



EVALUATION OF DIFFERENT SOYBEAN GENOTYPES IN TERMS OF ISOFLAVONES, ANTIOXIDANTS AND SOME QUALITY TRAITS

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Abstract: Soybean (*Glycine max* L.) varieties around the world have different quality characteristics that determine their use and nutritional value. In this study, the isoflavones (daidzein and genistein), isoflavone glycosides (daidzin and genistin), total flavonoid, total phenolic, free radical scavenging activity (DPPH), crude protein, crude fiber, fat, and condensed tannins contents of Turkish origin soybean genotypes were determined. The isoflavone contents were determined in the LC-MS/MS, antioxidants and condensed tannins content in the spectrophotometer, and other quality traits were determined in the NIRS device. The daidzein and genistein contents ranged between 0.035-0.446 and 0.308-1.188 ppm, respectively. The genistin content (0.254-8.906 ppm) was more variable than daidzin (0.388-1.006 ppm). Soybean genotypes exhibited high antioxidant characteristics. The crude protein contents were ranged from 36.127-40.603%. As a result, all genotypes examined were found to be rich in bioactive metabolites, therefore, high-quality raw materials for food production and human consumption.

Keywords: Soybean, Food, Protein, Secondary metabolite

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1. Introduction

Soybean (*Glycine max* L.) is native to Southeast Asia, widely grown for its edible seeds and young pods more than 3.000 years ago (Carter et al., 2004). Soybean consumption is increasing all over the world due to its high nutritional value and low cost. In 2019, world soybean cultivation was 128 million hectares, with 370 million tons of production, mostly in Brazil, the United States, and Argentina. In Türkiye, it is cultivated on 30 thousand hectares with an average yield of 4.44 tons of ha⁻¹ grain (Anonymous, 2023).

Soybean is also rich in secondary metabolites such as furanocoumarins, isoflavonoids, and pterocarpin, as well as antioxidant substances that positively affect the health of humans and animals. These substances play a role both in the defense mechanism of the plant and they also have beneficial effects on human physiology and diseases. The amount of secondary metabolites is genotype-dependent and give the plant a distinctive odor (Li et al., 2010; Tantasawat et al., 2011). Besides, condensed tannins in soybean are mitigating the risk of diabetes risk in humans by decreasing blood sugar levels (Kumari and Jain, 2015).

A large number of soybean varieties are available worldwide and naturally, they vary secondary

metabolites content. But the common feature of all is that it contains a high amount of protein and other essentials vitamins that play important role in our daily life (Chen et al., 2018). In this respect, the secondary metabolites, antioxidant traits, crude protein, crude fiber, and fat content in 12 different soybean genotypes were investigated in the current study.

2. Material and Methods

2.1. Materials

Soybean seeds, from eleven varieties (Altınay, Altınsoy, Arısoy, Atakişi, Atlas 3616, Cinsoy, Çetinbey, Umut 2002, Sarıgelin, Yemsoy and Yeşilsoy) and one local population, were used as plant material. Varieties were obtained from different institutions and private companies, and the local population was obtained from farmers.

2.2. Methods

2.2.1. Isoflavones (daidzein and genistein) and isoflavone glycosides analysis

Secondary metabolite analysis of the seed samples was determined with slight modification according to Carolina et al. (2021). Finely ground seed sample (2 g) was added to a glass vial (15 ml) containing 10 ml of methanol and 2 mL of aqueous 0.1 M HCl. The samples were sonicated for 3 minutes (×3) at room temperature.



Then, the samples were centrifuged at 5000 rpm for 5 minutes and the liquid on the collapsed samples was taken with the aid of a syringe. The finally, samples were adjusted to 2 mL of methanol and read on the LCMS/MS.

2.2.2. Total phenolic contents

The total phenolic contents of samples were determined with slight modification according to the Folin-Ciocalteu reagent (FCR) method of Singleton et al. (1999). Samples (200 μ L) were mixed with diluted FCR (200 μ L) and shaken vigorously for 3 min. Then, 200 μ L sodium carbonate (Na_2CO_3) solutions (20%) were added. Then samples absorbance of each sample was measured at a spectrophotometer at the absorbance value of 760 nm after incubating in dark at room temperature for 2 h. The total phenolic contents were expressed as mg equivalents of gallic acid (GAE) g^{-1} dry weight (DW) according to the equation obtained from the standard gallic acid graph and calculated from the calibration curve ($R^2= 0.9994$).

2.2.3. Total flavonoid content

The total flavonoid content was determined by using Arvouet-Grand et al. (1994) with some modifications. Each sample (200 μ L) was mixed with 100 μ L of aluminum nitrate (10%) and 100 μ L of potassium acetate (1 M). The total volume of the solution was adjusted to 5mL with ethanol. Similarly, a blank was prepared by adding methanol in place of the sample. Absorbance measurements were read at a spectrophotometer at the absorbance value of 417 nm after 40 min incubation at room temperature in dark conditions. Total flavonoid content was expressed as mg equivalents of quercetin (QE) g^{-1} DW according to the equation obtained from the standard quercetin graph and calculated from the calibration curve ($R^2= 0.9994$).

2.2.4. Free radical scavenging activity (DPPH)

The effect of each sample on 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) radical was identified according to Gezer et al. (2006). One hundred microliters from each sample in methanol were added to 3.9 mL of 0.004% methanol solution of DPPH. The absorbance of each sample was read at a spectrophotometer at the absorbance value of 517 nm after 30 min incubation at room temperature in dark.

2.2.5. Total condensed tannin

A 6 ml of tannin solution was added to 0.01 g of ground seed then placed in a tube and mixed on a vortex. The tubes were tightly capped and kept at 100 ° C for 1 hour, and the samples were allowed to cool. Then, they were read at a spectrophotometer at the absorbance value of 550 nm (Bate-Smith, 1975). Condensed tannins were calculated by the following formula: Absorbance (550 nm x 156.5 x dilution factor)/Dry weight (%).

2.2.6. Crude protein, crude fiber and fat content

Finely powdered seed samples were subjected to crude protein (CP), crude Fiber (CF) and fat analysis by using Near Reflectance Spectroscopy (NIRS, 'Foss XDS') with the software package program 'IC-0904FE'. These analyses were made in Yozgat (Türkiye) Bozok University Faculty of Agriculture Field Crops Laboratory.

2.3. Statistical Analysis

The data was expressed as mean \pm standard deviation and analyzed by analysis of variance (ANOVA). Duncan test was employed to draw the comparison between means and the significance was accepted at $P<0.05$. The correlations between examined parameters were determined by Pearson's correlation coefficient. The Biplot analysis was carried out with the help of the JMP package program.

3. Result and Discussion

Isoflavones (daidzein and genistein) and isoflavone glycosides (daidzin and genistin) contents of soybean genotypes were shown in Table 1. The genotype was significant ($P<0.01$) on daidzein, genistein, daidzin, and genistin contents. The highest daidzein and genistein content were determined in genotypes Yemsoy (0.446 ppm) and Çetinbey (1.188 ppm), respectively. Generally, the local population exhibited low isoflavone content compared to varieties. The daidzein and genistein are the most abundant isoflavone in soybean (Frank et al., 1999). These compounds are currently receiving more attention because of their potential benefit to human health especially in cancer treatment and prevention (Barnes, 2010; Bursaća et al., 2016). Wardlaw (2000) reported that consume half a cup of soybean a day is effective in cancer prevention. Previous studies showed that the daidzein and genistein content of soybean seed ranged between 0.08-2.35 mg g^{-1} and 0.02-0.83 mg g^{-1} , respectively (Malencic et al., 2012; Sumardi et al., 2017). The isoflavone contents of the soybean genotypes we studied were different from those reported in previous studies, which could be attributed to genetic variation.

The highest daidzin content was determined in the genotype of Altınay (1.006 ppm), while the lowest was genotype Yemsoy (0.395 ppm) and local population (0.388 ppm). Daidzin is an isoflavone glycoside that occurs naturally in soybean, it has antioxidant and anti-carcinogenic (Lu et al., 2009). Lojza et al. (2004) reported that daidzin content of soybean seed ranged between 0.249-0.534 mg g^{-1} . In the present study, the genistin content of soybean genotypes ranged between 0.254-8.906 ppm, and the highest genistin content was determined in the genotype Yeşilsoy. Choi et al. (2020) indicated that genistin is a popular ingredient with anti-adipogenic and anti-lipogenic properties. In other words, the genistin prevents excess weight by reducing fat accumulation in the body. Also, it is used to prevent weight gain after losing weight. Previous researchers indicated that genistin was the most abundant in soybean compared to the other isoflavone glycosides (Lee et al., 2005). These findings of the researchers were similar to our study, and genistin content of soybean seed was more than daidzin content (Table 1).

There were significant ($P<0.01$) differences in terms of total phenolic, total flavonoid, and DPPH-radical scavenging activity between genotypes (Table 2). With regards to total phenolic content, the genotype of

Çetinbey (20.469 mg GAE g⁻¹) showed the highest level, followed by the local population (18.177 mg GAE g⁻¹) and Cinsoy genotype (17.855 mg GAE g⁻¹). The phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity, while antioxidants show positive effects on improving health (Soobrattee et al., 2005; Glenville, 2006). Dajanta et al. (2011) reported that soybean total phenolic content ranged between 27.67-37.29 mg GAE g⁻¹. In the present study, the total phenolic contents examined soybean seeds were different from the previous study due to differences in the genetic material used. Some researchers stated that genotype is significant on the total phenolic content in soybean and that the differences in total phenolic content may be reflect genotypic

variability (Chung et al., 2008; Josipović et al., 2016). The highest total flavonoid content was determined in genotype Atakişi (6.738 mg QE g⁻¹), followed by genotype Atlas 3616 (6.062 mg QE g⁻¹) (Table 2). The flavonoids reduce blood lipid and glucose in humans, and they are good for heart patients Josipović et al. (2016) found that the average flavonoid content of 33 soybean genotypes was 0.511 mg CAE g⁻¹. The highest DPPH was determined in the genotypes Yeşilsoy (30.440%) and Yemsoy (29.150%), while the lowest was in the local population (9.676%). DPPH is one of the most important methods to evaluate the antioxidant properties of plants and desirable to be high. Zamindar et al. (2017) reported that DPPH of soybean ranged from 6.79-36.55%.

Table 1. Isoflavon and isoflavone glycoside contents of soybean genotypes

Genotypes	Daidzein (ppm)**	Genistein (ppm)**	Daidzin (ppm)**	Genistin (ppm) **
Çetinbey	0.190 ± 0.014 ^f	1.188 ± 0.060 ^a	0.552 ± 0.003 ^g	5.421 ± 0.345 ^c
Arısoy	0.258 ± 0.021 ^d	0.147 ± 0.030 ⁱ	0.666 ± 0.015 ^d	0.427 ± 0.025 ^g
Sarıgelin	0.385 ± 0.024 ^b	0.381 ± 0.020 ^g	0.605 ± 0.004 ^e	0.254 ± 0.117 ^g
Atakişi	0.260 ± 0.007 ^d	0.066 ± 0.003 ⁱ	0.484 ± 0.010 ^h	7.762 ± 0.184 ^b
Altınsoy	0.114 ± 0.009 ^g	0.308 ± 0.015 ^h	0.574 ± 0.010 ^f	1.799 ± 0.037 ^e
Yemsoy	0.446 ± 0.021 ^a	0.558 ± 0.002 ^e	0.395 ± 0.009 ⁱ	5.469 ± 0.150 ^c
Umut 2002	0.039 ± 0.002 ^h	1.139 ± 0.048 ^b	0.747 ± 0.001 ^b	5.253 ± 0.092 ^c
Cinsoy	0.183 ± 0.004 ^f	0.234 ± 0.021 ⁱ	0.708 ± 0.008 ^c	3.983 ± 0.158 ^d
Yeşilsoy	0.019 ± 0.001 ^h	0.604 ± 0.030 ^d	0.578 ± 0.018 ^f	8.906 ± 0.111 ^a
Altınay	0.220 ± 0.009 ^e	0.672 ± 0.002 ^c	1.006 ± 0.003 ^a	4.174 ± 0.184 ^d
Atlas 3616	0.321 ± 0.020 ^c	0.497 ± 0.003 ^f	0.424 ± 0.007 ⁱ	0.348 ± 0.015 ^g
Local population	0.035 ± 0.002 ^h	0.380 ± 0.001 ^g	0.388 ± 0.008 ⁱ	0.976 ± 0.077 ^f

** P<0.01, There is no difference between the same letters in each column (P<0.05).

Table 2. Antioxidant properties of soybean genotypes

Genotypes	TP (mg GA/g)**	TF (mg QE/g)**	DPPH (%)**
Çetinbey	20.469 ± 0.296 ^a	5.709 ± 0.015 ^{de}	11.012 ± 0.729 ^{ef}
Arısoy	15.678 ± 0.027 ^d	5.312 ± 0.029 ^f	14.980 ± 3.077 ^d
Sarıgelin	16.711 ± 0.553 ^c	5.621 ± 0.014 ^e	11.336 ± 0.486 ^{ef}
Atakişi	9.513 ± 0.269 ^g	6.738 ± 0.014 ^a	12.672 ± 0.121 ^e
Altınsoy	15.335 ± 0.523 ^d	5.782 ± 0.000 ^{cde}	24.534 ± 1.214 ^b
Yemsoy	13.997 ± 0.348 ^e	4.047 ± 0.029 ⁱ	29.150 ± 0.405 ^a
Umut 2002	14.389 ± 1.092 ^e	5.915 ± 0.044 ^{bcd}	20.972 ± 1.052 ^c
Cinsoy	17.855 ± 0.660 ^b	6.003 ± 0.044 ^{bc}	11.417 ± 0.648 ^{ef}
Yeşilsoy	15.683 ± 0.175 ^d	5.944 ± 0.044 ^{bcd}	30.040 ± 0.485 ^a
Altınay	13.949 ± 0.594 ^e	4.356 ± 0.515 ^h	20.810 ± 0.162 ^c
Atlas 3616	12.075 ± 0.512 ^f	6.062 ± 0.014 ^b	12.429 ± 0.688 ^e
Local population	18.177 ± 0.027 ^b	5.003 ± 0.015 ^g	9.676 ± 0.445 ^f

** P<0.01, There is no difference between the same letters in each column (P<0.05). TP= total phenolic content; TF= total flavonoid; DPPH= free radical scavenging activity.

Crude protein, crude fiber, fat, and condensed tannin contents of soybean genotypes were given in Table 3. Among to genotypes, significant differences were detected (P<0.01) in terms of crude protein, crude fiber, fat contents, while condensed tannin was not significant.

Among the genotypes, the rude protein content amongst was ranged from 36.127% (local population) to 40.603% (genotype Atakişi). The protein content of soybean is important for diabetics and cholesterol patients (Bhathena and Velasquez, 2002), and used to replace

animal proteins in the diet (Lindsay and Claywell, 1998). It has been suggested that soybeans are low in saturated fat and cholesterol and consuming 5 grams of soybean protein per day may be beneficial for heart health (Bolla, 2015). Kulan et al. (2017) indicated that a high variation in soybean seed for protein contents, and ranged between 36-40%. The fiber content of genotype Altınsoy (4.977%), Yemsoy (5.477%), Yeşilsoy (5.217%), and local population (5.690%) was higher than other genotypes. Ciabotti et al. (2006) found that the fiber content of soybean ranged between 7.09-7.56%. Soybean seed is low in saturated fat and naturally cholesterol-free (Bolla, 2015). Besides, some researchers indicated that fiber of soybean decreases serum cholesterol in patients with high cholesterol levels (Shorey et al., 1985)

The fat content in studied genotypes was ranged between 17.440-22.337% and was less in the local population than varieties. Kulan et al. (2017) reported that the fat content of 13 different soybean genotypes ranged from 19.2% to 23.1%. Tannin is a polyphenol that possesses various medicinal properties, as well as acts as an antioxidant. Tannins are divided into two groups as condensed and hydrolyzable tannins. Condensed tannins

are effective against asthma, hypersensitive pneumonitis, allergic rhinitis. Some researchers indicated that condensed tannins are anti-nutrients, but beneficial at low concentrations (2-3%) (Champ, 2002; Akindahunsi and Salawu, 2005). In this study, condensed tannins of soybean genotypes ranged from 0.219% (genotype Yeşilsoy) to 0.272% (genotype Umut 2002). El-Shemy et al. (2000) found an average of 0.029% concentrated tannin content in soybean seeds they examined. The correlations between the investigated traits in soybean genotypes are given in Table 4. The strong and negative correlations were noted between crude protein and crude fiber content (-0.676) followed by the correlations between total flavonoid and crude fiber (-0.592), total phenolic content and crude protein (-0.542), genistein and crude protein (-0.588). It was also determined that there was a negative correlation of daidzein with genistein, daidzin and genistin meaning that the increase in the daidzein content in soybean results the decrease in other isoflavones. On the other hand, there was a low and positive correlation of genistein with daidzin and genistin.

Table 3. Some quality traits of soybean genotypes

Genotypes	CP (%)**	CF (%)**	FAT (%)**	CT (%)
Çetinbey	36.173 ± 0.127 ^e	4.860 ± 0.167 ^{b-e}	20.150 ± 0.175 ^{cd}	0.263 ± 0.099
Arisoy	39.520 ± 0.315 ^b	4.297 ± 0.547 ^{de}	21.047 ± 0.076 ^b	0.257 ± 0.105
Sarıgelin	37.010 ± 0.760 ^{cde}	4.600 ± 0.668 ^{cde}	17.857 ± 0.087 ^g	0.237 ± 0.026
Atakişi	40.603 ± 0.411 ^a	4.047 ± 0.245 ^e	19.853 ± 0.176 ^d	0.254 ± 0.067
Altınsoy	36.640 ± 0.234 ^{de}	4.977 ± 0.240 ^{a-d}	18.910 ± 0.144 ^f	0.248 ± 0.096
Yemsoy	36.930 ± 0.520 ^{cde}	5.477 ± 0.405 ^{ab}	20.363 ± 0.110 ^c	0.248 ± 0.096
Umut 2002	37.303 ± 0.410 ^{cd}	4.417 ± 0.325 ^{cde}	19.873 ± 0.110 ^d	0.272 ± 0.090
Cinsoy	38.803 ± 1.037 ^b	4.857 ± 0.758 ^{b-e}	19.487 ± 0.092 ^e	0.257 ± 0.047
Yeşilsoy	37.653 ± 0.263 ^c	5.217 ± 0.359 ^{abc}	20.130 ± 0.308 ^{cd}	0.219 ± 0.067
Altınay	37.437 ± 0.603 ^{cd}	4.837 ± 0.498 ^{b-e}	19.257 ± 0.308 ^e	0.257 ± 0.088
Atlas 3616	37.217 ± 0.260 ^{cd}	4.560 ± 0.275 ^{cde}	22.377 ± 0.160 ^a	0.239 ± 0.081
Local population	36.127 ± 0.770 ^e	5.690 ± 0.785 ^a	17.440 ± 0.378 ^h	0.263 ± 0.076

** P<0.01, There is no difference between the same letters in each column (P<0.05). CP= crude protein, CF= crude fiber, CT= condensed tannin.

Table 4. The correlation values between quality traits in soybean genotypes

	Genistein	Daidzin	Genistin	TP	TF	DPPH	CP	CF	FAT	CT
Daidzein	-0.261	-0.185	-0.254	-0.295	-0.287	-0.109	0.157	-0.209	0.277	-0.170
Genistein		0.215	0.288	0.346	-0.153	0.183	-0.588*	0.137	0.111	0.271
Daidzin			0.071	0.048	-0.154	0.111	0.155	-0.306	-0.099	0.239
Genistin				-0.206	0.182	0.476	0.259	0.014	0.125	-0.129
TP					-0.196	-0.233	-0.542*	0.476	-0.395	0.177
TF						-0.347	0.432	-0.592*	0.173	-0.129
DPPH							-0.143	0.330	0.153	-0.394
CP								-0.676**	0.298	0.008
CF									-0.383	-0.155
FAT										-0.161

* P<0.05, ** P<0.01, There is no difference between the same letters in each column (P<0.05). TP= total phenolic; TF= total flavonoid; DPPH= free radical scavenging activity; CP= crude protein; CF= crude fiber; CT= condensed tannin.

The biplot graphic of the 12 soybean genotypes for investigated traits is present in Figure 1. PCA (Principle Component Analysis) shows the relationships between genotype and traits as a whole, and it has many advantages according to the correlation analysis which shows the relationship between two traits (Yan and Reid, 2008). The results of PCA revealed that the first 1 component (PCA 1) and the second (PCA 2) respectively exhibited 27.4% and 17.8% of the variation, a total of 45.2%. This analysis shows that what

genotype/genotypes have higher values in terms of the quality traits and that these traits are in a positive or negative relationship with each other. According to the biplot, crude fiber and DPPH with genistein are in the same direction, while total phenolic and condensed tannin with daidzin are in the same direction. In addition to, 1 (Çetinbey) and 12 (Local population) genotypes exhibited a higher value in total phenolic content compared to the other genotypes (Figure 1).

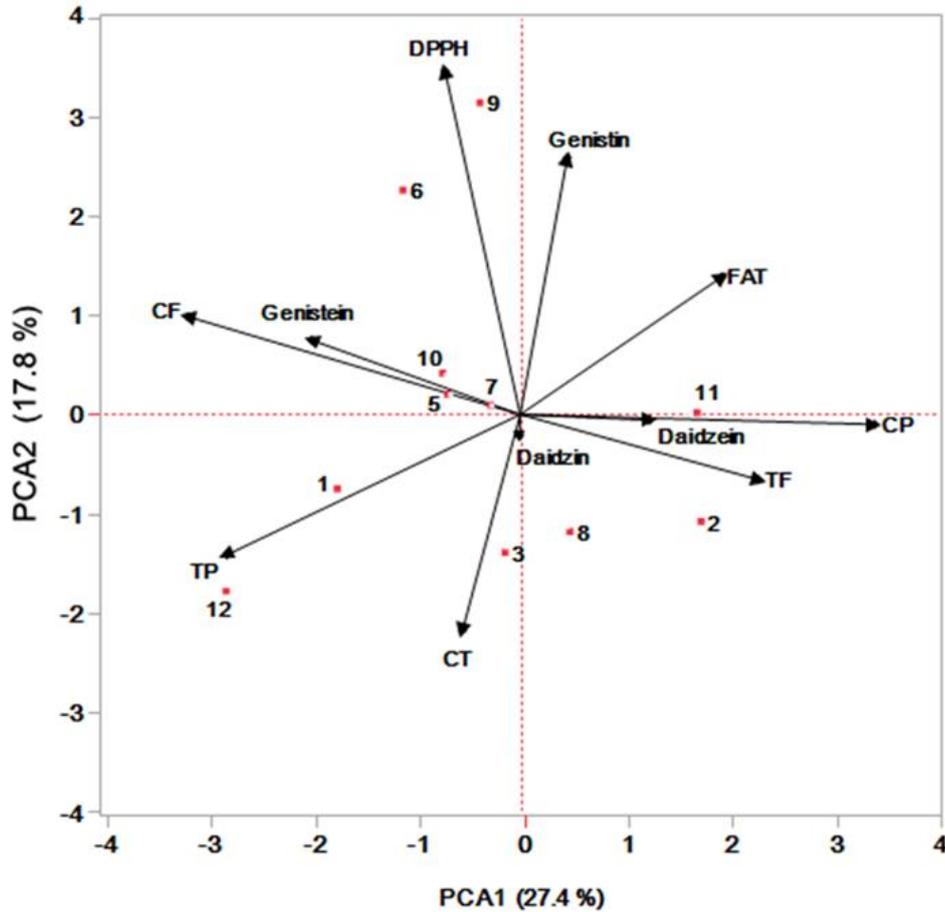


Figure 1. Principal component analysis of some quality traits of soybean genotypes. TP= total phenolic, TF= total flavonoid, DPPH= free radical scavenging activity, CP= crude protein, CF= crude fiber, CT= condensed tannin. G1= Çetinbey, G2= Arısoy, G3= Sarıgelin, G4= Atakişi, G5= Altınsoy, G6= Yemsoy, G7= Umut 2002, G8= Cinsoy, G9= Yeşilsoy, G10= Altınay, G11= Atlas 3616, G12= local population.

4. Conclusion

This study reveals the role of genotype selection in soybean in terms of healthy diets or the special demands of consumers. Isoflavonoids, antioxidants, and some quality traits in soybean seed were found to be different among the genotypes.

When isoflavonoid (daidzein, genistein) and isoflavone glycoside (daidzin and genistin) contents were compared, Yemsoy, Çetinbey, Altınay, and Yeşilsoy genotypes exhibited high value. Genotypes of Çetinbey, Atakişi, and Yeşilsoy antioxidant characteristics were more than other genotypes. On the other hand, all genotypes had sufficient nutritional value in terms of crude protein, crude fiber, fat, and condensed tannins

contents. Within respect, all genotypes used in the current study constituted high-quality raw materials for human consumption and food production.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	N.E.	E.G.	H.M.	U.B.	M.Ç.D.
C	20	30	20	20	10
D	30	40	30		
S		50	50		
DCP	20	40	20	10	10
DAI		40	30	30	
L	20	20	20	20	20
W	10	30	30	30	20
CR	20	20	20	20	20
SR	10	30	30	30	0

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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