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Characterization of flax genetic resources in Türkiye through variance analysis of antioxidant, phenolic compound and fatty acid contents

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Abstract

This study was conducted in 2021 using seeds from 10 different flax cultivars (Sarı 85, Cill 1351, Cill 1370, Cill 1400, Cill 1412, Larnaka, Milas, Newtürk, and Dilman) in the Ankara ecology. The study investigated the oil content, protein content, fatty acid composition, antioxidant capacities, and specific phenolic compound contents of these seeds. Correlations among the bioactive compound contents of flax seeds were elucidated using Principal Component Analysis (PCA) and Heatmap analysis. As a result of the research, statistically significant differences were found among the seed properties of the varieties. According to the PCA method, the correlation among fatty acids was determined as 71.2% (PC1+PC2), while the correlation between phenolic compounds and antioxidants was determined as 60.4% (PC1+PC2). In the study, the highest oil content was obtained in the Newtürk variety with 35.3%, while the lowest oil content was obtained in the Larnaka variety with 32.2%. The highest α-linolenic acid (C18:3) ratio of 53.9% was detected in the Newtürk variety, while the lowest α-linolenic acid (C18:3) ratio of 46.8% was obtained from the Cill1423 variety. Ferulic (Cill 1351: 18.51 µg/g) and protocatechuic (Cill 1423: 20.83 µg/g) acids were found to be the most abundant compounds in flax seeds. In the research, it was determined that the Cill 1351 (4.08 mg trolox/g) and Dilman (4.16 mg trolox/g) varieties had higher antioxidant capacities than the other varieties.

Keywords: Flax, Antioxidant, Phenolic compounds, Fatty acids, Protein

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INTRODUCTION

Today, people attribute increasing health problems to their dietary habits. Despite the growing human population and the increasing demand for staple food products, the reduction in available agricultural land day by day, as a result of which agricultural activities shift predominantly towards the production of staple food products, and the consideration of yield and main quality criteria in breeding efforts, have led to a decrease in food diversity and the nutritional content of consumed foods. As a result, cases of hidden hunger are observed in people. Those who want to eat healthily believe that returning to traditional dietary methods and adding new plant species to their diets will solve nutritional problems. Individuals who are conscious about nutrition and have access to the food they desire are aware that food is not only essential for satisfying hunger and meeting basic nutritional needs but also a crucial factor in preventing nutrition-related diseases and improving mental well-being. Recent studies have shown that many diseases are caused by improper or inadequate nutrition. Studies in this regard indicate that omega-3 fatty acids are effective in anti-ulcer activity, anti-secretory effect, renoprotection in lupus nephritis, anti-atherogenic effect, CVD prevention, and decreasing blood pressure (Harper et al., 2006; Dupasquier et al., 2007; Dugani et al., 2008; Rodriguez-Leyva et al., 2010; Caligiuri et al., 2014); plant proteins are effective in neurodegenerative disease prevention, controlling blood pressure, influencing hypertriglyceridemia, influencing diabetes mellitus, and having anti-hypertensive properties (Velasquez et al., 2003; Omoni and Aluko, 2006; Oomah et al., 2007); dietary fibers are effective in hunger suppression and reducing total cholesterol in the blood (Kristensen et al., 2012); and lignans are effective in controlling hypertension, protecting against cancer and diabetes, controlling dyslipidemia, reducing breast cancer growth, and affecting postmenopausal women's symptoms (Nowak et al., 2007; Adolphe et al., 2010; Simbalista et al., 2010; Dew and Williamson, 2013; Flower et al., 2014). The flax plant, which has recently become in demand for both healthy nutrition and dietary consumption, is a member of the Linaceae family and is an annual herbaceous plant. Its gene center is Ethiopia, Central Asia and India. (Habibollahi et al., 2016; Choudhary et al., 2017; Singh et al., 2017; Goudenhooft et al., 2018; Tchoumtchoua et al., 2019; Landoni et al., 2020; Nag et al., 2020; Xie et al., 2020; Talebi and Matsyura, 2021). It is reported that human beings began to benefit from flax as far back as 30,000 years ago (Balter, 2009; Kvavadze, 2009; Fu, 2011). Flax is a plant cultivated for many years in many countries of the world because of its fibers obtained from its stems and its oils obtained its seeds (Katar, et al, 2023). It is probable that humankind first used the seeds of flax and then began to benefit from its fiber.

The area under flax cultivation for seed production worldwide is 3,540,139 hectares, with a total production of 3.367.380 tons. The highest seed production is carried out by Kazakhstan with 1,058,247 tons, followed by Russia with 787,923 tons and Canada with 578,000 tons (FAO, 2020). People's pursuit of natural and healthy nutrition has led to the resurgence of highnutrient plants like flax. The high content of polyunsaturated and monounsaturated essential fatty acids, phenolic compounds, proteins, and minerals in flax seeds has led to its inclusion in diets. The fact that 30 grams of flax seeds have the potential to meet 7% to 30% of the daily intake of elements such as calcium, magnesium, and phosphorus has increased its importance in diets (Singh et al., 2011). Furthermore, due to its rich content, it has become a preferred ingredient in bird feed mixes. The seeds contain, varying from genotype to genotype, approximately 41% oil, 28% fiber, and 20% protein (Oomah, 2001; Pengilly, 2003; Flaxcouncil, 2022). Its oil is rich in alpha-linolenic acid, an important fatty acid for health (Sargi et al., 2013). Additionally, due to its content of polyunsaturated linolenic and linoleic fatty acids, it tends to oxidize quickly, so it should be consumed fresh. If consumed directly, it should be ground daily whenever possible (Sargi et al., 2013).

Phenolic compounds are substances found in plants that influence many quality criteria such as color, taste, and aroma (Dong et al., 2001; Cemeroğlu, 2007; Predieri et al., 2006; Gundogdu et al., 2021). Due to their antioxidant properties, these compounds are effective in plants' defense systems against environmental stress factors and play a role in many physiological processes (Colaric et al., 2005; Gündoğdu, 2019). Nowadays, there is a growing demand for products with high antioxidant content in human nutrition. Particularly, foods rich in phenolic compounds with anticancer properties are in the spotlight (Scalbert et al., 2005). Studies have shown that flax seeds contain a high amount of phenolic compounds. Phenolic compounds have anticancer and antioxidant properties. It is reported that flax seeds have three different types of phenolic compounds, namely phenolic acids, flavonoids, and lignans (Kajla et al., 2015). The major phenolic acids in flaxseed meal are ferulic acid (10.9 mg/g), chlorogenic acid (7.5 mg/g), and gallic acid (2.8 mg/g), along with other phenolic acids, low amounts of pcoumaric acid glucosides, hydroxycinnamic acid glucosides, and 4-hydroxybenzoic acid. The major flavonoids in flax seeds are Flavone C- and Flavone O-glycosides (Beejmohun et al., 2007; Mazza, 2008).

Flax is produced and consumed for its oil and fiber, and intensively used in several sectors. It is important industrial plants with several uses (Zuk et al., 2015; Yaşar, 2023). Flax is a versatile plant species widely used in various industries such as oil production, cosmetics, pharmaceuticals, and more. In oil industry production, the residue from seeds after oil extraction, known as flaxseed meal, is a valuable byproduct rich in protein and mineral content. Flaxseed meal has qualities suitable for both human and animal nutrition. This research has identified the bioactive compound contents of seeds from flax varieties commonly grown in our country and statistically defined the correlations among these compounds.

MATERIALS AND METHODS

Plant material, fatty acid, and protein analyses

In the study, various sources provided Sarı 85, Cill 1351, Cill 1370, Cill 1400, Cill 1412, Larnaka, Milas, Newtürk, and Dilman flax varieties (Table 1).

To obtain the seeds used in the research, a trial was established on April 5, 2021, under Ankara ecological conditions, in a Randomized Complete Block Design with 3 replications. Plots, each with an area of 4 x 1.2 = 4.8 m2, were established with 6 rows, a row length of 4 m, and a row spacing of 20 cm. Manual seeding was performed for the trial, and organic-mineral fertilization was applied at a rate of 10 kg per hectare. Weed control was carried out, and two irrigations were performed, one at the emergence stage and the other at the pre-flowering stage. The trial was manually harvested in the yellow ripening stage on August 20th. The total annual precipitation at the trial location was 297 mm, with the lowest average temperature during the vegetation period (9.3°C) occurring in April and the highest average temperature (16.5°C) in June. The annual precipitation was lower than the long-term average precipitation (318 mm). The soil at the trial site was determined to be clayey-loamy, low in organic matter (1.63%), with available phosphorus content at 9.7%, slightly alkaline (pH=7.7-7.8), lime-rich (28% lime), and low in salt content (1.18 dS/m). Fixed oil content analyses were conducted using a Soxhlet apparatus, fatty acid composition analyses were performed using the method recommended by Gölükcü et al. (2016), and protein content analyses were carried out using the Kjeldahl method (Balkan, 1978).

Table 1. Some information about the varieties.

Genotype name	1000 seeds Weight (g)	Flower Color	Seed Color	Oil Rate (%)	Country of Orjin	Growing Type
Sarı 85*	5.40	white	yellow	37.7	Türkiye	Spring
Larnaka*	5.40	blue	darkbrown	37.3	Pakistan	Winter
Milas*	6.20	blue	lightbrown	36.2	Türkiye	Winter
Newtürk*	5.40	blue	darkbrown	37.1	U.S.A	Spring
Dillman*	5.20	lightblue	brown	32.3	U.S.A	Winter
Clli-1351*	6.18	blue	lightbrown	34.8	Türkiye	Winter
Clli-1400*	6.18	blue	brown	38.2	Türkiye	Spring
Clli-1412*	6.15	lightblue	brown	35.3	Türkiye	Spring
Clli-1370*	5.69	blue	brown	35.0	Türkie	Spring
Clli-1423*	5.76	blue	brown	34.6	Türkiye	Spring

**The seeds used in the experiment were supplied by the United States Department of Agriculture (USDA). Yaşar,2023 and Yaşar ve Yetişsin, 2023.*

Phenolic compound analysis

Phenolics were extracted using a modification of the methods developed by Kosar et al. [1] and Trandafir et al. [2]. The seed samples were mixed with acetone and water (1: 4) and vortexmixed for 1 min. Trifluoroacetic acid (0.100 ml) was then added to the mixture followed by vortexmixing for 1 min and by incubation in a hot water bath at 60 °C for 60 min. After cooling, the extracts were filtered through a nylon membrane (pore size 0.45 µm, Merck). Extracts were analysed by HPLC with ultraviolet spectrophotometric detection using LC-20A system (Shimadzu, Tokyo, Japan). A reverse phase column Nucleosil C18 (25 cm × 3.2 mm, particle size 5 µm; Supelco) and a twosolvent system (A: formic acid-water, 2.5: 97.5, v/v and B: acetonitrile-water, 2.5: 97.5, v/v) were used. Detection was accomplished at 280–360 nm. Content of phenolics was expressed as milligrams per kilogram.

Antioxidant analysis

Determination of ABTS cation radical scavenging activity Determination of ABTS cation radical scavenging activity of methanolic extracts of seeds and sprouts was carried out according to methods of Pająk et al. (2017). ABTS cation radical was obtained in the reaction of 2mM phospate-buffered stock (PBS) solution of 2,2′-azinobis (3-ethylbenothiazoline-6sulfonic acid) diammonium salt (ABTS) with potassium persulphate. The mixture was left to stand for 24 h, until the reaction was completed and then ABTS solution was diluted by PBS to obtain the absorbance of 0.800 ± 0.03 at λ=734 nm. Fifty microliters of appropriately diluted methanolic extract of seeds or sprouts was mixed with 6 mL of the ABTS%+ solution and the absorbance of the resulting solution was measured after 15 min at λ=734 nm. Antioxidant activity (AA) was expressed as mg of Trolox equivalents per g of d.m. of seeds and sprouts.

Statistical analysis

The statistical analyses of the obtained values were conducted using the 'JUMP' statistical software program, and the differences between the means were tested using the Duncan multiple comparison method. One way analysis of variance – ANOVA and Tukey's HSD comparison of means of samples were used for analyzing variations. Correlations among studied traits were determined by Pearson's pairwise correlations using the "corrplot" package of R software. Interrelations of factors (storage periods and spermidine doses) and traits were determined by principal component analysis (PCA)with the "ggplot2" package of R software (Wickham, 2011). Heatmap analysis was performed with the R package "bioconductor" (Gentleman et al., 2004).

RESULTS AND DISCUSSION

Total fat and protein contents

In this study, it was observed that there were two distinct groups in terms of oil content values, with the highest oil content being 35.3% in the Newtürk variety and the lowest oil content being 32.2% in the Larnaka variety. When the variance analysis values of the trial are examined, it is observed that the difference in oil content values among the varieties is statistically significant at $p \leq 0.05$, while the difference in protein content is not statistically significant (Table 1). Čolovic et al. (2016) reported that in their study on the nutritional properties of 18 local flax varieties in Serbia, the oil content ranged from 34% to 40%, and the difference among varieties was significant, while the protein content ranged from 19% to 27%, and the difference among varieties was also significant. Similarly, Sauvant et al. (2004) and Sargi et al. (2013) found in their studies on different flaxseed genotypes that the protein content ranged from 21.8% to 22.6% and 23.2% to 24.4%, respectively, and the crude oil content ranged from 32.7% to 37.91% and 37.8% to 38.1%, respectively. This indicates that the differences in protein and oil content among flax varieties can vary depending on the variety and environmental conditions. There are some studies conducted to observe the health effects of flaxseed oil and protein. Dugani et al. (2008) reported in their study with rats that oil and mucilage obtained from flaxseed reduced the number and length of ethanol-induced gastric ulcers. Kaithwas & Majumdar (2010) also reported that flaxseed oil showed a significant inhibitory effect on gastric secretion/total acidity and aspirin-induced gastric ulceration in rats. Clark et al. (2001) investigated the effect of flaxseed oil acids on lupus nephritis disease and found that plasma lipids and serum viscosity did not change, but serum creatinine decreased in some patients. Dupasquier et al. (2007) conducted a study to see the anti-atherogenic effect of flaxseed in rabbits and reported that rabbits fed with ground flaxseed supplementation on a cholesterol-enriched diet had reduced plasma cholesterol and saturated fatty acids, increased ALA plasma content, inhibited plaque formation in the aorta and aortic sinuses, reduced circulating cholesterol levels, and inhibited atherosclerosis at the cellular level through antiproliferative and anti-inflammatory effects.

Table 2. The average values of protein and oil contents in the varieties, the formed groups, and the variance analysis

Varieties	Oil Content (%)	Protein Content (%)
Cill 1351	33.33 ± 0.75 ab	24.32 ± 0.66 a
Cill 1370	33.50 ± 0.61 ab	19.16 ± 2.73 a
Cill 1400	33.17 ± 0.67 ab	21.14 ± 3.67 a
Cill 1412	33.23 ± 0.29 ab	23.71 ± 0.14 a
Cill 1423	32.93 ± 1.22 ab	21.39 ± 2.27 a
Dilman	34.03 ± 0.50 ab	23.09 ± 2.82 a
Larnaka	32.20 ± 1.30 b	23.77 ± 1.96 a
Milas	34.07 ± 0.68 ab	23.69 ± 1.55 a
Newtürk	35.30 ± 0.46 a	23.28 ± 2.83 a
Sarı 85	33.53 ± 1.20 ab	23.20 ± 2.36 a
$HSD_{0.05}$		6,74
Varyans analizi		n.s

**indicates p≤0.05. ns: not significant.*

Like other plant products, flaxseed proteins also interact with other components and possess techno-functional properties in the food system. The amino acid distribution of protein in flaxseed is reported to be similar to that of soybean protein (Rabetafika et al., 2011). Flaxseed, like many other plant protein sources, is not recommended as a sufficient protein source because it lacks some essential amino acids. However, it can be recommended as a supplementary protein source. Flax seeds and flaxseed meal contain approximately 21% and 34% protein, respectively. It is reported that the protein content can vary depending on genetic and environmental factors (Chung et al., 2005).

Since there was no statistically significant difference in protein content among the varieties evaluated in this study, it can be said that variety preference will not lead to differences in the amount of protein intake. However, it may lead to differences in oil content. It can be suggested that consuming the seeds of flax varieties with higher oil content would be a more suitable option to derive greater biobenefits from flaxseed oil's beneficial effects (Table 1).

Fat acids contents

When the obtained findings are examined, it is observed that there is a high degree of variation among varieties in terms of fatty acid composition. Due to the difference in α-linolenic acid (C18:3) content, which stands out for its health effects, four different groups have been formed among the varieties. The highest α-linolenic acid (C18:3) content was found to be 53.9% in the Newtürk variety, while the lowest α-linolenic acid (C18:3) content was obtained from the Cill1423 variety with 46.8%. In light of the findings obtained in this study, it can be seen that variety preference is important to derive greater biobenefits from flaxseed oil's beneficial effects (Table 3). Čolovic et al. (2016) reported in their study with 18 local flax varieties in Serbia that they found α-linolenic acid (C18:3) content in the range of 42.9% to 61.0%. In other similar studies, Bean & Leeson (2002) found α-linolenic acid (C18:3) content in the range of 51.5% to 59.3% in a study with 23 flax genotypes, and El-Beltagi et al. (2007) reported α-linolenic acid (C18:3) content in the range of 46.0% to 50.7% in a study with five flax genotypes. The values obtained in this study fall within the range of values found by researchers, and it is also observed that there are genotypes with higher α-linolenic acid (C18:3) content among the genotypes examined.

When evaluated together with other foods containing α-linolenic acid (C18:3), which is important for human and animal health, such as fish and walnuts, it can be said that flaxseed would be a cheaper option. This is because fish and walnuts are not accessible in many countries, and they are also more expensive compared to other foods. Flax is a field crop that can be grown in almost every part of the world. In a study conducted by Bağcı et al. (2023) in the ecological conditions of Ankara (318 mm of rainfall), it is reported that the Cill 1412 variety yielded 180 kg/ha of seeds, 59 kg/ha of oil, and 42.9 kg/ha of protein. Since the αlinolenic acid (C18:3) content in the oil of the Cill 1412 variety is 47.3%, the amount of αlinolenic acid (C18:3) that can be obtained per hectare will be around 27 kg. The total omega3 fatty acid content in fish oil, which has an omega-3 fatty acid content of approximately 3366%, is around 1.95% for α-linolenic acid (C18:3) (Mattos et al., 2004; Malayoğlu et al., 2009). When the same amount of α-linolenic acid (C18:3) is desired to be obtained from fish oil, it is seen that 14 times more fish oil needs to be consumed. However, it is necessary to continue consuming fish to obtain other omega-3 fatty acids found in fish oil, such as stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid. The daily requirement for α-linolenic acid (C18:3) for an adult woman is 1.1 g, while this amount is around 1.6 g for adult men (Pandohee, 2022). Taking these amounts into account, consuming 7 g/day for women and 10 g/day for men from flaxseed, which contains 30% oil, will meet their α-linolenic acid requirements.

Table 3. Average values, formed groups, and variance analysis for fatty acid compositions (%)

Varieties	α-linolenic acid (C18:3)	Linoleic acid (C18:2)	Oleic acid (C18:1)	Palmitic acid (C16:0)	Stearic acid (C18:0)
Cill 1351	49.20 ± 0.60 bcd	13.50 ± 0.10 f	25.20 ± 0.50 bcd	6.53 ± 0.15 a	5.60 ± 0.10 cd
Cill 1370	46.00 ± 0.00 d	15.53 ± 0.06 b	25.93 ± 0.06 ab	6.30 ± 0.00 abc	6.20 ± 0.00 a
Cill 1400	47.90 ± 0.50 bcd	11.60 ± 0.10 h	28.50 ± 0.60 a	5.97 ± 0.06 cd	6.00 ± 0.00 abc
Cill 1412	47.33 ± 0.45 cd	16.13 ± 0.35 a	25.43 ± 0.55 bc	6.20 ± 0.10 abc	4.90 ± 0.20 e
Cill 1423	46.80 ± 0.30 cd	14.80 ± 0.20 cd	26.70 ± 0.10 ab	6.37 ± 0.15 ab	5.33 ± 0.06 d
Dilman	51.03 ± 0.85 ab	15.30 ± 0.10 bc	22.83 ± 0.65 cd	6.03 ± 0.06 bcd	4.80 ± 0.10 e
Larnaka	49.13 ± 0.15 bcd	14.03 ± 0.06 ef	24.80 ± 0.20 bcd	6.30 ± 0.00 abc	5.70 ± 0.00 bcd
Milas	49.70 ± 0.20 bc	12.67 ± 0.06 g	25.27 ± 0.35 bcd	6.33 ± 0.06 ab	6.03 ± 0.06 ab
Newtürk	53.90 ± 3.80 a	12.13 ± 0.35 gh	22.53 ± 2.85 d	5.83 ± 0.25 d	5.63 ± 0.35 bcd
Sarı 85	48.07 ± 0.25 bcd	14.33 ± 0.15 de	25.43 ± 0.06 bc	6.43 ± 0.06 a	5.70 ± 0.10 bcd
$HSD_{0.05}$	3.68	0.54	2.84	0.33	0.41
Varyans analizi	**	**	**	**	**

***indicates p≤0.001.*

Antioxidant and phenolic compound contents

In this study, statistically significant differences were observed among varieties in terms of phenolic compound and total antioxidant contents (p≤0.001). According to the research, Cill 1351 (4.08 mg trolox/g) and Dilman (4.16 mg trolox/g) varieties had higher antioxidant capacities compared to other varieties. Among flaxseed varieties grown under the same ecological conditions and cultural practices, Çili 1370 was found to have the lowest antioxidant capacity (Table 3). Pająk et al. (2019) reported antioxidant activities of flaxseeds ranging from 0.70 to 6.79 mg trolox/g. The same researchers emphasized that flaxseeds have high antioxidant activities and stand out in this regard compared to many other plants. Similar findings have been reported by other researchers as well (Wang et al., 2017; Kosiorowska et al., 2022). The findings obtained in these studies are consistent with our data, and it is evident that antioxidant values vary among varieties. When the phenolic compound contents of flaxseed varieties were examined, ferulic and protocatechuic acids were found to be the most abundant compounds in flaxseeds. In light of the results, the highest ferulic acid content was determined in Çili 1351 variety at 18.51 µg/g. Protocatechuic acid was detected as the highest in Çili 1423 variety at 20.83 µg/g (Table 3). Generally, caffeic acid was lower than other phenolic compounds. In a study by Wang et al. (2017), they reported that the ferulic acid content of Zhongya2 flaxseed variety was 21.13 µg/g, and the p-coumaric acid content was 9.29 µg/g. In another similar study, the ferulic acid content in flaxseeds was determined as 0.11 mg/100g (Pajak et al., 2019). Phenolic compounds are chemical compounds that perform many important biological functions in plants, such as growth, development, defense, and protection against environmental stresses. Phenolic compounds are an important source of antioxidants for plants (Kaviarasan et al., 2007; Pandey & Rizvi, 2009; Randhir et al., 2004). In this study, it was determined that flaxseed varieties commonly cultivated in Turkey are rich in phenolic compounds, and these varieties are genetically valuable materials in terms of biochemical content compared to the literature.

Table 4. Average values of phenolic compound (µg/g) and antioxidant (mg/ trolox/g dry weight) contents, groups formed and analysis of variance.

Cultivars	Caffeic	Ferulic	Gallic	P-Coumaric	Protocatechuic	Quercetin	Total Antioxidant
Cill 1351	1.56 ± 0.09f g	18.51 ± 0.70 a	6.14 ± 0.06 a	2.20 ± 0.13 d	3.26 ± 0.08 ı	3.11 ± 0.06 e	4.08 ± 0.11 a
Cill 1370	2.72 ± 0.15 e	15.11 ± 0.62 b	5.49 ± 0.09 b	5.15 ± 0.10 c	5.80 ± 0.14 h	2.53 ± 0.06 f	2.22 ± 0.08 d
Cill 1400	1.14 ± 0.05 g	9.69 ± 0.40 d	4.86 ± 0.10 c	6.48 ± 0.19 c	12.42 ± 0.32 c	6.07 ± 0.06 a	3.50 ± 0.08 b
Cill 1412	5.58 ± 0.12 b	12.01 ± 1.34 c	3.57 ± 0.09 de	13.96 ± 1.13 ab	7.45 ± 0.64 fg	4.84 ± 0.06 b	2.84 ± 0.11 c
Cill 1423	1.87 ± 0.16f	9.54 ± 0.13 d	2.84 ± 0.15f g	1.78 ± 0.06 d	20.83 ± 0.75 a	4.65 ± 0.09 bc	3.62 ± 0.06 b
Dilman	6.40 ± 0.11 a	11.60 ± 0.44c d	3.21 ± 0.12 ef	15.88 ± 0.90 a	15.28 ± 0.45 b	4.06 ± 0.10 d	4.16 ± 0.08 a
Larnaka	2.57 ± 0.18 e	6.48 ± 0.11 e	2.51 ± 0.11 g	6.11 ± 0.08 c	8.56 ± 0.21 ef	2.17 ± 0.07 g	3.67 ± 0.09 b
Milas	4.79 ± 0.23 c	5.82 ± 0.21 e	1.53 ± 0.06 h	12.84 ± 0.70 b	6.43 ± 0.18 gh	1.72 ± 0.08 h	2.89 ± 0.06 c
Newtürk	1.80 ± 0.13 f	12.40 ± 0.35 c	3.80 ± 0.14 d	12.01 ± 0.65 b	9.62 ± 0.16 de	4.43 ± 0.08 c	2.31 ± 0.08 d
Sarı 85	3.80 ± 0.11 d	11.78 ± 0.40 cd	3.50 ± 0.12 de	12.92 ± 0.35 b	10.61 ± 0.56 d	6.25 ± 0.18 a	2.79 ± 0.11 c
HSD $_{0.05}$	-0.55	-2.29	0.42	-2.23	-1.63	-0.36	-0.35
Varyans analysis	***	***	***	***	***	***	***

*** *indicates p≤0.001.*

Determination of interrelationships between bioactive compounds and flax cultivars by PCA and Hatmap

In the statistical analysis conducted to determine the correlation between the biochemical compound contents of flaxseed varieties, a positive correlation was found between total oil content and α-linolenic acid (r=0.72, p≤0.05). However, a negative correlation was observed between the dominant α-linolenic acid and oleic acid in flaxseeds (r=0.78, p≤0.01). Among the fatty acids, a parallel relationship was found between palmitic acid and stearic acid, oleic acid, and linoleic acid, while generally, a negative correlation was found among the other fatty acids (Figure 1). When phenolic compounds were examined, the highest positive correlation was determined between gallic acid and ferulic acid (r=0.87, p≤0.001). A statistically significant positive relationship was observed between caffeic acid and p-coumaric acid at the r=0.80 level. A positive relationship was found between total antioxidant and protein, while a negative correlation was found between total oil.

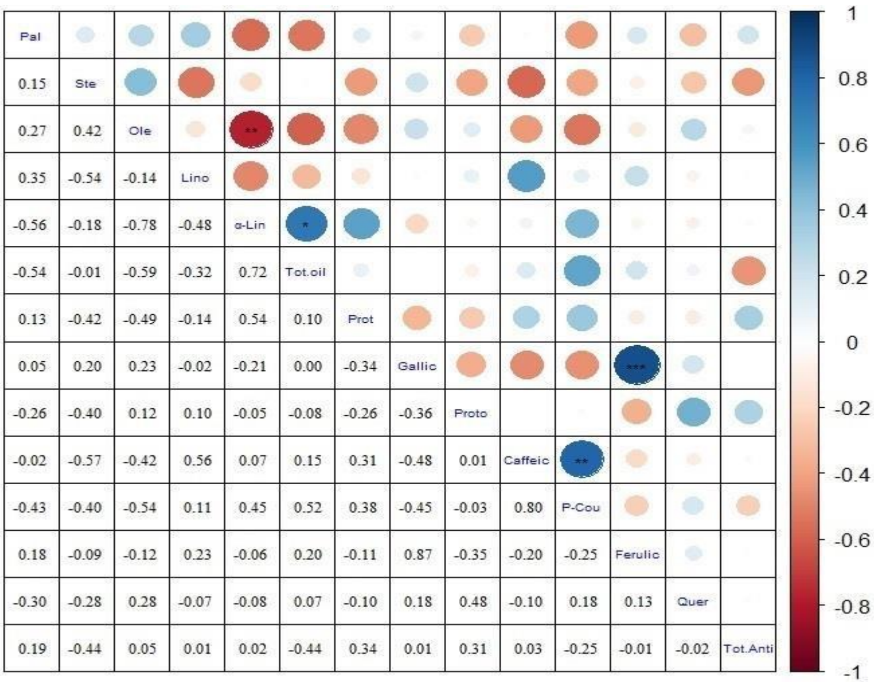

Figure 1. Variation among oil acids, protein, antioxidant and phenolic compounds. The color scale fading from red to blue indicates correlation values from -1 to +1, and the circle size illustrates the redundancy of the correlation. *,**, and *** indicates significance at p≤0.05, p≤0.01, and p≤0.001, respectively. Pal: palmitic, Ste: stearic, Lino: linoleic, α-Lin: α-linolenic acid, Tot.Oil: total oil, Prot: protein, Proto: protocatechuic acid, P-Cou: p-coumaric acid, Quer: quercetin, Tot.Anti: total antioxidant.

Principal Component Analysis (PCA) was conducted in this study to reveal the distribution of biochemical compounds according to flaxseed varieties (Figure 2). PCA is a statistical analysis method that reveals the statistical significance levels of study data and enables the evaluation of results from a scientific perspective. According to the PCA method, the correlation among fatty acids was measured as 71.2% (PC1+PC2). The correlation between phenolic compounds and antioxidants was determined as 60.4% (PC1+PC2). Newtürk and Dilman varieties stood out in terms of total fatty acid and total antioxidant content, respectively. In terms of protein content, Çilli 1351 variety exhibited superior characteristics compared to other varieties. The study found that the ferulic acid content of flaxseed varieties was higher than other phenolics, with Çilli 1351 and Çilli 1370 varieties standing out in terms of ferulic acid content.

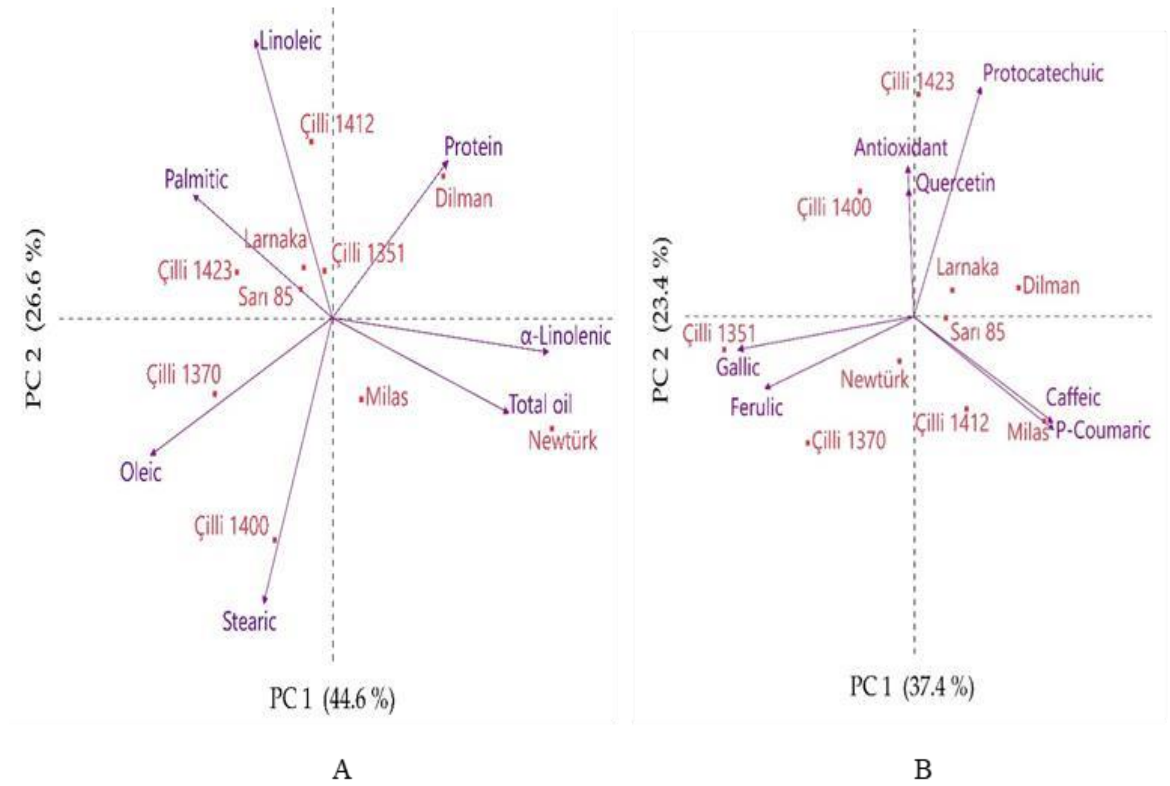

Figure 2. Characterization of the distribution of fatty acids, protein (A) and antioxidants, phenolic compounds (B) in different cultivars of flax using PCA.

According to the Hatmap analysis, in the cluster analysis of biochemical compounds and flaxseed varieties, the Newtürk variety formed a separate group from the other varieties. Çilli 1370 and Çilli 1351 were clustered together, while the other varieties formed a separate group (Figure 3). When looking at the distribution of biochemical compounds according to flaxseed varieties, it can be seen that essentially two groups have formed. Gallic acid and ferulic acid were classified in the group containing fatty acids, while total antioxidants and proteins were classified in the group containing phenolic compounds. In terms of total fatty acids and α-linolenic acid content, the Newtürk variety stood out. Regarding total antioxidant content, the Dilaman and Çilli 1351 varieties exhibited superior characteristics compared to the other varieties.

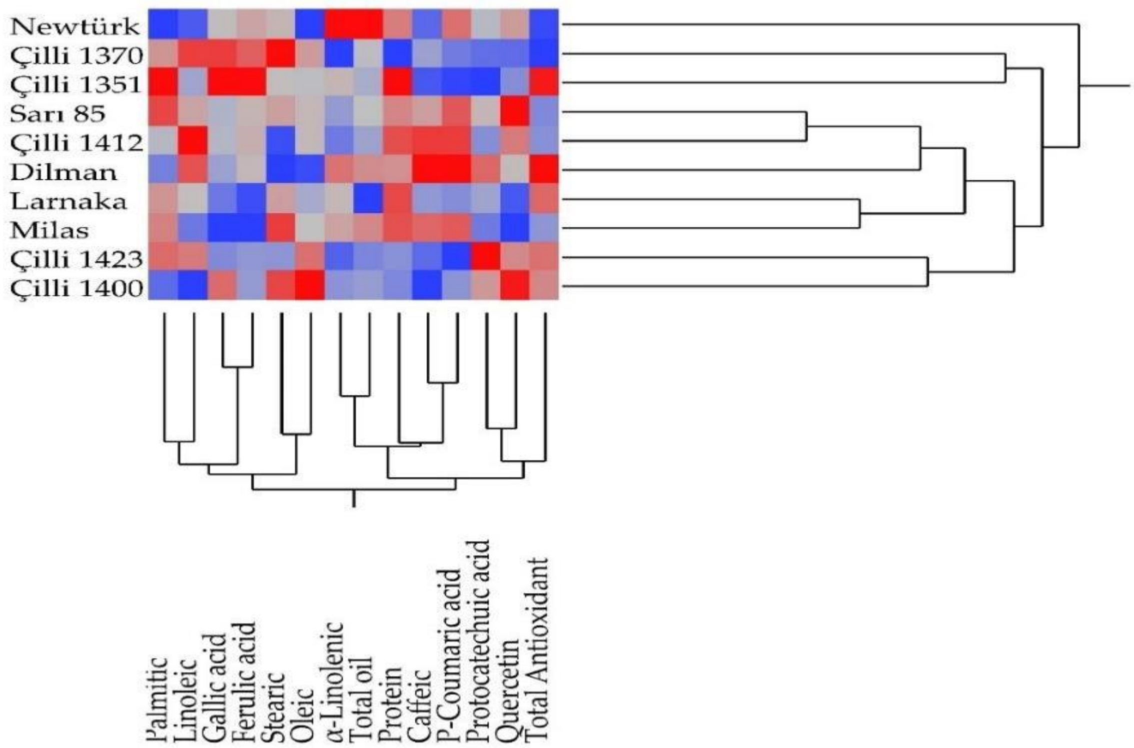

Figure 3. Correlations of biochemical compounds in different cultivars of flax using Hatmap analysis.

CONCLUSION

In this research where the biochemical compounds of flaxseeds, commonly used in various applications, were determined, Çilli 1351 variety showed promise in terms of phenolic compounds, total antioxidants, and protein content. The Newtürk variety stood out in terms of α-linolenic acid content, one of the most abundant fatty acids in flaxseeds. While no significant difference was found among varieties in terms of protein content according to the statistical analysis, it was determined that other biochemical contents showed significant variations among varieties. Ferulic acid and gallic acid, which are among the most abundant phenolics in flaxseeds, were classified in the group formed by fatty acids according to the Hatmap analysis. In conclusion, in this research conducted in the same ecological conditions and under the same cultural practices, the classification of flaxseed varieties was made in terms of biochemical contents. In this regard, especially the Newtürk and Çilli 1351 varieties showed superior performance.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that there is no conflict of interest between them.

Author contribution

The authors declare that they have contributed equally to the article and have not plagiarised.

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