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ORIGINAL PAPER

Investigating Some Physicochemical Properties and Fatty Acid Composition of Native Black Mulberry (*Morus nigra* L.) Seed Oil

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Abstract The physicochemical properties of seed and seed oil obtained from the native black mulberry (*Morus nigra* L.) were investigated in 2008 and 2009. The results showed that the seed consisted of 27.5–33% crude oil, 20.2–22.5% crude protein, 3.5–6% ash, 42.4–46.6% carbohydrate and 112.2–152.0 mg total phenolics/100 g. Twenty different fatty acids were determined, with the percentages varying from 0.02% myristic acid (C14:0) to 78.7% linoleic acid (C18:2). According to the GC analysis of fatty acid methyl esters, linoleic acid (C18:2), followed by palmitic acid (C16:0), oleic acid (C18:1) and stearic acid (C18:0) were the major fatty acids, which together comprised approximately 97% of the total identified fatty acids. High C18:2 content (average 73.7%) proved that the black mulberry seed oil is a good source of the essential fatty acid, linoleic acid. Linolenic acid (C18:3) was also found in a relatively lower amount (0.3–0.5%). The α-tocopherol content was found to be between 0.17 and 0.20 mg in 100 g seed oil. The main sterols in the mulberry seed oil were *β*-sitosterol, Δ5-avenasterol, Δ5, 23-stigmastadienol, clerosterol, sitosterol and Δ5, 24-stigmastadienol. The present study stated that the native black mulberry seed oil can be used as a nutritional dietary substance and has great usage potential.

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Introduction

Dietary antioxidants are important components because they protect against free radicals, such as reactive oxygen species in the human body. Free radicals are known to be the major contributors to degenerative diseases of aging and are recognized as major factors causing cancer, cardiovascular disorders and diabetes [1].

In the last few decades, natural fruits, fruit extracts and seeds have received much attention as sources of bioactive substances such as antioxidants, antimutagens and anticarcinogens [2–7]. A large quantity of oils and fats, whether for human consumption or for industrial purposes, is presently derived from plant sources [8]. Seed oils are the main source of dietary ingredients related to their fatty acid composition and tocopherol content [9]. Recently, cold-pressed edible seed oils, including black caraway, carrot, hemp, and cranberry seed oils, have become commercially available [10]. The seed oils from different plants are specially taking into consideration because of their mono- and polyunsaturated fatty acid content, especially for the existence of linoleic acid and linolenic acid. Linoleic acid, an "essential fatty acid", cannot be produced by the human body and is accepted as being an anticarcinogenic substance. Deficiency symptoms include dry hair, hair loss and poor wound healing [11]. From this point of view, mulberry seed oil, very rich in linoleic acid, may be a valuable source of dietary fat. Mulberry seed oil was rather poor in linolenic acid. Low levels of linolenic acid are desired in edible oils, because high levels of this fatty acid can cause unfavorable odor and taste in oil. Additionally, since

linolenic acid is simply oxidized due to having three double bonds on its hydrocarbon chain, the stability or shelf-life of an oil rich in linolenic acid would be too short. Because of its low quantities of linolenic acid, mulberry seed oil also has advantages in terms of human health and the shelf-life of the oil [12].

Mulberry (*Morus* L.) belongs to the family *Moraceae* and 3 species; *M. alba* L. (white mulberry), *M. nigra* L. (black mulberry) and *M. rubra* L. (red mulberry) are commonly cultivated for fruit production in Turkey [11, 13] as black mulberries are widespread in Anatolia. Cultivation of the mulberry in Turkey has been carried out for more than 400 years. Ninety five percent of mulberry trees in Turkey are white, 3% are red and 2% are black mulberries [14]. Mulberry fruits have been used for the production of some traditional Turkish food products (mulberry pekmez, mulberry pestil, mulberry kome), marmalades, juices, liquors and natural dyes [15].

Different researchers investigated certain properties of whole mulberry fruit, mulberry fruit extract and mulberry leaves. Ercisli and Orhan [11] investigated some physicochemical characteristics of black mulberry and determined the phenolic content (1,943–2,237 mg gallic acid equivalents/100 g fresh mass), vitamin C (14.9–18.7 mg/100 mL), antioxidant activity (63–76%) and malic acid content (123–218 mg/g). In another study where the chemical compositions of white, red and black mulberries were compared, it was stated that the highest total phenolic and flavonoid contents were observed in black mulberry [15]. Arabshahi-Delouee and Urooj [16] studied antioxidant properties of various solvent extracts of mulberry leaves and stated that the methanolic extract, with the highest amount of total phenolics, was the most potent antioxidant in all the assays used. However, to our knowledge, there are few reports on the mulberry seeds and mulberry seed oil [17–19], and this is the first study on the certain physicochemical properties and fatty acid composition of black mulberry seed oil. Therefore, the aim of the present study was to investigate some physicochemical properties and the fatty acid composition of black mulberry seed oil.

Materials and Methods

Obtaining and Preservation of Raw Material

Fresh black mulberry fruits (*M. nigra* L.) of five different trees were obtained from Pınarhisar, a town (latitude 41°37′ N, longitude 27°31′ E and altitude 193 m) on the south side of the Istranca Mountain Range, in Turkey, in the 2008 and 2009 January–July growing periods. According to the conditions of the region, the trees were known to be free of pesticides and had a high fruit yield. All berries were picked at the commercially ripe stage and selected according to uniformity of shape and color. The fruits were harvested by hand and transferred to the laboratory under refrigerated conditions (4 ± 1 °C) for 2 h. Seeds were separated from the fruits immediately, dried at 50 °C in vacuum conditions and stored for further analysis performed on the same day.

Determination of Some Physicochemical Properties of Black Mulberry Seeds and Seed Oils

A digital balance with a sensitivity of 0.0001 g was used to determine the 1,000-grain weight of the mulberry seeds. Oil extraction from the seeds was carried out by hexane extraction under the operating conditions specified in IUPAC method no. 1.121 [20]. Dried seeds were ground into fine powder using a laboratory mill.

For solvent extraction, 15 g of ground seeds were placed in cellulose paper cones and extracted using hexane in a Soxhlet extractor for 8 h [21]. The oil was then recovered by evaporating off the solvent using a rotary evaporator and the residual solvent was removed by drying in a vacuum oven at 60 °C [22]. The total oil contents of the samples are expressed as a percentage by mass of the sample. Protein and ash content of the samples was determined according to the AOAC methods 920.87 and 940.26, respectively [23]. The nitrogen conversion factor for crude protein calculation was 6.25. The percentage of the total carbohydrate was calculated by difference. The amount of total phenolics in the mulberry seeds was determined according to the Folin-Ciocalteu procedure [24]. Methanolic extracts of the samples (300 μL) were introduced into test tubes; 1.5 mL of Folin-Ciocalteu's reagent (diluted 1:10 v/v with water) and 1.2 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 90 min and absorbance values were measured using a spectrophotometer at 765 nm. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram dry material.

Moisture content, free fatty acid content, peroxide value and refraction index of oil samples were determined in accordance to the IUPAC methods 1.122, 2.201, 2.102 and 2.501, respectively [20]. Specific weights of the oil samples were determined according to AOAC [23].

Fatty Acid Analysis

Fatty acid methyl esters (FAME) were prepared from the mulberry seed oils after alkaline hydrolysis, followed by methylation in methanol plus BF_3 (14% boron trifluoride). The final concentration of the FAME was approximately 7 mg/mL in heptane [23]. Analyses of the FAME by capillary GLC were carried out on a Hewlett-Packard 6,890

chromatograph, equipped with a flame ionization detector (FID) on a split injector. A fused-silica capillary column was used, CP^{TM}-Sil 88, 50 m × 0.25 mm i.d., 0.2 μm film; Chrompack. The column was operated isothermally at 177 °C and injector and detector were kept at 250 °C. The carrier gas was helium at a flow rate of 1 mL/min.

The fatty acid methyl esters peaks were identified by comparing their retention times with individual standard FAMEs, approximately 99% pure (Supelco, USA) and analyzed using Total Chrom Workstation Software. The relative percentage of the fatty acid was calculated on the basis of the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

α-Tocopherol Analysis

Tocopherol analysis was performed by HPLC under isocratic conditions [25]. All solvents used were HPLC grade; all other reagents were analytical grade. Extracts (25 μl) were chromatographed on a 5-μm silica column (250 × 4.6 mm) using a mobile phase of ethyl acetate/acetic acid/hexane in a ratio of 1:1:198 (v/v/v). The flow rate was set at 1.5 mL min^{-1} and a fluorescence detector was used with the excitation and emission wavelengths at 290 and 330 nm, respectively. A calibration curve was generated using standard α-tocopherol (Sigma–Aldrich; 0 to 10 μg mL^{-1}; $R^2 = 0.999$) and identification was performed by retention time comparison with the standard.

Sterol Analysis

Oil (500 mg) was saponified with 25 mL methanolic potassium hydroxide (2 M) by boiling on a water bath for 1 h and the saponifiables were then extracted three times with hexane after water was added to the saponification mixture. Dry sodium sulfate (Na_2SO_4) was added and allowed to stand for 1 h. Samples (500 μl) was mixed with 100 μl of BSTFA (Bis(trimethylsilyl)trifluoroacetamide)/TMSCI (Trimethyl Chlorosilan) (4:1, v:v) mixture [26], and the sterols were silylated. 0.8 μl of the samples were analyzed on GC equipped with a CP-SİL 24 CB column (60 m × 0.32 mm × 1.00 μm). The temperature program was: 50 °C for (2 min); 60 °C min^{-1} ramp to 245 °C (1 min hold) and 3 °C min^{-1} ramp to 275 °C and then allowed to stand at this temperature for 35 min. Helium was the carrier gas with a flow rate of 0.8 mL min^{-1}. The injector and detector temperatures were set at 280 and 300 °C, respectively, and the samples were injected in a split mode (1:25).

Statistical Analysis

Each value is a mean of three replications. Values of different parameters were expressed as the mean ± standard deviation (means ± S.D.). The discussion is based on the one-way analysis of variance (ANOVA; $p < 0.05$). All statistical analyses were performed using the SPSS of the Windows Statistical Package (Release 8.0).

Results and Discussion

Physicochemical Properties of Mulberry Seeds

All results were calculated on a dry basis and are reported in Table 1. Physicochemical properties did not vary significantly ($p > 0.05$) between the different years. The results showed that the seeds contained 27.5–33.0% crude oil, 20.2–22.5% crude protein, 3.5–6.0% ash and 42.4–46.6% carbohydrate (by difference). According to Jiel et al. [18], mulberry seeds yielded 29.3% oil whereas Xiaolan et al. [19] reported a 30.7% oil yield for white mulberry seeds. While our results are in accordance with the previous reports on mulberry seeds, variations in oil yield and oil content may be due to the differences in variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds, location and the extraction method used [22, 27]. In a comparison, the oil content of approximately 30.0% proved that the black mulberry seed is a higher source of edible oil than grape seed (11.6–19.6%), cotton seed (15.2–22.0%), olive (12.0–28.0%) and raspberry seed (10.7%), as reported in previous studies [28–30]. Protein content of the seeds showed good correlation with the results of previous studies on mulberries [18, 19] in which 21.2 and 29.4% crude protein was found, respectively. As indicated by Kamel and Dawson [31], the protein content of grape seed was 8.2% and it was 10.12–11.81% according to Fantozzi [32] where both of the findings were lower than the protein content of mulberry seeds found in the present study. Additionally, the mulberry seed contains a protein content similar to cottonseed, which was found to be 22.32% by Sawan et al. [33]. The average ash content of the black mulberry seeds was found to be 4.5%. This finding was higher than the ash content of grapeseed (i.e. 2.2%) reported by Kamel and Dawson [31]. Plants absorb minerals during the growing season. The ash content of seeds depends not only on the variety, but also on the growing conditions such as soil and geographical conditions [34]. The 1,000-grain weight of the seeds was in the range of 2.029–2.305 g. The approximate total phenolic content of the seeds were determined to be 112.2–152.0 mg GAE/100 g. Our results are lower than the reported values for black mulberry fruits (1,943–2,237 mg GAE/100 g fresh mass, 1,422 mg GAE/100 g fresh matter), since the previous studies were conducted on whole fruits, not on mulberry seeds [11, 15].

Table 1 Physicochemical properties of native black mulberry (*Morus nigra* L.) seeds

Year	Samples	1,000-grain weight (g)	Oil content (%)	Protein content[a] (%)	Ash content (%)	Carbohydrate[b] (%)	Total phenolics (GAE)[c]
2008	1	2.284 ± 0.005	30.8 ± 0.7	22.5 ± 0.4	4.0 ± 0.6	42.7 ± 0.6	148.4 ± 8.2
	2	2.270 ± 0.006	30.2 ± 0.5	20.7 ± 0.5	3.7 ± 0.6	45.5 ± 0.7	143.5 ± 7.3
	3	2.301 ± 0.007	32.9 ± 0.7	21.1 ± 0.3	3.5 ± 0.5	42.4 ± 0.5	152.0 ± 8.0
	4	2.269 ± 0.005	29.6 ± 0.6	21.6 ± 0.4	5.1 ± 0.5	43.7 ± 0.5	112.2 ± 7.9
	5	2.029 ± 0.007	28.8 ± 0.6	20.9 ± 0.4	6.0 ± 0.5	44.4 ± 0.5	119.4 ± 8.4
	Average[Δ]	2.230 ± 0.006	30.5 ± 0.6	21.3 ± 0.4	4.5 ± 0.5	43.7 ± 0.6	135.1 ± 8.0
2009	1	2.305 ± 0.006	31.3 ± 0.8	22.0 ± 0.4	4.1 ± 0.4	42.7 ± 0.8	147.5 ± 7.6
	2	2.280 ± 0.005	30.3 ± 0.8	20.2 ± 0.4	3.8 ± 0.4	45.7 ± 0.8	144.3 ± 8.9
	3	2.292 ± 0.006	33.0 ± 0.7	20.6 ± 0.3	3.5 ± 0.4	42.9 ± 0.7	149.6 ± 6.3
	4	2.250 ± 0.004	30.1 ± 0.7	21.4 ± 0.4	4.9 ± 0.4	43.6 ± 0.8	114.2 ± 7.4
	5	2.050 ± 0.005	27.5 ± 1.0	20.2 ± 0.3	5.6 ± 0.4	46.6 ± 0.9	118.8 ± 7.6
	Average[Δ]	2.235 ± 0.005	30.4 ± 0.8	20.9 ± 0.4	4.4 ± 0.4	44.3 ± 0.8	134.9 ± 7.6
2008–2009		2.233 ± 0.006	30.5 ± 0.7	21.1 ± 0.4	4.5 ± 0.5	44.0 ± 0.7	135.0 ± 7.8
Year		NS	NS	NS	NS	NS	NS

NS not significant

All determinations were carried out in triplicate and mean values ± standard deviation (SD) reported

[Δ] Average values were calculated based on all 15 (5 samples × 3 replicates) data for each year with ± SD

[a] Protein content (%) = N (%) × 6.25

[b] Carbohydrate obtained by difference

[c] The total phenolic, expressed as garlic acid equivalents mg/100 g oil

Proximate Analysis of Mulberry Seed Oil

The physicochemical properties of crude mulberry seed oil are given in Table 2. The differences in the proximate composition of the oils belonging to the different growing periods were found to be insignificant ($p > 0.05$). Free fatty acid content of the samples was between 2.38 and 3.38% in the basis of oleic acid. The peroxide value (mequiv O_2/kg), refractive index (50 °C) and specific weight (25 °C) were in the ranges of 5.82–7.03, 1.464–1.472 and 0.950–0.975, respectively. Moisture content of the samples was between 0.016 and 0.024%. The refractive index of *M. alba* L. seed oil was in agreement with Xiaolan et al. [19] who found refractive index of 1.4750 at 20 °C.

As shown in Table 2, the average α-tocopherol content was found as 0.18 mg in 100 g seed oil for all samples collected in 2008 and 2009, where the highest value was found for the sample collected in 2009.

Sterols are known as biologically active phytochemicals and have important roles for human health. Table 2 shows the sterol composition of the black mulberry seed oil. The results indicated that the approximately 95% of the total sterols are composed of β-sitosterol, Δ5-avenasterol, Δ5,23-stigmastadienol, clerosterol, sitostenol and Δ5,24-stigmastadienol. Campesterol, stigmasterol, cholesterol and brassicasterol were also identified in the black mulberry seed oil but only in small amounts.

Fatty Acid Composition of Mulberry Seed Oil

The physicochemical and nutritional properties of any oilseed are affected by the fatty acid composition. A sample gas chromatogram obtained from the present study was supplied as Electronic Supplementary Material (ESM-Fig. 1). Fatty acid profiles of mulberry seed oil samples are presented in Tables 3, 4. According to the GC analysis of fatty acid methyl esters, linoleic acid (C18:2), followed by palmitic acid (C16:0), oleic acid (C18:1) and stearic acid (C18:0) were the major fatty acids, which together comprised approximately 97% of total identified fatty acids. C18:2 content of the mulberry oil varied in the range of 67.0–78.7%. It was followed by C16:0 and C18:1 in the ranges of 8.6–13.3 and 6.4–10.2%, respectively. When between-year effects on fatty acid composition of mulberry seed oils are compared, the differences in the all fatty acids were found to be insignificant (Tables 3 and 4). However, Gecgel et al. [27] reported that important factors influencing fatty acid composition are the variety and genetics of the seed. It was reported that environmental conditions influence fatty acid composition much more than the genotype of the variety does. Similarly, Lajara et al. [35] showed that temperature during the time elapsed between flowering and ripening is possibly the most important factor influencing fatty acid composition.

Table 2 Physicochemical properties of native black mulberry (*Morus nigra* L.) seed oils

Year	Samples	Free fatty acid (oleic acid %)	Peroxide value (mequiv O_2/kg)	Refractive index (50 °C)	Specific weight (25 °C)	Moisture content (%)	α-tocopherol (mg/100 g)	Sterol content and composition (% of total sterols)					
								Cholesterol	Brassicasterol	Campesterol	Stigmasterol	Δ7-stigmastenol	Others[a]
2008	1	2.42 ± 0.13	5.82 ± 0.20	1.471 ± 0.001	0.961 ± 0.009	0.018 ± 0.001	0.17 ± 0.01	0.20 ± 0.01	0.010 ± 0.001	2.68 ± 0.13	1.90 ± 0.15	0.180 ± 0.002	95.03
	2	2.68 ± 0.15	6.33 ± 0.35	1.464 ± 0.003	0.959 ± 0.008	0.024 ± 0.002	0.19 ± 0.01	0.30 ± 0.01	0.020 ± 0.001	2.56 ± 0.15	1.86 ± 0.18	0.200 ± 0.001	95.06
	3	3.15 ± 0.16	7.03 ± 0.19	1.471 ± 0.001	0.975 ± 0.009	0.020 ± 0.002	0.17 ± 0.01	0.30 ± 0.01	0.020 ± 0.002	2.65 ± 0.16	1.92 ± 0.18	0.190 ± 0.002	94.92
	4	2.75 ± 0.14	6.51 ± 0.19	1.471 ± 0.002	0.969 ± 0.007	0.016 ± 0.001	0.18 ± 0.01	0.20 ± 0.01	0.010 ± 0.002	2.68 ± 0.19	1.94 ± 0.17	0.170 ± 0.001	95.00
	5	3.08 ± 0.14	6.05 ± 0.28	1.464 ± 0.001	0.950 ± 0.009	0.022 ± 0.002	0.19 ± 0.01	0.30 ± 0.01	0.020 ± 0.001	2.64 ± 0.13	1.88 ± 0.15	0.180 ± 0.002	94.98
	Average[Δ]	2.82 ± 0.15	6.35 ± 0.24	1.468 ± 0.002	0.962 ± 0.009	0.020 ± 0.002	0.18 ± 0.01	0.26 ± 0.01	0.016 ± 0.001	2.64 ± 0.15	1.90 ± 0.17	0.184 ± 0.002	94.99
2009	1	2.38 ± 0.18	6.02 ± 0.17	1.472 ± 0.001	0.960 ± 0.006	0.019 ± 0.001	0.17 ± 0.01	0.20 ± 0.01	0.020 ± 0.001	2.66 ± 0.16	1.88 ± 0.18	0.160 ± 0.001	95.08
	2	2.63 ± 0.17	5.87 ± 0.18	1.471 ± 0.001	0.972 ± 0.005	0.020 ± 0.001	0.18 ± 0.01	0.30 ± 0.01	0.020 ± 0.002	2.58 ± 0.15	1.86 ± 0.18	0.180 ± 0.001	95.06
	3	3.38 ± 0.18	6.36 ± 0.19	1.469 ± 0.003	0.968 ± 0.008	0.022 ± 0.001	0.19 ± 0.01	0.20 ± 0.01	0.010 ± 0.002	2.63 ± 0.16	1.90 ± 0.15	0.200 ± 0.002	95.06
	4	3.10 ± 0.19	7.01 ± 0.20	1.472 ± 0.002	0.960 ± 0.004	0.018 ± 0.001	0.18 ± 0.01	0.30 ± 0.01	0.010 ± 0.001	2.66 ± 0.17	1.96 ± 0.16	0.170 ± 0.002	94.90
	5	3.13 ± 0.17	6.29 ± 0.19	1.466 ± 0.001	0.956 ± 0.006	0.020 ± 0.001	0.20 ± 0.01	0.20 ± 0.01	0.020 ± 0.001	2.62 ± 0.18	1.86 ± 0.15	0.200 ± 0.001	95.10
	Average[Δ]	2.92 ± 0.18	6.31 ± 0.19	1.470 ± 0.002	0.963 ± 0.006	0.019 ± 0.001	0.18 ± 0.01	0.24 ± 0.01	0.016 ± 0.001	2.63 ± 0.16	1.90 ± 0.16	0.182 ± 0.001	95.04
2008–2009		2.87 ± 0.17	6.33 ± 0.22	1.469 ± 0.002	0.963 ± 0.008	0.019 ± 0.002	0.18 ± 0.01	0.25 ± 0.01	0.016 ± 0.001	2.64 ± 0.16	1.90 ± 0.17	0.183 ± 0.002	95.02
Year		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS not significant

All determinations were carried out in triplicate and mean values ± standard deviation (SD) reported

[a] β-sitosterol + Δ5-avenasterol + Δ5,23-stigmastadienol + clerosterol + sitostenol + Δ5,24-stigmastadienol

[Δ] Average values were calculated based on all 15 (5 samples × 3 replicates) data for each year with ± SD

Table 3 Saturated fatty acid composition of native black mulberry (*Morus nigra* L.) seed oils

Fatty acid (%)	2008						2009						2008–2009	Year
	1	2	3	4	5	Average [Δ]	1	2	3	4	5	Average [Δ]		
Caproic acid C6:0	0.05 ± 0.003	0.06 ± 0.006	0.06 ± 0.003	0.04 ± 0.010	0.04 ± 0.008	0.05 ± 0.006	0.05 ± 0.008	0.04 ± 0.004	0.05 ± 0.006	0.06 ± 0.006	0.04 ± 0.004	0.05 ± 0.006	0.05	NS
Caprilic acid C8:0	0.05 ± 0.005	0.05 ± 0.007	0.06 ± 0.005	0.05 ± 0.006	0.03 ± 0.006	0.05 ± 0.006	0.05 ± 0.006	0.06 ± 0.006	0.06 ± 0.007	0.06 ± 0.005	0.04 ± 0.005	0.05 ± 0.006	0.05	NS
Myristic acid C14:0	0.02 ± 0.004	0.04 ± 0.005	0.04 ± 0.005	0.02 ± 0.006	0.04 ± 0.006	0.03 ± 0.005	0.03 ± 0.005	0.03 ± 0.005	0.03 ± 0.004	0.03 ± 0.004	0.04 ± 0.006	0.03 ± 0.005	0.03	NS
Palmitic acid C16:0	8.77 ± 0.040	12.58 ± 0.060	13.38 ± 0.066	8.80 ± 0.038	8.79 ± 0.056	10.46 ± 0.052	8.67 ± 0.036	11.54 ± 0.054	12.14 ± 0.062	8.70 ± 0.068	9.13 ± 0.038	10.04 ± 0.052	10.25	NS
Margaric acid C17:0	0.08 ± 0.002	0.11 ± 0.002	0.12 ± 0.001	0.08 ± 0.002	0.09 ± 0.001	0.09 ± 0.002	0.08 ± 0.002	0.13 ± 0.002	0.13 ± 0.001	0.09 ± 0.001	0.10 ± 0.002	0.11 ± 0.002	0.10	NS
Stearic acid C18:0	3.83 ± 0.085	5.50 ± 0.070	5.59 ± 0.046	3.70 ± 0.080	4.01 ± 0.050	4.53 ± 0.066	3.15 ± 0.070	5.78 ± 0.082	5.13 ± 0.048	3.98 ± 0.060	4.10 ± 0.058	4.43 ± 0.064	4.48	NS
Arachidic acid C20:0	0.13 ± 0.001	0.29 ± 0.003	0.33 ± 0.001	0.15 ± 0.005	0.22 ± 0.009	0.22 ± 0.004	0.16 ± 0.007	0.35 ± 0.005	0.32 ± 0.003	0.18 ± 0.005	0.22 ± 0.007	0.25 ± 0.005	0.24	NS
Behenic acid C22:0	0.04 ± 0.020	0.15 ± 0.012	0.16 ± 0.013	0.07 ± 0.018	0.08 ± 0.012	0.10 ± 0.015	0.05 ± 0.014	0.15 ± 0.012	0.16 ± 0.020	0.08 ± 0.018	0.08 ± 0.018	0.10 ± 0.016	0.10	NS
Lignoceric acid C24:0	0.03 ± 0.012	0.34 ± 0.006	0.26 ± 0.004	0.10 ± 0.012	0.05 ± 0.010	0.16 ± 0.009	0.03 ± 0.012	0.35 ± 0.012	0.25 ± 0.008	0.13 ± 0.010	0.06 ± 0.010	0.16 ± 0.010	0.16	NS
Total SFA	13.00	19.12	20.00	13.01	13.35	15.69	12.27	18.43	18.27	13.31	13.81	15.22	15.46	NS

All determinations were carried out in triplicate and mean values ± standard deviation (SD) reported

SFA saturated fatty acids, *NS* not significant

[Δ] Average values were calculated based on all 15 (5 samples × 3 replicates) data for each year with ± SD

Table 4 Monounsaturated and polyunsaturated fatty acid composition of native black mulberry (*Morus nigra* L.) seed oils

Fatty acid (%)	2008						2009						2008–2009	Year
	1	2	3	4	5	Average[Δ]	1	2	3	4	5	Average[Δ]		
Palmitoleic acid C16:1	0.05 ± 0.001	0.08 ± 0.001	0.13 ± 0.002	0.05 ± 0.001	0.08 ± 0.002	0.08 ± 0.001	0.05 ± 0.002	0.08 ± 0.001	0.13 ± 0.001	0.06 ± 0.002	0.08 ± 0.002	0.08 ± 0.002	0.08	NS
Heptadesanoic acid C17:1	0.03 ± 0.006	0.10 ± 0.007	0.14 ± 0.005	0.05 ± 0.007	0.05 ± 0.005	0.07 ± 0.006	0.04 ± 0.005	0.08 ± 0.026	0.14 ± 0.005	0.05 ± 0.005	0.04 ± 0.006	0.07 ± 0.009	0.07	NS
Oleic acid C18:1	6.53 ± 0.035	8.97 ± 0.045	10.27 ± 0.020	7.11 ± 0.040	7.93 ± 0.030	8.16 ± 0.034	6.48 ± 0.022	8.75 ± 0.040	9.79 ± 0.030	8.05 ± 0.028	8.13 ± 0.030	8.23 ± 0.030	8.20	NS
Linoleic acid C18:2	78.15 ± 1.278	69.09 ± 1.154	67.03 ± 1.098	77.40 ± 1.206	76.29 ± 1.173	73.59 ± 1.182	78.74 ± 1.325	69.84 ± 1.208	69.26 ± 1.254	76.13 ± 1.185	75.51 ± 1.200	73.89 ± 1.234	73.74	NS
Nonadecenoic acid C19:1	1.14 ± 0.056	1.28 ± 0.038	1.25 ± 0.060	1.32 ± 0.048	1.20 ± 0.050	1.24 ± 0.050	1.20 ± 0.050	1.35 ± 0.040	1.20 ± 0.036	1.30 ± 0.042	1.26 ± 0.050	1.26 ± 0.044	1.25	NS
Linolenic acid C18:3	0.48 ± 0.012	0.40 ± 0.013	0.42 ± 0.008	0.46 ± 0.010	0.54 ± 0.012	0.46 ± 0.011	0.48 ± 0.010	0.38 ± 0.010	0.43 ± 0.012	0.44 ± 0.008	0.53 ± 0.008	0.45 ± 0.010	0.45	NS
Eicosenoic acid C20:1	0.09 ± 0.004	0.28 ± 0.002	0.26 ± 0.002	0.09 ± 0.006	0.09 ± 0.004	0.16 ± 0.004	0.10 ± 0.006	0.31 ± 0.006	0.25 ± 0.004	0.09 ± 0.005	0.08 ± 0.005	0.17 ± 0.005	0.16	NS
Eicosadienoic acid C20:2	0.04 ± 0.005	0.12 ± 0.007	0.12 ± 0.010	0.05 ± 0.005	0.07 ± 0.007	0.08 ± 0.007	0.07 ± 0.005	0.24 ± 0.005	0.18 ± 0.006	0.08 ± 0.007	0.07 ± 0.006	0.13 ± 0.006	0.11	NS
Erusic acid C22:1	0.32 ± 0.004	0.33 ± 0.003	0.23 ± 0.010	0.31 ± 0.004	0.32 ± 0.008	0.30 ± 0.006	0.37 ± 0.005	0.36 ± 0.004	0.19 ± 0.010	0.30 ± 0.008	0.35 ± 0.006	0.32 ± 0.007	0.31	NS
Docosadienoic acid C22:2	0.14 ± 0.006	0.18 ± 0.006	0.11 ± 0.005	0.12 ± 0.005	0.04 ± 0.006	0.12 ± 0.006	0.15 ± 0.006	0.13 ± 0.004	0.13 ± 0.004	0.15 ± 0.005	0.08 ± 0.005	0.13 ± 0.005	0.12	NS
Nervonic acid C24:1	0.03 ± 0.014	0.05 ± 0.008	0.04 ± 0.006	0.03 ± 0.014	0.04 ± 0.012	0.04 ± 0.011	0.05 ± 0.012	0.05 ± 0.010	0.03 ± 0.008	0.04 ± 0.012	0.06 ± 0.014	0.05 ± 0.011	0.05	NS
Total MUFA	8.19	11.09	12.32	8.96	9.71	10.05	8.29	10.98	11.73	9.89	10.00	10.18	10.12	NS
Total PUFA	78.81	69.79	67.68	78.03	76.94	74.25	79.44	70.59	70.00	76.80	76.19	74.60	74.42	NS
Total USFA	87.00	80.88	80.00	86.99	86.65	84.30	87.73	81.57	81.73	86.69	86.19	84.78	84.54	NS

All determinations were carried out in triplicate and mean values ± standard deviation (SD) reported

PUFA Polyunsaturated fatty acids, *USFA* Unsaturated fatty acids, *NS* not significant, *MUFA* monounsaturated fatty acids

[Δ] Average values were calculated based on all 15 (5 samples × 3 replicates) data for each year with ± SD

Ercisli and Orhan [15] analyzed three mulberry species, (*M. alba* L., *M. rubra* L. and *M. nigra* L.) and reported that black mulberry fruits contained C18:2 as the dominant fatty acid (61.9%), followed by C16:0 (12.1%), C18:1 (14.8%), C18:0 acids (5.8%), C19:1 (1.3%) and C14:0 (1.1%). In another study of Ercisli and Orhan [11], C18:2 was also reported to be the dominant fatty acid (53.5–64.4%) followed by C16:0 (11.3–16.4%) in all black mulberry genotypes analyzed. C18:2 (13.5–44.4%), C16:0 (14.4–22.7%) and C18:1 (2.3–16.0%) were also reported to be the highest fatty acids found in fruits of three black mulberry (*M. nigra*) genotypes by Elmaci and Altug [36].

According to the results shown in Table 4, total polyunsaturated and total unsaturated fatty acids of mulberry seed oils were between 67.6–79.4% and 79.9–87.7%, respectively. The fatty acid profile of mulberry seed oil shows that it is a good source of the nutritionally essential fatty acid, linoleic acid. C18:2 may protect against ischemic stroke and lacunar infarction, possibly due to lowering of blood pressure levels and improvement in small-vessel circulation by means of reduced platelet aggregation and enhanced erythrocyte deformability [37]. Additionally, C18:2 perhaps the most potent anticancer fatty acid in that amounts $\leq$1% in the diet are sufficient to produce a significant protective effect [11]. This proved the importance of mulberry seed oil as a source of dietary ingredient. Linolenic acid (C18:3) was also found in relatively lower amounts. C18:3 is readily oxidized in oils such as soybean and canola to cause rancidity and off-flavors during storage or frying [27].

The levels of major fatty acids obtained in this study are in accordance with the results of the previous studies conducted on whole mulberry fruit [11, 15, 36]. However, the number of the fatty acids of black mulberry seed oil determined in the present study is higher than the number of fatty acids of the whole mulberry fruit reported in the previous studies. Ercisli and Orhan [15] reported that black mulberry fruits contained C18:2, C16:0, C18:1, C18:0, C19:1 and C14:0. However, a total of 20 fatty acids were determined in the present study. The 12 fatty acids other than the mentioned above are the minor fatty acids which are found in lower amounts than the others. Thus, the addition fatty acid observed in our study is the first report about the determination. It may be due to the difficulties in the detection of some trace fatty acids in the whole fruit. It is clear that the procedure of the oil extraction from the fruit and the fatty acid analysis in the oil enables the determination of some minor fatty acids. According to Ercisli and Orhan [15], behenic (C22:0) and palmitoleic (C16:1) acids were reported to be detected only in *M. alba* fruits (0.2 and 0.6%, respectively). In our study, C22:0 (0.1%) and C16:1 (0.1%) acids were also detected in black mulberry seed oil.

Conclusions

The present study showed that the mulberry seed with approximately 30% crude oil content has a good potential as an oil seed that can be used as a dietary source. Also, the present study showed that, according to the fatty acid composition, it seems possible to place mulberry seed oil among the oils of grape seed, safflower, sunflower, soybean, maize, cotton seed, poppy and tobacco, which are of the linoleic type. High C18:2 content (average 73.7%) also proved that black mulberry seed oil should be considered as an essential fatty acid source. α-Tocopherol and sterols as phytochemicals are also important components of the mulberry seed oil essential to prevent cardiovascular diseases.

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