



Total Phenolic Content, Antioxidant Capacity and Individual Phenolic Compounds of Defatted Kernel from Different Cultivars of Walnut

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Abstract

The aim of this study was to investigate the quality of defatted walnut kernel derived from oil extraction by Soxhlet method, to walnut genotypes. Antioxidant activity, total phenol and individual phenolic compounds were determined. Total phenolic content ranged from 3791.13–9408.6 mg GAE/100 g and antioxidant activity from 35.03–75.89 mmol Trolox/100 g, depending on the genotypes. Fifteen phenolic compounds were detected. The data provides evidence on high phenolic contents and high antioxidant potential of defatted walnut kernel and can be a basic ingredient in the food industry.

Keywords Defatted Walnut Kernel · Phenolic Content · Antioxidant Activity · Phenolic Compounds · Walnut

Gesamtphenolgehalt, antioxidative Kapazität und Einzelphenolverbindungen bei entöhlten Kernen verschiedener Walnuss-Sorten

Schlüsselwörter Entöhlte Walnusskerne · Phenolgehalt · Antioxidative Aktivität · Phenolische Verbindungen · Walnuss

Introduction

The English walnut (*Juglans regia* L.) has been studied and the research showed that walnut is highly appreciated for its food and therapeutic value. The seed propagated walnut trees can be very diverse and their nuts have a good potential for valuable sources of phytochemicals (Beyhan et al. 2016). Nutritional importance of walnut is related to the kernel. Kernel of walnut cultivars proved to be important sources of nutritive element (Cosmulescu et al. 2009) and serves as a good source of flavonoids and phenolic acids, compounds of considerable interest due to their antioxidant properties (Pereira et al. 2008). In terms of antioxidant capacity, Samaranayaka et al. (2008) consider that walnut with skin and skin of walnuts serve as good free radical

scavengers. As well walnut pressing nut is a rich source of bioactive compounds and is a new byproduct with potential use in the bakery industry as a supplement (Bakkalbasi et al. 2015). According to Slatnar et al. (2015), twenty-seven phenolic compounds were detected in kernels and pellets. A large variation in phenols and antioxidant capacity was found in the nuts evaluated in the study conducted by Abe et al. (2010); the antioxidant capacity varied a hundred times among the different nuts, from 1.2 to 120 mg.100 g⁻¹ (FW). The oil extraction residue is rich in proteins (unusually high in arginine, glutamic and aspartic acids) and has been employed in the formulation of various functional food products (Martinez et al. 2010). Ojeda-Amador et al. (2018) considers that polar phenolic compounds concentrate in the residual cake after separation of oily phase, which leads to a potential added value and applications as a source of bioactive compounds to this byproduct.

A great interest has been developed in walnut oil because of its essential fatty acid content, especially in the food and cosmetics industry, and the extraction of oil remains a residual, which is an important source of natural antioxidants. The aim of this paper was to analyze the quality of defatted walnut kernel and their total phenolics content, individual

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phenolic compounds content and antioxidant activity based on DPPH assay.

Materials and Methods

Chemicals and Reagents

Folin-Ciocalteu reagent (2N, Sigma-Aldrich), gallic acid (Sigma-Aldrich), anhydrous sodium carbonate (Sigma-Aldrich), methanol (Merck), aluminum nitrate (Sigma-Aldrich), potassium acetate (Sigma-Aldrich), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Merck), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Merck, Germany), standards of phenolic acids (gallic, vanillic, chlorogenic, syringic, *p*-coumaric, ferulic, sinapic, salicylic and elagic), flavonoids (catechin, epicatechin, rutin, myricetin and quercetin) and juglone (Sigma-Aldrich, Germany), acetic acid (HPLC grade, Merck, Germany) were used in this experiment.

Preparation of Samples

Fresh walnut (moisture <8%) from 20 genotypes was used as started material. All genotypes of walnut come from the area of Bechet, Romania (43°47'N/23°57'E). Whole kernels with their seed coats (1000g) were cut through a mechanical grinder and fried (UM200 Memmert, Germany at 1200 °C, for 30 min). Defatted walnut kernel, after removing oil in a Soxhlet apparatus using hexane as solvent, were used in our experiments. For extraction of phenolic fraction from walnut kernels, methanol was used. In the extraction of phenolic fraction the solid-to-liquid ratio was 1:10.

Total Phenolics Content

To colorimetrically determine total phenolics content in the extracts with Folin-Ciocalteu reagent, the method used was the one described by Cosmulescu and Trandafir (2012). The amount of 5 mL Folin-Ciocalteu reagent (diluted 1:10 with ultrapure water) was added to 1 mL extract (1:20 diluted with methanol). After 2 min, the amount of 4 mL of 7.5% sodium carbonate solution was added to the mixture and kept in the incubator for 2 h at the room temperature. The absorbance of mixture was determined to be 765 nm (Evolution 600 UV/VIS spectrophotometer, Thermo Scientific USA). From standard curves (0–250 mg/L) total phenolic content of each extract was determined using gallic acid as a standard. The results were expressed as gallic acid equivalents in milligrams per 100 g (mg GAE/100 g).

Antioxidant Activity Based on DPPH Assay

The extracts capacity of scavenging the 2,2-diphenyl-1-picrylhydrazyl free radical was monitored using the method defined by Cosmulescu and Trandafir (2012). Briefly, the amount of 50 µL of each extract was mixed with 3 mL of methanolic solution containing DPPH radicals (40 mg/L). The mixture was kept in the dark (for 30 min), and the absorbance was measured at 517 nm. All assays were conducted in triplicate. Standards of Trolox with various concentrations (0–200 mg/L) were used. Anti-oxidant capacity was expressed in mmol Trolox/100 g.

Individual Phenolics and Juglone

The extracts were centrifuged (8500 rpm, for 30 min, at ambient temperature), filtered and then stored at –20 °C. Chromatographic separation was achieved with Finningan Surveyor Plus HPLC system (Thermo Electron Corporation, San Jose, CA). According to Nour et al. (2013) and Trandafir et al. (2017) the mobile phase consisted of 1%

Table 1 Total flavonoid, total phenolics content and antioxidant capacity of the extracts^a

Sample	Total phenolics content ^b	Antioxidant capacity ^c
B1	4068.20 ± 3.52	37.38 ± 0.46
B2	5252.55 ± 40.75	56.6325 ± 0.01
B3	3993.00 ± 1.21	46.55 ± 0.91
B4	9408.6 ± 77.85	75.89 ± 0.02
B5	7335.87 ± 82.49	64.44 ± 0.27
B6	5227.2 ± 60.57	55.14 ± 0.27
B7	5156.1 ± 53.58	59.535 ± 1.47
B8	5534.14 ± 1.73	57.87 ± 0.57
B9	6003.38 ± 4.53	61.69 ± 0.29
B10	5769.04 ± 2.58	65.67 ± 0.02
B11	5272.87 ± 54.14	47.31 ± 0.36
B12	7030.75 ± 95.37	39.19 ± 0.02
B13	5484.80 ± 92.75	59.26 ± 0.42
B14	6933.75 ± 4.64	52.45 ± 0.40
B15	4161.89 ± 113.01	47.79 ± 0.03
B16	3987.22 ± 25.99	43.11 ± 0.02
B17	6446.54 ± 26.23	49.32 ± 0.02
B18	5215.81 ± 36.95	53.75 ± 0.04
B19	4737.51 ± 3.43	38.74 ± 0.06
B20	3791.13 ± 32.56	35.03 ± 0.33
Mean	5540.51 ± 1386.48	52.33 ± 10.71
Minimum	3791.13	35.03
Maximum	9408.6	75.89
CV% ^d	25	20.46

^aData expressed as means ± SD of three samples analyzed separately

^bmg GAE/100 g

^cmmol Trolox/100 g

^dcv% = coefficient of variation

aqueous acetic acid solution (A) and methanol (B). Samples were eluted with the following gradient: 90% A from 0–27 min, from 90–60% A in 28 min, 60% A for 5 min, from 60–56% A in 2 min, 56% A for 8 min, from 56–90% A in 1 min and 4 min 90% A to re-establish the initial conditions, before the injection of another sample. All gradients were linear. The flow rate was 1 mL/min and injection volume was 5 μ L. Column temperature was maintained at 20°C. Each compound was identified by its retention time and by spiking with standards under the same conditions. Detection was carried out with a photodiode array (PDA) detector by comparison with ultraviolet (UV) spectra of standards at 220–350 nm. Each compound was quantified according to peak area measurements, from calibration curves of corresponding standards. All samples were extracted and analyzed in triplicate. The content of investigated phenolic compounds was expressed in mg/100 g as mean values \pm standard deviations.

Statistical Analysis

Data were evaluated by one-way analysis of variance (ANOVA) using Statgraphics Centurion XVI software

(StatPoint Technologies, Warrenton, VA). Differences in content levels among the variants were estimated with a multiple range test based on Fisher's least significant difference (LSD) procedure at $P < 0.05$.

Results and Discussions

Total Phenolic Content and Antioxidant Activity

Total phenolics content and antioxidant capacity based on DPPH assay were determined in extracts made from defatted walnut kernel and the results are presented in Table 1. Total phenolics content and antioxidant capacity of alcoholic extracts of defatted walnut kernel varied between 3791.13 and 9408.60 mg GAE/100 g and 35.03–75.89 mmol Trolox/100 g, depending on the genotypes. The differences between genotypes are quite high, the variability coefficient having values 25% for the total phenolics content and 20.46% for the antioxidant activity (Table 1). A comparison of the average amount of total phenolic content showed that the difference between the genotypes studied was 2.48 times (3791.13 mg GAE/100 g) to the geno-

Table 2 Phenolic profile (flavonoids and juglone) of defatted walnut kernel ((mg/100 g)/Phenolics)^a

Genotyp	(+)-catechin hydrate	(-)-epicatechin	Rutin	Myricitin	Juglona	Quercitin
B1	44.99 \pm 0.61	130.24 \pm 0.50	45.06 \pm 0.44	25.30 \pm 0.47	18.03 \pm 0.59	0.69 \pm 0.09
B2	18.22 \pm 0.53	161.45 \pm 0.30	10.91 \pm 0.25	42.05 \pm 0.37	12.55 \pm 0.28	0.57 \pm 0.08
B3	21.93 \pm 0.59	25.83 \pm 0.50	14.13 \pm 0.30	38.99 \pm 0.62	11.94 \pm 0.64	0.78 \pm 0.05
B4	29.22 \pm 0.16	449.64 \pm 0.43	124.39 \pm 0.41	36.64 \pm 0.36	2.53 \pm 0.23	2.07 \pm 0.34
B5	146.65 \pm 0.37	458.11 \pm 0.37	90.22 \pm 0.30	28.79 \pm 0.47	16.47 \pm 0.35	1.48 \pm 0.35
B6	33.41 \pm 0.40	256.15 \pm 0.56	83.44 \pm 0.36	27.48 \pm 0.35	19.43 \pm 0.13	1.99 \pm 0.68
B7	47.29 \pm 0.46	130.36 \pm 0.20	50.84 \pm 0.51	25.16 \pm 0.56	19.13 \pm 0.23	1.33 \pm 0.31
B8	58.52 \pm 0.33	154.18 \pm 0.56	94.10 \pm 0.61	29.54 \pm 0.36	25.49 \pm 0.32	1.66 \pm 0.36
B9	125.11 \pm 0.59	380.82 \pm 0.49	78.44 \pm 0.32	34.28 \pm 0.46	21.27 \pm 0.27	0.44 \pm 0.02
B10	91.72 \pm 0.36	332.15 \pm 0.57	95.68 \pm 0.21	39.34 \pm 0.03	20.10 \pm 0.38	1.38 \pm 0.21
B11	60.12 \pm 0.58	192.80 \pm 0.54	73.33 \pm 0.46	26.61 \pm 0.42	15.79 \pm 0.33	0.53 \pm 0.04
B12	48.30 \pm 0.05	182.67 \pm 0.31	54.67 \pm 0.48	30.65 \pm 0.22	27.14 \pm 0.51	0.41 \pm 0.06
B13	55.98 \pm 0.51	422.68 \pm 0.46	69.46 \pm 0.41	37.59 \pm 0.42	19.30 \pm 0.27	0.35 \pm 0.21
B14	41.88 \pm 0.46	292.35 \pm 0.20	66.65 \pm 0.37	21.60 \pm 0.40	18.08 \pm 0.46	0.40 \pm 0.06
B15	89.61 \pm 0.35	396.92 \pm 0.63	87.08 \pm 0.15	36.29 \pm 0.29	17.04 \pm 0.42	0.44 \pm 0.02
B16	108.99 \pm 0.47	331.61 \pm 0.25	67.60 \pm 0.42	27.63 \pm 0.45	28.84 \pm 0.56	0.41 \pm 0.04
B17	92.82 \pm 0.49	345.68 \pm 0.46	53.97 \pm 0.67	45.62 \pm 0.25	25.85 \pm 0.58	0.45 \pm 0.01
B18	76.59 \pm 0.19	334.28 \pm 0.33	56.89 \pm 0.60	47.98 \pm 0.52	11.83 \pm 0.42	0.59 \pm 0.02
B19	49.41 \pm 0.38	188.69 \pm 0.47	78.16 \pm 0.58	29.73 \pm 0.49	12.99 \pm 0.67	0.44 \pm 0.01
B20	43.75 \pm 0.44	176.44 \pm 0.17	80.83 \pm 0.55	37.49 \pm 0.37	11.88 \pm 0.16	1.69 \pm 0.06
Mean \pm SD	64.23 \pm 34.88	267.15 \pm 123.58	68.79 \pm 26.75	33.44 \pm 7.25	17.78 \pm 6.29	0.90 \pm 0.59
Min	18.22	25.83	10.91	21.60	2.53	0.35
Max	146.65	458.11	124.39	47.98	28.84	2.07
CV% ^b	54.30	46.25	38.88	21.68	35.37	65.55

^aData expressed as means \pm SD of three samples analyzed separately

^bcv% = coefficient of variation

Table 3 Phenolic profile (phenolic acids) of defatted walnut kernel (mg/100 g/Phenolics)^a

Genotyp	Gallic Acid	Vanillic Acid	Chlorogenic Acid	Syringic Acid	<i>p</i> -Coumaric Acid	Ferulic Acid	Sinapic Acid	Salicylic Acid	Ellagic Acid
B1	48.96±0.49	22.65±0.25	11.58±0.54	1.68±0.10	5.55±0.05	10.07±0.18	30.81±0.42	128.57±1.03	17.13±0.29
B2	69.08±0.34	61.18±0.38	15.99±0.37	2.28±0.15	4.83±0.30	46.17±0.73	9.81±0.26	184.08±0.79	6.83±0.49
B3	51.90±0.67	48.04±0.34	10.53±0.15	1.70±0.13	4.13±0.14	42.48±0.31	9.21±0.18	121.42±0.31	14.23±0.38
B4	88.15±0.52	23.12±0.76	17.68±0.28	1.72±0.21	10.93±0.36	59.20±0.56	14.13±0.30	213.38±0.34	13.01±0.31
B5	81.70±0.37	18.28±0.68	13.59±0.56	1.31±0.25	7.73±0.35	24.77±0.63	26.33±0.24	200.91±0.62	104.98±0.37
B6	54.99±0.46	11.75±0.48	12.82±0.63	11.25±0.51	19.27±0.42	47.39±0.69	22.66±0.45	151.94±0.52	39.21±0.18
B7	72.37±0.28	51.00±0.51	29.68±0.24	0.57±0.07	7.88±0.14	32.93±0.55	4.42±0.27	139.09±0.32	9.97±0.60
B8	65.28±0.81	35.31±0.24	22.93±0.26	1.82±0.20	9.85±0.31	21.26±0.56	3.92±0.36	75.86±0.52	16.10±0.61
B9	64.99±0.24	21.78±0.24	15.37±0.47	1.29±0.25	7.17±0.18	52.86±0.46	10.02±0.38	160.02±0.44	8.99±0.58
B10	79.68±0.64	32.56±0.47	21.99±0.49	1.50±0.10	7.80±0.20	17.62±0.47	16.32±0.32	132.29±0.40	59.07±0.42
B11	56.02±0.35	18.34±0.28	13.85±0.96	1.81±0.02	5.79±0.39	9.90±0.34	5.28±0.35	88.72±0.49	23.64±0.23
B12	47.12±0.47	28.31±0.37	19.46±0.15	3.75±0.12	9.37±0.44	14.42±0.26	6.76±0.52	117.22±0.23	26.62±0.42
B13	65.35±0.49	49.56±0.56	35.05±0.26	6.44±0.32	17.58±0.43	24.91±0.38	26.75±0.44	280.99±0.30	58.73±0.46
B14	52.94±0.38	24.63±0.64	20.90±0.35	4.12±0.12	15.47±0.48	15.88±0.38	15.91±0.63	152.95±0.50	36.96±0.59
B15	40.69±0.56	14.17±0.29	12.67±0.65	4.10±0.24	14.26±0.41	23.10±0.32	27.70±0.45	165.39±0.39	71.80±0.55
B16	42.98±0.57	6.83±0.18	13.39±0.24	1.01±0.11	1.28±0.08	13.35±0.30	10.18±0.56	91.05±0.51	52.15±0.56
B17	53.61±0.34	22.43±0.24	27.29±0.39	4.23±0.12	12.84±0.32	22.08±0.47	18.59±0.40	155.90±0.47	59.10±0.48
B18	54.67±0.29	27.20±0.56	15.82±0.27	6.29±0.06	14.13±0.27	21.04±0.55	16.30±0.07	138.69±0.47	46.16±0.57
B19	49.36±0.71	8.51±0.18	9.44±0.34	9.37±0.11	10.54±0.21	12.80±0.27	14.59±0.39	41.42±0.09	22.18±0.56
B20	36.70±0.37	6.97±0.14	19.08±0.64	5.03±0.11	7.61±0.30	10.42±0.21	11.56±0.21	71.99±0.68	22.13±0.39
Mean ± SD	58.83±14.09	26.63±15.52	17.96±6.74	3.56±2.90	9.70±4.69	26.13±15.35	15.06±8.18	140.59±54.79	35.45±25.78
Min	36.7	6.83	9.44	0.57	1.28	9.90	3.92	41.42	6.83
Max	88.15	61.18	35.05	11.25	19.27	59.20	30.81	280.99	104.98
CV% ^b	23.95	20.72	37.52	81.46	48.35	20.47	54.31	38.97	72.72

^aData expressed as means ± SD of three samples analyzed separately^bcv% = coefficient of variation

type B20 compared to the genotypes B4 (9408.60 mg GAE/100 g). A comparison of the average total phenolic content achieved by Slatnar et al. (2015) showed that the walnut kernel contained less phenolic (7.7 mg GAE/g FW) compared to pellets (7.9 mg GAE/g FW). Similar variations in total phenolics content were observed by Ojeda-Amador et al. (2018); walnuts show a very high content in total phenolic compounds (10,045–12,474 mg/kg; as gallic acid). Wu et al. (2004) have reported a total phenolics value of 1556 mg GAE/100 g for lyophilized walnuts and Trandafir et al. (2017) found a phenolics content of 2089.2 mg GAE/100 g in sample extracted with methanol from defatted walnut kernel by Soxhlet method.

Antioxidant capacity based on DPPH assay are influenced by genotypes and varied between 35.03 mmol Trolox/100 g to B20 and 75.89 mmol Trolox/100 g to B4, the difference between varieties being 2.16 times. Several studies have concluded that walnut has a higher capacity of antioxidants than any other nuts and that skimmed matter has the greatest contribution. The results obtained by Arranz et al. (2008) showed that the defatted matter provided the bulk of the antioxidant capacity (estimated about 332 μ mol Trolox/g dm) of this nut, a major proportion derived from insoluble tannins. A high antioxidant activity was observed in hulls and walnut flour from whole kernels by Labuckas et al. (2008), between 47.7 and 186 ppm BHT equivalent in hull and between 14.0 and 44.3 ppm BHT equivalent in walnut flour from whole kernels.

Phenolic Profile of Defatted Walnut Kernel

The contents of individual phenolic compounds (mg/100 g) of the extracts from defatted walnut kernel are shown in Tables 2 and 3. Five flavonoids (catechin, epicatechin, rutin, myricetin and quercetin), nine phenolic acids (gallic, vanillic, chlorogenic, syringic, *p*-coumaric, ferulic, sinapic, salicylic and ellagic) and juglone were identified and quantified. There is various variability between genotypes, the variation coefficient giving value that is variable from 20.47–81.46%. The results revealed that (–)-epicatechin is found in the highest concentration, between 25.83 and 458.11 mg/100 g, with an average concentration of 267.15 mg/100 g. The difference between analyzed genotypes is high, 17.73 times between the highest and the lowest content (Table 2). The content of (+)-catechin hydrate varied between 18.22–146.65 mg/100 g. Remarkable amounts of (+)-catechin and (–)-epicatechin were quantified with averages of 5.70 and 4.46 g per kg DM, respectively, in the cashew nut testa by Trox et al. (2011). Rutin content varied between 10.91 mg/100 g in B2 genotype and 124.98 mg/100 g in B4 genotype, with an average 68.79 mg/100 g (Table 2). Higher amount of quercetin was found in B4 genotype (2.07 mg/100 g). Rutin and

quercetin have been described as cell-protecting agents because of their antioxidant, antinociceptive, and anti-inflammatory actions (Azevedo et al. 2013). Myricetin, one of the most common flavonols with potent antioxidant and free-radical scavenging activities ranged from 21.60 and 47.98 mg/100 g (Table 2). Previous experimental results (Trandafir et al. 2016, 2017) have indicated that flavonoids are major components of kernel and pellicle walnuts. There were significant differences among samples in all phenolic acids (Table 3). The amount of salicylic acid detected in our study is average of 140.59 mg/100 g within variation limits of 41.42–280.99 mg/100 g; the difference between the genotypes studied was 6.78 times. Gallic acid was the most abundant (88.15 mg/100 g) in B4 genotype. A gallic acid level between 5.3 and 9.5 mg/kg in walnut kernels of different varieties was found by Slatnar et al. (2015). Ellagic acid (35.45), vanillic acid (26.63), ferulic (26.13), chlorogenic acid (17.9), sinapic (15.06), *p*-coumaric (9.70), syringic (3.56), were detected in defatted walnut kernel in significantly amount. Colaric et al. (2005) reports that syringic acid, juglone, and ellagic acid are predominant components of kernel and pellicle walnuts, while ferulic and sinapic acid are found in lowest quantities. Juglone is known as the characteristic compound of walnut and in the present study it was found that the juglone content ranged between 2.53 and 28.84 mg/100 g.

In conclusion, the study provides evidence on high phenolic contents and high antioxidant potential of defatted walnut kernel and can be a basic ingredient in the food industry.

Conflict of interest I. Trandafir and S. Cosmulescu declare that they have no competing interests.

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