h drying at 60 °C.

Number of sample	Raw walnuts weight (g)	Dry walnuts weight (g)	Weight loss (%)
1	9.929	9.664	2.65
2	9.700	9.438	2.70
3	9.332	9.079	2.71
4	10.017	9.777	2.39
5	8.652	8.472	2.08
6	9.524	9.263	2.76

Fatty acid profiles of samples of raw (n=6) and dry walnuts (n=6) are shown in **Table 2**. Although content of unsaturated fatty acids was were high (about 90%) there were no significant change in fatty acids profiles of walnuts after 12 h of heating at 60 °C. The fatty acids found in the high amounts were linoleic, oleic and α-linoleic acid. The mean n-3/n-6 ratio was 0.171 ± 0.012.

The percentage of inhibition of DPPH (61.95 ± 0.38% for raw and 61.79 ± 0.27% for dried) and ABTS radicals (85.11 ± 0.94 for raw and 85.44 ± 0.72% for dried) in presence of methanolic extracts of defatted walnuts (dried and raw) is represented in **Figure 1**. Data obtained for dried walnuts were corrected for weigh loss of 2.55% and there was no significant differences between dried and raw walnuts measuring antioxidant activity by DPPH and ABTS test.

Table 2. Fatty acid composition of raw and dry walnuts.

	raw walnuts (n=6)	dry walnuts (n=6)
	%	%
16:0	7.06 ± 0.30	7.11 ± 0.26
16:1n-7	0.10 ± 0.01	0.08 ± 0.01
18:0	2.73 ± 0.38	2.81 ± 1.34
18:1n-9	17.31 ± 0.90	16.35 ± 0.60
18:1n-7	0.79 ± 0.05	0.75 ± 0.04
18:2n-6	61.38 ± 1.11	62.40 ± 0.99
18:3n-3	10.64 ± 0.46	10.49 ± 0.81
Unsaturated fatty acids	90.21 ± 0.13	90.07 ± 0.32

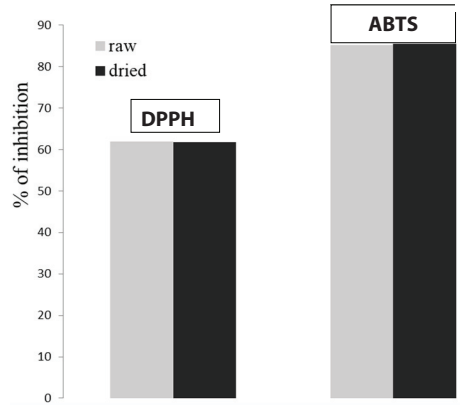

Figure 1. Antioxidant capacity of methanolic extracts of defatted raw and dried walnuts analyzed by 2,2 diphenyl-1-picrilhydrazyl test (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) test (ABTS).

DISCUSSION

Heat treatment of walnuts at 60 °C for 12 h did not alter their fatty acid composition and methanolic extracts of defatted walnuts showed no decrease measuring antioxidant capacity by DPPH and ABTS tests.

The fatty acid profiles of walnut samples found in this study is similar and comparable with the literature data for walnut oil compositions [9-11]. The unsaturated fatty acids content was about 90% similar to literature data from other studies [10]. Samples of walnuts contain the highest amounts of linoleic acid with 61.85% (in raw walnuts) and 62.4% (in dry walnuts) of total fatty acids that is comparable with 57.7-65.1% reported by Rabrenovic et al. [9] for five cultivar from Serbia. The found ALA content of about 10% is also comparable with literature data found in other studies [9, 10]. Finally, it is confirmed that SFA amounts in walnut oils are very low, with content of less than 10% of total fatty acids [9-11].

Heat treatment may induce changes in fatty acid composition and antioxidant status of nuts. Ozkan et al. [22] showed minor reduction in phenolic compounds and changes in fatty acid profiles of Brazilian nut and hazelnut after 20 minutes roasting at 130 °C. In this study the content of ALA was significantly reduced in both nuts after heat treatment. In study on the effect of roasting at 110 °C for 16 minutes on fatty acids and antioxidant capacity of almonds, pine, cashew and pistachio authors find increased antioxidative capacity using DPPH test after roasting in all nuts except for pistachios [23]. They suggest that the antioxidant capacity may increase due to the formation of new product showing antioxidant activity by Maillard reaction. Considering changes in fatty acid profiles, roasting generally has the greatest effect on ALA content, reducing it in almost all nuts in this study. Most of the nuts are dried following harvest on air or by heat treatment to prevent the microbial spoilage of nuts. Arcan et al. [12] found that there are not difference in total antioxidant activity determined by ABTS test of fresh and dry walnuts.

In our study we found no significant change in fatty acid profiles and ALA content, which is very high in walnuts. ALA decreasing in nuts was detected when they were treated on higher temperature 130 °C and 110 °C while in our study as we dried walnuts at 60 °C for 12 h. Literature data for effects of heat treatment on antioxidant activity in nuts are unconsented: reporting minor decrease of phenolic compounds, increase in DPPH activity and no significant effect. Our results are in line with Arcan et al. [12] study for fresh and dry walnuts.

CONCLUSION

In this work we found that fatty acid profiles of walnuts show no change after heat treatment at 60 °C for 12 h, so we suggest that oil fraction of dried and raw walnuts could show the same beneficial effects on health. Antioxidative capacity of methanolic extracts of dried walnuts showed no significant effect compared to raw walnuts. Further studies of polyphenols profiles are needed to clarify if naturally occurring antioxidants are destroyed during heat treatment 60 °C and if new product with antioxidant activity are produced. However, results of this study showed that total antioxidant status of walnuts is not significantly changed after drying at 60 °C for 12 h showing no difference in nutritional capacity.

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Profili masnih kiselina i antioksidativna svojstva sirovih i sušenih oraha

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Kratak sadržaj

Dijetarni unos oraha povezuje se sa brojnim pozitivnim efektima na zdravlje. Svoj efekat na zdravlje orasi ostvaruju zahvaljujući povoljnom masnokiselinskom sastavu i visokom sadržaju antioksidanasa. Zagrevanje oraha moglo bi dovesti do promena u masnokiselinskom sastavu i uticati na antioksidativni kapacitet. Cilj ovog rada bio je da se uporede masnokiselinski profili i antioksidativni status svežih i oraha sušenih na 60 °C u trajanju od 12 sati. Masnokiselinski sastav analiziran je gasnom hromatografijom. Antioksidativni kapaciteti uzoraka oraha određivani su DPPH i ABTS testovima. Utvrđeno je da nije bilo promena u masnokiselinskim profilima posle sušenja oraha na 60 °C u trajanju od 12 sati. Najzastupljenija masna kiselina bila je linolna sa sadržajem od 61.38 ± 1.11% u sirovim i 62.40 ± 0.99% kod sušenih oraha. Ulje oraha sadržalo je 10.64 ± 0.46% i 10.49 ± 0.81% α-linolenske kiseline (ALA) u svežim i sušenim orasima, respektivno. DPPH testom nije utvrđeno da postoje razlike u antioksidativnom potencijalu metanolnih ekstrakata oraha sušenih u odnosu na sveže kao ni sa ABTS testom. Naš eksperimet je pokazao da proces sušenja oraha nije smanjio njihov nutritivni kapacitet što je verovatno posredovano očuvanjem sadržaja masnih kiselina.

Ključne reči: *orasi, masne kiseline, linolenska kiselina, antioksidativni kapacitet.*