Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Nitrogen Fertilization Effects on Oil Content, Sucrose, α- Tocopherol, Fatty Acid and Aminoacid Compositions of Confectionary Sunflower Seed

Öner Canavar[a], Hatice Kübra Gören[a*]

[a] *Aydin Adnan Menderes University, Faculty of Agriculture, Department of Field Crops, 09970, Aydin, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Hatice Kübra Gören, E-mail: hkubra.goren@adu.edu.tr
Received: 29 April 2024 / Revised: 13 August 2024 / Accepted: 03 October 2024 / Online: 14 January 2025

Cite this article
Canavar Ö, Gören H K (2025). Nitrogen Fertilization Effects on Oil Content, Sucrose, α- Tocopherol, Fatty Acid and Aminoacid Compositions of Confectionary Sunflower Seed. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 31(1):230-241. DOI: 10.15832/ankutbd.1475152

ABSTRACT
This study investigates the effects of nitrogen fertilization on the composition of confectionary sunflower seeds, including parameters such as oil content, alpha-tocopherol, sucrose, amino acids and fatty acid composition. Nitrogen fertilization, surprisingly, had no significant effect on sunflower oil content, with remarkable differences observed between the genotypes. The α-tocopherol content, an important antioxidant, displayed responses that were dependent on the genotype upon application of nitrogen. The Somon Beyazı genotype consistently demonstrated higher oil content compared to the Ahmet Bey genotype. In addition, both genotypes showed a decrease in α-tocopherol levels as the application of nitrogen increased. Sucrose content was higher in the Somon Beyazı genotype and decreased significantly with increasing nitrogen doses. Significant variations were observed in fatty acid compositions, further emphasizing the impact of genotype and nitrogen application. The study also revealed diverse amino acid profiles, with notable concentrations of glutamine and asparagine. This comprehensive study highlights the complex interaction between genotype and nitrogen fertilization and provides valuable insights for optimising sunflower seed production and quality. The results emphasise the significance of integrating both genetic factors and nutrient management practices into crop cultivation for improved agricultural outcomes.

Keywords: Oil content, α-tocopherol, Sucrose, Amino acid profiles, Fatty acid composition

1. Introduction

Sunflower (*Helianthus annuus* L.), which has substantially wide range of adaptation with regard to farming production to varied latitudes, longitudes and photoperiods of the World, is one of the most a good candidate of oilseed crops for supply to the gap and demand of edible oil. Sunflower is used mainly in the edible oil industry and many other industries such as paint, cake, bread and in animal feeding meal and etc. Two types of sunflower are grown: oil seed purpose and non-oil seed sunflower for commercial market (Phanindra et al. 2018; Tan & Kaya 2019). Confectionary sunflowers are highly variable for morphological characters (Tan et al. 2016 and Tan et al. 2017). Non-oil seed sunflower is white or black striped, white or black color hull and comes from large-seeded varieties with low oil content and it is used in baking and snack applications. The improvement of nutrition facts and the quality of the oil is associated with its fatty acid composition and particularly the proportions of oleic, linoleic and linolenic acids (Jalilian et al. 2012). Here's a clearer version of the sentence:

Sunflower seeds are highly important due to their essential nutrients (Lie et al. 2017). As people increasingly demand healthier food options and are also suffering from cardiovascular diseases (Ryan et al. 2015), the nutritional value of sunflower seeds becomes even more significant. Consequently, cultivating promising genotypes with high yielding ability and applying favorable agricultural practices as planting date, irrigation, plant row spacing and nitrogen fertilization offer a great opportunity to improve seed yield and oil quality of sunflower seeds (Lie et al. 2017).

Although confectionary sunflower is a significant income earner in the World, it is generally considered together with oil sunflower in the world literature. Although it is evaluated separately for confectionary type and oil type on a country basis, confectionary sunflower statistics are not included in organizations in international agricultural organizations ISA, OECD, FAO and so on (Kaya et al.). Farmers have insufficient knowledge about breeding or some farm production techniques. This situation may cause low productivity and not reaching the desired level for high seed yield and quality in production. Poor quality and non-standard sunflower seed causes difficulties in processing and quality problems in the final product to be offered to the consumer. Appropriate cultivation techniques will be effective in terms of yield and quality in confectionary sunflower farming. One of the methods used in agriculture is nitrogen fertilization. It is possible to observe the effect of nitrogen fertilization in

sunflower physiologically and morphologically (Ghani et al. 2000; Valchovski 2002; Osman & Awed 2010; El Satar et al. 2017). For example, Scheiner et al. (2002) investigated nitrogen fertilization and the need for nitrogen in sunflower in their study in Pampas, Argentina. As a result of the research, the nitrogen fertilization increased the yield by 17% and nitrogen fertilization decreased the oil concentration in the seed. In this concern, Osman & Awed (2010) showed that 100 kg N ha^{-1} fertilization was suitable for sunflower seed and the higher rate 150 kg N ha^{-1} indicates a negative effect on the oil contents and seed yield.

Even though there is a classification rule in oil type sunflower in terms of sales and marketing, no any officially classification rules for confectionary sunflower type in many countries in the World. The most important problem in the production and marketing of confectionary sunflower is the lack of a certain quality standardization. When farmers market their products, they sell according to completely only physical and relative methods without any nutrition facts of seeds such as oil content, protein, amino acids, tocopherols, sugar content.

In this study, we conducted a research on the nutritional facts content of confectionary sunflower seed, which has two different shell colors, such as oil content, α-tocopherol, sugar ingredients, fatty acids and amino acid compositions under different nitrogen fertilization treatments in the field. In recent years, although there are a lot of studies, which explain the effects of nitrogen fertilization or non-fertilization on the nutrition facts of sunflower seeds, especially lacking of studies with regards to the changing of sucrose content and α-tocopherol under different nitrogen doses applications.

α-Tocopherol exhibits the maximum vitamin E activity (in vivo). But its in vitro activity is relatively low (Bramley et al. 2000). On the other hand, α-Tocopherol is a more effective free radical scavenger than α-Tocopherol in vitro (Duthie et al. 1991). These astonishing properties have made the tocopherols an incredibly essential nutrient and which must be present in edible oil in a significant amount. (Naz et al. 2011). The reports of Zhang et al. 2012 suggested that when the vitamin E (Toc) intake is lacked either from the diet or from supplements may reduce the risk of liver cancer. There may be associated between nitrogen and tocopherols (Hussein et al. 2014), because tocopherols and nitrogen are found mostly localizially in the same plant tissues, specifically in the plastids, a cell organelle containing chlorophyll and involved in photosynthesis (Gzyl-Malcher et al. 2010) and N is a major component of chlorophyll, amino acids, enhancing photosynthetic activity and other important biomolecules such as adenosine triphosphate (ATP) and nucleic acids (Wagner 2011).

The objectives of present study were to investigate how the effects of different nitrogen fertilization doses on oil content, tocopherol, sucrose, amino acids and fatty acid compositions of two different hull color confectionary sunflower seeds. In addation, we considered that the results of our study can provide a good data set in terms of chemical. nutrition facts of seeds for more comprehensive confectionary sunflower standardization studies that will be carried out in the future.

2. Material and Methods

2.1. Plant materials and the properties of experiment site

Somon Beyazı belongs to white hull color and Ahmetbey as a black hull color; confectionary sunflower genotypes were used in the experiment as a plant material. Two confectionary sunflower varieties were provided by the Trakya Agricultural Research Institute in Turkey. This research was conducted in the Aegean Region, with an altitude of 820m and between 38° 5 'north latitude 29° 36' east longitude. The location of the experimental area showed the transition between the Mediterranean climate dominant in the Aegean and the continental climate of Central Anatolia. The soil properties of field experiment showed Table 1. Average annual precipitation is 409.56 mm. The highest rainfall is in April and the lowest in August during the summer growing season. The values of climate conditions in field experiment were given in Figure 1.

Table 1- The soil properties of field experiment

Properties	***Results***	***Rating***
The texture of soil	*Silty Clay Loam*	
pH	8.23	Alkali
% Total Salt	0.020	Low
% Lime	38.67	Silty Clay Loam
% Sand	16.83	
% Silty	44.50	
% Organic matter	1.93	Low
% Marl	46.44	Extensive
Phosphorus (P) ppm	5.16	Low
Potassium (K) ppm	291.1	Very low
Calcium (Ca) ppm	4685	High
Magnesium (Mg) ppm	528.8	Very high
Iron (Fe) ppm	2.65	Critical level
Manganese (Mn) ppm	3.68	Enough suitable
Zinc (Zn) ppm	1.19	Enough suitable
Cuper (Cu) ppm	3.68	Enough suitable
Boron (B) ppm	0.41	Very low

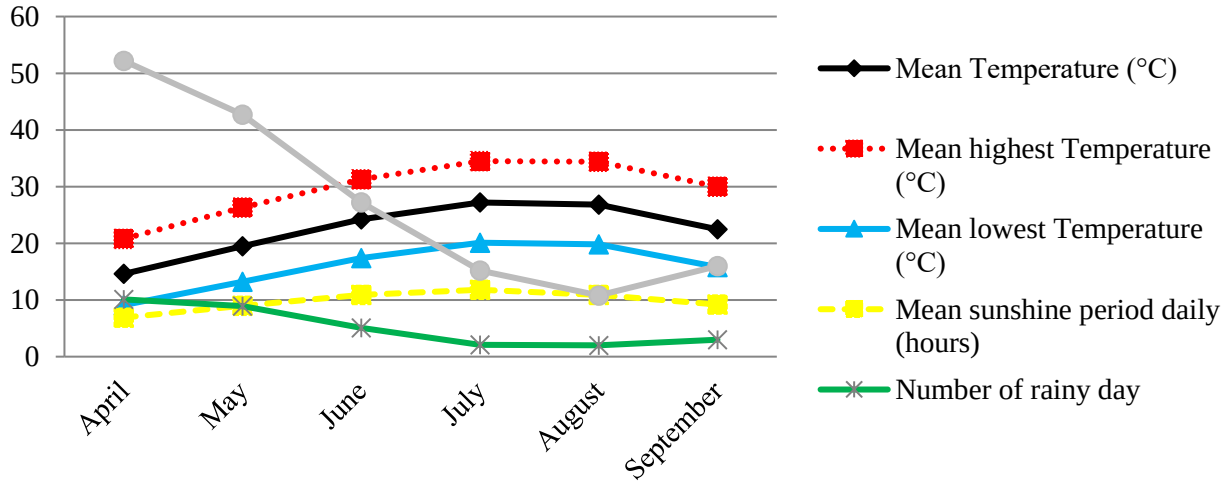


Figure 1- The climate conditions of study (long term means 1957 – 2019)

2.2. The application of fertilizer and design of field experiment

In the experiment, the main plots were set up with different nitrogen fertilizer doses of 0, 60, 120, 180 kg N ha^{-1}. *First fertilization during soil tillage before planting;* the application of different nitrogenous fertilizer dosage, which is one of the factors (main-plot) in the experiment, was carried out with the split-plot methods. Nitrogen fertilization process was applied in two different periods. During the planting preparation of the tillage soil, only for 60, 120 and 180 kg ha^{-1} nitrogen fertilization plots were fertilized by calculating Ammonium Sulphate (AS 21% N) fertilizer to provide 60 kg ha^{-1} Nitrogen (N) doses. 640 gr AS fertilizer was calculated and applied to each parcels (22.4 m^2). No nitrogenous fertilization was applied to the parcels to be treated with 0 kg ha^{-1} of nitrogen.

In addition, before planting in the experimental area, it was calculated also and applied from Triple Superphosphate (TSP 43-44% P_2O_5) fertilizer to provide 60 kg ha^{-1} pure Phosphate (P) fertilization for all parcels. 315 gr TSP fertilization was calculated and applied on all parcels of study. Potassium Sulphate (50% K_2SO_4) was also calculated and applied as 60 kg ha^{-1} pure Potassium (K) fertilization. 270 gr Potassium Sulphate fertilization was applied on all parcels.

Second fertilization before flowering of plants; Second fertilization was not applied to parcels with 0 and 60 kg N ha^{-1}. 295 gr UREA (46 % N) fertilization was fertilized on the parcels manured with 60 kg N ha to obtain 120 kg ha^{-1} Nitrogen doses application. 584 gr UREA was applied on the parcels manured with 120 kg ha^{-1} N to obtain 180 kg ha^{-1} Nitrogen doses.

The sunflower seeds were planted with a four-row pneumatic seed drill, with a distance between rows of 0.7 metres and a plant distance of 0.2 metres. 25 days after planting, the intra-rows were thinned with an inter-row as 40 cm (plant density was 3.5 plant m^{-1}). Plots consisted of four rows 8 m long × 2.8 m wide. The harvest area was 19.6 m^2 in each parcel. Furrow irrigation was applied and hand weeding was performed throughout the growing period when necessary.

2.3. Collecting plant samples

The seeds with shelled were randomly selected from sunflower plants in the middle each plot without any size classification during harvesting. The rate of sampling was approximately represented by 15 % of the seed yield in each parcel. The shell of sunflower seeds were immediately crushed by hand nippers to avoid contamination without waiting after harvested. Unshelled seeds were immediately stored at – 20 °C until analysis.

2.4. The analysis of oil content

Soxhlet method was used to determine the oil ration of unshelled confectionary sunflower seeds (Tesan et al. 2022)). After 250 mL balloons were tarred. 10g sample grounded was taken and surrounded with filter paper. Then 150 mL hexane was added and put into Soxhlet tubes. The Soxhlet tubes were immersed in the Soxhlet device for 4 hours. To remove left hexan in the tubes were put into oven for 30 min at 105 °C.

2.5. Determination of fatty acids composition using gas chromatography (GC)

First, the extracted crude sunflower oils underwent gas chromatography (GC) analysis following esterification. For sample preparation, 10 mg of extracted sunflower oil was mixed with 0.1 mL of 2 M methanolic potassium hydroxide (KOH) and blended. Then, the solution was mixed with 2 mL of isooctane. The final mixture was spun at 300 mL/minute for 5 minutes. One hundred µl was extracted from the upper layer using a micro-syringe and subsequently injected into the Gas Chromatography with Flame Ionization Detection (GC-FID) (Model X, Manufacturer Y).

To determine the peaks in the chromatogram, a standard mixture comprising each fatty acid was injected into the GC-FID, and their corresponding retention times (RT) were identified. Technical term abbreviations were always explained when used for the first time. The SP TM-256 Column, Macherey-Nagel, Duren, Germany, with a film thickness of 0.2 µm on a 100 m × 0.25 mm ID column was used. Helium served as the carrier gas and had a linear velocity of 1 mL m–1, while the split ratio was set at 40:1. The starting temperature of the column was set at 140 °C, with an air flow rate of 400 mL s–1 and an H_2 flow rate of 40 mL s–1, sustained for 10 minutes. Subsequently, the column temperature was raised by 4 °C per minute until it reached 240 °C. The fatty acid composition was analysed by computing the area percentage of each peak (Reed et al. 2004).

2.6. The analysis of sugar components (Glucose. Fructose. Maltose. Sucrose)

A 5 g portion of finely ground sunflower seeds was weighed and mixed with 40 mL of deionised water to dissolve in a 150 mL tube. Following this, 25 mL of methanol was added to the solution. Ultra-pure deionised water was then added to fill the remaining 100 mL of the solution. The mixture was passed through a 0.45 µm PTFE filter. Finally, 20 µL samples of the extract were injected into HPLC for analysis. A calibration curve was prepared by injecting a mixture of sugar components, comprised of Glucose, Fructose, Sucrose, and Sorbitol, at specific concentrations into the device.

The sugar components were analyzed using HPLC (with an RID detector) and the results were determined by substituting the values for the sugars in the sample onto the calibration curve. Technical abbreviation explanations were included where first used. The analysis was carried out using a mobile phase of Asetontril:dH2O (80:20) (V: V) with a column of NH2 Colon 5 µm, 4.6x250 mm operating at a flow rate of 1.5 mL/min at 30 °C.

2.7. The analysis of α-tocopherol using high performance liquid chromatography

α-Tocopherol content was determined using HPLC-FLD. Approximately 0.5 g of the sample was weighed and diluted to 10 mL with hexane, then vortexed. After appropriate dilution, 50 µL of the solution was injected into a Shimadzu HPLC system (mod. LC-10ADVP) with an FLD detector (mod. SPDM10AVP) and a reversed-phase column (Si 60, 5 µm, 4.0 x 250 mm). The detector was set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The mobile phase, consisting of hexane: 2-propanol (99.5:0.5), flowed at 0.8 mL/min with a column temperature of 30 °C.

Along with the sample, the standards of α-tocopherol prepared at different concentrations were injected into the device and the calibration curve was obtained. Quantification was performed by substituting the peak area value of α-tocopherol from the sample into the calibration curve.

2.8. Amino acid profiles of seeds

The amino acid composition of the prepared sunflower seed was determined using a High Performed Liquid Chromatograph (Shimadzu Nexara XR, HPLC system). 0.1 g ground samples were hydrolysed by adding 5 mL 6 N HCL, 250 µL 2 mM Phenol, 0.1 g Na_2SO_3. The solution was waited for 24 hours at 110 °C. The pH of the sample was adjusted to the range of 6.7 – 7.3 with 5 N NaOH and then centrifuged at 4000 rpm for 5 min at room temperature.

The solvent used (for derivatization) were: Borat tampon (Diluent); [Boric acid + KOH, concentration (0.4 M 100 mL, pH: 10.2], OPA solution; [OPA + Diluent (10 mg OPA, 100 µL MeOH, 900 µL Diluent, 10 µL 3-MPA) 10 mg mL^{-1} (1mL)], FMOC solution; [ACN (2.5 mg FMOC, 1mL ACN) 2.5 mg mL^{-1})].

2.9. Statistical analysis

The statistical analysis of the study investigated the effect of different nitrogen doses on two sunflower hybrid varieties using a split-plot design with three replications. In addition, the differences between sunflowers of different hull colours and the interaction of nitrogen x genotype were assessed using a two-way split-plot ANOVA for all parameters. This was carried out using the statistical software JMP Pro16 software (SAS Institute, Cary, NC, USA) and TARIST (Açıkgöz et al., 1994). Significant differences in the means of replications were evaluated by means of Fisher's least significant difference (LSD) approach. Regression analyses were performed using Microsoft Excel to assess the correlation between the oil content, α-tocopherol, sucrose of two genotypes, and nitrogen fertilisation doses (see Figure 1). All differences reported were statistically significant at 0.05.

3. Results and Dıscussıon

3.1. Effects of nitrogen fertilization on oil content

The effect of nitrogen fertilization was not statistically found on the oil content of sunflower seed (Table 2). It was found that there was a significantly difference in terms of oil content of two different hull color confectionary sunflower (Table 2). Generally, the oil content of Somon Beyazı (white hull color) confectionary sunflower seed was higher than that of Ahmet Bey genotype under all nitrogen fertilization doses (Figure 2). Especially, two different confectionary sunflower showed differences with regards to the changing of oil content of seeds under 60 kg N ha^{-1} fertilization (Figure 2). Somon Beyazı genotype showed an increase in oil content at a dose of 60 kg nitrogen fertilization, whereas Ahmet Bey genotype decreased. But the oil content of Somon Beyazı was decreased with subsequent increasing doses of nitrogen fertilization (Figure 2). Figure 2 showed that the oil content of two confectionary genotypes were decreased with the increasing nitrogen doses fertilization, even though there was not found statistically any differences the effect of nitrogen fertilization in field experiment. These results are consistent with those of Abdel-Sabour and AboEl-Seoud (1996), Nanjundappa et al. (2001), Munir et al. (2007), and Nasim et al. (2012), who observed decreases in oil percentages with increased N application. The oil content of the Somon Beyazı genotype decreased non-linearly by 1.1%, from 38.9% to 37.8%, as nitrogen fertilization increased from 0 kg N ha^{-1} to 180 kg N ha^{-1}. In contrast, the oil content of the Ahmet Bey genotype decreased linearly by 2.0%, from 36.8% to 34.8%, with increasing nitrogen fertilization doses. This might be due to the varying genetic potential of the hybrids. The effect of the year was also not significant. These results are consistent with the findings of Roche et al. (2010) and Bukhsh et al. (2011), who found that different sunflower variety exhibit distinct responses to oil content due to differences in their genetic makeup. In addition to Steer et al. (1984) reported that excessive amounts of nitrogen can decrease the concentration of sunflower oil as a result of increased protein concentration, without any parallel increase in biomass as evidenced.

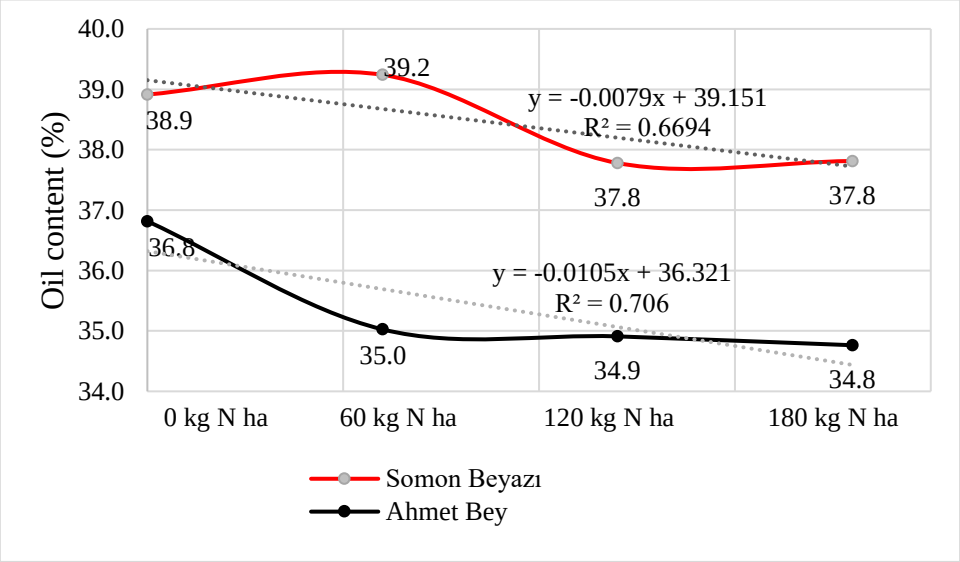


Figure 2- The effect of nitrogen on the oil content of two different hull colored confentionary sunflower seed

Table 2- The ANOVA results of oil content, α-Tocopherol and sucrose content of two confectionary sunflower under different nitrogen fertilization conditions

Variance Sources	*df*	*The Values of Mean Square*		
		Oil content (%)	*α-Tocopherol*	*Sucrose*
Replicate	2	2.0094	2783.51	0.02275
N Fert.	3	3.16594ns	43688.3ns	0.0023ns
Error 1	6	1.1609	15023.3	0.02852
Genotype	1	56.1204**	223494**	1.6485ns
N x G	3	1.32176ns	47802.6*	0.01029***
Error 2	8	3.60058	9092.9	0.028921
$LSD_{N <0.05}$		ns	ns	ns
$LSD_{G <0.05}$		2.727	2.908	ns
$LSD_{NxG <0.05}$		ns	5.257	0.335

*: P<0.05; **: P<0.01; ***: P<0.001, ns:non-significant

3.2. Effects of nitrogen fertilization on α-tocopherol

There were significant effects of genotypes and nitrogen fertilization × genotype for α-tocopherol content (Table 2). There were significantly differences between the α-tocopherol content of two genotype in all nitrogen fertilization doses (Table 2). Considering mean values, Ahmet Bey significantly higher content of α-tocopherol by 256.9 µg g^{-1} in 0 kg N ha^{-1}, 280.8 µg g^{-1} in 60 kg N ha^{-1}, 207,5 µg/g in 120 kg N ha^{-1} and 27.4 µg g^{-1} in 180 kg N ha^{-1} fertilization treatments than that of Somon Beyazı genotype. It was observed important considerable decrease of α-tocopherol content in both sunflower genotype with the increasing nitrogen fertilization doses (Figure 3). The reduction in α-tocopherol content was markedly 22.04% and 20.25% in the 120 kg N ha^{-1} nitrogen fertilization in Ahmet Bey and Somon Beyazı sunflower genotypes respectively. The study showed that application of different concentrations of nitrogen fertiliser can affect α-tocopherol levels in both sunflower genotypes to different rates.

Tocopherols play an important role in the quality of oil due to their antioxidant activity, which is more effective at relatively low concentrations (Beltrán et al. 2005). On the other hand, when α-tocopherol is present at relatively higher concentrations, a pro-oxidative effect can occur (Belizit & Grosch, 1986). In our study, there was no significant effect of the dose of nitrogen on the ratio of tocopherols. The difference observed was due solely to the genotypes, and the responses to the different doses of N were not uniform.

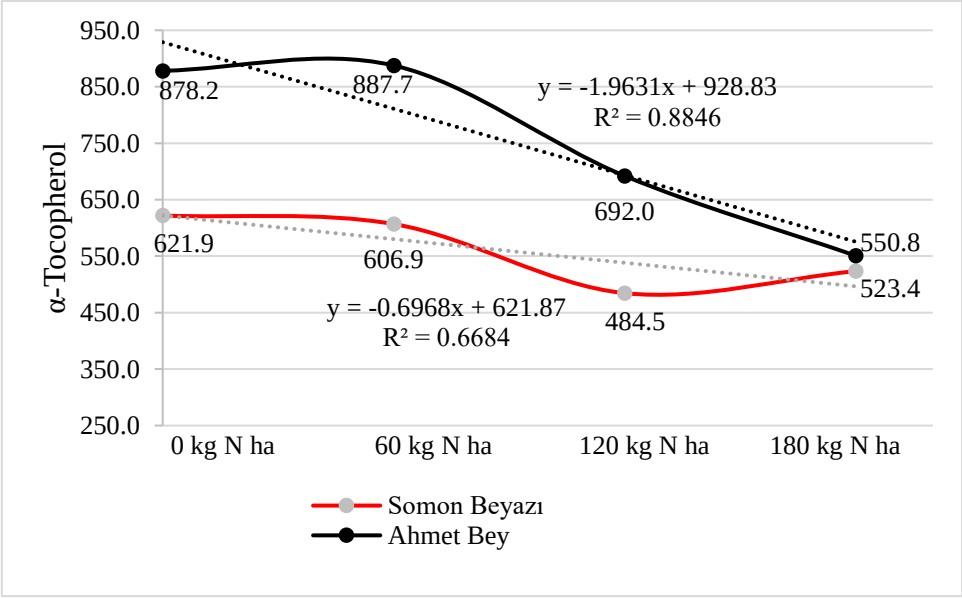


Figure 3- The effect of nitrogen on the α-Tocopherol of two different hull colored confentionary sunflower seed

3.3. Effects of nitrogen fertilization on sucrose

Figure 4 displayed that the change in sugar content of both genotypes in the application of different nitrogen dose fertilizers. The sugar content of the Somon Beyazı genotype showed higher values than the sugar content of Ahmet Bey sunflower genotype in

all nitrogen dose fertilizations (Figure 4). Furthermore, sucrose content of the two sunflower cultivars under different nitrogen doses fertilization had decreased by 19.77% at 15 DAF (Days After Flowering), 32.44% at 30 DAF, and 48.37% at 45 DAF, respectively, an average decrease of 33.53%.

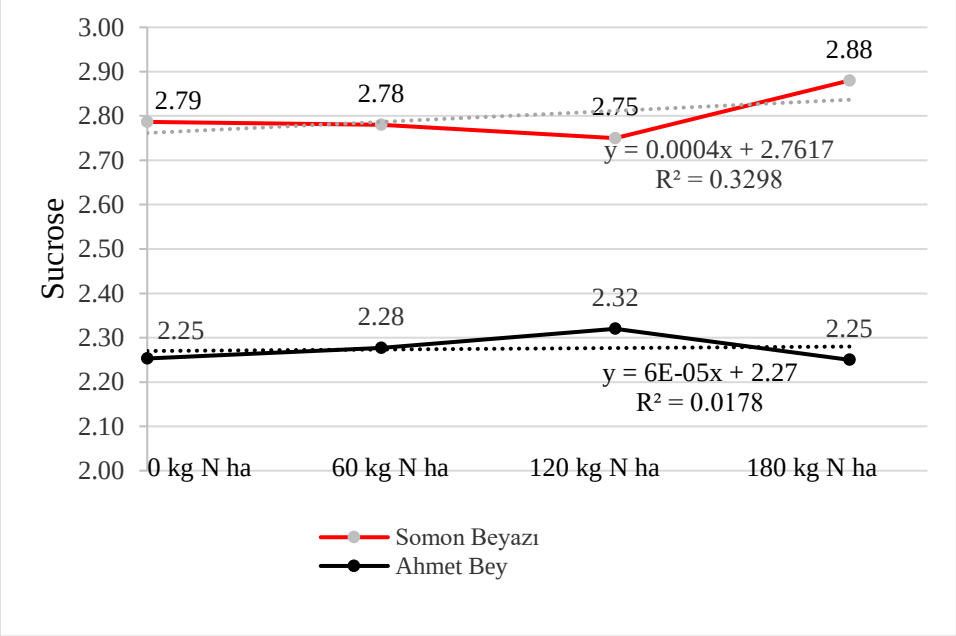


Figure 4- The effect of nitrogen on the sucrose content of two different hull colored confentionary sunflower seed

3.4. Effects of nitrogen fertilization on fatty acid compositions

Results of fatty acid composition for two confectionery sunflower genotypes under different nitrogen fertilization are shown in Table 5. Myristic acid, stearic acid, oleic acid, linoleic acid, gondoic acid, linolenic acid, behenic acid and lignoceric acid showed significant differences between genotypes. Moreover, there were significant differences in arachidic acid and triconoic acid for both N dose and genotypes, and significant effects of nitrogen fertilization × genotype interaction were observed for palmitic acid and gondoic acid. However, no statistical difference was found in palmitoleic acid and its parameters.

Table 5- ANOVA results of fatty acids composition of two confectionary sunflower genotype under different nitrogen fertilization

Variance Sources	*df*	*The Values of Mean Square*						
		Miristik Acid	*Palmitic Acid*	*Palmitoleic Acid*	*Heptadeconoic Acid*	*Stearic Acid*	*Oleic Acid*	*Linoleic Acid*
Replicate	2	9.04e-6	0.00031	0.0272	0.01395	0.00692	0.40744	0.37079
N Fert.	3	0.00002	0.01299**	0.02861	0.01421	0.05729	1.30025	1.54579
Error 1	6	5.87e-6	0.00092	0.0282	0.01431	0.02869	0.50338	0.35549
Genotype	1	0.00056***	0.70761***	0.03096	0.0187	0.9165***	35.9611***	71.3633***
N x G	3	0.00003	0.00934*	0.02861	0.01477	0.03923	1.0603	1.07065
Error 2	8	0.000010	0.001645	0.028573	0.014361	0.015964	0.52275	0.45873
$LSD_{N<0.05}$		ns	0.0141	ns	ns	ns	ns	ns
$LSD_{G<0.05}$		0.0058	0.0432	ns	ns	0.0574	0.6879	1.5556
$LSD_{NxG<0.05}$		ns	0.0561	ns	ns	ns	ns	ns

*: P<0.05; **: P<0.01; ***: P<0.001

Table 5 (Continue)- ANOVA results of fatty acids composition of two confectionary sunflower genotype under different nitrogen fertilization

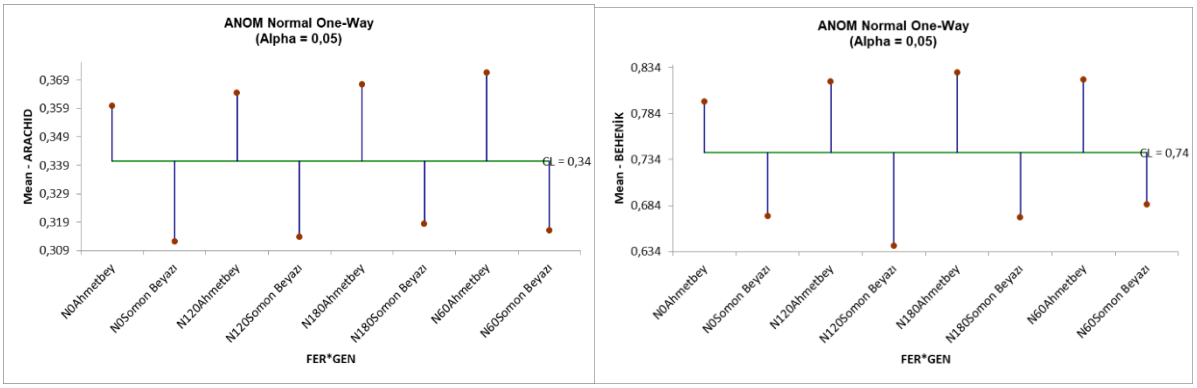
Variance Sources	*df*	*The Values of Mean Square*					
		Arachidic Acid	*Gondoic Acid*	*Linolenic Acid*	*Behenic Acid*	*Triconoic Acid*	*Lignoseric Acid*
Replicate	2	3.88e-6	3.79e-6	1.79e-6	0.00017	6.67e-7	0.00009
N Fert.	3	0.00008**	7.28e-6	8.04e-6	0.00078	2.94e-6*	0.00007
Error 1	6	6.82e-6	6.9e-6	3.29e-6	0.0011	4.44e-7	0.00002
Genotype	1	0.0154***	0.0035 ***	0.00081***	0.13336***	0.00001*	0.0373***
N x G	3	0.00002	0.00008 **	0.00001	0.00085	1.17e-6	0.00002
Error 2	8	0.000027	0.000007	0.000003	0.001053	0.0000012	0.000043
$LSD_{N<0.05}$		0.1155	ns	ns	ns	ns	ns
$LSD_{G<0.05}$		0,0387	0.0005	0.0028	0.0126	0.0010	0.0867
$LSD_{NxG<0.05}$		ns	0.0001	ns	ns	ns	ns

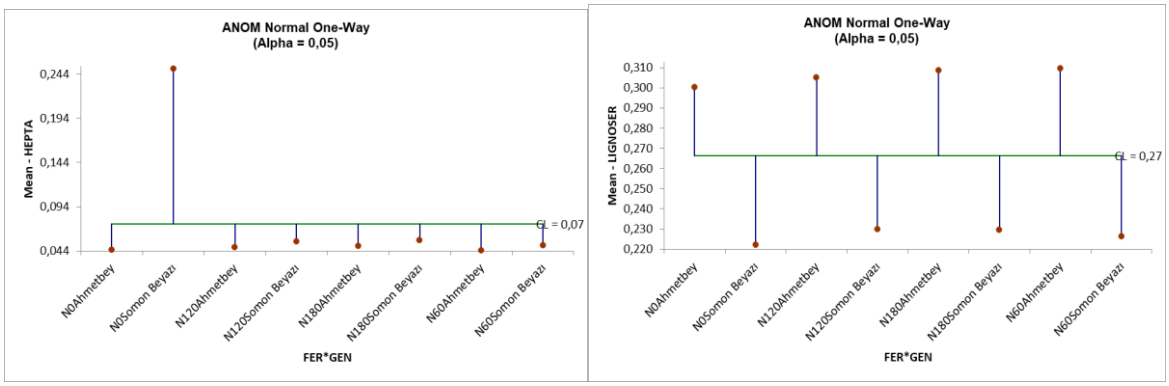
*: P<0.05; **: P<0.01; ***: P<0.001

The fatty acid composition analysis of two confectionery sunflower genotypes under varying levels of nitrogen fertilization is presented in Figure 5. The genotypic response to fertilization doses in the ANOM is visually illustrated in a decision chart resulting from mean comparisons via the Analysis of Mean (ANOM) method. When analysing the fatty acid compositions for different fertilizer doses, it was observed that the Ahmetbey variety had values higher than both the overall mean and the Somon Beyazı genotype for all nitrogen doses. Notably, highest values for fatty acids such as Myristic, Palmitic, Stearic, Linolenic, Tricona, and Lignoceric were recorded at a nitrogen dose of 180 kg/ha-1.

Palmitoleic, Heptadecenoic and Linoleic acids responded negatively to N application, whereas Oleic acid reached its highest concentration at 16 kg/ha-1 of nitrogen. These results are supported by Nanjundappa et al. (2001), Munir et al. (2007), and Boydak et al. (2010), who observed decreases in the composition of this fatty acid with increased N application.

On the other hand, arachidic, gadoleic and behenic acids showed their maximum values at a nitrogen dose of 120 kg/ha^{-1}.





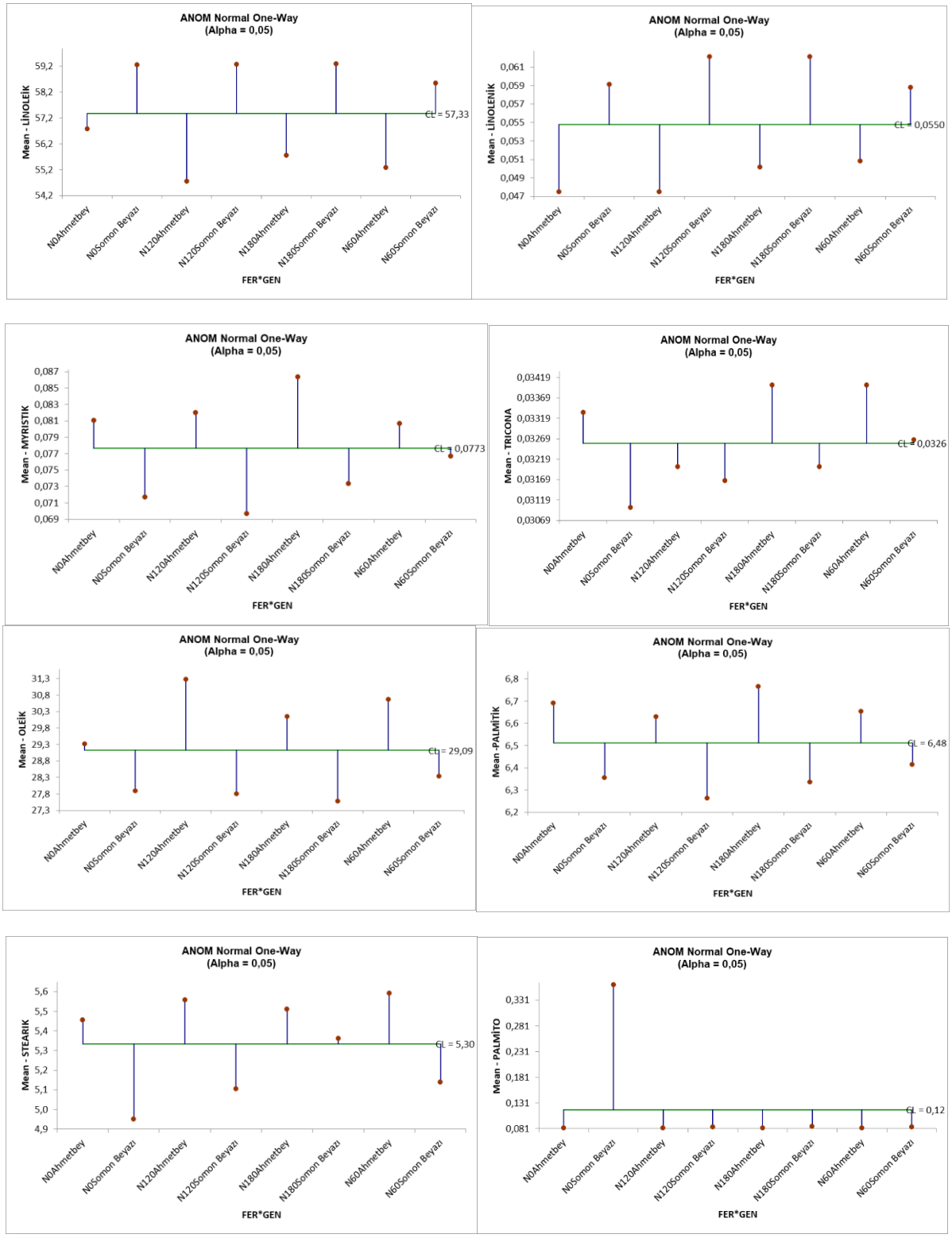


Figure 5- The Anom (Analysis of Means) of fatty acids composition of two confectionary sunflower genotype under different nitrogen fertilization

3.5. Effects of nitrogen fertilization on amino acid profiles

The ANOVA results for the amino acid profile of two confectionary sunflower varieties under nitrogen fertilization doses are presented in Table 3. The analysis revealed significant interactions between N dose*genotype for the levels of glutamine, serine, histidine, glycine, arginine, alanine, phenylalanine, lysine, leucine, and tyrosine. While differences were observed in Tryptophan, Valine, Isoleucine, and proline values across nitrogen doses, no statistically significant variation was found for Asparagine and Cysteine values (Table 4). The analysis demonstrates a diverse distribution of amino acids, with 16 different types detected at varying concentration levels. Particularly noteworthy is the high content of glutamine and Asparagine, followed by cysteine,

arginine, glycine, serine, lysine, valine, and phenylalanine, all exceeding 1 gram per 100 millilitres. Additionally, the presence of Histidine, Tryptophan, alanine, isoleucine, leucine, proline, and tyrosine was also confirmed. Glutamine, serine, histidine, glycine, arginine, alanine, phenylalanine, lysine, leucine, and tyrosine. The optimum nitrogen dose was determined as 120 kg N ha-1 and the variety with black hull colour was higher than the variety with white hull colour in all treatments. The distribution of amino acid composition to nitrogen doses was a quadratic distribution and this result scientifically revealed that the most suitable nitrogen dose was 120 kg N ha^{-1} (Table 4).

The use of reductive amination and transamination processes produces a greater amount of amino acids compared to fatty acids (Prasad 1990). This shift in metabolic focus has implications for oil content, as Singh & Quadri (1984) and Sharma & Gaur (1988) reported that high levels of nitrogen lead to a decrease in oil content (%) due to an increase in impurities. Consequently, managing nitrogen levels is crucial to balancing amino acid and fatty acid production, as well as maintaining oil quality. Protein synthesis is entirely reliant on the amount of nitrogen fertilizer accessible for plant use, as stated by Nasim et al. (2012). The use of nitrogen fertilizer also leads to an increased concentration of protein regardless of the irrigation method utilized, as noted by Jalilian et al. (2012).

Table 3- ANOVA results of aminoacids profile of two confectionary sunflower under different nitrogen fertilization conditions

Variance Sources	*df*	*F values*							
		Asp	*Glu*	*Ser*	*His*	*Gly*	*Thr*	*Arg*	*Ala*
Replicate	2	1.031	0.219	0.105	1.333	0.281	1.435	1.867	0.018
N Fert.	3	1.506ns	101.882**	334.629**	34.001**	124.350**	90.387**	29.617**	55.265**
Error 1	6	0.024	0.004	0.000	0.000	0.000	0.001	0.001	0.000
Genotype	1	2.053ns	0.631ns	4.012ns	0.127ns	0.033ns	14.003**	0.267ns	0.529ns
N x G	3	0.808ns	19.085**	32.887**	9.485**	37.224**	3.044ns	18.285**	21.678**
Error 2	8	0.025	0.006	0.000	0.000	0.001	0.001	0.001	0.000
$LSD_{N<0.05}$		ns	0.089	0.008	0.015	0.019	0.054	0.053	0.019
$LSD_{G<0.05}$		ns	ns	ns	ns	ns	0.029	ns	ns
$LSD_{NxG<0.05}$		ns	0.141	0.016	0.025	0.059	ns	0.065	0.032

*: P<0.05; **: P<0.01; ***: P<0.001. The values of errors were given as the mean of square. N: Nitrogen fertilization. G: Genotype. Asp: Asparagine. Glu: Glutamine. Ser: Serine. His: Histidine. Gly: Glycine. Thr: Tryptopthan. Arg: Arginine. Ala: Alanine

Table 3(continue)- ANOVA results of aminoacids profile of two confectionary sunflower under different nitrogen fertilization conditions

Variance Sources	*df*	*F values*							
		Cys	*Val*	*Phe*	*IsLe*	*Lys*	*Leu*	*Pro*	*Try*
Replicate	2	0.724	1.510	0.082	0.397	0.086	5.232	8.951	2.319
N Fert.	3	2.865ns	12.760**	18.250**	8.401*	22.958**	134.508**	507.960**	57.114**
Error 1	6	0.020	0.001	0.001	0.001	0.001	0.000	0.000	0.000
Genotype	1	0.317ns	1.103ns	0.116ns	0.004ns	0.238ns	0.629ns	7.948*	0.010ns
N x G	3	3.972ns	3.123ns	19.304**	1.369ns	12.762**	9.439**	3.902ns	9.254**
Error 2	8	0.016	0.001	0.000	0.002	0.001	0.000	0.003	0.000
$LSD_{N<0.05}$		ns	0.035	0.054	0.034	0.043	0.015	0.014	0.010
$LSD_{G<0.05}$		ns	ns	ns	ns	ns	ns	0.049	ns
$LSD_{NxG<0.05}$		ns	ns	0.015	ns	0.050	0.040	ns	0.027

*: P<0.05; **: P<0.01; ***: P<0.001. The values of errors were given as the mean of square. N: Nitrogen fertilization. G: Genotype. Cys: Cysteine. Val:Valine. Phe: Phenylalanine. Le: Leucine. Lys: Lysine. IsLeu: Isoleucine. Pro: Proline. Try:Tyrosine

There was a significant increase in protein contents with the increased N rates. Amjed and Ulah (2012) recorded protein contents for highest (225 kg N ha^{-1}) and lowest (15.33%) in N0 (control). There were similar results in 2011, which are in line with those of Nanjundappa et al. (2001) and Munir et al. (2007), who observed increased protein due to N application. Sunflower hybrids had a significant effect on protein content in achene. 'Ahmetbey' attained significantly higher protein content than 'Somon Beyazı' which might be due to varying genetic potential of the hybrids (Table 4) These results are in accordance with the findings of Roche et al. (2010) and Bukhsh et al. (2011) that different sunflower hybrids exhibit the differential response to protein content in due to their difference of genotype.

Table 4- The changing of amino acids profiles in two different hull color confectionary sunflower seeds by different nitrogen fertilization

Amino Acids (g 100m^{-1})	*0 kg N ha^{-1}*		*60 kg N ha^{-1}*		*120 kg N ha^{-1}*		*180 kg N ha^{-1}*	
	Somon Beyazı	*Ahmet Bey*	*Somon Beyazı*	*Ahmet Bey*	*Somon Beyazı*	*Ahmet Bey*	*Somon Beyazı*	*Ahmet Bey*
Asparagine	2.081	2.109	2.001	2.067	2.150	2.200	2.168	2.161
Glutamine	4.881	4.921	4.705	4.894	5.261	5.486	5.351	4.993
Serine	1.075	1.069	1.023	1.083	1.113	1.131	1.087	0.958
Histidine	0.480	0.475	0.466	0.502	0.526	0.544	0.517	0.476
Glycine	1.477	1.494	1.409	1.544	1.567	1.648	1.598	1.376
Tryptophan	0.720	0.734	0.708	0.647	0.598	0.509	0.419	0.367
Arginine	1.876	1.886	1.838	1.937	2.008	2.091	1.965	1.802
Alanine	0.990	0.996	0.952	1.002	1.027	1.048	0.990	0.893
Cysteine	2.052	2.119	1.878	2.087	2.176	2.019	2.338	2.103
Valine	1.071	0.987	1.008	1.032	1.086	1.116	1.059	1.025
Phenylalanine	1.018	1.042	1.009	1.020	1.135	1.178	1.070	0.983
Isoleucine	0.785	0.758	0.745	0.769	0.804	0.842	0.811	0.772
Lysine	1.361	1.367	1.322	1.378	1.455	1.488	1.401	1.284
Leucine	0.707	0.759	0.741	0.794	0.766	0.727	0.672	0.653
Proline	0.872	0.883	0.861	0.828	0.950	1.074	0.952	1.091
Tyrosine	0.550	0.541	0.537	0.581	0.574	0.577	0.542	0.502

4. Conclusıons

The study investigated how nitrogen fertilization influences the composition of sunflower seeds, focusing on sugar, amino acids, fatty acids, oil content, and α-tocopherol levels across different genotypes and nitrogen doses. It found that nitrogen fertilization did not significantly affect oil content, although variations between genotypes were observed, with the Somon Beyazı genotype showing higher oil content compared to Ahmet Bey. Both genotypes exhibited reduced α-tocopherol levels with increased nitrogen application, but the Ahmet Bey genotype maintained consistently higher α-tocopherol levels. Sucrose content also declined with higher nitrogen doses in both genotypes, with Somon Beyazı having higher sucrose levels than Ahmet Bey. Fatty acid compositions differed notably among genotypes and in response to nitrogen fertilization, indicating the influence of both genetic factors and nutrient management. Additionally, the amino acid profiles revealed significant variations, with high levels of glutamine and asparagine. Overall, the study provides valuable insights into the intricate relationships between genotype and nitrogen fertilization, suggesting that optimizing cultivation practices can enhance the nutritional value and quality of sunflower seeds while emphasizing the importance of integrating genetic and nutrient management factors in crop cultivation.

Acknowledgments

This study was financially supported by ZRF-20022 of Scientific Research Projects Unit at Aydın Adnan Menderes University. We thank to Agrefar – Environmental food analysis laboratory for their assistance in conducting the analysis.

References

Abdel-Sabour M F & Abo El-Seoud M A (1996). Effects of organic-waste compost addition on sesame growth, yield, and chemical composition. *Agriculture, Ecosystems & Environment* 60(2-3): 157-164

Açıkgöz N, Akba ME, Moghaddam A, Özcan K (1994). Turkish data-based statistics programmer for PC. *Turkey Field Crops Congress*, Ege University Press, pp. 264-267

Ali A & Ullah S (2012). Effect of nitrogen on achene protein, oil, fatty acid profile, and yield of sunflower hybrids. *Chilean Journal of Agricultural Research* 72(4): 564-569

Beltrán G, Aguilera M P, Del Río C, Sanchez S & Martínez L (2005**).** Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chemistry* 89(2): 207-215

Boydak E D, Karaaslan K & Türkoğlu H (2010). The effect of different nitrogen and irrigation levels on fatty acid composition of peanut oils. *Turkish Journal of Field Crops* 15: 29-33

Bramley P M, Elmadfa I, Kafatos A, Kelly F J, Manios Y, Roxborough H E, Schuch W, Sheehy P J A & Wagner K H (2000). Vitamin E. *Journal of the Science of Food and Agriculture* 80: 913-938

Duthie G G, Gonzalez B M, Morrice P C & Arthur J R (1991). Inhibitory effects of isomers of tocopherol on lipid peroxidation of microsomes from vitamin E-deficient rats. *Free Radical Research Communications* 15: 35-40

Ghani A, Hussain M & Qureshi M S (2000). Effect of different irrigation regimens on the growth and yield of sunflower. *International Journal of Agriculture and Biology* 2: 334-335

Gonzalez Perez P et al. (2015). Characterization of the amino acid composition of soils under organic and conventional management after addition of different fertilizers. *Journal of Soils and Sediments* 15: 890-901

Jalilian J, Modarres-Sanavy S, Saberali S F & Sadat-Asilan K (2012). Effects of the combination of beneficial microbes and nitrogen on sunflower seed yields and seed quality traits under different irrigation regimes. *Field Crops Research* 127: 26-34

Munir M A, Malik M A & Saleem M F (2007). Impact of integration of crop manuring and nitrogen application on growth, yield, and quality of spring planted sunflower (*Helianthus annuus L.*). *Pakistan Journal of Botany* 39: 441-449

Nanjundappa G, Shivaraj B, Janarjuna S & Sridhara S (2001). Effect of organic and inorganic sources of nutrients applied alone or in combination on growth and yield of sunflower (*Helianthus annuus L.*). *Helia* 24(34): 115-120

Nasim W A, Ahmad A, Bano R, Olatinwo M & Usman T (2012). Effect of nitrogen on yield and quality of sunflower (*Helianthus annuus L.*) hybrids under sub-humid conditions of Pakistan. *American Journal of Plant Sciences* 3: 243-251

Naz S, Sherazi S T H & Talpur N (2011). Changes of total tocopherol and tocopherol species during sunflower oil processing. *Journal of the American Oil Chemists' Society* 88: 127-132

Osman E B A & Awed M M M (2010). Response of sunflower to phosphorus and nitrogen fertilization under different plant spacing. *Assiut University Bulletin for Environmental Research* 13: 11-19

Phanindra K S, Toprope V N & Thakur N R (2018). Genetic variability in mutant confectionary sunflower (*Helianthus annuus L.*). *Journal of Research ANGRAU* 46(2): 15-20

Prasad D T (1990). Proteins of the phenolic extracted sunflower meal: I. Simple method for removal of polyphenolic components and characteristics of salt-soluble proteins. *LWT-Food Science and Technology* 23: 229-235.

Roche J, Alignan M, Bouniols A, Cerny M, Mouloungi Z, Vear F & Merah O (2010). Fatty acid profile composition in sunflower seeds as affected by genotypes and environmental conditions. *Food Chemistry* 121: 990-995.

Sharma S K & Gaur B L (1988). Effect of levels and methods of nitrogen application on seed yield and quality of sunflower. *Indian Journal of Agricultural Sciences* 33: 330-331

Singh C N & Quadri S J S (1984). Note on response of sunflower to rates and time of nitrogen application. *Journal of Agricultural Research* 10: 76-77

Steer B T, Hocking P J, Kortt A A & Roxburgh C M (1984). Nitrogen nutrition of sunflower (*Helianthus annuus L.*): Yield components, the timing of their establishment, and seed characteristics in response to nitrogen supply. *Field Crops Research* 9: 219-236

Tan A S & Kaya Y (2019). Sunflower (*Helianthus annuus L.*) genetic resources, production, and research in Turkey. *OCL* 26: 21

Tan A S, Aldemir M & Altunok A (2017). Yield potentials of some confectionery sunflower genotypes under ecological conditions of Menemen, Izmir. *ETAE Dergisi / Anadolu Journal of AARI* 1: 1-16 ((In Turkish)

Tan A S, Altunok A & Aldemir M (2016). Oilseed and confectionary sunflower (*Helianthus annuus L.*) landraces of Turkey. In *19th International Sunflower Conference* (pp. 556-566). Edirne, Turkey

Valchovski I (2002). Influence of heavy rate of nitrogen fertilizer on oil content and fatty acid composition of different varieties and hybrids. *Rasteniev dni Nauk* 39(516): 338-341

Wagner S C (2011). Biological nitrogen fixation. *Nature Education Knowledge* 2(11): 14