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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of Fatty Acids in Seeds of *Morus alba* L. (White Mulberry), *Morus nigra* L. (Black Mulberry) and *Morus nigra-pandula* L. (Flanking Mulberry) Grown in Şanlıurfa province

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Abstract: In this study, the fatty acid profiles of three mulberry species white (Morus alba L.), black (Morus nigra L.), and floppy mulberry (Morus nigra pandula L.) cultivated in Şanlıurfa in 2021 were analyzed. Oils were extracted from the seeds using the Soxhlet method, and the fatty acid compositions were identified through gas chromatography. A total of ten different fatty acids were detected in the mulberry seeds. Among them, linoleic acid was the most abundant across all three species, followed by significant amounts of palmitic acid, oleic acid, and stearic acid. The study also calculated the atherogenic and thrombogenic indices, finding that the values were well below the recommended limits. Overall, the results highlight that all three mulberry species are rich sources of linoleic acid, an essential fatty acid, and have potential applications in the food, pharmaceutical, and cosmetic industries.

Keywords: Black mulberry, fatty acid, flankıng mulberry, Şanlıurfa, white mulberry.

Şanlıurfa İlinde Yetiştirilen *Morus alba* L. (Beyaz dut), *Morus nigra* L. (Siyah dut) ve *Morus nigra-pandula* L. (Sarkık dut) Türlerinin Tohumlarında Yağ Asitlerinin Belirlenmesi

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Öz: Bu çalışmada 2021 yıllında Şanlıurfa'da yetiştirilen beyaz *(Morus alba* L.), siyah (*Morus nigra* L.) ve sarkık dut (*Morus nigra pandula* L.) türlerinin yağ asitleri kompozisyonu belirlenmiştir. Dut çekirdeklerinden sokshelet ekstraksiyon yöntemiyle yağlar ekstrakte edilmiş ve gaz kromatografisi yöntemiyle yağ asidi bileşimleri tespit edilmiştir. Dut meyvelerinde toplam on farklı yağ asidi tanımlanmıştır. Her üç türde de linoleik asit en yüksek oranda tespit edilen yağ asidi olmuştur. Palmitik asit, oleik asit ve stearik asit tespit edilen diğer önemli yağ asitleridir. Çalışmada ayrıca aterojenik ve
trombojenik indeksler de belirlenmiştir. Elde edilen değerlerin önerilen limitin oldukça altında olduğu saptanmıştır. Sonuçlar her üç dut çeşidinin de esansiyel bir yağ asidi olan linoleik asit bakımından önemli bir kaynak olduğunu ve gıda, ilaç ve kozmetik gibi alanlarda kullanılabileceğini göstermiştir.

Anahtar kelimeler: Beyaz dut, siyah dut, sarkık dut, Şanlıurfa, yağ asidi.

INTRODUCTION

Fatty acid composition in plants can vary even among individuals of the same species. An increase in temperature during seed development tends to reduce linoleic acid levels while raising the concentrations of oleic, palmitic, and stearic acids (Samanci & Ozkaynak, 2003). Research has shown that seeds produced in colder climates generally contain higher levels of linoleic acid (Nagaraj & Reddy, 1997), whereas seeds from warmer climates have elevated oleic acid levels (Pritchard et al., 2006). High temperatures negatively impact the synthesis of linoleic and linolenic acids but enhance the production of oleic acid (Weiss, 1983; Stryer, 1986; Röbbelen et al., 1989). In sesame populations, stearic and oleic acid contents increase from northern to southern latitudes, while palmitic and linoleic acid contents decrease. These shifts are largely attributed to environmental differences such as climate and soil conditions across different ecological regions (Uppstrom, 1995; Cuniberti et al., 2004; Fatwa & Namzer,

2023). The fatty acid profiles of seeds are influenced by a variety of factors, including ecological, morphological, physiological, and agricultural conditions, as well as genetic variation (Anastasi et al., 2000). Furthermore, the ongoing changes in fatty acid distribution from seed formation through maturation are referred to as ontogenetic variability (Baydar, 2000; Baydar et al., 2006). The Moraceae family includes 24 species of mulberry. Plants of the *Morus* genus are either monoecious or dioecious, milky-sapped, and can be either deciduous trees or shrubs. Their leaves are typically alternate, though they can occasionally be opposite, simple, or palmately lobed. The flowers are arranged in cymes, and they produce aggregate fruits of various shapes (Davis, 1982). Mulberries thrive in temperate, tropical, and subtropical climates, adapting to a wide range of environmental and soil conditions. They have become naturalized across Asia, Europe, and the Americas, with major cultivation regions including the Middle East, East and Southeast Asia, Africa, Europe, and the Americas (Ercisli & Orhan, 2007; Hussain et al., 2017; Zhang et al., 2018; Ustun-Argon et al., 2019). In Turkey, mulberry cultivation has a history spanning over 400 years, with *Morus alba* (white mulberry), *Morus nigra* (black mulberry), and *Morus rubra* (red mulberry) being the most commonly grown species. Mulberries are enjoyed fresh and are also processed into products such as molasses, pestil (fruit leather), köme (a walnut-filled fruit roll), marmalade, juice, and liqueur (Aydin et al., 2015; Gecgel et al., 2011; Hussain et al., 2017; Ustun-Argon et al., 2019). Between 2017 and 2021, Turkey's annual mulberry production across 15 provinces averaged 73,383 tons, with specific yearly outputs of 66,647 tons in 2018, 69,317 tons in 2019, 70,620 tons in 2020, and 69,475 tons in 2021.

Mulberry fruits have long been used in traditional medicine across Turkey and many other countries for various purposes, including as an antihelminthic, treatment for toothaches, expectorant, laxative, emetic, and remedies for sore throat, fever, thirst, dysentery, and oral lesions (Turan et al., 2017; Ustun-Argon et al., 2019). They are also recognized for their potential pharmacological benefits in managing cholesterol, diabetes, oxidative stress, and obesity, owing to their rich content of polyphenolic compounds (Chan et al., 2016; Turan et al., 2017; Zhang et al., 2018). Mulberry seed oil is a source of linoleic and linolenic acids essential fatty acids that humans cannot synthesize and must obtain through diet. These fatty acids are vital for building cell membranes, supporting brain and nervous system development and function, and regulating hormone production (Sonta et al., 2021; Ercisli & Orhan, 2007; Hussain et al., 2017). The purpose of this study was to analyze the fatty acid profiles, as well as the atherogenicity (AI) and thrombogenicity (TI) indices, of oils extracted from three mulberry varieties White mulberry (BD) (*Morus alba* L.), Black mulberry (KD) (*Morus nigra* L.), and Flanking mulberry (SD) (*Morus nigra-pendula* L.) and to assess their potential health impacts

MATERIAL AND METHOD

On May 20, 2021, samples of white mulberry, black mulberry, and drooping mulberry were collected from trees located at the Osmanbey Campus of Harran University. The collected plants were assigned collector numbers: white mulberry as Aslan 5044, black mulberry as Aslan 5045, and drooping mulberry as Aslan 5046. Photographs of the mulberry samples are shown in Figure 1. The sampling area, Osmanbey settlement, lies north of the Harran Plain, where the plain meets the Germüş Plateau (Benek et al., 2016), as shown in Figure 2. Seeds were separated from the mulberry fruits by washing with water and subsequently dried. A sample of 5–10 grams of dried and ground seeds was placed into a filter paper cartridge and loaded into an extractor connected to a balloon. The cartridge was set up to enable the circulation of petroleum ether. After six hours of extraction, the petroleum ether was recovered through distillation. The resulting oil, contained in a glass flask, was placed in an oven at 103°C to evaporate any remaining ether, then cooled in a desiccator and weighed. The oil content was calculated relative to the dry weight of the seeds (Uylaşer and Başoğlu, 2000). The oils from the ground seeds of the mulberry varieties were extracted using the Soxhlet extraction method.

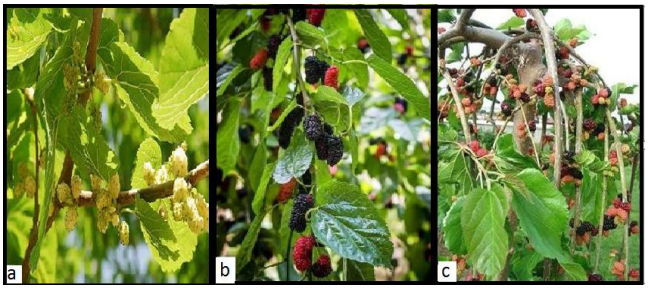

Figure 1. *Morus alba* (White mulberry), b; *Morus nigra* (Black mulberry) c; *Morus nigra-pandula* (Flankıng mulberry)

All laboratory analyses in this study were conducted at the Central Laboratory of Harran University (HUBTAM). A 100 µL oil sample was placed into a 20 mL test tube, and 2 mL of isooctane was added. The tube was sealed and shaken thoroughly. Next, 200 µL of 2 N KOH solution in methanol was added to the tube and vortexed for 1 minute. After approximately 30 minutes, the upper clear layer was transferred into a vial. A 1 µL sample was extracted using an injector and injected into the GC-FID device (TS-EN ISO 12966-2, 2017). The analysis was performed using a Thermo Trace GC Ultra model gas chromatograph with FID.

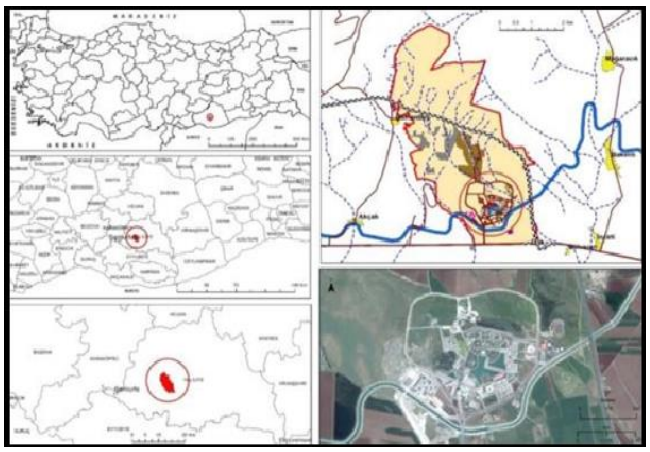

Figure 2. Osmanbey campus area where mulberry samples were taken (Map by Benek et al., 2016)

A Flame Ionization Detector (FID) was used for the analysis. A 60 m HP-88 column was employed to separate the fatty acids. The temperatures for the detector and injection block were set at 280°C and 250°C, respectively. A temperature program was applied to the column, starting at an initial temperature of 100°C. This temperature was raised by 10°C/min to 180°C, then by 5°C/min to 220°C, where it was held for 5 minutes. The split ratio was set at 1/100, and the injection volume was 1 µL.

Atherogenicity (AI) and thrombogenicity (TI) indices are used to evaluate the potential effects of fatty acids on cardiovascular health. These indices are widely recognized and reliable for assessing fats in both solid and liquid forms. Analyzing these indices provides valuable insight into the different impacts of individual fatty acids on human health, particularly concerning atherosclerosis, blood clot formation, increased risk of atheroma, and thrombus development (Tilami, 2022). The indices were calculated using the formulas proposed by Ulbricht (1991).

$$AI=(4\times C14:0+C16:0)/(\sum MUFA+\sum PUFA);$$

$$TI=[(C14:0+C16:0+C18:0)/(0.5\times\sum MUFA+0.5\times\sum n6\ PUFA+3\times\sum n3\ PUFA+(n3/n6))].$$

A computer program was employed to statistically analyze the results. The outcomes from three replicates are presented as the mean value ($\bar{x}$) and standard deviation (SD). Fatty acids were evaluated using analysis of variance (ANOVA), and mean comparisons were conducted using the Tukey test. Differences between means were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The fatty acid components of the 3 mulberry varieties used in our study are given in Table 1.

Table 1. Fatty acid compositions and AI and TI indices of mulberries of BD, KD and SD cultivars.

Fatty acids (%)	White mulberry (BD) *($\bar{x}$±ss)*	Black mulberry (KD) *($\bar{x}$±ss)*	Drooping mulberry (SD) *($\bar{x}$±ss)*
Myristic Acid (C14:0)	0.057±0.012	0.047±0.006	0.050±0.010
Palmitic Acid (C16:0)	10.843±0.230^{c}	10.287±0.225^{b}	8.777±0.129^{a}
Palmitoleic Acid (C16:1)	0.090±0.010^{a}	0.073±0.015^{ab}	0.057±0.006^{b}
Heptadecanoic Acid (C17:0)	0.063±0.006	0.073±0.012	0.073±0.006
Heptadecanoic Acid (C17:0)	0.063±0.006	0.073±0.012	0.073±0.00
Heptadecanoic Acid (C17:0)	0.063±0.006	0.073±0.012	0.073±0.006
Heptadecenoic Acid (C17:1)	0.037±0.006^{b}	0.027±0.006^{a}	0.030±0.000^{ab}
Stearic Acid (C18:0)	3.520±0.070^{ab}	3.393±0.086^{a}	3.560±0.070^{b}
Oleic Acid (C18:1n9c)	8.003±0.675^{a}	9.280±0.735^{b}	9.463±0.225^{b}
Linoleic Acid (C18:2n6c	76.733±0.469	7.,307±0.856	76.987±0.180
α-Linolenic Acid (C18:3n3)	0.443±0.021^{a}	0.413±0.015^{a}	0.813±0.050^{b}
Arachidic Acid (C20:0)	0.147±0.006^{b}	0.090±0.020^{a}	0.137±0.006^{b}
SFA	14.630±0.140^{c}	13.890±0.123^{b}	12.597±0.047^{a}
MUFA	8.130±0.671^{a}	9.380±0.755^{b}	9.550±0.229^{b}
PUFA	77.177±0.461	76.720±0.862	77.800±0.226
ω3	0.443±0.021^{a}	0.413±0.015^{a}	0.813±0.050^{b}
ω6	76.733±0.469	76.307±0.856	76.987±0.180
ω3/ ω6	0.006±0.000^{a}	0.005±0.000^{a}	0.011±0.001^{b}
AI	0.130±0.003^{c}	0.122±0.003^{b}	0.103±0.002^{a}
TI	0.329±0.004^{c}	0.311±0.004^{b}	0.271±0.002^{a}

Sybols: a-b: Different letters indicate significant differences in column SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids PUFA: Polyunsaturated fatty acids among species.

A total of 10 distinct fatty acids were found in mulberry seed oils. The most prevalent fatty acids were linoleic acid, palmitic acid, oleic acid, and stearic acid. Linoleic acid had the highest concentration among the species, ranging from 76.307% to 76.987%. The linoleic acid content in BD (76.733%) aligns with the values reported by Ustun-Argon, İlhan, Gökyer, Ozturk, and Koparal, Rahman (2019) and Sánchez-Salcedo et al. (2016), but is higher than those found by Ercisli & Orhan (2007). The linoleic acid levels in KD are similar to those reported by Gecgel et al. (2011) and Sánchez-Salcedo et al. (2016), though they exceed the values from Ercisli & Orhan (2007).

The palmitic acid content was highest in SD (10.843%) and lowest in BD (8.777%). The BD values in this study align with those reported by Rahman and Sánchez-Salcedo et al. (2016). However, Ustun-Argon et al. (2019) found lower values, while Ercisli & Orhan (2007) observed higher values. The KD values in this study were consistent with those of Gecgel et al. (2011), but lower than those reported by Ercisli & Orhan (2007) and Sánchez-Salcedo et al. (2016). The lowest oleic acid content was found in BD (8.003%), and the highest in SD (9.463%). In comparison, Zhang et al. (2010), Ustun-Argon et al. (2019), and Sánchez-Salcedo et al. (2016) reported lower values for BD, while Ercisli & Orhan

(2007) reported higher values. For the KD species, the oleic acid levels reported by Gecgel et al. (2011) and Sánchez-Salcedo et al. (2016) were lower, while those from Ercisli & Orhan (2007) were higher. Stearic acid values were similar across species, averaging around 3.5%. In BD species, the values from Ustun-Argon et al. (2019) and Sánchez-Salcedo et al. (2016) matched those of this study, while they were lower than those reported by Rahman and Ercisli & Orhan (2007). For KD species, the values were similar to those of Sánchez-Salcedo et al. (2016) but lower than those from Gecgel et al. (2011) and Ercisli & Orhan (2007). No significant differences in polyunsaturated fatty acids (PUFA) were found between species, although saturated fatty acids (SFA) were higher in BD, and monounsaturated fatty acids (MUFA) were higher in SD.

The AI and TI indices are values that reflect the interactions between various fatty acids and their impact on human coronary health. The AI value represents the potential to prevent plaque aggregation, reduce cholesterol and phospholipid levels, thereby protecting against coronary diseases. On the other hand, the TI index indicates the blood's tendency to clot (Ulbricht, 1991; Busova & Dorko, 2021). Consuming foods with low AI and TI values is advantageous for cardiovascular health, with recommendations suggesting these values should be below 1 (Ouraji et al, 2009; Karslı, 2021). The AI and TI indices for the current study are provided in Table 1, which shows that both indices are below the recommended value of 1. The AI index was lowest in SD (0.103) and highest in BD (0.130). Similarly, the TI index was lowest in SD (0.271) and highest in BD (0.329). Our literature review did not find any studies on this topic related to mulberry seed oils. However, Tilami (2022) found that the AI and TI values for most seed oils were under 1 in his research.

CONCLUSION

This study analyzed the fatty acid profiles of white, black, and drooping mulberry varieties, as well as their atherogenicity (AI) and thrombogenicity (TI) indices. The main fatty acids identified were linoleic, palmitic, oleic, and stearic acids, with linoleic acid—an essential polyunsaturated fatty acid—being the most prevalent, making up over 76% of all varieties. The AI and TI values were notably lower than the recommended threshold of 1, suggesting positive health implications. These results imply that mulberry varieties could be a valuable natural source of essential fatty acids, particularly linoleic acid. In comparison, previous studies on fig and mulberry seed oils reported different fatty acid compositions, including palmitic acid (6–7%), oleic acid (15-16%), linoleic acid (29-31%), and linolenic acid (41-42%) (Duman & Yazıcı, 2018). In contrast, this study found higher levels of linoleic acid (76-77%) and linolenic acid (44-52%) in mulberry seeds, while palmitic and oleic acid levels were slightly higher and lower, respectively. These findings suggest a distinct fatty acid composition in mulberry kernel oil compared to fig kernel oil, with mulberry oil being especially rich in linolenic acid. Overall, the fatty acid profile and the balance between saturated and unsaturated fats are consistent with existing research. Linoleic acid, known for its cardiovascular benefits, is particularly noteworthy (Boelhouwer, 1983; Slama et al., 2020; Akgün & Başhan, 2024). Similar variability was observed in the seed oil of Opuntia ficus indica, which contains 9.32% palmitic, 3.11% stearic, 16.8% oleic, and 70.3% linoleic acid (Ennouri et al., 2005). This highlights that fatty acid profiles can vary among plant species and may change depending on various factors. Understanding these differences is crucial for evaluating oil quality and determining the best uses. Thus, knowledge of fatty acid composition can aid in cultivating specific mulberry varieties suited for particular applications and environmental conditions.

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