Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 54, Issue 3, Year 2026, Article Number 15167
https://doi.org/10.15835/nbha54315167
Journal homepage: https://www.notulaebotanicae.ro

Article

Evaluation of Fatty Acid Profiles and Lipid Quality Indices in Hulled Wheat (*Triticum monococcum* and *T. dicoccum*) Genotypes from Türkiye

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Abstract

This study aimed to determine the grain fatty acid composition and nutritional lipid quality indices of 17 hulled wheat genotypes (*Triticum monococcum* and *Triticum dicoccum*) collected from different regions of Türkiye. Oils extracted from wheat grains were converted into fatty acid methyl esters (FAMEs) via transesterification and analysed using gas chromatography (GC-FID). Results revealed that unsaturated fatty acids-particularly linoleic and oleic acids-predominated in all genotypes, while saturated fatty acids such as palmitic acid were present in lower proportions. Total saturated fatty acids (∑SFA) ranged between 14.20% and 18.53%, whereas total unsaturated fatty acids (∑UFA) varied from 79.42% to 83.79%. Monounsaturated fatty acids (∑MUFA) were detected in the range of 24.60% to 32.13%, and polyunsaturated fatty acids (∑PUFA) were found to be between 50.59% and 55.83%. These findings highlight a highly favourable lipid profile, particularly in certain genotypes. In addition, nutritional quality indices, including atherogenic index (AI), thrombogenic index (TI), hypocholesterolemic/hypercholesterolemic ratio (h/H), PUFA/SFA, and n-6/n-3 ratios, differed among genotypes. Principal Component Analysis (PCA) and hierarchical cluster analysis further demonstrated the underlying genetic diversity in fatty acid profiles. These results suggest that certain genotypes with superior lipid composition may serve as valuable genetic resources in wheat breeding programs or in the development of functional food products.

Keywords: einkorn wheat; emmer; fatty acid composition; functional food; nutritional index

1 Introduction

Hulled wheats, namely einkorn (*Triticum monococcum*) and emmer (*Triticum dicoccum*), are among the earliest domesticated cereal species. Archaeological and genetic evidence indicate that einkorn wheat was domesticated approximately 10,000 years ago during the Neolithic period in the Karacadağ region of Southeastern Anatolia, Türkiye (Zaharieva and Monneveux, 2014). Similarly, emmer wheat, a tetraploid species and the domesticated form of *Triticum dicoccoides*, is reported to have been domesticated in the Fertile

Received: 17 Mar 2026. Revised: 11 Jul 2026. Accepted: 31 Jul 2026. Published: 21 Aug 2026.

Crescent, most likely in Southeastern Anatolia or the Levant, during the early Neolithic era (Ozkan et al., 2002).

The nutritional composition and functional properties of einkorn and emmer wheats have been reported to be superior to those of modern wheat species in several respects. Numerous studies have demonstrated that ancient wheats contain higher levels of protein, vitamins, and minerals than contemporary wheat cultivars (Biel et al., 2021). For instance, whole-grain einkorn wheat has been reported to contain protein at 15%-22%, whereas bread wheat typically contains 11%-15% protein (Arzani and Ashraf, 2017; Biel et al., 2021; Cetiner et al., 2023; Alkan et al., 2025). Similarly, the ash content of einkorn and emmer wheats is higher; in whole-grain flour, ash content is approximately 2.5% in einkorn, compared with around 1.5% in bread wheat (Biel et al., 2021). This characteristic reflects the greater capacity of ancient wheats to accumulate essential micronutrients, such as iron, zinc, and selenium, compared with modern wheat varieties (Tekin et al., 2018).

The carbohydrate profile and dietary fibre content of ancient wheats also differ markedly from those of modern wheat species. Whole grains of einkorn and emmer contain higher levels of dietary fibre, particularly hemicellulose, cellulose, and lignin fractions concentrated in the bran layer, compared with modern wheats (Biel et al., 2021). As a consequence, products made with einkorn flour are suggested to have a lower glycemic index, promoting prolonged satiety and a more gradual postprandial increase in blood glucose levels. Indeed, several studies have reported that einkorn wheat displays reduced starch digestibility and a lower glycemic response, attributable to its primitive starch structure and elevated fibre content (Arzani and Ashraf, 2017).

In addition to their carbohydrate characteristics, these wheats are notable for their antioxidant constituents. Whole-grain hulled wheats are rich sources of bioactive phytochemicals, including phenolic acids, flavonoids, and carotenoids. Phenolic compounds such as ferulic acid, which are predominantly concentrated in the wheat bran, are well recognized for their strong antioxidant and anti-inflammatory properties; adequate intake of these compounds has been associated with reduced oxidative stress and a lower risk of coronary heart disease, stroke, and certain types of cancer (Abdel-Aal and Rabalski, 2008). Several studies have demonstrated that the total antioxidant capacity of einkorn and emmer wheats is higher than that of modern wheat varieties, largely due to their richer phytochemical profiles (Arzani and Ashraf, 2017; Abdel-Aal and Rabalski, 2008). For instance, einkorn wheat is distinguished by its yellowish flour colour, which reflects its high carotenoid content; notably, the predominant carotenoid in einkorn grains, lutein, has been reported to occur at substantially higher levels than in bread wheat (Abdel-Aal and Hucl, 2014).

Although cereal grains consist predominantly of starch and other carbohydrate fractions (accounting for more than 80% of the grain), the lipid fraction is present in relatively smaller amounts. Nevertheless, hulled wheats exhibit notable differences in both lipid content and fatty acid composition when compared with modern wheat species. Whole grains of einkorn and emmer typically contain 3-4% oil, higher than the typical lipid content of bread wheat grains (approximately 2%). The nutritional value of wheat oil is largely determined by the composition and relative proportions of its constituent fatty acids. Oils derived from hulled wheats are characterized by a high proportion of unsaturated fatty acids, with particularly elevated levels of polyunsaturated fatty acids. In einkorn wheat oil, linoleic acid (C18:2, ω-6) has been identified as the predominant fatty acid, accounting for nearly half of the total fatty acids, followed by oleic acid (C18:1, ω-9), which represents approximately 25–30% of the total fatty acid content (Brandolini and Hidalgo, 2011). In contrast, the proportion of saturated fatty acids is relatively low. For example, palmitic acid (C16:0), the principal saturated fatty acid in wheat oil, typically constitutes less than 15% of the total fatty acids (Relina et al., 2022). This fatty acid profile indicates that oils from einkorn and emmer wheats possess a more favourable nutritional distribution. Indeed, diploid einkorn wheat populations have been reported to exhibit a high unsaturated-to-saturated fatty acid ratio of approximately 5.3, and an omega-6/omega-3 ratio of

approximately 9:1 (Relina et al., 2022). These findings suggest that the lipid quality of hulled wheats may be advantageous for the balance of essential fatty acids.

The primary hypothesis of this study is that the grain fatty acid composition and the corresponding lipid quality indices of *T. monococcum* and *T. dicoccum* genotypes collected from different ecological regions of Türkiye vary significantly due to genotypic differences. It is further hypothesized that certain hulled wheat genotypes exhibit a lipid profile enriched in unsaturated fatty acids, which may be reflected in nutritionally favourable lipid quality indicators, including lower atherogenic and thrombogenic index values and higher hypocholesterolemic/hypercholesterolemic ratios. Within this framework, the study aims to elucidate the determining role of genetic variation in fatty acid biosynthesis and lipid quality indices.

Total oil content is an important quality parameter; however, fatty acid composition allows lipid quality to be evaluated in a more detailed and nutritionally meaningful manner. In particular, the relative proportions of saturated, monounsaturated, and polyunsaturated fatty acids directly influence health-related lipid quality indices and determine the potential functional value of wheat-based foods. Therefore, evaluating fatty acid composition in hulled wheat genotypes may provide more meaningful information for nutritional characterization and breeding programs than determining oil quantity alone.

2 Materials and Methods

2.1 Wheat Samples

The wheat genotypes used in this study were obtained from the Türkiye Seed Gene Bank (Ankara, Türkiye). The genotypes originated from accessions collected across nine provinces of Türkiye and conserved in the Türkiye Seed Gene Bank. Detailed information regarding the geographical origins of the wheat samples is provided in Table 1.

Table 1. Wheat genotypes used in the study.

Sample No.	Species	Location	Sample No	Species	Location
B1	*Triticum monococcum*	Kütahya	B10	*Triticum monococcum*	Şanlıurfa
B2	*Triticum monococcum*	Kütahya	B11	*Triticum monococcum*	Aksaray
B3	*Triticum monococcum*	Samsun	B12	*Triticum monococcum*	Bolu
B4	*Triticum monococcum*	Samsun	B13	*Triticum dicoccum*	Karabük
B5	*Triticum monococcum*	Samsun	B14	*Triticum dicoccum*	Karabük
B6	*Triticum monococcum*	Eskişehir	B15	*Triticum dicoccum*	Kastamonu
B7	*Triticum monococcum*	Samsun	B16	*Triticum dicoccum*	Karabük
B8	*Triticum monococcum*	Karabük	B17	*Triticum dicoccum*	Kars
B9	*Triticum monococcum*	Türkiye	-	-	-

2.2 Extraction and Preparation of Fatty Acid Methyl Esters

For each analysis, 100 grams of wheat samples were ground into a fine powder, and 10 grams of the homogenized material were weighed. The samples were extracted three times with 100 mL of hexane using an ultrasonic bath for 30 minutes per extraction. The combined extracts were filtered and evaporated under reduced pressure to remove the solvent. From the resulting crude oil, 50 mg was accurately weighed and dissolved in 5 mL of hexane. Then, 5 mL of 1 M potassium hydroxide prepared in methanol was added, and the mixture was vortexed for 1 minute to facilitate transesterification. After phase separation, 1 mL of the upper hexane phase containing fatty acid methyl esters (FAMEs) was transferred into GC vials for analysis (Telci et al., 2014).

2.3 Gas Chromatographic Conditions

Fatty acid methyl esters were analysed using a gas chromatograph (Thermo Scientific Trace 1310-Thermo Scientific, Milano, Italy) equipped with a flame ionization detector (FID-6850 A) and a TR-Fame capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were set at 250 °C. The analysis was performed in split mode with a 50:1 split ratio, using helium as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program began at 100 °C, increased at 5 °C/min to 180 °C, and was then held for 10 minutes. It was then raised to 250 °C at a rate of 3 °C/min and held for an additional 20.7 minutes. Identification of individual fatty acids was achieved by comparing retention times with those of a certified standard mixture (FAME MIX CRM47885, Sigma-Aldrich), containing 37 components. The results were expressed as the relative percentage of each fatty acid based on peak area data obtained from the FID (Telci et al., 2014).

2.4 Lipid Quality Indices

The nutritional quality of the lipids in the samples was evaluated using several lipid quality indices calculated from the fatty acid percentages determined by gas chromatography. In this context, the atherogenic index (AI) and thrombogenic index (TI) were calculated according to the equations proposed by Ulbricht and Southgate (1991). These indices provide a quantitative measure of the balance between saturated fatty acids, particularly those known to promote atherosclerotic and thrombogenic processes (mainly C12:0, C14:0, and C16:0), and monounsaturated and polyunsaturated fatty acids, which are considered to exert anti-atherogenic effects.

The atherogenic index (AI) and the thrombogenic index (TI) were calculated using the following Eq. 1 and 2, respectively.

$$AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0) / \Sigma UFA \quad \text{(Eq. 1)}$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times \Sigma MUFA + 0.5 \times \Sigma n\text{-}6 + 3 \times \Sigma n\text{-}3 + (\Sigma n\text{-}3 / \Sigma n\text{-}6)] \quad \text{(Eq. 2)}$$

To assess the potential effects of the samples on cholesterol metabolism, the hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated as described by Santos-Silva et al. (2002). This method is based on the ratio between hypocholesterolemic fatty acids primarily C18:1n-9, C18:2n-6, and C18:3n-3-and hypercholesterolemic fatty acids (C14:0 and C16:0). In addition, the PUFA/SFA ratio and the n-6/n-3 ratio were calculated to evaluate the overall nutritional quality of the fatty acid profiles, following standardized methodologies widely accepted in the literature (Simopoulos, 2002; Wood et al., 2008). The PUFA/SFA ratio was obtained by dividing the total polyunsaturated fatty acids by the total saturated fatty acids, whereas the n-6/n-3 ratio was calculated as the ratio of total linoleic acid (C18:2n-6) to total linolenic acid (C18:3n-3)

3 Results and Discussion

The fatty acid composition exhibited considerable variation among the 17 hulled wheat genotypes examined (Table 2). When the overall distribution of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) was evaluated, unsaturated fatty acids were found to predominate in all genotypes. Total saturated fatty acid content ranged from 14.20% (B11) to 18.53% (B13), whereas total unsaturated fatty acids varied between 79.42% (B13) and 83.79% (B11), indicating that hulled wheat genotypes generally possess lipid profiles rich in unsaturated fatty acids. Notably, genotype B11, which exhibited the lowest ΣSFA value, also showed relatively higher ΣMUFA and ΣPUFA proportions.

Table 2. Fatty acid compositions of selected wheat cultivars.

RT	Fatty Acid	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17
19.26	Myristic acid methyl ester (C14:0)	0.11	0.09	0.09	0.07	0.08	0.07	0.11	0.04	0.08	0.08	0.08	0.09	0.11	0.1	0.1	0.09	0.09
22.2	Pentadecanoic acid methyl ester (C15:0)	0.05	0.06	0.06	0.06	0.06	0.05	0.07	0.09	0.05	0.06	0.06	0.05	0.06	0.05	0.04	0.06	0.05
25.19	Palmitic acid methyl ester (C16:0)	15.72	16.82	14.07	14.56	13.91	13.9	15.2	14.29	14.67	14.64	13.87	16.93	18.11	17.03	17.25	16.27	15.07
25.77	Palmitoleic acid methyl ester (C16:1)	0.13	0.17	0.19	0.21	0.19	0.15	0.21	0.16	0.21	0.18	0.16	0.22	0.21	0.21	0.21	0.15	0.16
30.7	Elaidic acid methyl ester (C18:1n9t)	0.33	0.45	0.49	0.42	0.44	0.37	0.57	0.45	0.46	0.46	0.35	0.54	0.51	0.58	0.58	0.55	0.43
31.12	Oleic acid methyl ester (C18:1n9c)	28.45	22.32	27.56	27.79	26.94	27.87	28.31	27.49	29.65	28.49	27.49	22.96	22.61	24.08	22.87	26.72	25.7
32.56	*cis*-linoleic acid methyl ester (C18:2n6c)	47.33	51.51	49.58	48.79	50.41	49.86	47.32	49.45	47.57	48.55	50.18	50.55	49.84	49.36	51.03	48.89	50.88
34.12	Linolenic acid methyl ester (C18:3n3)	3.26	4.32	3.19	3.94	3.77	3.66	4.03	3.93	3.34	3.62	3.86	4.22	4.14	4.33	3.94	4.22	3.95
35.7	Arachidic acid methyl ester (C20:0)	0.28	0.21	0.24	0.23	0.21	0.24	0.23	0.21	0.25	0.22	0.19	0.26	0.25	0.26	0.25	0.25	0.18
36.12	Eicosenoic acid methyl ester (C20:1)	1.69	1.66	1.76	1.71	1.67	1.68	1.87	1.71	1.81	1.72	1.69	1.96	2.05	1.77	1.66	1.66	1.58
-	∑Saturated	16.16	17.18	14.46	14.92	14.26	14.26	15.61	14.63	15.05	15	14.2	17.33	18.53	17.44	17.64	16.67	15.39
-	∑Unsaturated	81.24	80.49	82.83	82.92	83.48	83.64	82.38	83.28	83.09	83.08	83.79	80.5	79.42	80.38	80.33	82.25	82.75
-	∑MUFA	30.6	24.6	30	30.13	29.24	30.07	30.96	29.81	32.13	30.85	29.69	25.68	25.38	26.64	25.32	29.08	27.87
-	∑PUFA	50.59	55.83	52.77	52.73	54.18	53.52	51.35	53.38	50.91	52.17	54.04	54.77	53.98	53.69	54.97	53.11	54.83

Among the saturated fatty acids, palmitic acid (C16:0) was the dominant component. The palmitic acid proportion ranged from 13.87% (B11) to 18.11% (B13), clearly exceeding the levels of other SFA components across all genotypes. Myristic acid (C14:0) was present at very low levels in all genotypes, ranging from 0.04% to 0.11%. Similarly, pentadecanoic acid (C15:0) showed a narrow range of variation, with values between 0.04% and 0.09%. Arachidic acid (C20:0) was detected at levels ranging from 0.18% to 0.28% and did not exhibit pronounced differences among genotypes. Mandrioli et al. (2024) investigated the lipid and fatty acid composition of twenty-two durum wheat landraces and modern cultivars and reported that the most abundant fatty acids, in decreasing order, were linoleic acid (C18:2, cis,cis-9,12), oleic acid (C18:1, cis-9), palmitic acid (C16:0), α-linolenic acid (C18:3, all cis-9,12,15), and stearic acid (C18:0).

The low levels of these minor SFA components indicate that the total saturated fatty acid content was largely determined by palmitic acid. With respect to monounsaturated fatty acids, total MUFA values ranged from 24.60% (B2) to 32.13% (B9). Oleic acid (C18:1n9c), the predominant component within the MUFA fraction, was detected at levels ranging from 22.32% (B2) to 29.65% (B9) across the genotypes. These findings indicate that oleic acid proportions were particularly elevated in genotypes such as B8, B9, B10, and B11.

Elaidic acid (C18:1n9t) was present at relatively low levels, ranging from 0.33% to 0.58%, with limited variation among genotypes. Palmitoleic acid (C16:1) was detected in the range of 0.13-0.22% and contributed only marginally to the MUFA fraction due to its low abundance. Eicosenoic acid (C20:1) ranged from 1.58% to 2.05%, with relatively higher levels observed in certain genotypes, particularly B13 and B14.

A clear predominance was observed within the polyunsaturated fatty acid (PUFA) fraction. ΣPUFA values ranged from 50.59% (B1) to 55.83% (B2), representing the largest proportion of the total fatty acid composition in all genotypes. cis-Linoleic acid (C18:2n6c) was the dominant PUFA, with values ranging from 47.32% (B7) to 51.51% (B2). The consistently high proportion of linoleic acid across all genotypes indicates that hulled wheats are characteristically rich in this fatty acid. In contrast, linolenic acid (C18:3n3) was present at lower levels, ranging from 3.19% (B3) to 4.33% (B2), with relatively higher concentrations observed in genotypes B2, B4, B5, and B16.

When the overall fatty acid profiles were considered, the high proportion of unsaturated fatty acids, particularly the predominance of linoleic and oleic acids, emerged as a defining feature of the hulled wheat genotypes. Genotypes such as B8, B9, B10, and B11, which exhibited comparatively higher proportions of both MUFA and PUFA, appear to be nutritionally more favourable for lipid quality. In contrast, genotype B13 exhibited a distinct profile, with a higher SFA content (18.53%) and a relatively lower PUFA proportion (53.98%). This variability highlights the pronounced genetic diversity in lipid composition among hulled wheat genotypes. Zengin et al. (2017) characterized the fatty acid composition of seven wheat cultivars and

reported substantial variation among cultivars in total saturated fatty acids (SFA; 37.76-45.20%), monounsaturated fatty acids (MUFA; 18.33-22.03%), and polyunsaturated fatty acids (PUFA; 32.77-43.41%). The PUFA contents observed in the hulled wheats examined in the present study were notably higher than those reported for the cultivars studied by Zengin et al. (2017). Similarly, Suchowilska et al., (2009) investigated the variability in fatty acid composition of 50 spring accessions of *T. monococcum*, *T. dicoccum*, and *T. spelta* from diverse origins, reporting MUFA and PUFA contents of 26.85% and 56.55% for *T. monococcum*, and 25.20% and 55.89% for *T. dicoccum*, respectively. These findings are consistent with the results of the present study. Relina et al. (2021) detected trace amounts of 2 minor fatty acids in wheat accessions: eicosanoic (arachidic) acid and eicosenoic acid. Their contents were found to be 0.1% in most of the species investigated (below 0.5% across all species) and were characterized by wide variability. In the present study, Eicosenoic acid methyl ester (C20:1) was higher than the values reported by Relina et al. (2021), ranging from 1.03 to 2.05. Arachidic acid methyl ester (C20:0) was found to be similar to the results reported by Relina et al. (2021), which ranged from 0.13 to 0.28.

The fatty acid composition of wheat, particularly the balance of saturated and unsaturated fatty acids, plays a crucial role in modifying AI values (Table 3).

Table 3. Fatty acid–derived health indices (AI, TI, h/H, PUFA/SFA, and n6/n3) of different genotypes.

Genotype	AI		TI	h/H	PUFA/SFA	n6/n3
B17	0.186	0.296	5.312	3.563	12.881	B17
B16	0.202	0.316	4.88	3.186	11.585	B16
B15	0.22	0.346	4.486	3.116	12.952	B15
B14	0.217	0.335	4.54	3.079	11.4	B14
B13	0.234	0.364	4.204	2.913	12.039	B13
B12	0.215	0.335	4.567	3.16	11.979	B12
B11	0.169	0.27	5.844	3.806	13.0	B11
B10	0.18	0.291	5.48	3.478	13.412	B10
B9	0.18	0.295	5.462	3.383	14.243	B9
B8	0.174	0.278	5.643	3.649	12.583	B8
B7	0.19	0.298	5.203	3.29	11.742	B7
B6	0.17	0.274	5.826	3.753	13.623	B6
B5	0.17	0.273	5.798	3.799	13.371	B5
B4	0.179	0.285	5.504	3.534	12.383	B4
B3	0.174	0.286	5.673	3.649	15.542	B3
B2	0.213	0.331	4.622	3.25	11.924	B2
B1	0.199	0.324	4.993	3.131	14.518	B1

AI: The atherogenic index; TI: Thrombogenic Index; h/H: The hypocholesterolemic/hypercholesterolemic ratio; PUFA/SFA: the total polyunsaturated fatty acids / the total saturated fatty acids; n6/n3: total linoleic acid (C18:2n-6) / total linolenic acid (C18:3n-3).

Wheat typically has lower saturated fatty acid (SFA) content while being a source of healthier monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), which are known to be beneficial for cardiovascular health (Popa et al., 2012; Chen and Liu, 2020). Alterations in dietary fatty acid intake have been linked to changes in AI; diets higher in PUFAs (found in whole grains) than in saturated fats result in lower AI values (Popa et al., 2012; Moreira et al., 2021). Genetic diversity within wheat genotypes plays a crucial role in determining the AI values through the biosynthesis of fatty acids. Research by Chen et al. (2012) indicates significant genetic variation among wheat cultivars, suggesting that genetic diversity can lead to differential expression of genes involved in fatty acid synthesis, thereby affecting the nutritional profile of wheat, including its lipid content, which relates to AI values. The fatty acid derived lipid quality indices calculated for the hulled wheat genotypes exhibited pronounced differences among genotypes. The

atherogenic index (AI) values ranged from 0.169 (B11, B6, and B5) to 0.234 (B13), with the lowest AI values predominantly observed in genotypes such as B11, B6, B5, B8, and B3. In contrast, genotypes including B13, B15, B14, and B12 were characterized by higher AI values, indicating relatively higher proportions of C14:0 and C16:0 fatty acids.

Thrombogenic Index (TI) is a critical measure in the field of food science and nutrition, particularly when assessing the health impacts of dietary fats and oils. TI values associated with wheat species/varieties are essential for understanding their nutritional quality and potential cardiovascular implications. Genetic diversity among wheat cultivars influences their TI values. Studies indicate that polymorphisms within wheat species can affect levels of fatty acids and bioactive compounds, thereby influencing TI values (Medini et al., 2005). The thrombogenic index (TI) ranged from 0.270 in genotype B11 to 0.364 in genotype B13. The lowest TI values were observed in genotypes B11, B6, and B5, whereas the highest TI values were recorded in genotypes B13, B15, and B14.TI values in bread wheat have been reported to range from 0.08 to 0.20 in many studies (Agarwal et al., 2005; Medini et al., 2005; Taran et al., 2017; Ratusz et al., 2018; Ullah et al., 2024). The results of the *T. monococcum* and *T. dicoccum* samples in the present study were higher than the T. aestivum results reported in the literature. The similar distribution patterns observed for both AI and TI indicate that pro-atherogenic and pro-thrombogenic fatty acids varied in parallel among the genotypes. The hypocholesterolemic/hypercholesterolemic (h/H) ratio ranged from 4.204 (B13) to 5.844 (B11). Genotypes exhibiting the highest h/H ratios, including B11, B6, B5, B8, and B3, were characterized by higher proportions of hypocholesterolemic fatty acids, whereas genotypes with the lowest h/H ratios, such as B13, B15, and B14, reflected relatively higher proportions of hypercholesterolemic fatty acids.

The PUFA/SFA ratio is a widely accepted dietary marker for assessing the fatty acid profile of foods. Lower ratios may contribute to higher cholesterol levels and an increased risk of chronic diseases, including cardiovascular disease (CVD). Emphasizing a higher intake of PUFAs relative to SFAs in the diet is particularly vital for managing cholesterol levels and mitigating cardiovascular risks. The PUFA/SFA ratio varied among the genotypes, ranging from 2.913 (B13) to 3.806 (B11), with the highest values observed in genotypes B11, B6, B5, and B8. Notably, genotype B13, which exhibited the highest proportion of saturated fatty acids, also displayed the lowest PUFA/SFA ratio. Mandrioli et al. (2024) analyzed twenty-two tetraploid accessions, comprising modern elite cultivars and landraces, and reported that the PUFA/SFA ratio varied between 2.84 and 3.56. Another study reported a PUFA/SFA ratio of 3.8 in wheat (Wang et al., 2021).

The n-6/n-3 ratio reflects the dietary intake of essential fatty acids critical for human health. A higher n-6/n-3 ratio, common in Western diets, has been linked to adverse health outcomes, while a balanced ratio is generally recommended for better health. Many modern diets exceed a 15:1 ratio, contributing to inflammatory conditions (Wall et al., 2010). This disproportionate intake has fuelled interest in dietary adjustments to better balance these fatty acids. The n-6/n-3 ratio ranged from 11.400 (B14) to 15.542 (B3), exhibiting a wide variation among the genotypes. The highest n-6/n-3 values were observed in genotypes B3, B1, and B9, whereas the lowest values were detected in genotypes B14, B16, B2, and B7. Wang et al. (2021) reported that the n-6/n-3 ratio varied between 4.9 and 15.0 for wheat.

Overall, genotypes such as B11, B6, B5, B8, and B3 exhibited more favorable fatty acid profiles, characterized by higher h/H and PUFA/SFA ratios, whereas genotypes including B13, B15, and B14 displayed relatively less advantageous profiles, as reflected by their higher AI and TI values. These findings demonstrate substantial variation in the nutritional quality of fatty acid composition among hulled wheat genotypes.

The heatmap illustrating the distribution of fatty acid components among genotypes revealed pronounced variation across all fatty acids in the hulled wheat genotypes examined (Figure 1). Among the saturated fatty acids, palmitic acid (C16:0) exhibited clear predominance, reaching the highest values across all genotypes. Palmitic acid levels were notably higher in genotypes B13, B3, and B2, whereas lower in genotypes B11 and B10. Other saturated fatty acids, including myristic acid (C14:0), pentadecanoic acid

(C15:0), and arachidic acid (C20:0), were present at low concentrations in all genotypes and were represented by lighter colour intensities in the heatmap.

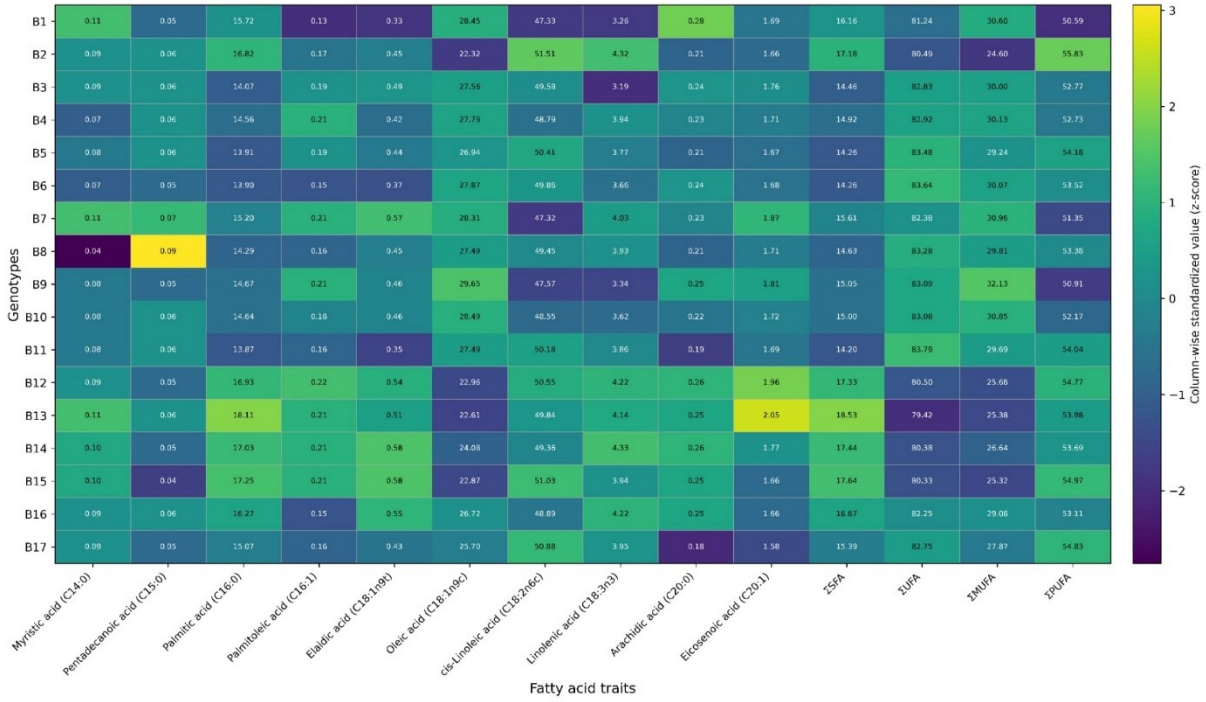


Figure 1. Heatmap of fatty acid methyl ester (FAME) compositions across wheat oil samples, indicating the relative abundance (%) of individual fatty acids and grouped categories (ΣSaturated, ΣUnsaturated, ΣMUFA, ΣPUFA).

Within the monounsaturated fatty acids, oleic acid (C18:1n9c) emerged as one of the components exhibiting the widest variation across genotypes. Higher oleic acid intensities were observed particularly in genotypes B8, B9, B10, B11, and B1, whereas lower values were detected in genotypes such as B2 and B3. In contrast, elaidic acid (C18:1n9t) and palmitoleic acid (C16:1) were present at relatively low levels, with limited variation among genotypes. Eicosenoic acid (C20:1) showed comparatively higher values in certain genotypes, notably B13 and B3, and was represented by moderate colour intensities in the heatmap. Among the polyunsaturated fatty acids, linoleic acid (C18:2n6c) was the most prominent component, displaying high colour intensity across all genotypes. Linoleic acid levels were particularly elevated in genotypes B2, B1, B16, and B3, whereas genotypes B7 and B9 exhibited relatively lower intensities compared with the others. Linolenic acid (C18:3n3) ranged from low to moderate levels across all genotypes, with higher values observed especially in genotypes B2, B16, and B4.

Total saturated fatty acids (ΣSaturated) were represented in the figure by a gradient ranging from light blue to yellowish tones, with the lowest values observed in genotypes B11 and B10 and the highest values in genotypes B13, B3, and B2. In contrast, total unsaturated fatty acids (ΣUnsaturated) were depicted with consistently high color intensities across all genotypes, reaching particularly elevated levels in genotypes B11, B10, B9, and B8. The distribution patterns of MUFA and PUFA followed similar trends: MUFA intensities were higher in genotypes B9, B10, B8, and B11 and lower in genotype B2, whereas PUFA levels were consistently high across all genotypes, with the highest intensities observed in B2, B1, and B16. Overall, the heatmap clearly demonstrates substantial diversity in fatty acid composition among the genotypes. While certain genotypes (e.g., B8, B9, B10, and B11) exhibited more intense colour distributions for both MUFA and PUFA, others (e.g., B13 and B2) were characterized by a more pronounced dominance of saturated fatty

acids. This visual pattern provides clear evidence of strong genetic variation in lipid composition among the hulled wheat genotypes.

The principal component analysis (PCA) revealed a clear separation of the 17 hulled wheat genotypes based on their fatty acid profiles (Figure 2). The first two principal components accounted for a substantial proportion of the total variation among genotypes, and the score plot demonstrated distinct clustering patterns along both axes. Genotypes B2, B12, B13, B14, and B15 were positioned on the positive side of the PC1 axis, separating them from the remaining genotypes and indicating a similar directional trend in their fatty acid composition. In contrast, genotypes B8, B11, and B6, located on the negative side of the PC1 axis, exhibited a structurally distinct fatty acid profile compared with the other genotypes. Along the PC2 axis, genotypes B1, B7, and B9 were positioned in the upper region of the score plot, clearly separated from the remaining genotypes. Conversely, genotypes B5, B6, B8, B11, and particularly B17 clustered in the lower region, suggesting that these genotypes shared similarly low PC2 component loadings. Genotypes B3, B4, B10, and B16, located near the central region of the plot, exhibited a relatively balanced distribution with respect to both principal components. Overall, the PCA plot demonstrates pronounced variation in fatty acid profiles among the genotypes and highlights that certain genotypes (e.g., B2, B12, B13, and B14) are distinctly separated from the others. This separation indicates a strong genetic basis underlying the observed differences in fatty acid composition.

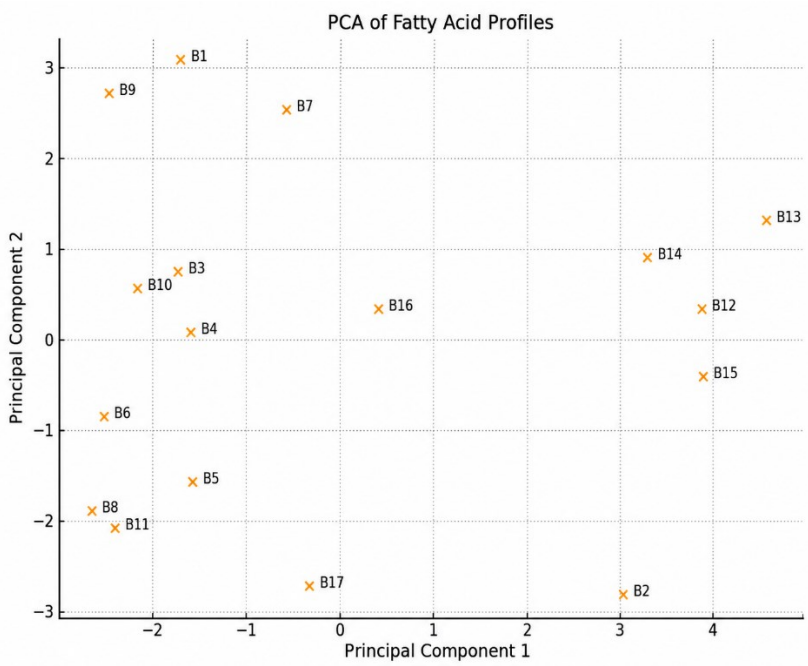


Figure 2. Principal Component Analysis (PCA) of fatty acid methyl ester compositions in wheat oil samples.

The hierarchical clustering dendrogram demonstrated that the 17 hulled wheat genotypes were separated into two main clusters based on their fatty acid profiles (Figure 3). The first major cluster comprised genotypes B2, B13, B15, B14, and B12, which were highly similar to one another. Within this cluster, the closest association was observed between genotypes B14 and B12, followed by B15, whereas B13 and B2 were more distantly linked. The second major cluster displayed broader variability and included genotypes B8, B17, B6, B11, B5, B1, B9, B3, B10, B4, B16, and B7. Within this cluster, the initial close association was observed between genotypes B6 and B11, followed by the subsequent inclusion of B5. Genotypes B1, B9, and B3 were positioned closer to each other, while genotypes B10 and B4 were linked to this subgroup at greater distances.

Notably, genotype B7 was located at the most distant position within this cluster, indicating that it has the most distinct fatty acid profile among the genotypes. Overall, the dendrogram highlights pronounced differences in fatty acid composition among the genotypes and demonstrates that certain genotypes, particularly B2, B13, and B7, exhibit distinct clustering patterns, reflecting marked divergence in their lipid profiles.

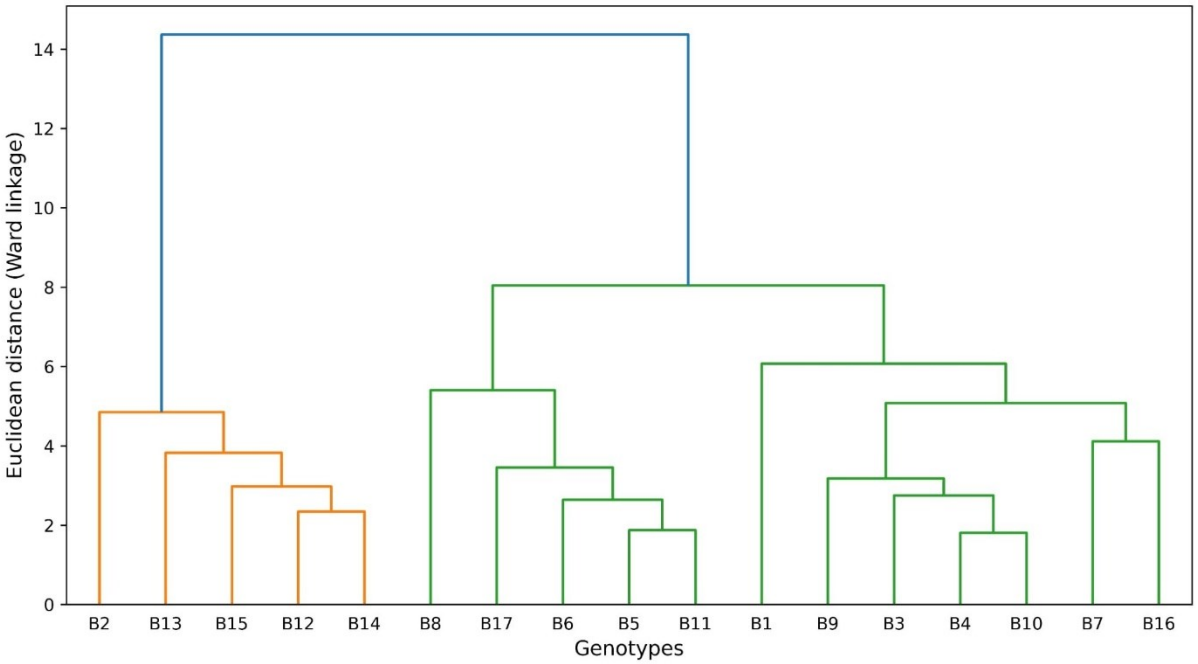


Figure 3. Hierarchical clustering dendrogram of wheat oil samples based on their fatty acid methyl ester compositions.

This study evaluated the grain lipid composition and associated nutritional quality indices of 17 hulled wheat genotypes (*T. monococcum* and *T. dicoccum*) from the Turkish gene pool, with the aim of assessing the breeding and functional food development potential of these ancient cereal species. The findings are consistent with the general cereal lipid profile reported in the literature, indicating the predominance of unsaturated fatty acids (UFAs) across all genotypes, particularly high levels of linoleic acid (C18:2n6c) and oleic acid (C18:1n9c) (Kan, 2015). The proportion of total unsaturated fatty acids reached at least 79.42% even in the genotype with the lowest value and increased to 83.79% in the highest-performing genotypes.

These proportions demonstrate that hulled wheats offer a more favourable lipid profile for human health compared with animal-derived foods, which are typically characterized by higher proportions of saturated fatty acids (Khalili Tilami and Kouřimská, 2022). For instance, the consistently high linoleic acid content, ranging from 47.32% to 51.51% across all genotypes and representing the dominant fatty acid, clearly indicates that these wheats constitute a rich source of essential fatty acids (EFAs) (Demir et al., 2015; Relina et al., 2022). This observation is also consistent with widely accepted reports indicating that linoleic acid is the predominant fatty acid in other cereal species, including common wheat, where it typically accounts for approximately 59.10% of total fatty acids (Kan, 2015).

Palmitic acid (C16:0) was identified as the principal component of the saturated fatty acid fraction and largely determined the total saturated fatty acid content in genotypes exhibiting values ranging from 14.20% to 18.53%. The presence of short-chain saturated fatty acids, such as myristic acid (C14:0), at negligible levels (<0.11%) minimizes the potential adverse effects of this lipid profile on cardiovascular health, particularly by limiting the contribution of pro-atherogenic fatty acids (Khalili Tilami and Kouřimská, 2022). Moreover, the

fatty acid profiles of hulled wheat species (e.g., *T. monococcum* and *T. dicoccum*) reported in previous studies, characterized by the predominance of linoleic acid (51.9-55.9%) and oleic acid (19.4-24.8%), further support the notion that this lipid composition represents a species-specific, nutritionally advantageous, and relatively stable trait (Demir et al., 2015).

The pronounced variation observed among genotypes is of considerable importance for both nutritional quality and technological utilization, underscoring the necessity of employing a broad genetic base in wheat breeding programs (Tekin et al., 2018; Mandrioli et al., 2024). In particular, genotype B11 (*T. monococcum*), which exhibited the lowest saturated fatty acid content (14.20%) and the highest unsaturated fatty acid content (83.79%), represents the most favourable example within the collection in terms of lipid quality. In contrast, genotype B13 (*T. dicoccum*) exhibited the opposite profile, with the highest saturated fatty acid content (18.53%) and a relatively lower proportion of unsaturated fatty acids. This wide range of variation suggests the presence of genetic alleles within the hulled wheat gene pool that optimize desirable fatty acid biosynthetic pathways, a notion further supported by the PCA and hierarchical clustering analyses, which confirm that this differentiation is strongly rooted in genetic factors. Such variation in fatty acid composition is particularly critical from a technological perspective, especially regarding the balance between oleic acid (MUFA) and linoleic acid (PUFA) contents (Mandrioli et al., 2024). High oleic acid content, such as that observed in genotype B11 (27.49%), combined with a low proportion of saturated fatty acids, may enhance the oxidative stability of flour and flour-based products, thereby extending shelf life (Mandrioli et al., 2024). Recent studies on durum wheat lines have likewise confirmed that genotypes with higher oleic acid/linoleic acid ratios are valuable for improved shelf life and technological sustainability (Mandrioli et al., 2024). These findings emphasize that the fatty acid profile of wheat influences not only its nutritional value but also its industrial processing quality. Nevertheless, the fact that the genotypes were collected from diverse ecological regions of Türkiye suggests that the observed variation may be attributable not solely to genetic control but also to genotype-by-environment (G × E) interactions (Brankovic et al., 2015). Previous studies have reported that G x E interactions can exert an even stronger influence than genotype alone on quality-related traits in wheat, including antioxidant capacity, phenolic compounds, and phytic acid content (Brankovic et al., 2015). Fatty acid biosynthesis is likewise known to be sensitive to climatic factors, particularly temperature and moisture conditions during the grain-filling stage (Khalili Tilami and Kouřimská, 2022). Therefore, to determine whether the superior fatty acid profiles of these genotypes are maintained across diverse environmental conditions, it is essential to implement multi-environment trials (METs) to clarify the heritability and breeding value of the identified elite genetic materials (Brankovic et al., 2015).

The lipid quality indices calculated to quantitatively substantiate the nutritional superiority of the genotypes provide valuable insights into their potential to reduce cardiovascular disease (CVD) risk. The atherogenic index (AI) and thrombogenic index (TI) values ranged from 0.169 to 0.234 and from 0.270 to 0.364, respectively, among the genotypes, reflecting parallel variation with differences in the concentrations of fatty acids known to exert pro-atherogenic and pro-thrombogenic effects, particularly C14:0 and C16:0. Genotype B11, which exhibited the lowest AI and TI values (0.169 and 0.270, respectively), displayed a lipid profile with a strong potential to minimize CVD risk.

These indices are widely used as standard metrics for evaluating the nutritional quality of plant-derived lipids, with lower values representing a more desirable balance. Notably, the AI value of 0.169 observed in genotype B11 is remarkably low compared with many conventional seed oils, and low AI and TI values have been consistently associated with cardioprotective benefits in the literature (Khalili Tilami and Kouřimská 2022; Gaeini et al., 2025). The hypocholesterolemic/hypercholesterolemic (h/H) ratio ranged from 4.204 (B13) to 5.844 (B11), highlighting the substantial influence of genotypic selection on cholesterol metabolism. A higher h/H ratio indicates that hypercholesterolemic fatty acids (C14:0 and C16:0) are largely counterbalanced by hypocholesterolemic fatty acids (MUFA and PUFA), thereby suggesting a favorable effect on cholesterol metabolism (Khalili Tilami and Kouřimská, 2022). The approximately 39% higher h/H

ratio observed in genotype B11 compared with B13 represents a quantitative measure of the tangible nutritional advantage conferred by this genotype. Considering that dietary fat quality indices are associated not only with CVD risk but also with other metabolic parameters, including type 2 diabetes and prediabetes (Gaeini et al., 2025), the development and utilization of genotypes exhibiting higher h/H ratios (e.g., B11, B6, B5, and B8) may contribute to broader improvements in metabolic health.

The polyunsaturated/saturated fatty acid (PUFA/SFA) ratio also varied among the genotypes, ranging from 2.913 (B13) to 3.806 (B11). General dietary guidelines recommend a PUFA/SFA ratio greater than 1.0 to support cardiovascular health (Khalili Tilami and Kouřimská 2022); the present results demonstrate that all examined genotypes substantially exceed this critical threshold. This high PUFA/SFA ratio confirms the potential of hulled wheats to contribute meaningfully to dietary PUFA intake, which is often insufficient in modern diets, given that dietary lipids constitute a major source of essential PUFAs (Khalili Tilami and Kouřimská 2022). However, another important aspect of the nutritional profile of these cereals is the balance of essential n-6 and n-3 fatty acids. Among the studied genotypes, the n-6/n-3 ratio ranged from 11.400 (B14) to 15.542 (B3). Although an n-6/n-3 ratio close to 4:1 is generally recommended to reduce inflammation in modern diets, the relatively high ratios observed here represent an inherent characteristic of cereal lipids, as linoleic acid, the predominant PUFA in wheat, occurs at substantially higher levels than linolenic acid (Khalili Tilami and Kouřimská, 2022). Notably, the extent of genotypic variation itself (11.400-15.542) provides critical insights for wheat breeding programs. In particular, genotypes such as B14 and B16, which exhibited relatively lower n-6/n-3 ratios, as well as genotypes with comparatively higher linolenic acid contents (e.g., B2, B4, and B5), can be regarded as promising genetic materials for nutritional enhancement strategies. This differentiation offers plant breeders a tangible framework for developing wheat cultivars with a more balanced essential fatty acid profile by targeting increased n-3 fatty acid biosynthesis (Relina et al., 2022).

The genetic diversity underlying the fatty acid profiles is strongly supported by the results of principal component analysis (PCA) and hierarchical clustering, as these multivariate statistical approaches effectively captured and grouped the variation among genotypes. The ability of PCA to explain a substantial proportion of the total genotypic variation and to clearly cluster genotypes according to their fatty acid compositions indicates that the examined lipid traits are largely under genetic control. If environmental effects had been predominant, such clear and consistent clustering patterns among genotypes would not be expected (Brankovic et al., 2015).

This strong indication of heritability confirms the high breeding value of genotypes with superior lipid profiles, such as B11, for reliably transferring targeted traits to subsequent generations in breeding programs (Tekin et al., 2018; Relina et al., 2022). Hierarchical clustering further highlighted that genotypes B11 and B6, which were superior in nutritional indices such as AI and TI, were grouped into a distinct main cluster, separate from genotypes B13 and B15, which were characterized by higher saturated fatty acid content. This separation demonstrates that not only individual fatty acid proportions but also derived indicators of nutritional quality are strongly genetically determined. Consequently, genotypes such as B11 are recommended as primary breeding parents, not only for biodiversity conservation efforts but also for direct utilization in functional food production or for the introgression of health-promoting lipid profiles into modern wheat cultivars (Tekin et al., 2018; Relina et al., 2022).

4 Conclusions

Grain fatty acid compositions and corresponding lipid quality indices of 17 hulled wheat genotypes (*T. monococcum* and *T. dicoccum*) collected from different regions of Türkiye were evaluated in the current study. Variations were recorded among the genotypes, with profiles rich in unsaturated fatty acids, notably linoleic and oleic acids. The predominance of total unsaturated fatty acids over saturated fatty acids indicates that these wheats have a lipid profile advantageous for cardiovascular health. Nutritionally important parameters,

including the atherogenic index, thrombogenic index, hypocholesterolemic/hypercholesterolemic (h/H) ratio, PUFA/SFA ratio, and n-6/n-3 ratio, differed among genotypes, indicating that certain genotypes exhibit superior nutritional lipid profiles. Furthermore, principal component analysis and hierarchical clustering discriminated genetic diversity based on fatty acid composition, indicating strong genotypic differentiation. In conclusion, hulled wheats such as einkorn and emmer represent promising genetic resources not only from the perspectives of traditional agriculture and biodiversity conservation but also for functional food development and healthy nutrition. These genotypes may be regarded as valuable genetic material for future wheat breeding programs aimed at developing cultivars with improved, health-promoting lipid profiles.

Author Contributions

Conceptualization: F.R.A., B.Ç.; Data curation: F.R.A., H.A., S.Ş.; Formal analysis: F.R.A., H.A., S.Ş., B.Ç.; Investigation: F.R.A., B.Ç., S.Ş.; Methodology: F.R.A., B.Ç., H.A.; Supervision: F.R.A.; Writing - original draft: F.R.A., B.Ç., H.A., S.Ş.; Writing - review and editing: F.R.A., B.Ç.

All authors read and approved the final manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflicts of interest.

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