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Research Article

Comparison of the fatty acid composition of pecan and walnuts from various regions and the development of a rapid analytical method

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ABSTRACT

The study determined the fatty acid profiles of pecan and walnut samples from various regions of Türkiye and proposed a rapid method for profiling fatty acids. Twenty walnut samples (Ahırlı, İncesu-Seydişehir and Derbent-Konya, Yenişarbademli and Yalvaç-Isparta, Burdur, Göksun and Çağlayancerit-Kahramanmaraş, Ezine-Çanakkale, Belen-Hatay, Denizli, Mut-Mersin, Ermenek-Karaman and Osmaniye) and one pecan sample (Narlıca-Hatay) were analysed. Fatty acid methyl esters (FAME) were prepared using direct methylation of 0.1-gram samples, and compositions were determined via gas chromatography. The study compared direct methylation to other extraction methods (ASE and Bligh & Dyer) using the “Maraş-18” cultivar. Results showed that linoleic acid levels in walnuts ranged from 48.5 to 64.1%, with an average of 57.16%. Oleic acid content ranged from 13.6 to 33.4%, with a mean of 20.5%. Alpha-linolenic acid varied between 7.6 and 17.1%, with the highest levels in the “Native” cultivar from Yenişarbademli. The pecan sample had high oleic acid (59.6%) but low α-linolenic acid (1.14%). While the “Maraş-18” cultivar showed consistent fatty acid profiles across methods, minor statistical differences in individual fatty acid percentages were observed. Due to its speed, efficiency, and alignment with green chemistry, the direct methylation method may be recommended for fatty acid analysis in walnut kernels.

Keywords: Walnut, *Juglans regia.* L, Fatty acid, Gas chromatography

Introduction

Walnuts (*Juglans regia.* L) are the most common nut tree in the world. They are distributed in Asia, Europe, North America, North Africa, South Africa, Australia, New Zealand, Chile, and Argentina. China, the United States, Iran, Türkiye, and Mexico are leading producers (Kithi et al., 2023). This ranking may change depending on the seasonal effect on the countries' annual production. In 2023, walnut production in Türkiye was 360 thousand tons, a 7.5% increase compared to 2022 (TÜİK, 2023). In Türkiye, walnuts have been traditionally incorporated into various culinary practices, particularly in preparing traditional sweets such as baklava, aşure, Turkish delight, walnut paste, walnut jam, and walnut sausage.

Walnut has economic, nutritious, and medicinal values (Vu et al., 2020). Walnuts are used not only for consuming the kernel as a nutrient but also for biomedical and industrial applications (Gao et al., 2024). In recent times, consumers have become increasingly aware of the nutritional benefits of nuts in general and the positive impact of a healthy diet on their well-being (Ni et al., 2022). Walnut kernels typically contain approximately 60% lipid and are particularly rich in α-linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6) (Zheng et al., 2020). Alfa-linolenic acid is critical for improving cardiovascular health, supporting cognitive function, and reducing inflammation (Ojha et al., 2024). Walnuts are a significant source of antioxidants and anti-inflammatory substances, offering a rich array of phytochemicals. As a result, walnuts have been known as a “superfood” and are highly recommended to include in daily diets (Romano et al., 2022).

It has been reported that different cultivars, genotypes and walnut-growing regions affect the food composition of walnuts (Kafkas et al., 2020; Ma et al., 2023). Significant variations in the contents of linoleic, α-linolenic, and oleic acids in walnut oil are naturally occurring due to differences in genotype. Although these variations are genetically determined, environmental factors such as latitude, temperature, and drought also play a crucial role in influencing fatty acid composition. Most of the variation observed in the fatty acid profile can be attributed to the geographical location of cultivation (Yang et al., 2022).

The study aims to determine the fatty acid profile of pecan and walnut samples grown in various regions of Türkiye, with particular attention to the distribution of monounsaturated and omega-3 fatty acids essential for human health. Furthermore, the study seeks to develop a rapid method for profiling fatty acids without requiring oil extraction, providing an alternative to traditional approaches.

Materials and Methods

Sample Preparation

A total of 20 walnut samples (place*cultivar) were collected from Konya (Ahırlı, İncesu-Seydişehir and Derbent), Isparta (Yenişarbademli and Yalvaç), Burdur, K.Maraş (Göksun and Çağlayancerit), Ezine (Çanakkale), Hatay (Belen), Denizli, Mersin (Mut), Karaman (Ermenek) and Osmaniye. The pecan sample is grown in Hatay (Narlıca-Antakya). Nine of the walnuts are “Native”, 4 from “Chandler”, 2 from “Bilecik”, 1 from “Maraş18”, 1 from “Şebin”, 1 from “Kepir”, 1 from “Fernor” and 1 from “Kaman”.

Walnuts were cracked, and approximately 100 g of walnut kernel was homogenised with a blender. Fatty acid methyl esters (FAMEs) of the samples were prepared using the direct methylation method. To test the effect of direct methylation versus FAME from extracted lipid, the “Maraş 18” cultivar was selected for direct methylation, and FAME was prepared from 30 mg lipid.

Lipid Extraction by Accelerated Solvent Extraction (ASE Thermo Scientific Dionex ASE 350)

Approximately 10 g of homogenised walnut kernel samples were placed into a stainless-steel cell (SST), and it was closed tightly then, following extraction program was applied (Thermo Scientific™ Dionex™ ASE™ 350). Hexane was used as a solvent, and the temperature was set at 90ºC and heated for 5 minutes. Static time was set for 5 minutes and cycled for three; rinse volume was 60% and purged for 100 seconds. Lipid was collected into a glass bottle. The remaining solvent was evaporated under the vacuum using a rotary evaporator (Heidolph, Germany).

Lipid Extraction by Bligh and Dyer Method

Approximately 5 g of grounded walnut kernel sample was weighed into a glass flask, and 20 mL of methanol and 20 mL of chloroform were added. Then, the flask was immersed in an ice bath homogenised for 1 minute. Distilled water (10 mL) was added and homogenised for 30 seconds. Homogenate was divided into a glass tube and centrifuged at 2000 rpm for 20 minutes. Methanol and water layer were sucked, lipid-containing 10 mL of chloroform was taken into preweighed flak, and chloroform was evaporated by using a rotary evaporator under the vacuum at 40°C. The remaining lipid was used for fame analysis. Lipid was calculated and expressed as a percentage.

Preparation of Fatty Acid Methyl Esters

Direct methylation: Approximately 0.1 g (0.0001 g) homogenised sample was weighed into a screw-capped test tube (15 mL), 2 mL of 0.5 M methanolic KOH was added, then capped tightly boiled at 115ºC using a block heater for 5 minutes. Samples were allowed to cool down to room temperature, and 1.5 mL of methanolic boron trifluoride (BF_3, 14%) was added and then boiled further for 5 minutes. Test tubes were allowed to cool down to room temperature, and fatty acid methyl esters were extracted with 2 mL of iso-octane into a brown amber vial.

Determination of fatty acid methyl esters: Separation of fatty acid was achieved using Teknokroma (TRCN100, TR-882192 serial number, 100% (Biscyanopropyl polysiloxane) column and gas chromatography (Shimadzu GC-2025 FID). The injection temperature was set at 280°C, and the detector temperature was set at 300°C. The oven temperature was initially adjusted to 120°C, kept at his temperature for 5 minutes, then raised to180°C with a 10°Cramp rate and kept at this temperature for 5 minutes. The column temperature was increased to 220°C with a 10°C ramp rate held for 10 minutes, and finally, it reached 240°C with the same ramp rate and was held at this temperature for 3 minutes.

The fatty acids were identified using FAME mix 37 and comparing their retention time. Results were expressed as percentage areas of individual fatty acids.

Statistical Analyses

The data was analysed using the SPSS version 22.0 software. The fatty acid composition of walnuts from different regions was compared using an ANOVA test. Univariate analysis was employed to compare the various methods used in determining the fatty acid composition of the "Maraş 18" walnut cultivar. Statistical significance is defined at the level of $p< 0.05$.

Results and Discussion

Walnut contains a considerable amount of lipid in its kernel, and the amount of oil ranges between 45-72% (Table 1). In this research, only the "Maraş 18" cultivar was subjected to lipid extraction (ASE method), and its lipid content was 55.76%. The chemical composition of walnuts changes with cultivar, soil quality, irrigation, season, and probably altitude. Literature value shows that walnuts are rich in oil and contain 11-23% of protein in the kernel. This energy-dense nut provides vitamins, minerals, bioactive compounds and essential fatty acids such as linoleic and α-linolenic acid. Among the plant-based edible oils, flaxseed oil is the richest source of omega-3 fatty acids (Öksüz et al., 2015), followed by walnut. However, using flaxseed in human nutrition is not as common as walnuts. Walnuts are the best source of omega-3 fatty acids among nuts, such as almonds, pistachios, chestnuts, and peanuts (Hayes et al., 2016; Ros and Mataix, 2006).

Table 1. Walnut proximate composition from different sources

	Total fat %	Kernel Moisture %	Protein %	Ash %	Reference
Walnut	52.5-64.8	1.00-3.80	14.0-22.8	1.00-2.50	(Muradoğlu and Balta, 2010)
Walnut	65-70	3.0-3.5	16.23-17.47	1.90-2.26	(Doğan and Akgül, 2005)
Walnut	68.8-72.1	3.85-4.50	14.38-18.0	3.3-4.3	(Pereira et al., 2008)
Walnut	51.98-68.3	-	14-18.1	-	(Kömür et al., 2024)
Walnut	61.9-70.9	3.3-3.9	15.2-19.2	1.3-2.1	(Özkan and Koyuncu, 2005)
Walnut	62.5-70.2	-	-	-	(Ada et al., 2020)
Walnut	45-70	-	11-16	-	(Geng et al., 2021)
Walnut	50.6-70	-	14-19.9	-	(Goodarzi et al., 2023)
Walnut	36.7-63.7	-	13.1-21.7	1.4-2.1	(Davarkhah et al., 2024)
Pecan	52.7-69.6	4.6-5.5	7.3-9.3	1.1-1.7	(Reis Ribeiro et al., 2020)
Pecan	71.97	3.52	9.17	1.49	(Hussain et al., 2021a)

The fatty acid composition of lipids determines their nutritional quality. The oil-rich walnut kernel fatty acid composition is displayed in Table 2. The fatty acid composition of walnuts consists of palmitic, palmitoleic, stearic, oleic, linoleic, α-linolenic, arachidic and gadoleic acids. Major fatty acids present in walnut as follow; 18:2 n-6>18:1 n-9>18:3 n-3>16:0>16:1>20:1>20:0 (<0.05%). This order was almost similar in the literature both data obtained from Türkiye (Aydın & Güven, 2024; Bayazıt & Sümbül, 2012; Gündeşli et al., 2023; Kırca et al., 2014; Yıldız et al., 2021) and internationally (Bada et al., 2010; Kafkas et al., 2020; Liu et al., 2020; Yang et al., 2022). There are also some exceptions that α-linolenic acid content is either very close to the oleic acid level or, in some cases, greater than oleic acid (18:1 n-9). However, there is a contradicting result in oleic and palmitoleic acid (16:1 n-7) contents (Okatan et al., 2022); the oleic acid might mistakenly replace the palmitoleic acid result and vice versa in the former authors' result. Apart from these fatty acids, 18:1 n-7 fatty acid was determined to be nearly 0.7% in walnuts in diluted samples. However, in the concentrated sample (>30 mg lipid for FAME or >100 mg sample), 18:1 n-7 fatty acid overlapped with C18:1 n-9. Therefore, these two fatty acids are expressed as 18:1 n-9 fatty acids. Only a few literature mentioned about 18:1 n-7 fatty acid in walnuts (Nogales-Bueno et al., 2021), and most of the literature includes this fatty acid in 18:1 n-9 (Gao et al., 2019; Greve et al., 1992; Hayes et al., 2016; Özkan & Koyuncu, 2005). Our findings in fatty acid composition correlated well with the French monovarietal walnut oils study by Le Dréau et al. (2024).

Table 2. Relative percentages of fatty acids contents of walnut cultivars and pecan (Mean ± SD)

District	Cultivar	C16:0	C16:1 n-9	C18:0	C18:1n-9c	C18:2n-6c	C20:1n-9	C18:3n-3
Ahırlı-Konya (n:3)	Native	6.96±0.04^{b-e}	0.14±0.01	1.96±0.01^{f}	21.93±0.22^{cde}	57.68±0.40^{abc}	0.16±0.00	11.16±0.15$^{d-ı}$
İncesu-Seydişehir (n:3)	Native	6.28±0.02^{c-h}	0.10±0.01	2.98±0.02^{a-e}	22.69±0.02^{cde}	54.85±0.06^{b-e}	0.17±0.01	12.96±0.01^{b-g}
Derbent-Konya (n:3)	Native	6.21±0.02^{d-h}	0.03±0.00	3.74±0.03^{a}	15.28±0.07^{de}	60.40±0.09^{ab}	0.18±0.01	14.17±0.04^{a-e}
Yenişarbademli-Isparta (n:3)	Native	6.63±0.01^{b-g}	0.06±0.03	2.67±0.01^{c-f}	24.20±0.28^{bcd}	49.37±0.19^{de}	0.19±0.00	16.89±0.22^{a}
Yalvaç-Isparta (n:3)	Native	6.02±0.05^{e-h}	0.07±0.00	3.30±0.05^{abc}	20.47±0.11^{cde}	59.97±0.13^{ab}	0.19±0.01	9.97±0.09$^{f-ı}$
Burdur (n:3)	Chandler	6.81±0.08^{b-f}	0.09±0.02	2.96±0.03^{a-e}	23.11±0.30^{b-e}	59.13±0.12^{abc}	0.19±0.01	7.71±0.12^{ı}
Market-Konya (n:3)	Chandler	5.98±0.0^{fgh}	0.06±0.01	3.04±0.02^{a-e}	14.04±0.33^{de}	63.80±0.24^{a}	0.22±0.00	12.86±0.01^{b-g}
Göksun-K.Maraş (n:3)	Native	5.69±0.10^{gh}	0.04±0.00	3.14±0.10^{a-d}	16.64±0.27^{de}	59.27±.15^{abc}	0.23±0.01	14.74±0.43^{a-d}
Çağlayancerit-K.Maraş (n:5)	Maraş 18	6.10±0.01^{e-h}	0.07±0.01	2.94±0.02^{b-e}	28.50±0.16^{bc}	52.24±0.15^{cde}	0.22±0.00	9.80±0.10^{ghı}
İncesu-Seydişehir (n:3)	Bilecik	7.98±0.03^{a}		2.79±0.03^{b-f}	22.72±0.10^{cde}	52.81±0.08^{b-e}	0.18±0.01	13.20±0.07^{b-f}
İncesu-Seydişehir (n:3)	Şebin	5.43±0.01^{h}	0.06±0.00	2.78±0.02^{b-f}	19.09±0.24^{de}	59.92±0.24^{ab}	0.22±0.01	12.34±0.05^{b-h}
Ezine-Çanakkale (n:3)	Chandler	7.30±0.22^{ab}	0.09±0.01	3.05±0.08^{a-e}	15.59±0.39^{de}	59.05±0.09^{abc}	0.20±0.01	13.88±0.28^{a-e}
Belen-Hatay (n:3)	Native	6.31±0.02^{c-h}	0.10±0.01	2.67±0.02^{c-f}	33.27±0.10^{b}	48.60±0.08^{e}	0.08±0.01	8.92±0.08^{hı}
Denizli (n:6)	Kepir	7.11±0.07^{bc}	-	2.93±.80^{bcd}	21.92±5.89^{cde}	55.88±3.47^{bcd}	0.20±0.02	11.91±3.29^{d-h}
Yalvaç-Isparta (n:3)	Chandler	6.71±0.02^{b-}	-	2.87±0.01^{a-e}	13.64±0.04^{e}	60.69±0.07^{ab}	0.23±0.01	15.85±0.02^{ab}
Mut-Mersin (n:3)	Native	5.90±0.05^{fgh}	-	3.62±0.04 ^{ab}	21.52±0.09^{cde}	54.72±0.17^{b-e}	0.22±0.01	14.02±0.27^{a-e}
Ermenek-Karaman (n:3)	Bilecik	6.50±0.06^{b-g}	-	3.39±0.05^{abc}	21.36±0.29^{cde}	57.69±0.34^{abc}	0.21±0.00	10.91±0.09$^{e-ı}$
Ermenek-Karaman (n:6)	Fernor	6.97±0.40^{bcd}	0.10±0.07	2.30±0.43^{ef}	15.84±1.70^{de}	59.44±0.62^{ab}	0.20±0.01	15.21±1.81^{abc}
Konya (n:3)	Kaman	6.25±0.14^{d-h}	0.06±0.00	2.84±0.00^{b-f}	18.00±0.53^{de}	59.96±0.67^{ab}	0.24±0.00	12.22±0.16^{c-h}
Osmaniye (n:3)	Native	6.27±0.15^{c-h}	0.10±0.00	2.80±0.00^{b-f}	18.00±0.53^{de}	59.93±0.65^{ab}	0.20±0.00	12.23±0.15^{c-h}
Mean		6.51	0.08	2.91	20.50	57.16	0.20	12.56
Min. – Max		5.43-7.98	0.03-0.14	1.96-3.62	13.64-33.27	48.60-63.80	0.08-0.24	7.71-16.89
Narlıca-Antakya (n:6)	Pecan*	6.56±0.92^{b-f}	0.10±0.00	2.49±0.20^{def}	59.64±9.17^{a}	29.74±7.58^{f}	0.31±0.02	1.14±0.57^{j}

*Pecan values were excluded from the calculation of mean and min-max values.

The fatty acid composition of 20 walnut cultivars varied within the range of literature values. Oleic acid levels changed between 13.6 to 33.4% with a mean value of 20.50%. Linoleic acid content changes between 48.5-64.1%, having 57.16% mean values. These values showed that walnut oil was rich in linoleic acid and contained a moderate amount of oleic acid compared to pecan. Alfa-linolenic acid content varied between 7.6-17.1%. The highest α-linolenic acid (17.1%) was found in the "Native" cultivar obtained from Yenişarbademli, and the minimum level was observed in the "Chandler" (7.6%) brought from Burdur. Alfa-linolenic acid contents of different cultivars as follows; "Native" cultivars between 9.92-14.74%, "Chandler" from different location 7.71-15.85%, "Maraş-18" 9.18%, "Bilecik" 10.91-13.20%, "Şebin" 12.34%, "Kepir" 11.91%, "Fernor" 15.21%, and "Kaman" 12.22%. With this respect, similarities and differences in fatty acid composition were displaced in Tables 3 and 4. In our study, the α-linolenic acid content of "Fernor" samples was found to be much greater than that of research done in various regions in Tunceli (Aydın and Güven, 2024). However, in another research conducted in Uşak, Türkiye, "Chandler" demonstrated the highest α-linolenic acid content (13.81%) among "Fernor" (9.59%), "Şebin" (13.56%), "Kaman" (9.47%), and "Maraş-18" (10.16%) (Yildiz et al.,2021). The walnut samples' total polyunsaturated fatty acid (PUFA) content ranged from 56.21 to 62.59%. It was emphasised that the differences between the samples could be attributed to the variability in climate conditions across the districts (Gündeşli et al., 2023).In another study on the fatty acid composition of various walnut cultivars in Argentina, the "Chandler" variety was found to contain a higher proportion of linoleic acid (60.9%) compared to other varieties (Cittadini et al., 2020).

Walnuts are the most α-linolenic acid rich types of nuts. Consequently, consuming walnuts in adequate quantities may reduce the dietary n-6:n-3 ratio. Therefore, one serving of walnuts (30 grams) supplies over 20% of the recommended intake of α-linolenic acid, thereby establishing walnuts as a significant source of this essential fatty acid (FDA, 2024; Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü, 2022).

Several factors affect the composition of the walnut. As mentioned by Greve et al. (1992) even in an environment with a single geographic location, maturity, irrigation rate, shade, and sun exposition are the main factors affecting walnut composition. Among these factors, maturity had the most significant impact on PUFA content, positively influencing it, while deficit irrigation negatively affected PUFA levels. (Greve et al., 1992). Various literature has shown that many factors, other than the cultivar, may affect the chemical composition of walnut oils, including the harvesting area and year, storage conditions or extraction process (Le Dréau et al., 2024).

Our findings indicate that walnuts might be a well-balanced n-6:n-3 ratio with a mean value of 4.74. The n-6:n-3 ratio was close to 5, which is the ideal ratio for a good diet (Le Dréau et al., 2024). A high n-6/n-3 ratio has been demonstrated to activate inflammatory pathways within the organism. Furthermore, the condition increases the risk of developing certain metabolic diseases, including insulin resistance, type 2 diabetes, and cardiovascular disease (Bishehkolaei & Pathak, 2024). Consuming walnuts in adequate quantities could reduce the dietary n-6:n-3 ratio.

According to the Türkiye Nutrition Guide-2022, it is recommended that α-linolenic acid should contribute 0.5% to daily energy intake (Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü, 2022). Based on this guideline, an adult with a daily energy requirement of 2000 kcal should consume approximately 1.1 grams of α-linolenic acid. Data from Turkomp indicate that 100 grams of walnuts contain an average of 6.2 grams of α-linolenic acid (TürKomp, 2015). According to literature, researchers have pointed out that in the studied walnut genotypes, the kernel ratio was 40–60% (Balcı et al., 2001) and 46–67% (Baojun et al., 2010). Yarılgaç et al. (2001) Reported that the kernel ratio was 45.09-59.27 % in their study. Our findings showed that kernel yields in "Chandler," "Maraş-18," and "Fernor" were 45.5, 48.4, and 56.7%, respectively. Consequently, it may be advised that an individual consume about 17-18 grams of walnuts to achieve the recommended intake of 1.1 grams of α-linolenic acid. This is almost equivalent to 3-4 whole walnuts to fulfil the requirement.

Walnut kernels should be recommended as a snack to school-age children, who has not nut allergy, to meet their omega-3 requirements and partially provide many essential nutrients.

In Türkiye, the pecan nut is known as "pika cevizi" because its kernel resembles walnuts. Assuming that the pecan was a kind of walnut, it was included in this research as a different kind of walnut. However, the content of α-linolenic acid, linolenic acid, and oleic acid of the pecan was unlike that of 20 different walnut samples. These findings made us question whether the pecan is a kind of walnut or not.

Table 3. Ration of n-6:n-3 and relative percentages of total SFA, MUFA and PUFA contents of walnut cultivars and pecan (Mean ± SD)

District	Cultivar	n-6:n-3	Tot SFA	Total MUFA	Total PUFA
Ahırlı-Konya (n:3)	Native	5.17±0.11^{a}	8.92±0.03^{cde}	22.24±0.22^{cde}	68.84±0.25^{a-d}
İncesu-Seydişehir (n:3)	Native	4.23±0.01^{a}	9.26±0.00^{b-e}	22.96±0.03^{cde}	67.80±0.06^{a-d}
Derbent-Konya (n:3)	Native	4.27±0.01^{a}	9.96±0.05^{a-d}	15.48±0.07^{de}	74.56±0.12^{abc}
Yenişarbademli-Isparta (n:3)	Native	2.92±0.04^{a}	9.30±0.01^{b-e}	24.45±0.26^{bcd}	66.26±0.31^{cde}
Yalvaç-Isparta (n:3)	Native	6.02±0.04^{a}	9.32±0.09^{b-e}	20.73±0.12^{cde}	69.94±0.21^{a-d}
Burdur (n:3)	Chandler	7.68±0.12^{a}	9.78±0.11^{a-d}	23.39±0.30^{b-e}	66.83±0.19^{a-e}
Market-Konya (n:3)	Chandler	4.96±0.02^{a}	9.02±0.08^{b-e}	14.33±0.33^{de}	76.66±0.24^{a}
Göksun-K.Maraş (n:3)	Native	4.02±0.12^{a}	8.83±0.21^{de}	16.91±0.27^{de}	74.01±0.46^{abc}
Çağlayancerit-K.Maraş (n:5)	Maraş 18	5.33±0.05^{a}	9.04±0.02^{cde}	28.80±0.15^{bc}	62.04±0.20^{de}
İncesu-Seydişehir (n:3)	Bilecik	4.00±0.02^{a}	10.78±0.02^{a}	23.03±0.10^{b-e}	66.00±0.14^{a-d}
İncesu-Seydişehir (n:3)	Şebin	4.86±0.02^{a}	8.21±0.02^{e}	19.36±0.25^{de}	72.26±0.26^{abc}
Ezine-Çanakkale (n:3)	Chandler	4.25±0.09^{a}	10.35±0.18^{ab}	15.88±0.39^{de}	72.93±0.20^{abc}
Belen-Hatay (n:3)	Native	5.45±0.04^{a}	8.98±0.04^{cde}	33.44±0.09^{b}	57.52±0.15^{e}
Denizli (n:6)	Kepir	4.94±1.07^{a}	10.03±0.87^{abc}	22.11±5.91^{cde}	67.78±6.75^{bcd}
Yalvaç-Isparta (n:3)	Chandler	3.83±0.01^{a}	9.58±0.03^{a-d}	13.87±0.04^{e}	76.54±0.06^{ab}
Mut-Mersin (n:3)	Native	3.90±0.09^{a}	9.52±0.06^{a-e}	21.74±0.09^{cde}	68.74±0.11g^{a-d}
Ermenek-Karaman (n:3)	Bilecik	5.29±0.08^{a}	9.90±0.11^{a-d}	21.50±0.36^{cde}	68.60±0.26^{a-d}
Ermenek-Karaman (n:6)	Fernor	3.96±0.52^{a}	9.28±0.38^{b-e}	16.11±1.67^{de}	74.66±2.11^{abc}
Konya (n:3)	Kaman	4.91±0.11^{a}	9.09±0.14^{b-e}	18.30±0.53^{de}	72.18±0.59^{abc}
Osmaniye (n:3)	Native	4.90±0.10^{a}	9.10±0.10^{b-e}	18.30±0.53^{de}	72.17±0.6^{abc}
Mean		4.74	9.42	20.75	69.71
Min.-Max.		2.92-7.68	8.21-10.78	13.87-33.44	57.52-76.66
Narlıca-Antakya (n:6)	Pecan*	29.36±7.96^{b}	9.05±1.07^{de}	60.00±9.24^{a}	30.88±8.15^{f}

*Pecan values were excluded from the calculation of mean and min-max values.

Therefore, we thought that pecan might not be a kind of walnut. Our further investigation about the pecan uncovers that although pecan and walnut belong to the same family, pecan belongs to the genus Carya and the species *Carya illinois*, while walnut belongs to the genus Juglans L. and the species *Juglans regia L.* (Hussain et al., 2021b). The fatty acid profile of pecans was like that of walnuts, but their level varied with different percentages. The lowest concentration of α-linolenic acid in our walnut samples was considerably higher than its highest concentration in our pecan nut sample (Figure 1.). The abundance of fatty acid in pecan was as follows: C18:1 n-9>C18:2 n-6>C16:0>C18:0>C18:3 n-3>C20:1>C16:1. Pecan was rich in oleic acid with a level of 59.6 %, compared to walnut 13.6-33.2%. The oleic acid content of pecans harvested from Antalya was found to be approximately 52%, while the linoleic acid content was determined to be 36%. Additionally, the α-linolenic acid content was determined to be 1.5% (unpublish data Ulutaş & Öksüz 2024). Pecan contains a much lower level of linoleic acid (29.6%) than walnut 48.6-60.69%, and with this respect, it contains low n-6 fatty acid. The findings revealed that palmitic acid 6.65%, palmitoleic acid 0.1%, stearic acid (18:0) 2.49%, oleic acid 59.64%, linoleic acid 29.74%, gadoleic acid (20:1 n-9) 0.31% and α-linolenic acid 1.14% were present in pecan kernel. The aforementioned findings were corroborated by a study investigating the ratios of oleic acid, linoleic acid and α-linolenic acid in 26 pecan cultivars grown in Türkiye. The results indicated that these ratios ranged between 55.91-71.27, 19.38-33.45 and 0.79-1.55%, respectively (Yıldız-Turgut & Özdemir, 2023). However, in the research conducted by Çelik (2024) on pecan samples grown in Serik, Antalya, the ratio of oleic acid was found to be lower (50.57%), while the ratio of linoleic acid (38.73%) and α-linolenic acid (1.49%) were found to be higher. Pecans were low in saturated fatty acid (9.1%), mostly palmitic, and high in MUFA (60%) and PUFA (30.9%). Oleic acid contributes almost 99.4% of total MUFA in pecan. Linoleic acid contributes most of the PUFA, having 96.2% of total PUFA, and a small amount of α-linolenic acid (1.14%) contributes 3.7% of PUFA. Our results were consistent with an average value of sixteen pecan cultivars studied in Uruguay (Ferrari et al., 2022), but having lower SFA, higher MUFA and lower PUFA than Southern Brazil varieties (Siebeneichler et al., 2024). The fatty acids profile of pecans showed the same order in terms of abundance but with different levels in different cultivars and geographic origins. The linoleic acid content of pecans in this research showed similarities with those of Eliot, Stuart, Cape Fair, and Success cultivars. However, its α-linolenic acid content was much lower than all cultivars harvested from Southern Brazil (Siebeneichler et al., 2024) and higher than Uruguay cultivars (Ferrari et al., 2022). Both international literature and our findings are consistent in that pecan oleic acid is much higher and α-linolenic acid content much lower than walnuts; it is a good chemical indicator to distinguish between processed products of pecan and walnuts. Our preliminary study showed that the FTIR spectrum of walnut oil and pecan oil were similar, and there was no distinctive spectrum to distinguish them from one another (Figure 4). Therefore, rapid fatty acid analysis will be a good indicator for discriminating between pecan and walnut products they make, such as baklava, nut paste, etc.

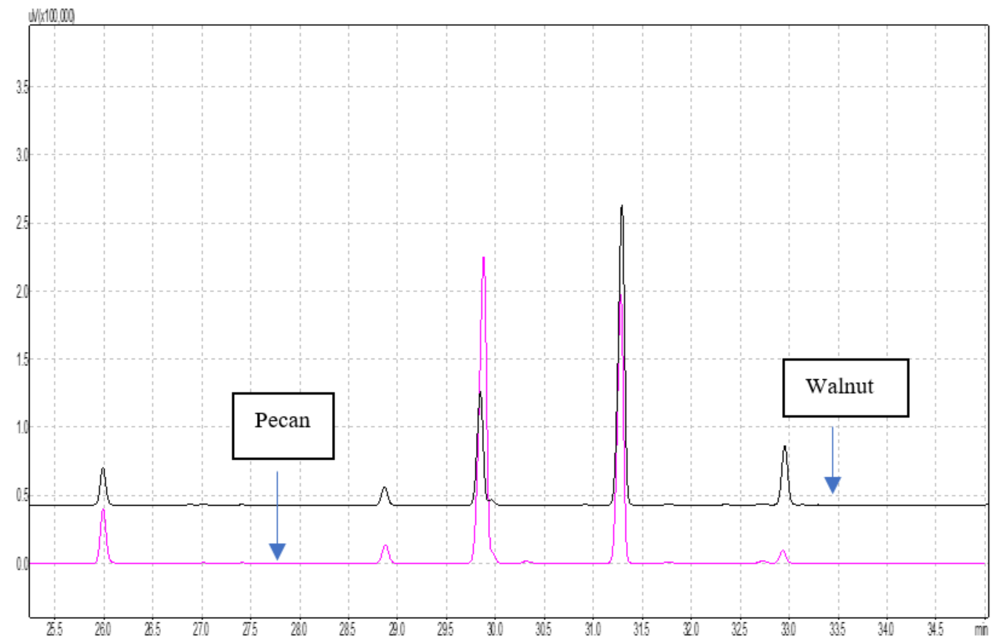

Figure 1. Comparative illustration of the fatty acid chromatogram of pecan and walnut

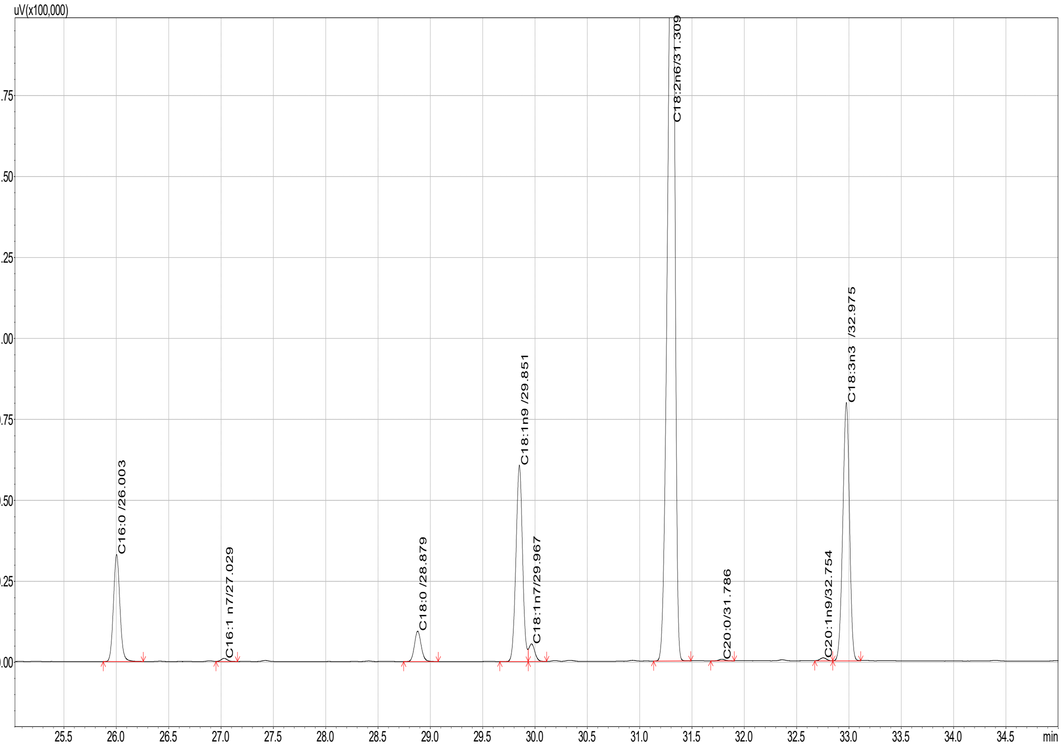

Figure 2. Fatty acid chromatogram of walnut sample

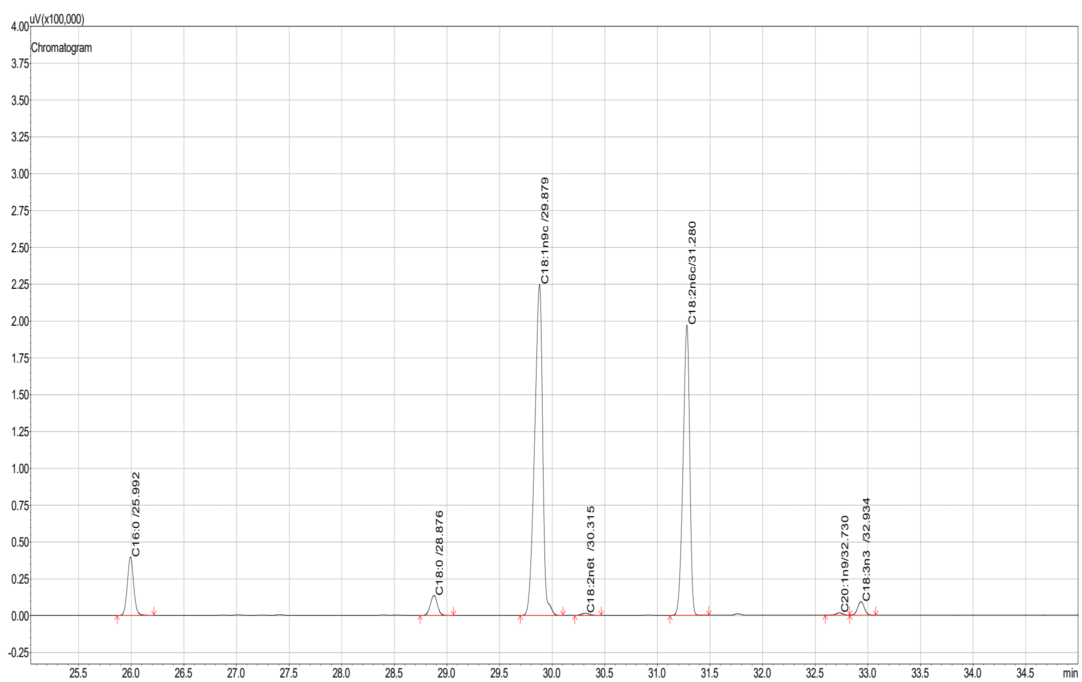

Figure 3. Fatty acid chromatogram of pecan sample

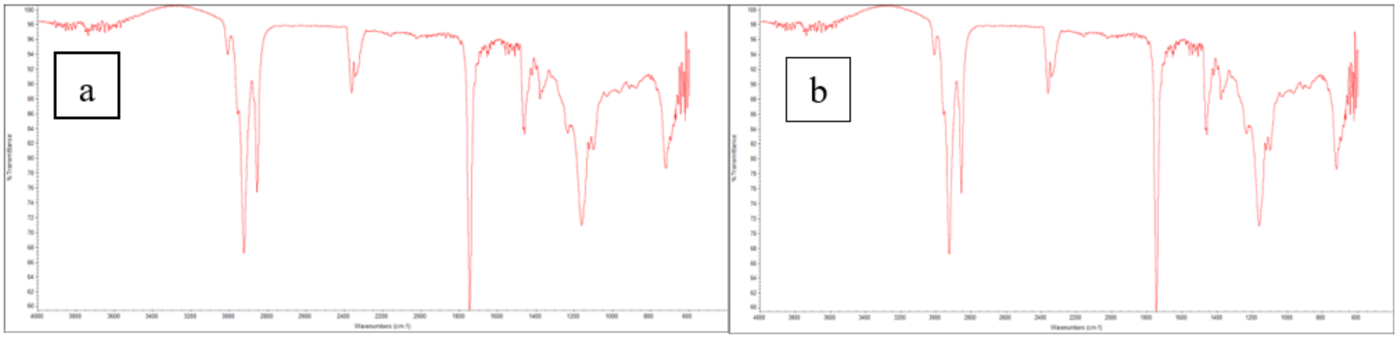

Figure 4. a) Walnut oil FITIR spectrum b) Pecan oil FTIR spectrum

Traditionally, fatty acid analysis is done by extracting lipids from the intended food and then converting them into fatty acid methyl esters under alkali conditions. This study prepared FAMEs without any lipid extraction from a homogenised sample. Initially, the 0.5-gram sample was converted to its fatty acid methyl esters as indicated under the direct methylation heading. However, this amount was too much and reduced to 100 mg for better separation.

Table 4. Comparison of fatty acid composition of walnut oils obtained by different methods (Mean ± SD)

FAME	ASE[1] X̄±SS	Bligh & Dyer[2] X̄±SS	Direct methylation X̄±SS	p
C16:0	6.38±0.03[a]	6.02±0.09[b]	6.10±0.01[b]	0.001
C18:0	3.17±0.02[a]	2.84±0.05[b]	2.94±0.02[b]	<0.001
C18:1 n-9	24.65±0.09[a]	27.15±0.59[b]	28.50±0.16[c]	<0.001
C18:2 n-6	54.35±0.07[a]	52.80±0.49[b]	52.24±0.15[b]	<0.001
C20:0	0.097±0.001[a]	0.088±0.006[ab]	0.092±0.001[b]	0.032
C20:1 n-9	0.20±0.01[a]	0.18±0.00[b]	0.22±0.00[c]	<0.001
C18:3 n-3	10.90±0.1[a]	10.50±0.1[b]	9.80±0.1[c]	<0.001

[1] ASE method lipid, and [2] Bligh & Dyer method.

This proposed method is rapid if the main concern is the fatty acid profile of any nuts since they are rich in oil. As indicated in Table 4, FAMEs were prepared from oil extracted by ASE, Bligh Dyer and the proposed method. The fatty acid profile of nuts was the same in all methods, but there were some statistical differences in the percentage of individual fatty acids. Percentages of C16:0, C18:0, C18:2 n-6, and C20:0 fatty acids were similar in the Bligh Dyer method and direct methylation method, and C18:1 n-9, C18:3 n-3 percentages were different in all methods. However, these differences were not great. Similarly, in the study conducted by Elouafy et al. (2022), walnut kernel oil was extracted using three different methods, and their fatty acid compositions were compared. The ratios of other fatty acids were found to be similar except for 18:3 n-3 fatty acids. In this research, due to its rapid, less time-consuming, and relatively contributing green chemistry applications, the proposed direct methylation method can be recommended for all kinds of nut fatty acid analysis. Moreover, the oven temperature program used in this study can be extended for 7 minutes to separate long-chain fatty acids such as DHA in fish and algae.

Conclusion

The fatty acid composition of walnut samples varied based on cultivars and locality. Major fatty acids in walnut samples were mainly unsaturated, such as linoleic, oleic and α-linolenic acids. Saturated fatty acid was present at an average of 9.42%. With this respect, walnuts are a good source of unsaturated fats, particularly omega-3 fatty acids, and are low in saturated fatty acids. Pecan was rich in oleic acids, nearly similar to low-grade olive oil, and lowered the effect of omega-6 fatty acids.

Walnut provides a significant source of α-linolenic acid required by the body, and almost consuming an average size of 3-4 walnuts is sufficient to fulfil this requirement. However, it should not be considered a single omega 3 fatty acid source. Along with walnuts, consuming seafood is also recommended to obtain balanced omega-3 fatty acids such as EPA and DHA since the body cannot synthesise enough EPA and DHA from α-linolenic acid.

Compliance with Ethical Standards

Conflict of interest: The author(s) declares that they have no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: Data will be made available on request.

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Disclosure: -

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