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Fatty acid and organic acid compositions of some Türkiye registered flax (*Linum usitatissimum* L.) varieties grown under alkaline soils

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Abstract

Flax (*Linum usitatissimum* L.) is an industrial plant that is used for multi-purposes in the world with its oil and fibers properties and have commercial importance. Flaxseed oil, besides being an alternative oil product, is an important additive in functional foods and animal feeds. In addition, it is rich in α-linolenic acid (ALA), lignans, proteins, dietary fibers and organic acids. Owing to its significant functions, the present study was designed to investigate the fatty acid and organic acid composition of flax cultivars (Karakız, Milas, Beyaz Gelin, Sarı-85, Konya Kahve, Clli1392, Clli1355) under alkaline stress conditions (≈pH:9,70). Accordingly, with respect to oil yield, highest yield was noted for Sarı-85 with a value of 2.28 g, whilts the lowest value (0.84 g) was recorded for Clli1392. Regarding oil components, in parallel to the yield, the highest percentage of α-linolenic acid was observed in Sarı-85 (60.51%) and the lowest value of the relevant compound was ascertained in Karakız (39.49%). In relation the profile of organic acid compounds; Clli 1355 were rich in succinic acid (46.705 ng/ul), lactic acid (35.238 ng/ul) and acetic acid (176.494 ng/ul), whilst Konya Kahve and Sarı-85 were found to be rich in propionic acid, 214.232 ng/ul and butyric acid, 32.895 ng/ul, respectively. In order to reduce the dimension, correlate and visualize the assayed parameters, the relevant data of the study was subjected to principal component analysis and heat-map clustering. The clear discrimination and scattering among the cultivars corresponding to the parameters were observed.

Keywords

Linseed, Salt stress, Alkaline soil, Abiotic stress, α-linolenic acid (ALA)

Introduction

The food sources needed by human beings cause a serious decrease due to the increasing world population and the pollution of natural resources. Because of the continuation of the current status, human beings have changed their tendencies concerned with the required/desired nutritional needs. For this reason, the need for herbal products is increasing rapidly (Van Der Roest et al., 2007; Bennett et al., 2012; Moghaddam et al., 2018; Koçak, 2021). Herewith, in order to for people to develop the agriculture-related industry and to meet their needs to, the raw material must be met at a sufficient level from the available resources, as well as the plant variety to be selected should be suitable (Miller, 2011; Mert, 2017; Eryılmaz and Kılıç, 2018). From the past to the present, human beings have naturally switched to agricultural production for their absolutely necessary activities when the sustainability of herbal products and the needs of the increasing population density cannot be met (Ruttan, 1999; Rocha et al., 2021). In present situation, the production started with many plants and one of them, the flax, started to be cultivated.

The flax cultivation plant dates back to 3500 and 4000 BC in Mesopotamia and Egypt (Richards et al., 2003; Duguid et al., 2007; Rocha et al., 2021). Flax is an annual, herbaceous and self-pollinating industrial plant belonging to the Linaceace family with 22 genera and 300 species and it is a diploid species with (2n=30) chromosomes distributed in temperate regions (Choudhary et al., 2017a; Goudenhooft et al., 2018; Tchoumtchoua et al., 2019; Landoni et al., 2020; Talebi and Matsyura, 2021; Saroha et al., 2021). Flax can be called by many different names. In Türkiye, local names such as "bezir, bızıktan, cimit, kön, siyelek and zeyrek" are used for the flax plant (Dumanoğlu, 2020).

With respect to the researches on fatty acid composition, a quite number of findings have been reported (El-Beltagi et al., 2007; Nitrayová et al., 2014; Göre and Kurt, 2021; Koçak, 2021). Accordingly, flax, an alternative oil plant and contains 35-65% oil in its structure and is used in different industries (preparation of varnish, paint, soap, paste and polymers) apart from food use. In addition to being rich in oil compositions such as flax, α-linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid, it also has high amounts of lignan, protein and fiber (Wang et al., 2017; Xie et al., 2020; Göre and Kurt, 2021; Koçak et al., 2022). Therewithal, it is used in cancer treatments (breast, blood, colon and skin, etc.) as it is an important source such as omega-3, omega-6, lignan and protein in its structure (Singh et al., 2017; Tarhan et al., 2021; Toulabi et al., 2021; Hamed et al., 2022).

Whole plants are affected by known environmental conditions resulting from their development. Nevertheless, they are exposed to biotic and abiotic stress factors throughout their life span. They develop an alternative defense mechanism that includes a wide variety of secondary metabolites to cope with different stress conditions. Inadequate mechanisms then pose serious threats to agricultural practices and global food security due to these factors (Khare et al., 2020; Shah and Smith, 2020). Increasing experimental evidence over the last 20 years has associated organic acid metabolism with plant tolerance to environmental stress. Its indicate that organic acids act not only as intermediates in carbon metabolism, but also as key components in the mechanisms some plants use to cope with nutrient deficiencies, metal tolerance, and plant-microbe interactions operating at the root-soil interphase (Lopez-Bucio et al., 2000).

Salinity and alkalinity, which are abiotic stress factors, are one of the main factors that impose great limitations on the growth, development, grain productivity and quality of crops in agriculture worldwide (Acosta-Motos et al., 2020; Kulak et al., 2020; Umar et al., 2021; Koçak et al., 2022). Soil is an essential component of Earth's terrestrial ecosystems. Significantly acidic or alkaline soils take up a significant portion of the earth's land area. In addition, in alkaline soils, Na^+, K^+, Cl^- and NO^- ionic forms predominate with increasing bicarbonate, sulfate, and bicarbonate forms. Soil salinity includes saline, sodic, and alkaline soils characterized by high salt content, high sodium (Na^+) content, high pH, respectively. In addition, in alkali-saline, alkali-saline-sodic and alkali-sodic soils, the predominance of bicarbonate and carbonate types cause toxicity effects on plants. (Rengasamy, 2010; Zewd and Siban, 2021). Herewith, as in saline-alkaline soils, salinity soil causes an account of high osmotic stress and ionic stress in plants, which leads to nutrient variability, but also reduces the photosynthesis capacity of the plant. As a result, it causes plant growth and development to stop (Zhang et al., 2022).

In the present study, it was aimed to determine the fatty acid compositions and yield/rates of organic acids of Türkiye registered flax cultivars in the trial area with high alkalinity and pH and low organic matter content.

Materials and Methods

Plant material and cultivation

For the study; some registered cultivars, *viz.* Karakız, Milas, Beyaz Gelin, Sarı-85, Konya Kahve, Clli1392, and Clli1355 were used and grown under high alkaline conditions. The relevant field trials were carried out at Igdir University Agricultural Research and Application Center (Figure 1). The experimental area is characterized with strongly alkaline (pH: 7.50-9.70), very salty (total salt 33.0-82.44) (%), organic matter (very little) (0.425-1.06) %, light (moderate) chalky ($CaCO_3$ amount 1.32-9.29) (%) (Şimşek et al., 2016; Karaoğlu and Yalçın, 2018; Temel and Keskin, 2022).

Figure 1. Soil structure of the trial area

Oil extraction from flaxseed

The oil extraction was done according to the method of Khattab and Zeitoun (2013) with some minor modifications. Briefly, 5 g of dried and powdered seed samples were extracted using 15 ml hexane.

Oil yield

The oil yield extracted from flaxseed was calculated as follows.

Yield (%) = $\frac{We}{Wt}$ x 100 (Gutte et al., 2015; Capar et al., 2021).

W_e = weight of extracted oil and W_t = weight of sample taken for extraction.

Detection of fatty acid composition by gas chromatography flame ionization detector (GC-FID)

The samples prepared for analysis were taken from 0.2 g of the oils of the flax samples into 15 ml capped centrifuge tubes in Igdir University Research Laboratory Application and Research Center (ALUM), and 10 ml of hexane was added to them and shaken well. Subsequently, KOH prepared by dissolving in 0.2 mL of 1 N methanol was added in the tubes containing the samples. Afterwards, it was shaken well, phase separation was observed, and it was kept in the dark for 2 hours until the upper phase became clear. After clarification, some amount of the upper phase was taken into vials and fatty acids were analyzed by Agilent 7820 A GC-FID device with SP 2560 100m*0.25mm*0.2µm capillary column in Gas Chromatography Flame Ionization Detector (GC-FID). Injection port and FID temperature is 240 ºC, 1/10 split ratio at 400 ml/min pressure in split injection mode. After waiting for 5 minutes at 140 ºC, the column temperature increased by 4 ºC per minute to reach 250 ºC, and after waiting for 15 minutes, it was increased to 260 ºC. Helium carrier gas was used as 41 cm/sec (Hydrogen). Samples injected with 1 µL into the device were compared with the GC-FID chromatogram obtained in the analysis of the "Supelco ® 37 Component FAME Mix-Sigma-Aldrich" standard mixture for a total of 37.75 minutes.

Organic acid extraction in flaxseed

The organic acid extraction was done according to the method of Pellegrini et al. (2018) with some minor modifications. Briefly, 5 g seeds were weighed, milled and extracted using methanol.

Detection of organic acid compounds by high performance liquid chromatography (HPLC)

The prepared flaxseed samples were filtered through 0.45 µm filter discs before High Performance Liquid Chromatography (HPLC) analysis of the methanol extracts of the samples at Igdir University Research Laboratory Application and Research Center (ALUM). The mean values of organic acids such as (succinic acid, lactic acid, acetic acid, propionic acid and butyric acid) were determined in the analysis. In this context, a (High Performance Liquid Chromatography (HPLC)) system (Agilent 1260; Agilent, Santa Clara, CA, USA) equipped with a diode array detector was used. The results were obtained by separating organic acids, analyzes with 10 µL of extract in a thermostated column (Hi-Plex H, 7.7 x 300 mm, 8 µm; Germany) at 70 °C.

Statistical analysis

The data of the study was subjected to principal component analysis (PCA) (Jamovi-Stats) and heat-map clustering (ClustVis) to visualise, correlate and discriminate the parameters (fatty acid and organic acids, as well as cultivars).

Results and Discussion

Oil ratio and fatty acid composition of the cultivars are collectively presented in Table 1, with their chromatograms in Figure 2, principal component analysis (Figure 3) and heat map clustering (Figure 4). Similarly, organic acid findings are given in Table 2, and Figure 5-7.

Crude oil yield;

In most living species, carbohydrates, proteins and fats, which are essential for the survival of living organisms, are important building blocks and the most important energy sources. The relevant oil has significant uses in raw materials in the food and industrial industry. Furthermore, it is also used animal feed. Corresponding to the critical functions, the species has gained a great attention with respect to the its cultivation. Flaxseed, like many oil seeds, is a product with a high oil content. (Turin et al., 2021). Along with the study, the average values of oil yield for cultivars were 25.28%. Considering the yield, the highest oil yield was recorded in Sarı-85 with a value of 45.6% (2.28 g/ 5 g and the lowest values (16.8%; 0.84 g/ 5 g) was noted for Clli1392 (Table 2). The present findings are consistent with the former reports (Tanman, 2009; 40.9%; Bayrak et al., 2010; 48.08%; Endes, 2010; 45.4%). In particular, Sarı-85 variety is the first registered one and therefore, is the most investigated. The current findings are similar to the previous reports (Kurt et al., 2006; 41.78%; Endes, 2010; 45.4%; Tayınmak, 2019; 47.29%; Keskin et al., 2020; 34.1%). However, yield per decare is lower than the previous reports (Tunçtürk and Tunçtürk, 2021). The decline might be explained with high level of alkalinity, poor organic and inorganic content.

Fatty acids composition;

Oil samples in flaxseeds are mostly known as monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids. Fatty acids have been used in many studies due to their significant effects and multiple biochemical roles, as well as the potential to reduce significant disease states (Orsavova et al., 2015; Xie et al., 2020). Generally, in addition to oil plants; the flax is also characterized by fatty acids such as α-linolenic acid (C18:3), linoleic acid (C18:2), oleic acid (C18:1), palmitic acid (C16:0) and stearic (C18:0). In addition, it is known that the saturated or unsaturated properties of fatty acids and their ratio to each other are of great importance in both human consumption and adaptation of the plant to the environment (Suri et al., 2020; Wu et al., 2020).

In the present study, α-linolenic acid was determined to be highest in Sarı-85 (60.51%), and the lowest in α-linolenic acid maintenance in Karakız (39.49%). According to previous studies, it has been determined that Sarı-85 is high and Karakız is low (Symoniuk et al., 2017; 44.90%; Liang et al., 2021; 41.21%; Njembe et al., 2021; 55.35%). In addition, linoleic acid ratio was 17.4% for Konya Kahve variety. Those findings are consistent with previous reports (Zhang et al., 2011; 15.37%; Hatanaka et al., 2021; 14.72%).

Table 1. Oil and fatty acids ratios of flax cultivars

Variety	Oil content (g)	Crude oil yield (%)	Palmitic acid (C16.0)	Stearic acid (C18.0)	Oleic acid (C18.1)	Linoleic acid (C18.2)	α-Linolenic acid (C18.3)
Karakız	1.03	20.6	11.64	7.12	24.75	14.83	39.49
Milas	2.09	37.8	6.55	7.31	26.17	15.24	44.71
Beyaz Gelin	0.87	17.4	8.04	7.68	20.99	17.18	46.26
Sarı-85	2.28	45.6	6.64	4.84	15.89	12.09	60.51
Konya Kahve	0.96	19.2	8.86	4.67	20.79	17.4	46.58
Clli1392	0.84	16.8	6.54	6.38	26.91	14.81	45.77
Clli1355	0.98	19.6	6.19	4.76	26.41	12.27	50.1

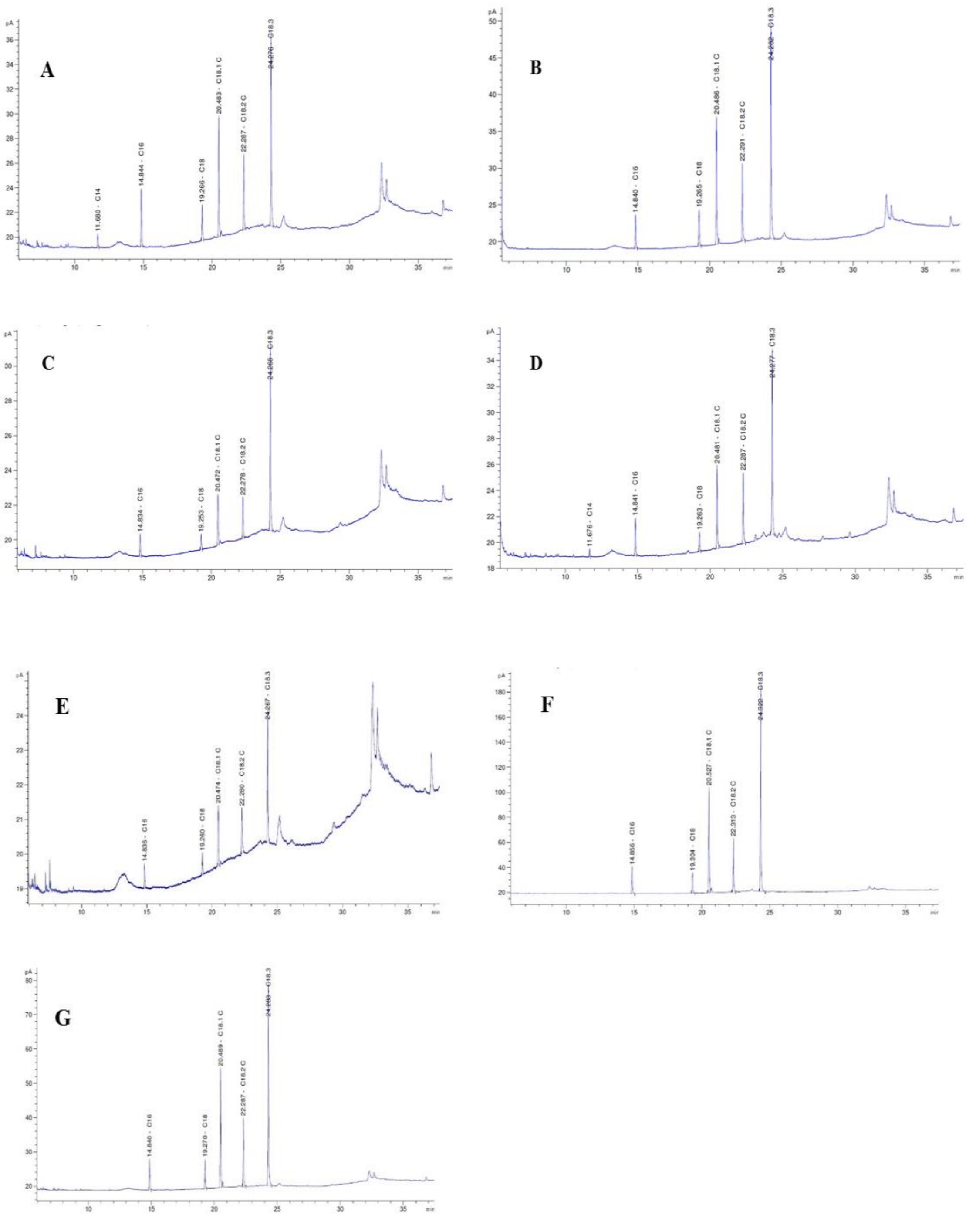

Figure 2. GC-FID fatty acids chromatograms of flax cultivars (A) Karakız, (B) Milas, (C) Sarı-85, (D) Konya Kahve, (E) Beyaz Gelin, (F) Clli 1355 and (G) Clli 1392

Oil yield and fatty acid compositions of flax cultivars with the help of principal component analysis (PCA) and heatmap

According to the Principal Component Analysis (PCA) analysis results, it is seen that the 14 variables included in the analysis are grouped under three factors with an Eigen value greater than 1. The first factor (PC1 $_{\text{Eigen value}}$:3.675) of the factors determined as important explains 52.5% of the total change in fatty acid composition, and the second factor (PC2 $_{\text{Eigen value}}$:1.281) explains 18.3% (Figure 3). These two factors explain 70.8% of the total change. Such a high disclosure rate indicates that fatty acid components can be differentiated according to varieties. In addition, similarly correlated cultivars or investigated parameters are concentrated in similar regions/axes. The oil yield and content are of great industrial importance. In our present findings, the highest oil yield was obtained in Sarı-85 and Milas cultivars. In addition to PCA analysis; according to the clustering results made with the help of heat map, two main clusters were observed (Figure 4). According to the evaluation of the varieties; While Konya Kahve, Karakız and Beyaz Gelin are in the same cluster, other varieties are grouped together. In the evaluation of fatty acid components; While oleic acid, stearic acid, linoleic acid and palmitic acid are in one group, parameters such as crude oil content, oil amount and α-linolenic acid are gathered in one group.

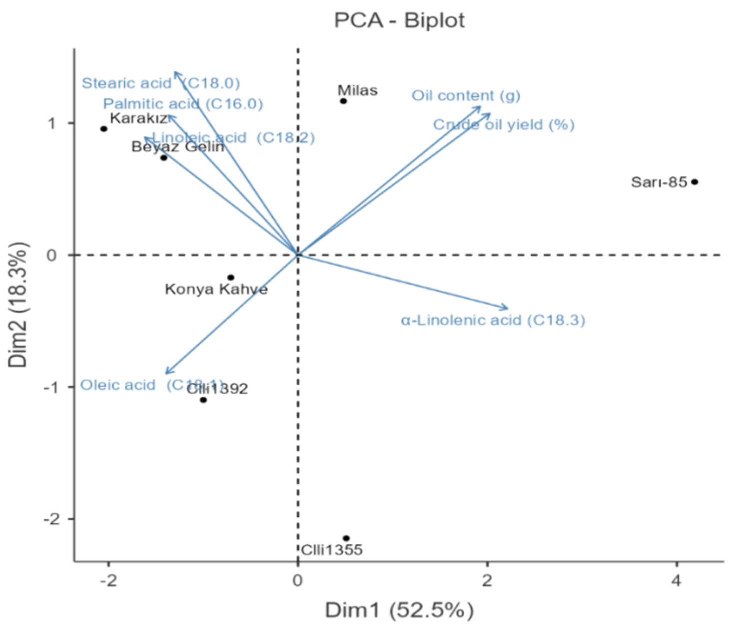

Figure 3. Principal component analysis of flax cultivars

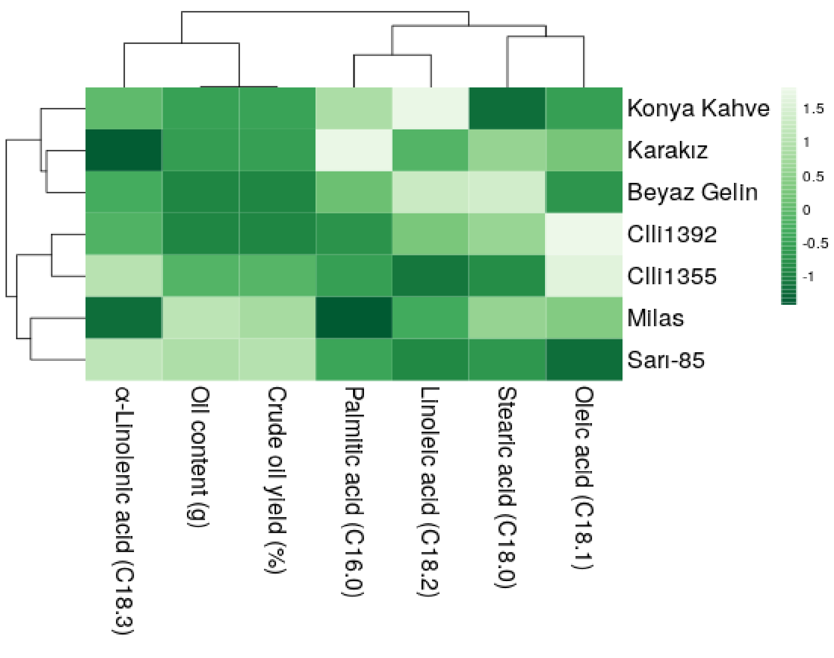

Figure 4. Heatmap clustering of oil and fatty acids of flax cultivars

Organic acids

Organic acids support many and varied functions in plants, exerting critical roles in response to the environmental stimuli as well as fruit formation and maturation. In particular, its substantial functions regarding nutrient deficiencies, some metal tolerance, and plant-soil organism interactions working between root and soil have been well-documented (Lopez-Bucio et al., 2000; Batista-Silva et al., 2018). Herewith the study, content of succinic acid, lactic acid, acetic acid, propionic acid, and butyric acid were quantified. According to the current findings, the highest average value was determined in acetic acid (105.53%). Among the average values in the organic acid profile of the related cultivars, the highest Clli 1523 (85.942%) was obtained. Butyric acid (32.895%) was determined as the highest value in Sarı-85 variety and propionic acid (214.232 %) was determined as the highest value in Konya Kahve in the current ratios. Considering the average values, the average major values of succinic acid and lactic acid of the related cultivars were determined as (24.802; 18.781%), respectively. Porokhovinova et al. (2022) reported that the average major values of succinic acid and lactic acid were 1.63%; 2.93%, respectively.

Organic acids and flax cultivars with the help of principal component analysis (PCA) and heatmap

According to the Principal Component Analysis (PCA) results given, it is seen that the 12 variables included in the analysis are grouped under 2 factors with an Eigen value greater than 1. However, the first factor ($PC1_{\text{Eigen value}}$: 2.953) of the important factors explains 59.1% of the total change in organic acid composition, and the second factor ($PC2_{\text{Eigen value}}$: 1.324) explains 26.5% (Figure 6). In addition, these two factors explained 85.6% of the total change. Such a high disclosure rate of the results indicates that the organic acid components can be separated according to the respective cultivars. At the same time, it is seen that the varieties with similar correlations or the investigated parameters are concentrated in similar regions/axes. In the evaluation of organic acids in our present findings, it was obtained that succinic acid, lactic acid and acetic acid were the highest in Clli 1355, respectively. In addition, it has been observed that Konya Kahve is high in propionic acid, while Sarı-85 is in butyric acid. In addition to the PCA analysis; According to the clustering results made with the help of the heat map, two main clusters were observed (Figure 7). In the related varieties; While Sarı-85, Milas and Clli 1392 cultivars were in the same cluster, it was determined that other cultivars were collected in a group. In the evaluation of organic acid components; while succinic acid and lactic acid are in a group; acetic acid and butyric acid were evaluated in the same group, while propionic acid was in a separate group, while other parameters were collected in one group.

Table 2. Organic acids and ratios of flax cultivars

Variety	Succinic acid	Lactic acid	Acetic acid	Propionic acid	Butyric acid
Karakız	19.258	14.636	69.233	35.16	6.199
Milas	15.349	11.769	66.668	40.014	14.117
Beyaz Gelin	20.122	15.022	59.986	86.869	10.535
Sarı-85	25.62	18.293	166.693	80.567	32.895
Konya Kahve	18.524	15.293	90.398	214.232	13.121
Clli 1392	28.04	21.218	109.268	14.052	31.2
Clli 1355	46.705	35.238	176.494	158.439	12.834

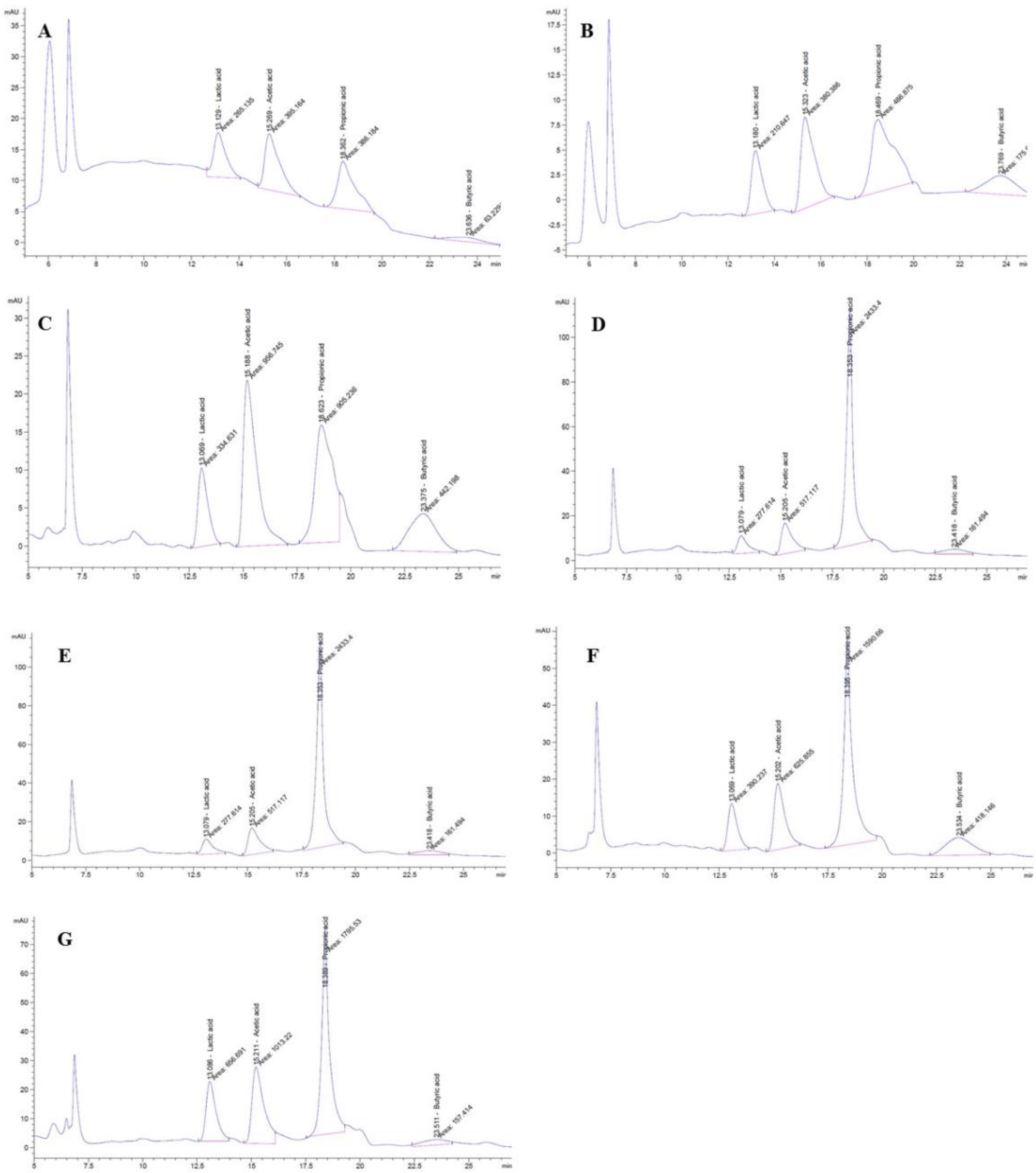

Figure 5. HPLC organic acid chromatograms of flax cultivars (A) Karakız, (B) Milas, (C) Sarı-85, (D) Konya Kahve, (E) Beyaz Gelin, (F) Clli 1355 and (G) Clli 1392

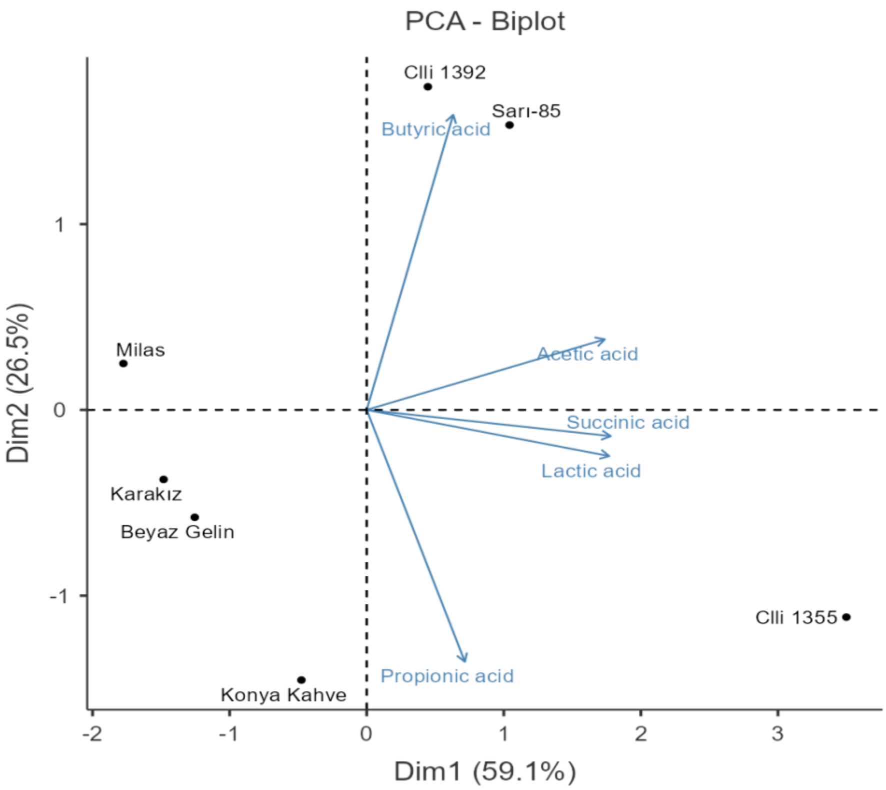

Figure 6. Principal component analysis of organic acids of flax cultivars

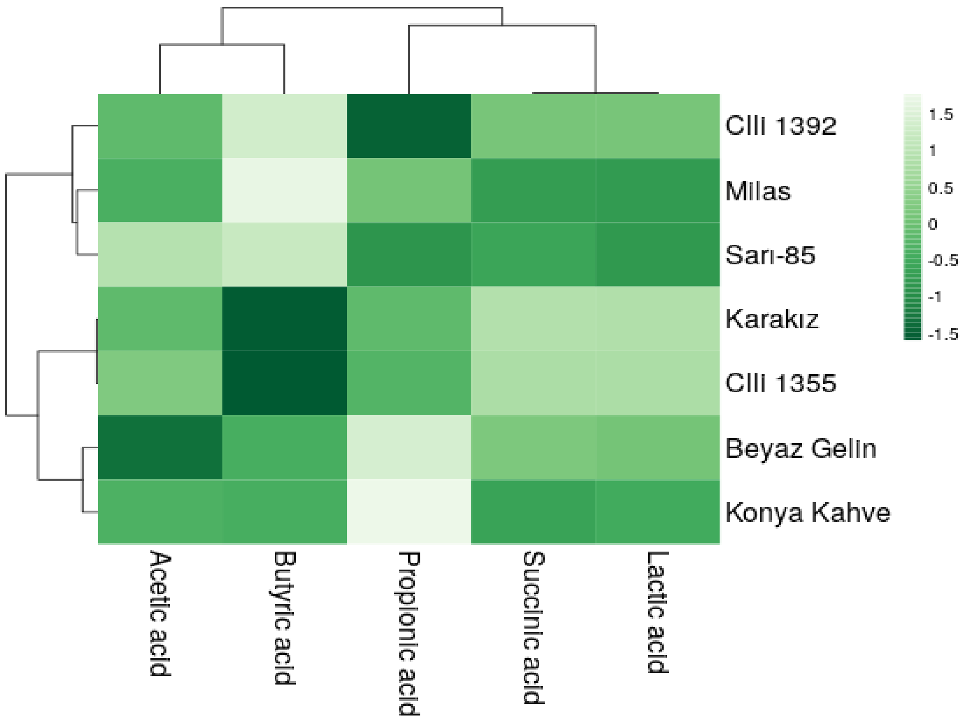

Figure 7. Heatmap clustering of organic acids of flax cultivars

Conclusion

Flax seeds are rich in fatty acids, organic acids and dietary fiber, protein, major phenolic compounds and flavonoids. However, it has faced the problem of losing its importance gradually. In order to increase the importance of the flax plant and to increase its production, the selection of flax varieties with oil properties suitable for the region is important in terms of diversifying markets for our country, which is a vegetable oil importer, and increasing the product range to be given to the farmer. In many countries, including our country, the fact that the harvested product obtained per unit area is low, as well as the wrong/deficiencies in the cultivation practices and the low market have reduced the importance of the flax plant. Our country is insufficient in the production of oilseed crop. For this reason, it is very clear that incentives and supports should be increased in order to reduce the crude oil deficit, prevent foreign dependency, increase the foreign exchange contribution to the country in order to export products with high added value, and produce oilseeds in order to be an important source of income for the farmer.

Compliance with Ethical Standards

Author contribution

The author verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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