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Characterization of bioactive content and aroma compounds of geographical indication İspir, Narman, and Hınıs dry beans

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Abstract: This study aimed to define the aroma components, antioxidant activity, total phenol, and sugar contents of three geographically indicated dry beans: İspir Dry Bean, Narman Sugar Bean, and Hınıs Bean. Significant differences were found among three different dry beans in terms of total phenolic content, total antioxidant capacity (2,2-diphenyl-1-picrylhydrazil test), sugar content, and volatile compounds. The total phenolic content ranged from 14.09 mg gallic acid equivalents per 100 g dry bean sample in Narman to 36.73 mg in Hınıs. The highest total antioxidant activity was observed in İspir Bean with 8.38%, while the lowest was in Narman Bean with 6.02%. Additionally, the total sugar contents of İspir, Narman, and Hınıs beans were determined to be 6.46%, 5.60%, and 4.22%, respectively. Forty different aroma volatiles, including 10 alcohols, five terpenes, 11 aldehydes, four esters, six acids and four ketones, were identified in the bean seed samples. Narman Bean had higher levels of total alcohols, total acids, and total esters compared to İspir and Hınıs beans, while İspir Bean had higher levels of total terpenes and total ketones and Hınıs Bean had higher levels of total aldehydes. Furthermore, PCA and heatmap analysis revealed that the dry beans were divided into two main groups and that Narman beans were separated from İspir and Hınıs beans. In conclusion, this study showed that the geographically indicated İspir, Narman, and Hınıs dry bean varieties differed from each other in terms of flavor and nutritive characteristics, including bioactive and volatile compounds.

Key words: Common bean, volatile compounds, total antioxidant, total phenol, PCA, heatmap

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is produced in many countries due to its affordability as a protein source, its role in meeting nutritional needs of the population, its contribution to the livelihood of rural population, its presence in traditional culinary cultures, particularly in Latin American countries, and its involvement in international trade (Moreno-Jiménez et al., 2014; Alzuaibr, 2023; Çilesiz et al., 2023; Nadeem and Baloch, 2023). As the most economically significant species within the genus *Phaseolus*, dry beans are produced in numerous regions worldwide(Nadeem et al., 2021). Dry beans, which hold an important place in Turkish cuisine, accounted for 22% of all legume consumption according to the 2022 data from the Turkish Statistical Institute.[1] The annual consumption of dry beans in Türkiye is 282,000 t, and the annual consumption of dry beans per capita varies between 3.3 and 3.5 kg. In 2021, Türkiye produced 305 thousand tons of dry beans in an area of 108,000 ha, accounting for 1.10% of world dry bean production (27.72 Mt of 35.92 Mha in 2021). Additionally, Türkiye's dry bean exports (0.18 Mt in 2021) represent 3.78% of the world's dry bean exports (4.78 Mt in 2021).[2]Legumes are a good source of bioactive phenolic compounds for humans, as these compounds play a significant role in many physiological and metabolic processes. They function as bioactive compounds and are

[1]Turkish Statistical Institute (2022). TURKSTAT [online]. Website http://www.tuik.gov.tr [accessed 10 June 2023].

[2]Food and Agriculture Organization of the United Nations (2021). FAOSTAT [online]. Website https://www.fao.org/faostat/en/ [accessed 10 June 2023].

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important determinants of color, taste, and flavor of foods. Additionally, they exhibit free radical-scavenging capacity and have the ability to interact with proteins (Singh et al., 2017). However, common bean has a wide range of phenolic content, with values comparable to other widely consumed legumes such as pea, chickpea, lentil, and soybean (Gan et al., 2017), highlighting the importance of beans as a source of phenolic compounds. Moreover, common beans contain carbohydrates, proteins, lipids, vitamin B, fiber, minerals, and bioactive substances with significant antioxidant activity, including flavonoids, anthocyanins, polyphenols, tannins, and flavones (García-Díaz et al., 2018). Through their antimutagenic, vasodilatory, antiinflammatory, and anticancer properties, these functional chemicals have been associated with the prevention of chronic degenerative diseases, such as diabetes, obesity, cardiovascular disease, and cancers of colon, breast, intestine, and ovary (Oomah et al. 2010; Xu and Chang, 2012; García-Lafuente et al., 2014; García-Díaz et al., 2018).

Legumes exhibit significant genotypic and phenotypic diversity due to their high adaptability. In addition to genetics, geographical location, growing seasons, and environmental factors also play important roles in the nutritional content of dry beans (Sathe et al., 1984; Florez et al., 2009). The common bean was introduced to Türkiye in the 17th century and, despite its foreign origin, it has been effectively adapted and spread throughout the country (Bozoğlu and Sözen, 2007).

Türkiye, with its different geographical formations and climates, has various natural reserves with genetic diversity and richness. However, the rapidly increasing population, advancing technology, changing consumption habits, and the drive to achieve greater profits with less investment have increased the use of highly productive varieties in production, resulting in a rapid loss of genetic diversity and natural wealth (Colak et al., 2018; Bozhuyuk, 2022; Topçu, 2022). Therefore, it is crucial to register and protect local genetic resources with high market value.

In many regions of Türkiye, local dry bean populations have become identified with the area where they are grown and have started to be considered among the traditional varieties. Additionally, some dry bean populations are registered as varieties within the Geographical Indication System in Türkiye (e.g., İspir Dry Bean, Hınıs Bean, Narman Sugar Bean), and there are also dry bean dishes registered as food. The consumers view a brand as the most important aspect of a food product, and thus, branding can enhance a product's value and recognition (Topcu, 2004). Consumers who consistently purchase the same brand know that they will receive the same features, benefits, and quality with each purchase (Topcu and Demir, 2012).

On the other hand, the reasons for preferring dry beans are influenced by factors such as acceptability, wetting properties, cooking quality, and nutritional value according to the consumer. Acceptability characteristics include a wide variety of properties such as grain size, shape, color, appearance, stability under storage conditions, cooking properties, quality, and taste (Reyes-Moreno et al., 1993).

In Erzurum Province, located in the northeast of Türkiye, three dry beans (İspir Dry Bean, Hınıs Bean, and Narman Sugar Bean) have been registered with geographical indication certificates. However, producers face challenges in marketing due to the perceptions that these three sugar beans have the same properties. Given that market demands extend beyond Türkiye's borders, it is crucial for both producers and consumers to understand the differences between these local genotypes. Therefore, the aim of this study was to comprehensively characterize the total phenolic content, total antioxidant activity, sugar compounds, and aroma compounds of geographical indication sugar beans (İspir Dry Bean, Hınıs Bean, and Narman Sugar Bean), which have high commercial value for the region.

2. Materials and methods

2.1. Plant material

In this study, İspir, Narman, and Hınıs beans with geographical indications were used as research material. The geographical indication (GI) name, registration date, applicant, and geographical borders of the investigated common beans are presented in Table 1. Additionally, certain physical and physicochemical properties of İspir Dry Bean, Narman Sugar Bean, and Hınıs Bean, as listed in the geographical indication registration certificate, are presented in Table 2. Dry bean samples were collected from production areas within the geographical borders in autumn (Figure 1). The samples were stored in a cool, dry place to maintain their nutritional value until used in the study.

2.2. Biochemical parameters

2.2.1. Extract preparation

Dry bean seeds were ground into a fine powder using a coffee bean grinder. The ground beans were then air-dried in a ventilated oven at 40 °C for 24 h, ground again, and passed through a standard sieve (number 18, with an open ring size of 1.00 mm). The flours (5 g) were mixed with 10 mL of 80% aqueous methanol (V/V) and shaken continuously for 2 h at room temperature using an orbital shaker set to 150 rpm. Afterward, the mixture was centrifuged at 5000 rpm for 10 min. Supernatants were filtered through filter paper (Whatman No. 1) and the residue was reextracted with 10 mL of 80% aqueous methanol (V/V) for 1 h. Finally, the volume of the collected extracts was adjusted to 20 mL with 80% aqueous methanol (V/V), resulting in a

Table 1. The geographical indication (GI) name, registration date, applicant, and geographical borders of İspir, Narman, and Hınıs beans.

GI name	Registration date	Applicant	Geographic borders
İspir Dry Bean	January 31, 2011	İspir Chamber of Tradesmen and Craftsmen	Borders of İspir district
Hınıs Bean	December 27, 2016	East Anatolian Agricultural Research Institute	Hınıs district and its villages
Narman Sugar Bean	July 20, 2020	East Anatolian Agricultural Research Institute	Erzurum Province, Narman district

Table 2. Certain physical and physicochemical properties of İspir, Narman, and Hınıs beans in geographical indication registration certificate.

Properties	İspir Dry Bean	Hınıs Bean	Narman Sugar Bean
Growth habit	Semiclimbing	Climbing	Climbing
Plant height (cm)	150–170	98	90–170
First pod height (cm)	11–15	16.5	13–30
Seed yield (kg/da)	230–305	200–250	150–200
100 seed weight (g)	30–32.5	48.06–49.58	55.5–61.2
Number of seeds per pod	4–5	4–10	-
Number of pods per plant	40–50	-	-
Number of seeds per plant	-	32.2	-
Pod length (cm)	7–12	-	-
Seed shape	Circular-slightly kidney	Circular-oval	Circular-oval
Maturity period (days)	130–140	-	150
Germination period (days)	8		
Flowering period (days)	56–61		
Wet weight (g)	-	96.96–101.30	116.2–119.1
Dry weight (g)	-	-	55.6–60.6
Water absorption capacity (g/grain)	-	0.48–0.51	-
Water absorption index (%)	-	1.01–1.05	-
Dry volume (mL)	-	139–140	143–147
Wet volume (mL)	-	236–240	254–256
Swelling capacity (mL/grain)	-	0.47–0.50	-
Swelling index (%)	-	2.20–2.25	-
Cooking time (min)	-	40–46	-
Protein (%)	-	23.4–25.1	-

final concentration of 0.25 g mL^{-1}). The extracts were kept at −20 °C until analysis. Two extractions were performed per sample. The supernatant was used as the extract for estimation of total phenol content and antioxidant activity.

2.2.2. Determination of total phenol content

Total phenols were analyzed using the Folin-Ciocalteu reagent procedure of Heimler et al. (2005) with minor modifications. Briefly, 15 µL of extract, 1.5 mL of double-distilled water, and 150 µL of diluted Folin-Ciocalteu (Sigma) were added to a 2-mL tube. The mixture was subsequently included with 150 µL of saturated 20% sodium carbonate (Na_2CO_3) (w/v) and shaken. The samples were incubated for 2 h at room temperature and in the dark. For the control and blank groups, 15 µL of double-distilled water and 15 µL of 80% aqueous methanol (V/V) were used, respectively. In 250 µL of each sample, the solution absorbance at 765 nm was measured using a spectrophotometer (Multiskan Go, Thermo Scientific, MA, USA). A calibration curve was created using methanolic solutions of gallic acid, and the results were expressed as gallic acid equivalents (GAE). Quantification was performed by measuring milligrams of gallic acid equivalents (GAE) per 100 g of dry matter.

2.2.3. Determination of total antioxidant activity

Radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging

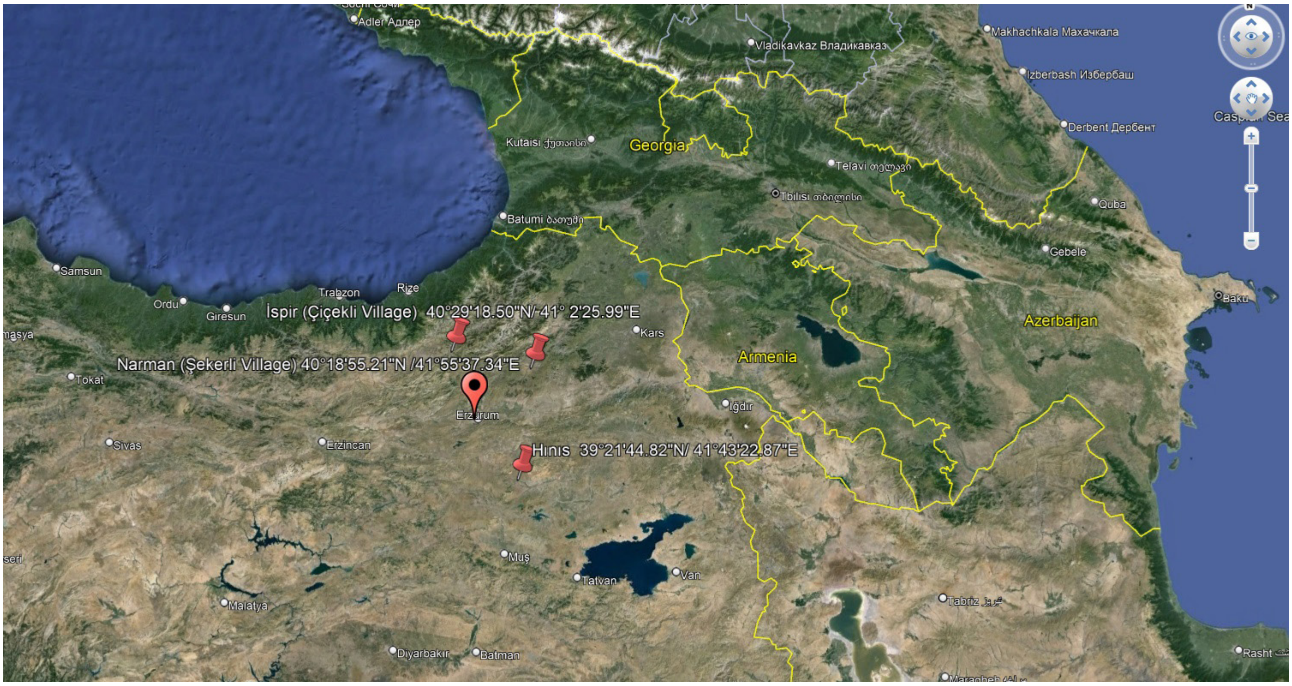

Figure 1. Districts where geographically indicated common beans are collected.

potential in each bean sample according to the method of Brand-Williams et al. (1995), with some modifications. First, 100 µL of the extract was added to a 2-mL centrifuge tube, and then 1900 µL of DPPH in methanol (95%) was added to the tube under darkroom conditions. The shaken mixture was incubated at 24 °C for 60 min in a dark room. When the reaction reached a steady state, the absorbance values of the mixture were measured at 515 nm using a spectrophotometer (Multiskan Go, Thermo Scientific). For each of the control and blank groups, double-distilled water and methanol were used, respectively, instead of seed extract. The results were calculated using the following formula:

$$DPPH\ inhibition\ (\%) = (Abs_{control} - (Abs_{control} - Abs_{blank} / Abs_{sample}) \times 100)$$

$$Percentage\ of\ reduction\ power\ (\%) = ((Abs_{control} - Abs_{sample}) / Abs_{control}) \times 100$$

2.2.4. Determination of sugar content

Glucose, fructose, xylose, sucrose, and total sugar content in the dry bean samples were determined according to the methods developed by Giannoccaro et al. (2008) and Hou et al. (2009). Briefly, ground bean seeds (0.15 g) were placed in 2-mL Eppendorf microcentrifuge tubes containing 1.5 mL distilled deionized water. The mixture was vortexed and incubated at room temperature at 200 rpm for 15 min on a horizontal shaker. It was then placed in an ultrasonic bath and sonicated at 80 °C for 15 min. After sonication, the mixture was centrifuged at 5500 rpm for 15 min and filtered using Whatman nylon syringe filters (0.45 µm, 13 mm diameter) before HPLC analysis. The high-performance liquid chromatographic device (Shimadzu LC 20A VP, Shimadzu, Kyoto, Japan) consisted of a controller connected to a refractive index detector, outfitted with an in-line degasser, pump, and autoinjector, all interfaced to a PC running Class VP chromatography manager software. The separations were conducted on a reverse-phase Ultrasphere Coregel-87 C analytical column (Transgenomic) with dimensions of 300 mm × 7.8 mm i.d., 5 µm particle size, and a flow rate of 0.6 mL min^{-1}. Isocratic elution was performed using ultrapure water. Individual sugars were quantified using their standards and reported as a percentage of dry weight (DW).

2.2.5. Determination of aroma compounds

Flour of İspir, Narman, and Hınıs beans was sieved using a 100-mesh sieve. Three different samples were analyzed for each type of bean. Headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) was performed with modifications to the methodology of Urün et al. (2021), including adjustments in sample preparation, incubation, volatile extraction, injection, and GC-MS analysis. Method parameters were optimized before analysis to capture a wide variety of volatile compounds. The samples were weighed (0.5 g) and placed into the headspace vial. Next, 1 mL of saturated $CaCl_2$ was added to increase the solution's ionic strength and drive the legume volatiles into the headspace vial. A PTFE-coated silicone septum screw cap was then used to close the vial firmly. The mixture was incubated at 40 °C for 30 min with agitation at 250 rpm. For volatiles extraction, SPME fiber

(85 µm CAR/ PDMS; carboxene/polydimethylsiloxane; gray) was utilized. The adsorbed aroma compounds of the bean seed samples were analyzed using a Shimadzu GC-2010 Plus gas chromatography-mass spectrometer (GC/MS). The GC oven temperature was initially held at 40 °C, then programmed to rise to 260 °C at a rate of 5 °C/min, and held at 260 °C for 40 min. The injector and detector were both set to 250 °C. The ionization voltage was set to 70 eV and scanned between 35 and 350 amu. The carrier gas (helium) flow rate was 1 mL/min. Aroma compounds were identified by comparing the mass spectra of standard compounds from the Wiley, NIST, and Flavor GC–MS Libraries with the mass spectra of unknown compounds. The mass spectra were also matched to those of reference compounds and confirmed using retention indices from published sources. Total ion chromatograms were used to calculate the relative percentage quantities of the separated compounds.

2.2.6. Statistical analysis

The raw data obtained in three replicates were summarized in Microsoft Excel. Differences in phenolic content, antioxidant activity, and sugar content among seeds from three geographically indicated bean genotypes were compared using a one-way analysis of variance (ANOVA). Duncan's multiple-range test at the 95% confidence level was used to find differences among means. The program used for these analyses was SPSS (version 27, IBM Corporation, Armonk, NY, USA, 2020). Volatile compound data are presented as an average of three measurements. Heatmap with hierarchical clustering analysis and principal component analysis (PCA) were performed on the variability of total phenolic, total antioxidant, total sugar, and aroma components (including total alcohols, total terpenes, total aldehydes, total esters, total acids, and total ketones) across genotypes. Heatmap with hierarchical clustering and PCA plots were generated using BioVinci (version 3.0.9, BioTuring Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Total phenol content and antioxidant capacity

When the geographical indication bean cultivars were compared with respect to total phenolic levels, the differences between genotypes were found to be significant at a level of $p < 0.05$ (Figure 2). The total phenolic contents, from the highest to the lowest, were 36.73, 24.78, and 14.09 mg GAE/100g DW, obtained from Hınıs, İspir, and Narman beans, respectively. Phenolic compounds are one of the most important families of phytochemicals present in beans. These molecules play a significant role in human health because they possess antioxidant activity associated with antidiabetic, antiobesity, antiinflammatory, antimutagenic, and anticarcinogenic properties. Based on the World Health Organization's recommendation to consume 400 g of vegetables daily, a study shows that the average daily intake of total phenolics from this source can range from 100 to approximately 460 mg (Pérez et al., 2023). The total phenolic content varies greatly among and within market classes of common beans, depending on several factors, including genetic, environmental, and the analytical methods used for measurement (Broughton et al., 2003; Häkkinen and Törrönen, 2006). Cardador-Martínez et al. (2002) reported that the content of phenolic compounds in common bean seeds was approximately 145 mg/g and represented approximately 11% of the total seed weight. In a study conducted by Sahasakul et al. (2022), the total phenolic content of 10 common bean cultivars from three different genera was found to be between 0.72

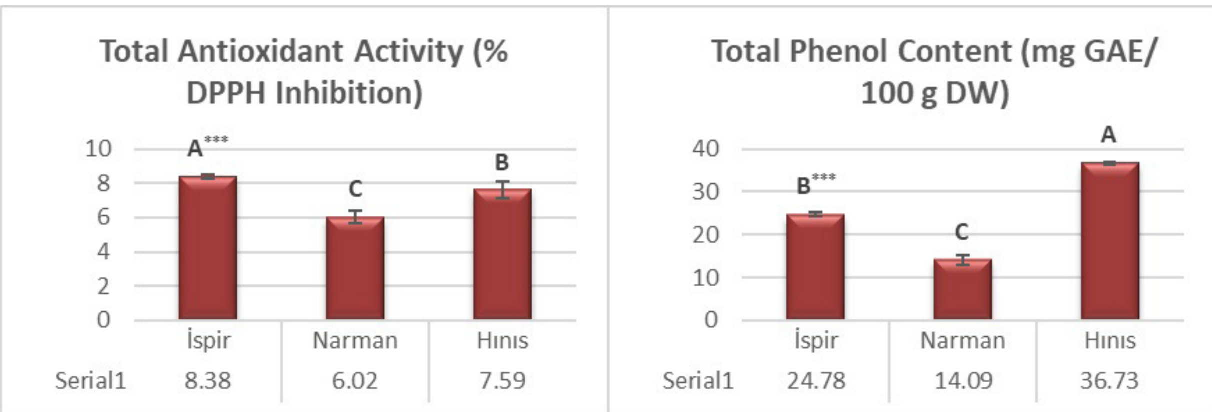

Figure 2. Values of the total phenol content (mg GAE/100 g) and total antioxidant activity (%) in the seeds of geographical indication common bean genotypes. Values are expressed as mean ± standard deviation (n = 3). Results with different letters are significantly different at a level of $p < 0.05$ (***: $p \leq 0.001$).

and 3.12 mg GAE/g DW. Kan et al. (2017) reported that the total phenolic content of 26 kidney beans ranged from 0.25 to 3.79 mg GAE/g DW. According to another study by Chávez-Mendoza et al. (2018), the total phenol content of the examined bean cultivars ranged from 0.69 to 3.32 mg GAE/g in the seed coat and from 0.44 to 0.99 mg GAE/g in the cotyledon. In another study investigating the total phenolic content in different parts of the bean (leaves, pods, and seeds), it was reported that the total phenol content in seed methanol extracts was 6.87 mg GAE/g (Ha et al., 2020). Yao et al. (2011) reported that common beans contain 8.59 mg GAE/g total phenol. Additionally, Ombra et al. (2016) reported that the total phenolic content of beans ranges from 0.14 to 1.29 mg GAE/g.

Numerous studies have used the DPPH method, which can easily measure bioactive compounds in beans, to determine their antioxidant activity (Ranilla et al. 2009; Ombra et al. 2016; Kajiwara et al. 2022). The total antioxidant activities of the dry bean varieties examined in the study were evaluated using the DPPH method. The varieties exhibited statistically significant differences in DPPH values at a level of $p < 0.05$. Total antioxidant activity ranged between 6.02% and 8.38% in DPPH inhibition. İspir Bean variety had the highest antioxidant activity, with 8.38% DPPH inhibition, while Hınıs and Narman bean varieties had 7.56% and 6.02% DPPH inhibition, respectively (Figure 2). In a conducted study by Kajiwara et al. (2022), it was found that the DPPH inhibition values of 14 Andean bean genotypes with white, red, and variegated seeds ranged from 6.46% to 71.23% . Chávez-Mendoza et al. (2018) found the antioxidant capacity of 12 common bean varieties from different regions of Mexico ranged from 23.86% to 84.10% in the seed coat and from 0.66% to 29.77% in the cotyledon. Ha et al. (2020) reported that the DPPH inhibitory percentages of methanol extract from common bean seeds, pods, and leaves were 5.12%, 14.19%, and 45.60%, respectively.

The phytochemical and antioxidant content of plant materials is significantly affected by the method used for their extraction and purification (Nawaz et al., 2020). In this context, the differences among the research findings are thought to be largely due to the differences in the extraction and analysis methods.

3.2. Sugar content

Dry beans provide an important source of plant-based protein and carbohydrates in both animal and human diets (Messina, 2014). The taste and flavor of the beans are enhanced by the presence of simple sugars such as glucose, fructose (a reducing sugar), and sucrose (a nonreducing sugar) (Susi et al., 2021). *P. vulgaris* genotypes identified as sweet are favored in panelist tests, and sweetness has even been used to differentiate between cultivars (Mkanda et al., 2007). The sugar components of beans were analyzed using chromatographic method (Rupérez, 1998; Sánchez-Mata et al., 1998). In the study, glucose, sucrose, xylose, fructose, and total sugar contents of beans were found to be statistically different at a level of $p < 0.05$ (Figure 3). Hınıs Bean had the highest sucrose content with 5.30%, while Narman and İspir beans had sucrose contents of 4.45% and 3.62%, respectively. Additionally, the highest glucose and xylose contents were obtained from Hınıs Bean, with 0.39% and 0.47%, respectively, followed by Narman Bean with 0.36% and 0.35%, and İspir Bean with 0.27% and 0.18%. The fructose content of Narman Bean was higher with 0.45% compared to other bean varieties, it was followed by Hınıs Bean with 0.29% and İspir beans with 0.15%. When the total sugar content of the beans was compared, it was determined that the highest sugar content was in Hınıs Bean with 6.46%, while the total sugar content of Narman and İspir beans was 5.60% and 4.22%, respectively. According to the study carried out by Rupérez (1998), the total sugar content of different legume species was reported to range from 6.69% to 9.99%. In another study, Jacinto-Hernández et al. (2019) reported that the percentage of total sugar in *P. vulgaris* genotypes ranged from 6.2% to 6.5%. In another study analyzing carbohydrates in five different legumes, the total sugar was found to be 5.61%, 6.71%, 4.99%, 6.08%, and 7.22% for navy bean, pinto bean, lentils, faba bean, and mung bean, respectively. Additionally, the study reported that navy bean and pinto bean flours contained 2.23 ± 0.04% and 2.82 ± 0.09% sucrose and glucose, respectively (Naivikul and D'Appolonia, 1979).

3.3. Aroma compounds

Unprocessed legumes have a distinctive odor due to their natural plant metabolism. It has been revealed that the main causes of these odors are volatile substances such as hexanal and 1-octen-3-ol (Roland et al., 2017; Rajhi et al., 2021). Aroma compounds determined by HS/SPME/GC–MS techniques for beans, namely İspir, Narman, and Hınıs, are presented in Table 3. A total of 40 aroma volatile substances, including 10 alcohols, five terpenes, 11 aldehydes, four esters, six acids and four ketones, were detected in the seed samples of three common beans. According to the results, aldehydes were found as the main volatiles in bean seeds. According to Khrisanapant et al. (2019), aldehydes are the most prevalent volatile chemicals in legumes, which is consistent with the findings of our study. Uncooked dry bean seeds (red and white) isolated by vacuum steam distillation at 50 °C with continuous solvent (hexane) extraction contained 26 volatile chemicals. In the study of Buttery et al. (1975), 1-octen-3-ol (1.4 μg/kg) and (Z)-3-hexenol were identified as the most important flavor components of uncooked beans. In another study, 23 compounds, mainly aliphatic and aromatic alcohols and aldehydes, were detected in dry beans. The researchers

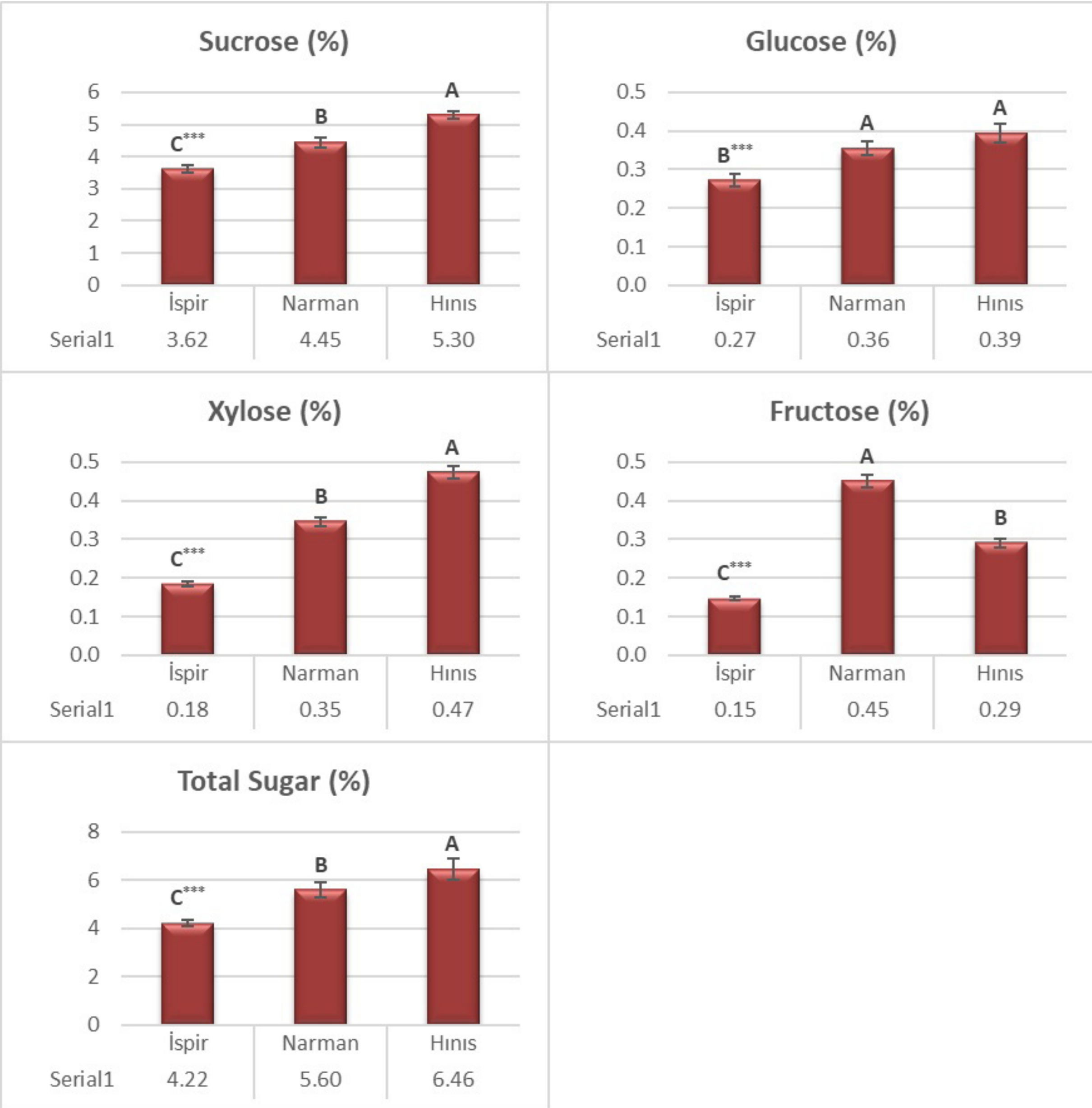

Figure 3. Values of the sucrose (%), glucose (%), xylose (%), fructose (%), and total sugar (%) in the seed of geographical indication common bean genotypes. Values are expressed as mean ± standard deviation (n = 3). Results with different letters are significantly different at a level of $p < 0.05$ (***: $p \leq 0.001$).

observed significant differences in unusual volatile compounds among different bean samples, suggesting a possible association with variation in taste (Lovegren et al., 1979). As shown in Table 3, the percentage of volatile compounds ranged from 16.73% to 23.11% for alcohols, from 2.00% to 7.72% for terpenes, from 32.80% to 40.48% for aldehydes, from 10.24% to 15.52% for esters, from 8.30% to 10.49% for acids, and from 10.58% to 18.58% for ketones. Additionally, the amount of total alcohol, terpene, aldehyde, ester, acid, and ketone aroma compounds varied among the cultivars. Rajhi et al. (2021) reported that the total identification percentages of volatiles extracted from different legume varieties ranged from 94.2% to 99.7%. The researchers obtained a total of 104 different aroma compounds from legumes. Additionally, in the study, seven monoterpene hydrocarbons, 10 oxygenated monoterpenes, 13 sesquiterpene hydrocarbons, four phenylpropanoids, two apocarotenes, nitrogen/sulfur derivatives, and 20 nonterpene derivatives from volatile chemical classes, were obtained from the flours of common bean varieties

Table 3. List of volatile compounds of İspir, Narman, and Hınıs bean seeds (expressed as a percentage).

Volatile compounds	Bean cultivars		
	İspir	**Narman**	**Hınıs**
Alcohols			
Ethanol	n.d.	4.89	n.d.
1-penten-3-ol	1.3	1.87	0.64
1-pentanol	1.28	2.36	2.33
1-hexanol	0.94	n.d.	0.99
1-octen-3-ol	0.95	0.75	n.d.
L-menthol	7.26	6.57	7.87
3-cyclohexen-1-ol, 4-methyl-1	n.d.	0.71	n.d.
(+)-Neomenthol	0.52	n.d.	n.d.
Cyclohexanol, 5-methyl-2	4.97	4.91	4.39
Benzenemethanol	n.d.	1.05	0.51
Total alcohols	**17.22**	**23.11**	**16.73**
Terpenes			
Benzene, 1-methyl-4	0.78	n.d.	0.68
Tridecane	1.36	n.d.	1.32
Heptane, 2,2,4,6,6-pentamethyl	1.21	n.d.	n.d.
Benzene, methyl	3.84	4.15	n.d.
Benzene, 1,2-dimethoxy	0.53	n.d.	n.d.
Total terpenes	**7.72**	**4.15**	**2.00**
Aldehydes			
Pentanal	2.30	2.81	3.77
Hexanal	24.78	26.14	32.11
2-pentenal	1.25	n.d.	0.67
2-heptenal	0.71	0.84	0.89
2,4 heptadıenal	0.83	n.d.	0.57
Benzaldehyde, 2,5-dimethyl	0.58	n.d.	n.d.
Benzaldehyde	1.30	2.28	1.47
2,4-heptadienal	1.05	1.92	n.d.
Nonanal	n.d.	2.17	n.d.
2 octenal	n.d.	n.d.	0.50
2,4-dimethyl benzaldehyde	n.d.	n.d.	0.50
Total aldehydes	**32.80**	**36.15**	**40.48**
Esters			
Capryl acetate	0.84	1.46	0.92
Tiglate	2.34	1.60	n.d.
1.2-benzenedicarboxylic acid, diethyl ester	12.20	10.69	9.33
Butyrate	n.d.	1.77	n.d.
Total esters	**15.38**	**15.52**	**10.24**
Acids			
Butanoic acid	0.90	n.d.	0.52
Hexanoic acid	2.02	3.68	2.62
Acetic acid	3.95	4.14	4.80
Nonanoic acid	1.43	1.97	1.42
Octanoic acid	n.d.	0.71	0.48
Butanoic acid, 2-methyl	n.d.	n.d.	0.62
Total acids	**8.30**	**10.49**	**10.45**

Table 3. (Continued.)

Ketones			
2-Cyclohexen-1-one, 2-methyl-5	16.12	9.01	8.39
Cyclohexanone, 5-methyl-2	1.66	1.57	1.99
Pulegone	0.80	n.d.	0.78
Gamma-hexalactone	n.d.	n.d.	0.56
Total Ketones	**18.58**	**10.58**	**11.72**
Total Other Compouds	**0.00**	**0.00**	**8.38**

n.d.: not detected.

with white, red and black seeds. Moreover, the researchers highlighted that the three bean varieties differed in the relative abundance of volatile compounds released.

Seven alcohol volatile compounds were found in İspir Bean, with higher levels of L-mentol (7.26%) and cyclohexanol 5-methyl-2 (4.97%) compared to other alcohol derivatives. Among the eight volatile alcohol compounds found in Narman Bean, L-mentol (6.57%), cyclohexanol 5-methyl-2 (4.91%) and ethanol (4.89%) were the most abundant alcohol derivatives. Six volatile alcohol compounds were provided in Hınıs Bean, and L-menthol (7.87%) and cyclohexanol 5-methyl-2 (4.39%) alcohol derivatives were more abundant than the others. On the other hand, five different terpene components were obtained in İspir Bean and benzene methyl (3.84%) component was higher than the others. However, only benzene methyl (4.15%) terpene compound was recorded in Narman Bean, while tridecane (1.32%) and benzene 1-methyl-4 (0.68%) terpene compounds were found in Hınıs Bean. İspir, Narman, and Hınıs's beans, respectively, contained eight, six, and eight different aldehyde volatile compounds. Additionally, hexanal was identified as the primary aldehyde component in all bean genotypes. In a prior study, the volatile compounds in eleven different varieties of legume seeds, including soybean, pea, chickpea, orange lentil, mung, broad, cowpea, adzuki, kidney, navy, and black beans, were analyzed. In the study, the most abundant aldehyde was found to be hexanal in all samples except navy beans (Khrisanapant et al., 2019). For esters, which is another volatile compound, three, four, and two ester derivatives were detected in İspir, Narman, and Hınıs beans, respectively. Meanwhile, the most abundant ester component in all bean samples was 1.2-benzenedicarboxylic acid diethyl ester. When the acid volatile components were examined, four different acid components were detected in İspir and Narman seeds, and six different acid components in Hınıs seeds, and the most abundant acid derivatives in all seed samples were acetic acid and hexanoic acid. The ketone volatile component of İspir, Narman, and Hınıs beans contained three, two, and four different components, respectively. For all bean seed samples, the 2-cyclohexen-1-2-methyl-5-ketone volatile component has been found to be more abundant.

3.4. Principal component analysis (PCA) of İspir, Narman, and Hınıs beans

In the study, differences on aroma profile and the bioactive content of İspir, Narman, and Hınıs seeds were investigated by PCA analysis. The first (PC1) and second (PC2) principle components accounted for 89.32% and 8.19% of the total variance, respectively (Figure 4). Aldehyde groups, particularly hexanal, were the most abundant group in each bean sample. On the other hand, alcohol volatile compounds, with 1-menthol and cyclohexanol 5-methyl-2, were the second most abundant volatile compounds after aldehydes in bean samples. Principal component analysis (PCA) and heatmap analysis, which show the differences of bioactive and volatile compounds between legume genotypes, allow us to understand how these genotypes show variations in terms of nutritional value and flavors (Granato et al., 2018; Bulut et al., 2023). Bioactive compounds are associated with the positive health effects of beans and often contain antioxidant, antiinflammatory, and anticancer properties. Volatile compounds, on the other hand, make up the aroma profile of the bean and can vary significantly between genotypes. In a study examining the volatile compounds of dry bean (*Phaseolus vulgaris* L.) genotypes representing three market classes (black, dark red kidney and pinto), a total of 62 volatile compounds were detected, including aromatic hydrocarbons, aldehydes, alkanes, alcohols, and ketones. In the study, it was observed that bean varieties differ in terms of volatile substance amount and profile. The combination of 18 compounds containing a common profile was reported to explain 79% of the variance between cultivars according to principal component analysis (PCA). Aroma compounds and bioactive contents of geographically indicated bean genotypes discussed in the study were subjected to HCA heatmap and used to reveal the differences between beans (Figure 5). The results of the analysis showed that the beans were divided into two main groups, and Narman beans were collected in a

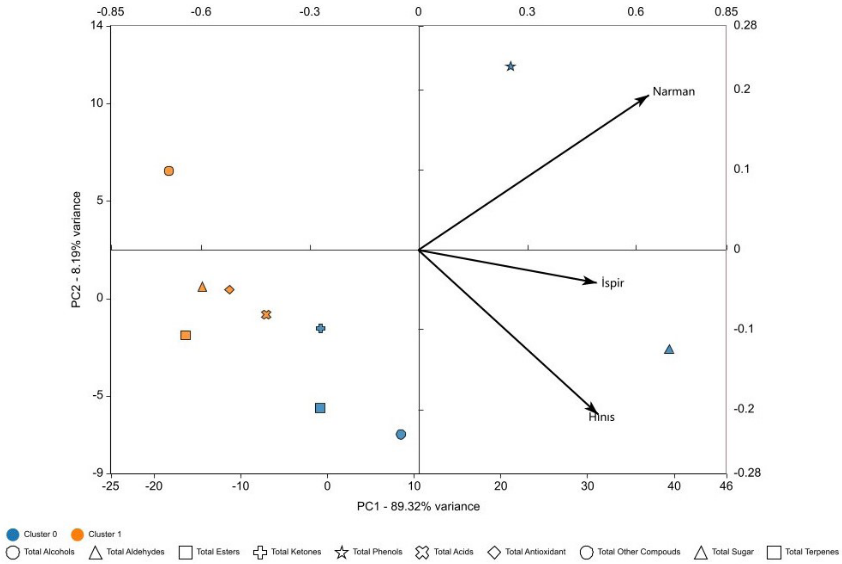

Figure 4. PCA graph for total phenol, total antioxidant, total sugar, total alcohols, total terpenes, total aldehydes, total esters, total acids, total ketones, and total other compounds affected by İspir, Narman, and Hınıs beans.

different cluster from İspir and Hınıs beans. According to the obtained dendrogram and heatmap, the Narman genotype differed from the other two bean genotypes in terms of containing ethanol, nonanal and butyrate components. However, unlike the other bean samples, it was observed that Narman Bean did not contain butanoic acid, 2-pentenal, 2,4-heptadienal, pulegone, tridecane, 1-hexanol, and benzene 1-methyl-4 components. Additionally, the heatmap derived from two-way heatmap with hierarchical clustering analysis (HCA) showed that all biochemical parameters were grouped into two major clusters (Figure 5). These findings were in agreement with PCA analysis, demonstrating that HCA is a useful tool for confirming the rationality of clustering and identifying the distinctive characteristics of each group. In a study investigating how soybeans (Glycine max) grown in different geographical regions can be distinguished using multivariate data analysis based on volatile metabolite profiles, multivariate data analysis techniques such as partial least squares-discriminant analysis (PLS-DA) and hierarchical clustering analysis (HCA) were implemented. With these analysis methods, researchers have managed to distinguish soybeans according to their geographical origin. In particular, 25 volatile compounds (terpenes such as limonene, myrcene; esters such as ethyl hexanoate, butyl butanoate; aldehydes such as nonanal and heptanal) have been identified, which distinguish soybeans grown in China from those grown in other regions (Kim et al., 2020).

4. Conclusion

Dry beans have distinct flavors and aromas, which are influenced by the presence of volatile compounds. These compounds contribute to the overall sensory experience of consuming beans. Additionally, dry beans contain bioactive compounds that provide potential health benefits. The bioactive contents and aroma values of the geographical indication İspir, Narman, and Hınıs dry beans examined in the study differ significantly. This result may be interesting for gastronomy tourists. The highest total phenolic content, 36.73 mg GA per 100 g of dry beans, was obtained from Hınıs beans. However, the highest total antioxidant activity (8.38%) was found in İspir bean samples. In addition, the total sugar contents of Hınıs, Narman, and İspir beans were determined to be 6.46%, 5.60%, and 4.22%, respectively. Furthermore, 40 distinct aroma volatiles, including 10 alcohols, five terpenes, 11 aldehydes, four esters, six acids, and four ketones, were identified from bean seed samples. Aldehydes, particularly hexanal, were found to be the most abundant volatile compounds in dry bean samples. Furthermore, Narman Bean showed higher percentage of total alcohols, total acids, and total esters compared to Ispir and Hınıs beans. Hınıs Bean contained higher total aldehydes and Ispir Bean contained higher total terpenes and total ketones. Additinoally, PCA and HTC heatmap analyses on the bioactive content and aroma components of dry bean samples revealed that Narman beans were collected in a separate bunch from İspir and Hınıs beans.

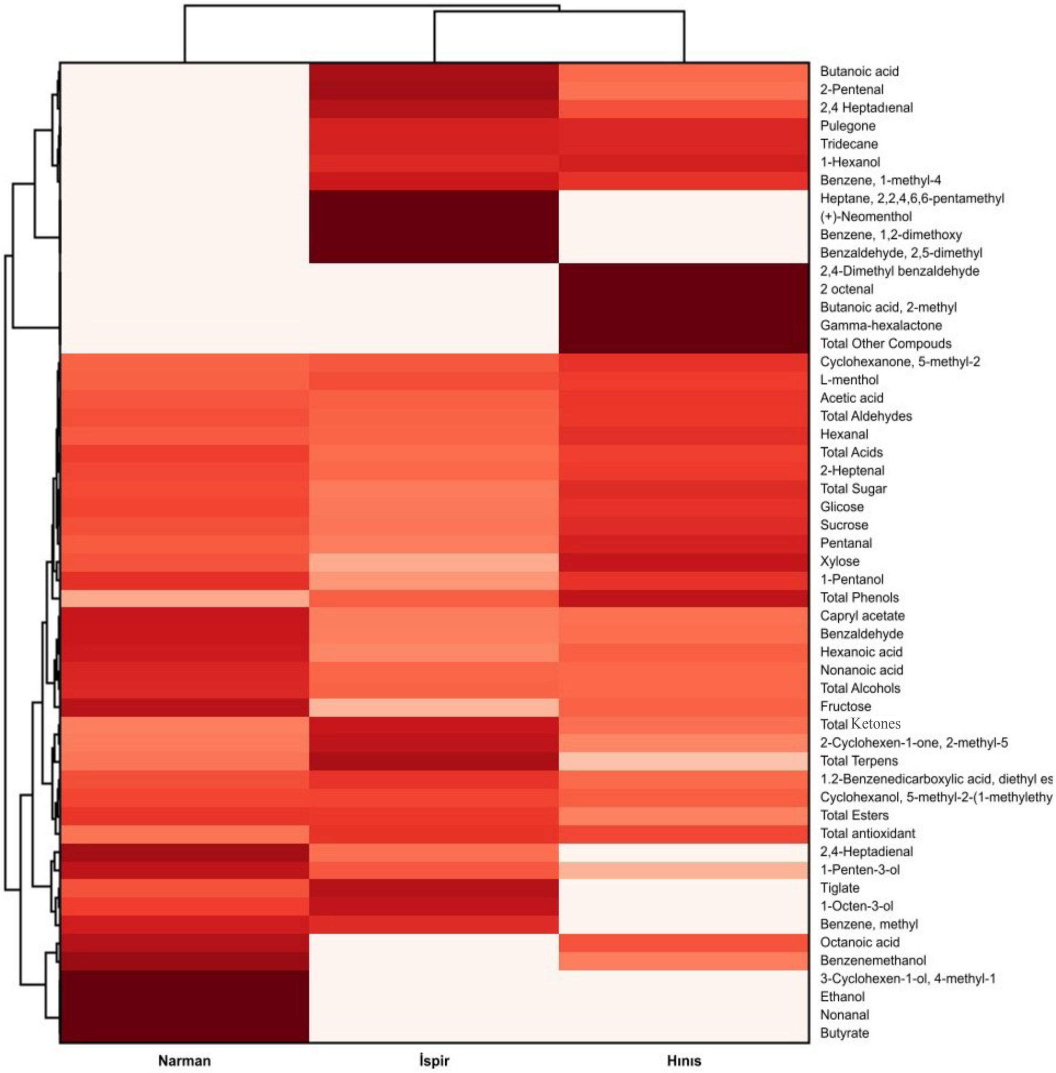

Figure 5. Heatmap with hierarchical clustering analysis (HCA) of aroma components and bioactive contents of İspir, Narman, and Hınıs bean samples. The amount of components is indicated by the colored cells on the map, with samples in the rows and compounds in the columns.

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Data availability
All data needed to conduct this study are provided within the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.

Ethical approval
All authors have read the study and expressed their willingness to publish it. This manuscript does not contain any research activity involving animals or human participants performed by any of the authors.

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