Effects of Cold-Press and Soxhlet Extraction Systems on Antioxidant Activity, Total Phenol Contents, Fatty Acids, and Tocopherol Contents of Walnut Kernel Oils

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Abstract: In this study, physico-chemical properties, fatty acid composition, and tocopherol contents of several walnut kernel oils obtained through cold-press and Soxhlet extractions were investigated. The acidity, peroxide, and unsaponifiable matter of oil samples extracted in the Soxhlet system were found higher. Total phenol contents of the oils obtained in cold press and Soxhlet extraction systems were 121.9 mg GAE/100g (Kaman-2) and 154.6 mg GAE/ 100g (Büyükoğa), and between 135.9 mg GAE/100g (Kaman-2) and 163.8 mg GAE/100g (Büyükoğa), respectively ($p < 0.05$). In addition, antioxidant activity values of walnut oils obtained in cold press and Soxhlet extractions varied between 17.3% (Kaman-2) and 19.7% (Kaman-5), and between 18.4% (Kaman-2) and 23.8% (Büyükoğa), respectively ($p < 0.05$). Linoleic acid contents of the oil samples extracted in cold-press varied between 55.19% (Kaman-5) and 56.71% (Kaman-2), while that extracted from Soxhlet extraction system varied between 54.47% (Kaman-2) and 55.93% (Büyükoğa). γ -Tocopherol contents of walnut oils extracted in cold press and Soxhlet extraction ranged between 9.41 mg/100g (Büyükoğa) and 10.83 mg/100g (Kaman-2), and 8.76 mg/100g (Kaman-5) and 9.33 mg/100g (Kaman-2), respectively, and were statistically significant ($p < 0.05$).

Key words: walnut oil, total phenol, antioxidant activity, fatty acids, tocopherols, GC, HPLC

1 INTRODUCTION

Walnut (*Juglans regia* L.) is considered as one of the oldest fruits cultivated in different parts of the world including Turkey. Most nuts are rich in monounsaturated fatty acids, but walnuts are also high in two polyunsaturated fatty acids (linoleic and α -linolenic)^{1, 2)}. Nuts have special common properties such as high oil content when compared to other oilseed species, and they are part of healthy diets³⁾. Walnut kernel contains about 52 - 75 % of oil depending on the variety, cultivation and irrigation of walnut trees. Regarding the fatty acid composition, unsaturated fatty acids such as oleic, linoleic, and linolenic acids dominate⁴⁻⁷⁾. Among the tocopherol fraction, the dominant tocopherol is γ -tocopherol, comprising 88 % of the total tocopherols^{5, 8)}. Some commonly used methods for oil production include pressing, Soxhlet extraction, and combined pre-pressing and solvent extraction⁹⁾. Walnut kernel oil obtained by cold-press has higher amount of essential fatty acid and different bioactive compounds than those of some

other common vegetable oils, which can serve as a promising alternate edible oil. Cold-pressing does not need both heat and chemical treatments, and hence, it does not destroy the beneficial properties of oils. Because of these properties, the demand for cold-press oils is increasing for natural and safe food products¹⁰⁾. Recently, there was an increasing demand to consume cold-pressed plant oils because of better nutritive properties. Cold-pressing is also environment friendly as it does not require much energy¹¹⁾. Although extraction with cold-pressing has been proposed, solvent extraction is the most widely used procedure on an industrial scale. However, limited information is available about qualitative comparison of walnut oils obtained by cold-press and solvent systems. The current study reports the experimental results about walnut oils obtained using petroleum ether-assisted Soxhlet extraction and cold pressing. The objective of this study was to determine the effect of cold-press and Soxhlet extraction techniques on physico-chemical characteristics, and fatty acid and to-

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Accepted October 31, 2018 (received for review July 20, 2018)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

copherol contents of the kernel oils obtained from three walnut varieties.

2 MATERIAL AND METHODS

2.1 Materials

Walnut fruits (Kaman-2, Kaman-5, and Büyükoba varieties with commercial importance) were collected from 20 selected walnut trees from Kırşehir Province, Turkey, in September 2017. The fruits were air-dried, hulls were removed, and the walnut kernels were homogenized, and stored at 4°C for further analyses.

2.2 Methods

2.2.1 Cold-press

The oil of walnut kernel was obtained by using cold-press extraction process after removing the broken or damaged walnut kernels and other impurities such as stem, and skin as they can have negative effects on the oil quality. Whole kernel was extracted with cold-press (2–6 L/h capacity) without heat treatment. Once pressed, the oil was allowed to sediment for a week to remove solid impurities. After sedimentation, the oil was filtrated, and purified oil was kept in a hermetically closed colored bottle under nitrogen at 4°C.

2.2.2 Soxhlet extraction

Walnut kernels were ground and extracted for 6 h using petroleum ether (50°C) in a Soxhlet extractor followed by evaporation of the solvent under reduced pressure. The extracted oil was kept in sealed glass bottles at –18°C for before further analysis.

2.2.3 Physico-chemical properties

Standard AOAC⁽¹²⁾ methods were used to determine the acid, peroxide value, density, iodine value, refractive index, and saponifiable and unsaponifiable values of oil samples.

2.2.4 Sample extraction

Total phenol content and antioxidant activity analyses of oils were carried out by extraction method as reported by Talhaoui *et al.*⁽¹³⁾ with some modifications. Walnut kernels were ground, and 4 g from each sample was mixed with 20 mL of methanol followed by 15-min sonication and 10-min centrifugation at 5000 rpm. The extraction was carried out in two cycles and centrifuged supernatants were separated and concentrated at 37°C in a rotary evaporator under vacuum. The extracted volume was made up to 25 mL using methanol.

2.2.5 Total phenolic content

Folin-Ciocalteu (FC) reagent methods as reported by Yoo *et al.*⁽¹⁴⁾ was used for evaluating the phenolic contents of the extracts. Folin-Ciocalteu (1 mL) and extract sample (0.5 mL) were combined and mixed for 5 min followed by the addition of 10 mL Na₂CO₃ (7.5%) and making the volume to 25 mL using distilled water. The samples were mixed well

and kept for 1 h, after which the absorbance was measured at 750 nm in spectrophotometer (Shimadzu, Japan). Gallic acid (GA) was used as standard for making the calibration curve and the results were given as mg GAE/100 g.

2.2.6 Antioxidant activity

Methanolic solution of DPPH (1,1-diphenyl-2-picrylhydrazyl)⁽¹⁵⁾ was used for assessing the antioxidant activity in oil samples. First, 1 mL of the extract was mixed with 2 mL methanolic solution of DPPH followed by vigorous shaking, 30-min incubation at room temperature, and measurement of absorbance values at 517 nm using a spectrophotometer.

2.2.7 Fatty acid composition

Walnut oil samples were first esterified using the ISO-5509⁽¹⁶⁾ procedure followed by identifying fatty acid methyl esters through comparison of retention time with those obtained from the samples and standards. The fatty acid methyl esters were injected in a gas chromatography system (Shimadzu GC-2010) equipped with a capillary column (Tecnocroma TR-CN100, 60 m × 0.25 mm, film thickness: 0.20 µm) and a flame-ionization detector (FID). The injection block and detector temperatures were set at 260°C and nitrogen at a flow rate of 1.51 mL/min was used as the mobile phase. The total flow rate was 80 mL/min, whereas the split rate was 1/40. The column temperature was set as 120°C for 5 min followed by an increment of 4°C/min until it reached 240°C where it was held for 25 min.

2.2.8 Tocopherol content

A 20 µL sample (obtained by solubilizing 250 mg of oil in 25 mL of *n*-heptane) was directly injected to a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) at a flow rate of 1.3 mL/min. The contents of tocopherol in either cold-pressed or Soxhlet extraction oil samples were determined following the method of Spika *et al.*⁽¹⁷⁾. The HPLC system for tocopherol analysis consisted of Shimadzu-HPLC equipped with a PDA detector and Li-ChroCART Silica 60 (4.6 × 250 mm, 5 µm; Merck, Darmstadt, Germany) column. Standard solutions of tocopherols (α-, β-, γ-, and δ-tocopherol) were used at 0–100 mg/L concentrations for comparison and quantification. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99/1, v/v).

2.3 Statistical Analyses

All analytical measurements were carried out in triplicate and a complete randomized split plot block design was used for carrying out the experiment. The obtained data were analyzed using analysis of variance (ANOVA) and performed in JMP version 9.0 (SAS Inst. Inc., Cary, N.C.U.S.A.). The results were expressed as means ± standard deviation of independent walnut oil samples⁽¹⁸⁾.

3 RESULTS AND DISCUSSION

The physicochemical properties, total phenol contents, and antioxidant activities of walnut oils obtained through cold-press and Soxhlet extraction are presented in Table 1. The acidity values of the oil samples obtained from cold-press were between 0.37 mgKOH/g (Büyükoğa) and 0.48 mg KOH/g (Kaman-2), while the acid values of walnut oils extracted using the Soxhlet apparatus ranged between 0.45 mg KOH/g (Büyükoğa) and 0.54 mg KOH/g (Kaman-5). The higher acid values of walnut kernel oils extracted by Soxhlet extraction could be attributed to the high action of lypolytic enzyme. The peroxide values of walnut oil samples obtained from cold-press were between 1.89 meq O₂/kg (Büyükoğa) and 2.68 meq O₂/kg (Kaman-5) ($p < 0.05$), while that obtained from Soxhlet apparatus ranged between 2.09 (Büyükoğa) and 2.87 meq O₂/kg (Kaman-5). The walnut oil obtained from the Büyükoğa variety extracted from cold-press and Soxhlet apparatus had the lowest peroxide values of 1.89 and 2.09 meq O₂/kg, respectively. However, the increase in the peroxide values of walnut oils extracted by Soxhlet apparatus can be attributed to the solvent used, heat application, and oxygen contact during extraction process. Generally, acidity and peroxide values of walnut oils extracted in the Soxhlet system were higher than those of walnut oils obtained using a cold-press system. The saponification values of walnut oils obtained in cold-press varied between 104.6 (Kaman-2) and 109.9 (Kaman-5), while that extracted from the Soxhlet system were between 103.9 (Kaman-2) and 108.2 (Kaman-5). Also the unsaponification matter values of walnut kernel oils obtained from cold-press varied between 0.41% (Kaman-5) and 0.48% (Kaman-2), while that from the Soxhlet system were between 0.87% (Kaman-2) and 0.93% (Kaman-5). Increase in the unsaponifiable matter of walnut kernel oils obtained in the Soxhlet apparatus can be attributed to the conversion of more matter into oil from the seeds due to the solvent used. Significant differences were observed among the physicochemical and bioactive properties of walnut oil

samples extracted from the cold-press and Soxhlet apparatus. In addition, acidity values of Kaman 2 and Büyükoğa and refractive index values of Kaman 2 and Kaman 5 walnut oils obtained through cold-press were found to be statistically similar. Özcan *et al.*²⁾ determined 3.18–3.53 meq O₂/kg peroxide value, 0.35–0.56% acidity, 102.09–114.6 saponification value, and 1.534–1.537 refractive index in the walnut oils extracted in the Soxhlet system. The total phenol contents of walnut kernel oils obtained from cold-press ranged between 121.9 mg GAE/100g (Kaman-2) and 154.6 mg GAE/100g (Büyükoğa), while that extracted through Soxhlet extraction ranged between 135.9 mg GAE/100g (Kaman-2) and 163.8 mg GAE/100g (Büyükoğa) ($p < 0.05$). The highest total phenol content (163.8 mg GAE/100g) was found in Büyükoğa walnut oil extracted from the Soxhlet system. The total phenol contents of oil samples extracted by the Soxhlet apparatus were observed to be high, which might be due to the presence of more bioactive components. While the antioxidant activity values of walnut oils obtained by cold-press varied between 17.3% (Kaman-2) and 19.7% (Kaman-5), the antioxidant activity values of walnut kernel oils extracted using the Soxhlet apparatus were between 18.4% (Kaman-2) and 23.8% (Büyükoğa). The antioxidant activity values of oil samples increased in parallel with the total phenol. Abe *et al.*²⁰⁾ reported that walnut kernels contained 2499 mg GAE/100g of total phenol and 120 µmol Trolox/g (fw) of antioxidant capacity. The results showed some differences compared to literature values^{1, 2, 7, 19, 20)}. The quality properties of walnut oils changed depending on the genotype and climatic conditions and maturity^{21–23)}.

Fatty acid compositions of walnut oils extracted in both cold-press and Soxhlet extraction systems are presented in Table 2. The fatty acid compositions of oils changed depending on the walnut varieties and extraction types. The most abundant fatty acids of walnut oils obtained in cold press and Soxhlet apparatus were linoleic acid, followed by oleic and linolenic acids. The palmitic acid contents of walnut oils obtained in from cold-press varied between

Table 1 Physico-chemical and bioactive properties of walnut oils obtained by cold press and Soxhlet extraction.

Parameters	Cold press			Soxhlet extraction		
	Kaman-2	Kaman-5	Büyükoğa	Kaman-2	Kaman-5	Büyükoğa
Acid value (mgKOH/g)	0.48 ± 0.03 _a	0.41 ± 0.05 _b	0.37 ± 0.09 _a	0.51 ± 0.07 _b	0.54 ± 0.01 _a	0.45 ± 0.09 _c
Peroxide value (meq O ₂ /kg)	2.27 ± 0.21 _{b**}	2.68 ± 0.13 _a	1.89 ± 0.19 _c	2.51 ± 0.27 _b	2.87 ± 0.18 _a	2.09 ± 0.15 _c
Saponification value (mg KOH/g)	104.60 ± 1.17 _c	109.90 ± 1.23 _a	106.80 ± 1.24 _b	103.90 ± 1.32 _c	108.20 ± 1.45 _a	105.92 ± 1.52 _b
Density (g/cm ³ ; 25°C)	0.927 ± 0.013 _b	0.938 ± 0.021 _a	0.917 ± 0.017 _c	0.951 ± 0.011 _c	0.962 ± 0.009 _a	0.959 ± 0.015 _b
Unsaponifiable value (%)	0.48 ± 0.03 _a	0.41 ± 0.07 _c	0.43 ± 0.01 _b	0.87 ± 0.09 _c	0.93 ± 0.07 _a	0.89 ± 0.06 _b
Iodine value (gI ₂ /100 g oil)	130.10 ± 1.53 _b	137.30 ± 1.62 _a	129.60 ± 1.49 _c	128.30 ± 1.61 _b	131.60 ± 1.47 _a	128.0 ± 1.64 _b
Refractive Index (n _D ²⁰)	1.542 ± 0.009 _b	1.547 ± 0.007 _b	1.549 ± 0.003 _a	1.539 ± 0.011 _a	1.531 ± 0.005 _c	1.538 ± 0.003 _b
Total pheno l(mgGAE/100 g)	121.90 ± 2.34 _c	138.70 ± 1.67 _b	154.60 ± 3.42 _a	135.90 ± 3.67 _c	147.10 ± 1.78 _b	163.80 ± 3.86 _a
Antioxidant activity (%)	17.30 ± 1.17 _c	19.70 ± 1.24 _a	18.50 ± 1.13 _b	18.40 ± 1.08 _c	21.60 ± 1.12 _b	23.80 ± 2.34 _a

*mean ± standard deviation; ** Values within each row followed by different letters are significantly different ($p < 0.05$).

Table 2 Fatty acid compositions of walnut oils obtained by cold press and Soxhlet extraction (%).

Fatty acids	Cold press			Soxhlet extraction		
	Kaman-2	Kaman-5	Büyükoba	Kaman-2	Kaman-5	Büyükoba
Myristic	0.06 ± 0.01 _a	0.05 ± 0.01 _b	0.04 ± 0.01 _c	0.03 ± 0.01 _b	0.04 ± 0.01 _a	0.02 ± 0.00 _c
Palmitic	6.81 ± 0.32 _{a**}	6.67 ± 0.18 _b	6.28 ± 0.09 _c	6.58 ± 0.17 _a	6.55 ± 0.21 _b	6.51 ± 0.13 _b
Palmitoleic	0.14 ± 0.03 _b	0.16 ± 0.01 _a	0.16 ± 0.03 _a	0.11 ± 0.01 _c	0.12 ± 0.01 _b	0.13 ± 0.03 _a
Stearic	2.83 ± 0.17 _b	2.87 ± 0.32 _a	2.64 ± 0.24 _c	2.91 ± 0.21 _b	2.95 ± 0.19 _a	2.88 ± 0.13 _c
Oleic	20.64 ± 0.56 _c	25.89 ± 0.61 _a	23.55 ± 0.39 _b	19.71 ± 0.57 _c	24.93 ± 0.28 _a	22.78 ± 0.55 _b
Linoleic	56.71 ± 0.89 _a	55.19 ± 0.54 _b	56.48 ± 0.28 _a	54.47 ± 0.64 _b	54.88 ± 0.48 _b	55.93 ± 0.51 _a
Linolenic	15.79 ± 0.13 _a	15.23 ± 0.17 _a	16.48 ± 0.63 _b	14.63 ± 0.41 _b	14.98 ± 0.57 _b	15.77 ± 0.13 _a
Eicosenoic	0.15 ± 0.03 _b	0.14 ± 0.01 _c	0.16 ± 0.05 _a	0.18 ± 0.02 _a	0.16 ± 0.03 _c	0.17 ± 0.01 _b

*mean ± standard deviation; ** Values within each row followed by different letters are significantly different ($p < 0.05$).

6.28% (Büyükoba) and 6.81% (Kaman-2), and that obtained in Soxhlet apparatus ranged between 6.51% (Büyükoba) and 6.58% (Kaman-2). Stearic acid contents of walnut oils obtained through cold-press were between 2.64% (Büyükoba) and 2.87% (Kaman-5), while that obtained from Soxhlet extraction system ranged between 2.88% (Büyükoba) and 2.95% (Kaman-5). The oleic acid contents of oil samples obtained from cold-press were determined between 20.64% (Kaman-2) and 25.89% (Kaman-5), while that extracted in Soxhlet extraction system ranged between 19.71% (Kaman-2) and 24.93% (Kaman-5) ($p < 0.05$). Linoleic acid contents of oil samples extracted through cold-press method varied between 55.19% (Kaman-5) and 56.71% (Kaman-2), while that extracted through Soxhlet extraction system varied between 54.47% (Kaman-2) and 55.93% (Büyükoba). The highest linoleic acids were found in Kaman-2 and Büyükoba walnut oils (56.71 and 55.93%) extracted in cold press and Soxhlet extraction systems, respectively. Linolenic acid contents of walnut oils obtained from cold-press were between 15.23% (Kaman-5) and 16.48% (Büyükoba), while that oils extracted through Soxhlet extraction varied between 14.63% (Kaman-2) and 15.77% (Büyükoba). As the impurities in the oils obtained from the Soxhlet Soxhlet system were probably higher, linoleic and linolenic acid contents were higher in the cold-pressed oils. The highest content of eicosenoic acid (0.18%) was found in Kaman-2 walnut oil extracted in Soxhlet apparatus ($p < 0.05$). Significant differences were observed among the fatty acid compositions of walnut oil samples extracted through cold-press and Soxhlet apparatus. However, palmitoleic and linolenic acid contents of Kaman 5 to Büyükoba and Kaman 2 to Kaman 5 walnut oils (respectively) obtained in cold press were found to be statistically similar. In addition, linoleic and linolenic acid contents of Kaman 2 and Kaman 5, and palmitic acid contents of Kaman 5 and Büyükoba walnut oils obtained by Soxhlet extraction were found to be statistically similar. Fatty acids showed some differences depending on walnut types and extraction methods. Some of these differences were owing to the extraction method used ($p < 0.05$).

Generally, fatty acids of walnut oils obtained from the cold-press system were found to be higher than the oil obtained by the Soxhlet extraction system. Özcan *et al.*²⁾ reported that walnut oil contained 6.3-6.5% palmitic, 2.5-2.6% stearic, 20.5-26.4% oleic, 49.7-55.5% linoleic and 14.5-14.8% linolenic acids. Özkan and Koyuncu¹⁾ reported that the main fatty acids of walnut genotype oils were 5.24-7.62% palmitic, 2.56-3.67% stearic, 21.18-40.20% oleic, 43.94-60.12% linoleic, and 6.91-11.52% linolenic acids. Kırbaşlar *et al.*¹⁹⁾ determined 0.04% myristic, 7.18% palmitic, 3.07% stearic, 13.55% oleic, 63.42% linoleic, and 12.22% linolenic acids in walnut oil extracted using the Soxhlet apparatus. In another study, walnut oil contained 6.46% palmitic, 2.65% stearic, 15.61% oleic, 64.14% linoleic, and 10.77% linolenic acids⁷⁾. Bujdoso *et al.*²⁴⁾ reported that the oil of some walnut cultivars contained 6.09-7.14% palmitic, 1.94-2.90% stearic, 16.18-30.14% oleic, 50.10-62.66% linoleic, and 8.35-14.19% linolenic acids. In another study, the minimum and maximum values of polyunsaturated fatty acids linoleic acid and α -linolenic acids in walnut oils were found to be 53.24% to 64.56% and 9.50% to 13.26%, respectively²⁵⁾. Mereles *et al.*²⁶⁾ determined 0.2-18.4 mg/100g α -tocopherol, 7.01-12.80% palmitic, 3.47-5.64% stearic, and 33.90-46.61% oleic acids in of *Macadamia integrifolia* nuts cultivated in Paraguay. These results are consistent with those reported by Bujdoso *et al.*²⁴⁾ and Kırbaşlar *et al.*¹⁹⁾, Özkan and Koyuncu¹⁾, Özcan *et al.*²⁾, Kafkas *et al.*²⁵⁾ and Mereles *et al.*²⁶⁾.

The tocopherol contents of walnut oils extracted using cold-press and Soxhlet extraction systems are presented in **Table 3**. All the tested walnut variety oils are rich in α - and γ -tocopherols. While α -tocopherol contents of oil samples obtained using the cold-press system ranged between 4.75 mg/100 g (Büyükoba) and 5.17 mg/100 g (Kaman-2), the α -tocopherol contents of walnut oils extracted through Soxhlet apparatus varied between 4.27 mg/100 g (Büyükoba) and 4.64 mg/100 g (Kaman-2) ($p < 0.05$). The γ -tocopherol contents of walnut oils extracted in cold-press ranged between 9.41 mg/100 g (Büyükoba) and 10.83 mg/100 g (Kaman-2), while that obtained from Soxhlet ex-

Table 3 Tocopherol contents of walnut oils obtained by cold press and Soxhlet extraction (mg/100 g).

Tocopherols	Cold press			Soxhlet extraction		
	Kaman-2	Kaman-5	Büyükbaba	Kaman-2	Kaman-5	Büyükbaba
α -tocopherol	$5.17 \pm 0.28_{a^*}$	$4.98 \pm 0.17_b$	$4.75 \pm 0.41_b$	$4.64 \pm 0.38_a$	$4.31 \pm 0.23_b$	$4.27 \pm 0.15_c$
β -tocopherol	$0.34 \pm 0.03_{a^{**}}$	$0.29 \pm 0.05_c$	$0.31 \pm 0.07_b$	$0.19 \pm 0.03_c$	$0.21 \pm 0.01_b$	$0.24 \pm 0.07_a$
γ -tocopherol	$10.83 \pm 0.35_a$	$9.67 \pm 0.28_b$	$9.41 \pm 0.17_b$	$9.33 \pm 0.25_a$	$8.76 \pm 0.31_b$	$9.13 \pm 0.11_a$
δ -tocopherol	$0.83 \pm 0.09_b$	$0.87 \pm 0.11_a$	$0.79 \pm 0.03_c$	$0.81 \pm 0.07_b$	$0.83 \pm 0.05_a$	$0.72 \pm 0.03_c$

*mean $\pm$ standard deviation; ** Values within each row followed by different letters are significantly different ($p < 0.05$).

traction varied between 8.76 mg/100 g (Kaman-5) and 9.33 mg/100 g (Kaman-2). The highest β -tocopherol and δ -tocopherol contents were determined in Kaman-2 (0.34 mg/100 g) and Kaman-5 (0.87 mg/100 g) obtained from cold-press ($p < 0.05$). Tocopherol contents were higher in the cold-pressed oils because of the probable higher impurities in the oils obtained from the Soxhlet system. Significant differences were observed among the tocopherols of walnut oil samples extracted in cold-press and Soxhlet extraction systems ($p < 0.05$). However, α -tocopherol and γ -tocopherol contents of Kaman 5 to Büyükbaba walnut oils obtained by cold-press were found to be statistically similar. Uzunova *et al.*⁷⁾ reported that walnut oils contained 4.4–5.7% α -tocopherol, 0.0–0.2% β -tocopherol, 85.1–88.2% γ -tocopherol, and 6.1–9.7% δ -tocopherol. Kornsteiner *et al.*²¹⁾ determined 12.4–32.8% mg/100 g β - and γ -tocopherol and 2.3–5.4 mg/100g δ -tocopherols in walnut oils. Kafkas *et al.*²⁵⁾ reported that tocopherol and its isomers such as α , $\gamma + \beta$, and δ tocopherol content of experimental walnut varieties were different. α -Tocopherol content of cultivars varied between 28.33 μ g/g (Howard) and 38.76 μ g/g (Sen), $\beta + \gamma$ tocopherol content varied between 161.01 μ g/g (Howard) to 312.19 μ g/g (Sen) and as for the δ -tocopherol content varied between 17.35 μ g/g (Serr) to 40.77 μ g/g (Sen) oil²⁵⁾. Mereles *et al.*²⁶⁾ determined 0.2–18.4 mg/100 g α -tocopherol in three cultivars of *Macadamia integrifolia* nuts cultivated in Paraguay. In a previous study, the tocopherol compositions analyzed by using high-performance liquid chromatography have shown that α - and γ -tocopherols were the predominant tocopherol homologs present; however, δ - and β -tocopherols were also detected in some samples²⁸⁾. In another study, Lavedrine *et al.*²⁹⁾ studied two walnut varieties (Franquette and Hartley) from two geographical origins (France, and USA), and the amounts of α , γ , and δ tocopherols of walnut oils ranged from 1.08 to 4.05, from 21.78 to 26.46, and from 2.51 to 4.23 (mg/100 g), respectively. Also, Bada *et al.*³⁰⁾ reported that γ -tocopherol was the major component of the total tocopherols in the walnut sample under study (from 289.01 ± 6.02 – 76.52 ± 5.98 mg/kg oil). In addition, Oliveira *et al.*³¹⁾ determined 303.2 and 405.7 μ g/g of total tocopherol in walnut oil. Maguire *et al.*³²⁾ reported that α -tocopherol was the most prevalent tocopherol except in walnuts. These results were somewhat different from those reported by

Uzunova *et al.*⁷⁾, Kafkat *et al.*²⁵⁾, Mereles *et al.*²⁶⁾, Kornsteiner *et al.*²⁷⁾, Lavedrine *et al.*²⁹⁾, Bada *et al.*³⁰⁾, Oliveira *et al.*³¹⁾ and Maguire *et al.*³²⁾

4 CONCLUSIONS

While the acid value, peroxide value, density, and unsaponifiable matter values of oil samples obtained in cold-press increased compared to the results of oils extracted in Soxhlet apparatus, the saponification value, iodine value, and refractive index decreased. The total phenol contents and antioxidant activity values of walnut oils extracted in cold-press were found to be lower than the values obtained using the Soxhlet apparatus. The linoleic acid was the dominant fatty acid of walnut oils obtained from both cold-press and Soxhlet extraction systems. The oil samples obtained from cold-press had higher contents of fatty acids and tocopherol contents than those extracted from Soxhlet apparatus, probably because of the presence of more impurities in the oil extracted from the Soxhlet apparatus method. The most abundant fatty acid present in both cold-press and solvent extraction oils was linoleic acid, followed by oleic and linolenic acids. In addition, the cold-pressing technique excludes the use of organic solvents which can yield an oil product free from chemical contaminants such as those used in Soxhlet technique. Walnuts oils enhance the nutritional value of the human diet because of its beneficial properties. The composition of walnut oil can change depending on the fruit variety, origin place, harvest year, and agro-technical measures.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no. RG-1439-80. Technical support of RSSU at King Saud University is also well appreciated.

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