Variations on the Chemical Compositions of Chestnut Fruits Collected at Different Locations

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Chestnut is an essential food source in many countries. Nutritional quality and potential health benefits of Anatolian chestnut trees (*Castanea sativa* Mill.) have led to increased concern and interest in chestnut production. However, knowledge of the factors that influence the chemical content of chestnut fruits still needs to be improved. Thus, the chemical compositions were evaluated for Anatolian chestnut fruits collected at 14 different locations in northern Türkiye, which is one of the biggest chestnut producers in the world. The effects of latitude, longitude, altitude, aspect, mean annual temperature (°C) (TMA), and mean annual precipitation (mm) (PMA) of the study locations on the chemical compositions of chestnut fruits were monitored. The effects of these parameters on several chestnut characteristics were examined using a mixed-effects multiple regression model. Latitude, longitude, TMA, and PMA were correlated with the mean concentrations of sucrose, free amino acid, glycine betaine, nitrogen (N) (%), and total carbon (C) (%) of the chestnut samples. The moisture content of the fruits was affected by longitude. The antioxidant and mineral content of the chestnut samples also varied by location. These findings may be helpful in site selection, production, and conservation of chestnut cultivars.

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INTRODUCTION

The chestnut tree has a wide distribution range across the northern hemisphere. Its edible nut (hereafter, fruit) is highly valued and frequently utilized as a source of food in many countries across Europe, America, and Asia (Özden Keleş *et al*. 2023). Over the last few decades, nutritional components used to enhance human health have gained prominence (Gibson 2008). Because of its high nutrient content, chestnut is also considered important to human nutrition due to its nutritional properties (Morini and Maga 1995), especially given that it includes vitamins, minerals, amino acids, and antioxidant phenolic compounds (Alasalvar and Shahidi 2008). In addition to its nutritional quality, it is recognized for its many potential health benefits (Yang *et al.* 2015). Chestnuts can be used as a component of gluten-free diets, and they are linked to a lower risk of cardiovascular disease (Sabaté *et al.* 2002; Pazianas *et al.* 2005).

The predominant ingredient of chestnut fruit is carbohydrates, mainly starch and sucrose (Ertürk *et al.* 2006; Akbulut *et al.* 2017). A significant role is played by amino

acids, whether they are present in free form or come through the metabolism of dietary proteins (Borges *et al.* 2008). Because they serve as both energy sources and building blocks for proteins and other significant molecules, amino acids are physiologically active and have a variety of biological roles. Plant tissues include free amino acids, such as those found in the fruits of *Castanea sativa* (De Vasconcelos *et al.* 2007). Moreover, chestnut fruit contains vitamins, particularly ascorbic acid, polyphenol compounds, and fibers (Gold *et al.* 2005; De Vasconcelos *et al.* 2007). Ascorbic acid and polyphenols have been associated with various positive health effects, including antioxidant and antimicrobial properties (Yang *et al.* 2015). Antioxidants slow or stop oxidative stress by impeding oxidative reactions (Willcox *et al.* 2004). Chestnuts also contain many trace elements (Borges *et al.* 2008). The nuts usually contain a considerable amount of Ca, Mg, P, and K contents (Ertürk *et al.* 2006). Chestnut consumption in a daily diet can provide numerous benefits in protecting against diseases, as they are nutrient-rich, cholesterol-free, and low-fat.

Previous studies indicate that the chemical content of chestnuts is usually variable (Şengül and İlgün 2017). For example, in two different studies conducted in northwest and northeast Türkiye, the mean total carbohydrates were found to be 65 and 80 g 100 g^{-1} (Ertürk *et al*. 2006; Mert and Ertürk 2017). Even different clones of a particular variety may have differing chemical characteristics (Pinnavaia *et al.* 1993). In addition to location, it is evident from several previous studies that different factors, such as meteorological circumstances (*i.e.*, temperature and precipitation), impact plant fruits (De Vasconcelos *et al.* 2010). Another factor that may influence the chemical composition of chestnut fruits is the harvesting year (Ferreira-Cardoso *et al.* 2005). Given previous research findings, uncertainty about the factors that influence the chemical content of chestnut fruits seems to remain. Additional research to quantify and understand these factors is still needed for regions where chestnut trees prevail.

The growing concern over food and health impacts has encouraged more studies on the chestnut fruit, primarily concentrating on its chemical composition (De Vasconcelos *et al.* 2009). Evaluation of the primary nutrients, minerals, and non-nutritive bioactive constituents in chestnut fruits has been a part of these studies (De Vasconcelos *et al.* 2010). Despite the previous research, the knowledge about the nutritional attributes related to chestnut trees' geographic locations still needs to be improved (Yang *et al.* 2015). Previous research has examined the nutritional composition of *Castanea sativa* across Europe (Fernandez-Agullo *et al.* 2014). To our knowledge, no study has been done on the compositional variations between chestnuts grown in various ecological zones of northern Türkiye, one of the biggest chestnut producers worldwide. Moreover, previous research regarding the chemical content of chestnut fruits has usually been carried out on fruits from orchards (Neri *et al.* 2010), while few studies have conducted such analysis of chestnut fruits from natural populations (Mert and Ertürk 2017). Thus, the main objective of this study is to examine the chemical compositions of chestnut fruits collected at 14 different locations from natural stands in northern Türkiye. The specific objective was to determine the factors that influence the chemical compositions of the fruits.

EXPERIMENTAL

Study Area

This study was conducted in Bartin, Kastamonu, and Sinop cities, located in northern Türkiye (Fig. 1). The study area is located within the Euro-Siberian phyto-geographic region. A typical Black Sea climate with cool springs and hot summers mainly characterizes the study area. However, from the sea towards the south, the influence of the continental climate may be observed. Oriental beech (*Fagus orientalis* Lipsky), black pine (*Pinus nigra* Arnold), and oaks (*Quercus* spp.) are other main tree species of the region. The mean annual precipitation of the study region ranged from 366 to 1132 mm, while the mean annual temperature varied between 12.4 and 15.2 (°C). Climatic data for the study locations were attained from the General Directorate of Meteorology in Türkiye. Chestnut fruits were collected from 14 study sites distributed across Bartin, Kastamonu, and Sinop cities (Table 1). The sites are located within the natural distribution range of *Castanea sativa* Mill. (hereafter, chestnut) (Conedera *et al.* 2016). Chestnut fruits were collected from natural stands.

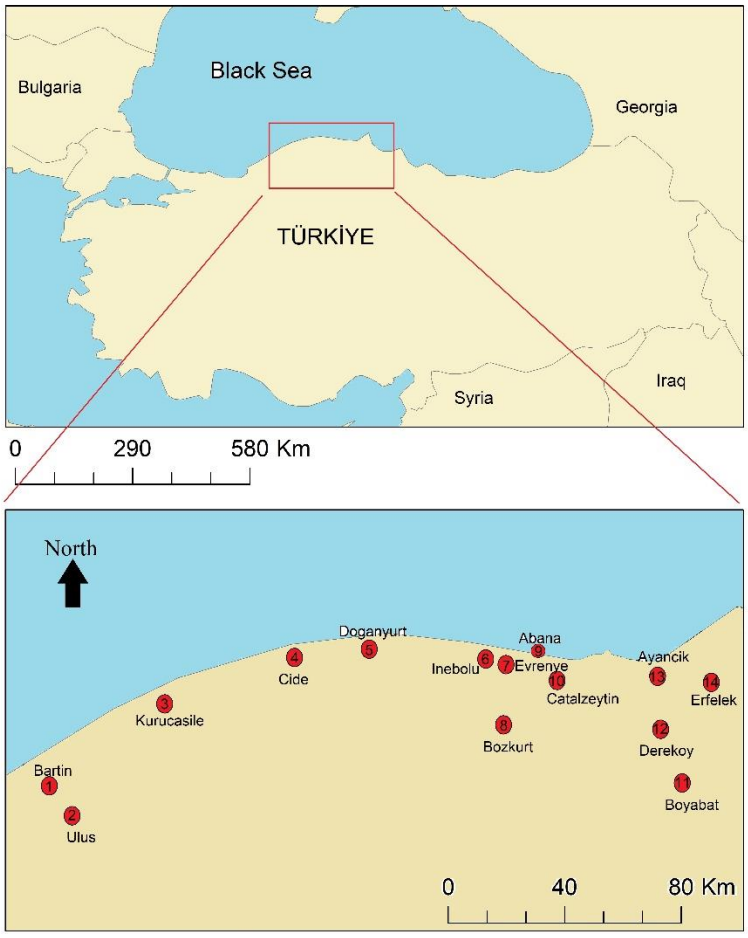

Fig. 1. Location of the study sites

Table 1. Location and Climate Data of the Study Sites

Location	Latitude	Longitude	Altitude Range (m)	Aspect	Temperature (C°)	Precipitation (mm)
Bartin	41°38´55"	32°20´30"	250 to 500	NW	12.4	1050
Ulus	41°28´21"	32°26´25"	250 to 500	NE	12.6	1020
Kurucasile	41°50´25"	32°42´50"	0 to 250	NW	13.1	1100
Cide	42°00´41"	33°21´54"	0 to 250	NW	14.1	1132
Doganyurt	42°00´25"	33°26´49"	250 to 500	NE	14.1	1081
Inebolu	41°58´05"	33°52´54"	250 to 500	NE	13.5	1023
Evrenye	41°57´25"	33°53´51"	0 to 250	NE	13.5	1023
Bozkurt	41°46´21"	34°03´28"	250 to 500	NW	15	890
Abana	41°58´25"	34°02´55"	0 to 250	NW	15.1	828
Catalzeytin	41°54´11"	34°10´17"	0 to 250	NE	15.2	936
Boyabat	41°29´50"	34°36´21"	0 to 250	N	13.2	366
Derekoy	41°46´15"	34°38´15"	250 to 500	NE	14.5	440
Ayancik	41°56´35"	34°36´10"	250 to 500	N	14.4	946
Erfelek	41°54´35"	34°51´45"	250 to 500	NW	14.5	440

Biochemical and Mineral Analyses

The sucrose in samples was determined using an anthrone reagent by the Pearson method (Egan *et al*. 1981). The fresh samples (1 g) were homogenized in 5 mL of 80% ethanol twice and filtered with the Whatman No. 2 filter paper. The collected extracts were diluted with deionized water to 50 mL and used for the glucose assay. The remaining sample was incubated at 4 °C in 50 mL of 80% ethanol for 24 h. Then, all solutions were filtered with the Whatman No. 2 filter paper. These samples were used for the sucrose assay. A total of 1 mL of each solution was poured into a test tube, and then 2 mL of anthrone reagent was added. The mixture was incubated for 20 min at 40 °C. The wavelength was read at 490 nm for glucose and 620 nm for sucrose by a spectrophotometer (SPECTRO Analytical Instruments, Kleve, Germany). The quantity of glucose was detected from the glucose standard curve, while sucrose was attained from the sucrose standard curve. The glucose and sucrose content of samples was expressed as mg g^{-1} FW.

To determine the moisture content, the chestnut exterior shell was carefully removed, and the fruits were sliced approximately to 4 to 6 mm thickness. Approximately 5 g of chestnut sliced samples was placed in drying containers brought to a constant weight and placed in an oven set at 105 ± 2 °C. When constant weight was reached (5 to 6 h), the drying containers were cooled in a desiccator and the samples were weighed again. The amount of moisture (%) in the samples was calculated according to Eq. 1 below (Cemeroğlu 2007),

$$\%\, Moisture = \left[\frac{M1-M2}{m}\right] 100 \quad (1)$$

where M_1 is the weight of sample taken + weight of drying container brought to constant weighing (mg), M_2 is the weight of the drying container brought to a constant weighing of the dried sample (mg), and m is the weight of sample taken (mg).

For free amino acid content (FAA), powdered chestnut (0.5 g) was boiled in 10 mL of 80% ethanol. The extract obtained was centrifuged at 800 g for 15 min. The supernatant was diluted to 10 mL with 80% ethanol. Then, 1 mL of extract was transferred into a 25-mL test tube, and 0.1 N NaOH was added using methyl red. A total of 1 mL of ninhydrin reagent was added, and the mixture was boiled for 20 min. Afterwards, 5 mL of ninhydrin reagent was added, and it was cooled. The mixture was diluted to 25 mL with distilled

water. The standard was prepared with glycine, and the absorbance was read at 570 nm (Moore and Stein 1948). The total free amino acid content was expressed as mg g^{-1} dry weight.

Proline content was determined according to the protocol of Bates *et al*. (1973). Then, 500 mg of fresh chestnut tissues were extracted in a solution of sulfosalicylic acid (10 mL, 3%). Acid-ninhydrin and glacial acetic acid solutions, each 2 mL, were poured into the filtrate (2 mL), and then the reaction mixture was placed in an incubator at 100 °C for 1 h. Thereafter, the mixture was properly cooled, and then 4 mL of toluene was dropped into it. The absorbance of the final solutions was recorded at 520 nm.

The glycine betaine content (GB) of samples was determined following the method described by Grieve and Grattan (1983). The optical density of the organic layer was recorded at 365 nm. Fresh tissue (500 mg) was extracted with warm distilled water (70 °C). The extract (0.25 mL) was mixed with 0.25 mL of 2 N HCl and 0.2 mL of potassium triiodide solution. The contents were shaken and cooled in an ice bath for 90 min. Then, 2.0 mL of ice-cooled distilled water and 20 mL of 1,2-dichloromethane (cooled at -10 °C) were added to the mixture. The two layers were formed in the mixture. The upper aqueous layer was discarded, and the optical density of the organic layer was measured at 365 nm. The concentrations of glycine betaine were calculated and expressed as mg g^{-1} fresh weight.

The amount of ascorbic acid (AA) was determined by following Klein and Perry's method (1982). The dried methanolic extract (100 mg) was extracted with 10 mL of 1% metaphosphoric acid for 45 min at room temperature and filtered through the Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 2,6-dichlorophenolindophenol, and the absorbance was measured within 30 min at 515 nm against a blank. The ascorbic acid content was calculated based on the calibration curve of authentic L-ascorbic acid (0.020 to 0.12 mg/mL). The assays were carried out in triplicate; the results were calculated as mean values ± standard deviations and expressed as mg g^{-1} of extract.

The amount of total polyphenols (TP) in the samples was determined following the method of Madhaiyan *et al*. (2004). Folin-Ciocalteu reagent was used with tannic acids as the standard polyphenol compound. About 0.5 mL of an extract was introduced into test tubes, followed by 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3. The absorbance was recorded at 660 nm, and total polyphenol content was determined using tannic acid standard curves. Polyphenol content was expressed as milligrams of tannic acid equivalent (TA) per gram of fresh weight (FW) of samples as mg TA g FW^{-1}.

To determine elemental analysis (%N, %C, Mg, P, S, K, Ca, Mn, Fe, Ni, Zn, Cu, and Se), first, the collected samples were dried at 65 °C because higher temperatures may disrupt the chemical structure of proteins and other molecules (Cemeroğlu 2007). Next, the samples were powdered to determine the elemental analysis in Kastamonu University's Central Research Laboratory using an inductively coupled plasma - optical emission spectroscope (ICP-OES -SpectroBlue II) device. The characteristics of the ICP-OES used were: plasma power (1200 W), nebulizer flow (0.8 L min^{-1}), coolant flow (13 L min^{-1}), auxiliary gas flow (0.8 L min^{-1}), and sample pump speed (30 rpm). Each sample was analyzed in triplicate. The pH values of the soil samples were determined using a digital pH meter (Thermo Scientific, Orion Star A111).

Statistical Analyses

The effects of altitude, latitude, longitude, aspect, mean annual temperature, and mean annual precipitation (*i.e.*, independent variables) on several chestnut characteristics were examined using a mixed-effect multiple regression model that was defined by Eq. 2,

$$R_v = \beta_0 + R_e + X^T + E \quad (2)$$

where R_v is the response variable, β_0 is the intercept, R_e is the random effect, X^T is the transposed matrix of the fixed effects, and E is the error term. Mean concentrations of sucrose, moisture, free amino acid (FAA), glycine betaine (GB), nitrogen (N) (%), total carbon (C) (%), and proline were used as the response variable (*i.e.*, dependent variable) in the model. The location where the chestnuts were collected was treated as a random effect. In this investigation, the model-fitting process was begun using all the aforementioned parameters. Variables having p-values higher than 0.05 were then sequentially eliminated from the model, and the model was re-fit after each eliminated variable. The iterative approach was repeated until the model only contained the variables with p-values lower than 0.05. With the use of residual analysis, the data's normality and homogeneity of variance were examined, and no deviations from these model assumptions were discovered. The variance inflation factor was used to analyze the multicollinearity among the chosen parameters; a VIF larger than 10 denotes substantial inter-variable collinearity (Dormann *et al.* 2013). Moreover, the differences among the locations where the chestnuts were analyzed using the analysis of variance (ANOVA) (α-level = 0.05). During the statistical analyses, "lme" and "aov" functions of R-Statistical software (R Development Core Team 2021) were utilized. It should be noted that this study did not aim to create predictive model, instead, it aimed to present statistical relationships between the dependent and independent variables.

RESULTS

In this study, the mean sucrose content of chestnut fruits ranged from 33.7 to 46.8 mg g^{-1} across the study sites, while the moisture content changed between 36.8 and 46.7% (Table 2). The highest FAA content was observed in Cide, while it was lowest in Ayancik. The average proline was 34.1 (µmol/g) across the study sites. The concentration of N (%) ranged from 0.238 to 0.367 indicating a pattern that increased from east to west. The mean content of GB changed between 25.2 and 36.4 mg g^{-1} among the study locations, while total C (%) ranged from 9.64 to 10.9 (Table 2).

Table 2. Mean Concentrations of Moisture (%), Sucrose (mg/g), Free Amino Acid (FAA) (mg g^{-1}), Glycine Betaine (GB) (mg g^{-1}), N (%), Total C (%), and Proline (µmol g^{-1}) of the Chestnut Samples by Location

Location	Moisture	Sucrose	FAA	Proline	N (%)	GB	Total C
Bartin	45.7	45.4	3.54	34.7	0.367	32.6	10.8
Ulus	42.7	46.8	3.58	36.4	0.294	33.4	10.3
Kurucasile	45.8	46.8	3.58	33.2	0.362	31.6	10.6
Cide	45.9	45.9	3.86	35.8	0.367	32.7	10.9
Doganyurt	38.8	41.7	3.24	33.4	0.287	28.8	9.9
Inebolu	48.7	41.4	3.27	32.8	0.288	27.4	10.0

Evrenye	39.4	42.4	3.17	34.7	0.278	27.6	10.7
Bozkurt	39.7	43.2	2.88	38.6	0.294	28.6	10.1
Abana	46.7	46.6	3.66	32.4	0.359	36.4	11.0
Catalzeytin	41.6	45.3	3.47	37.7	0.354	35.8	10.9
Boyabat	37.3	33.7	2.32	32.2	0.254	25.2	9.64
Derekoy	38.5	35.8	2.71	32.6	0.246	25.9	9.93
Ayancik	36.8	35.7	2.23	28.9	0.238	25.4	9.89
Erfelek	38.8	34.8	2.69	34.7	0.243	26.3	9.79

Table 3. Multiple Regression Analyses on the Concentrations of Sucrose, Moisture, Free Amino Acid (FAA), Glycine Betaine (GB), N (%), Total C (%), and Proline of the Chestnut Samples by Location. LAT and LONG refer to latitude and longitude, respectively

Models	p-value	AIC
Sucrose = 237.30157 – (7.279 LONG) + (0.086 LAT : TEMP)	< 0.001	-130.5
Moisture = 134.8 – (2.75 LONG)	< 0.001	-56.66
FAA = 22.371 – (0.706 LONG) + (0.0079 LAT : TEMP)	< 0.001	-255.8
Proline = non-significant	> 0.05	
N (%) = 2.023 – (0.065 LONG) + (0.0008 LAT : TEMP)	< 0.001	-380.3
GB = 7948.3– (4.3 LONG) – (187.1 LAT) –(572.4 TEMP)+ (13.8 LAT : TEMP)	< 0.001	-44.14
Total C (%) = 23.454 – (0.519 LONG) + (0.007 LAT : TEMP)	< 0.001	15.77

The mean concentration of moisture, sucrose, FAA, GB, N (%), and total C (%) of the chestnut samples significantly changed among the study sites ($p < 0.05$). In contrast, the differences between the sites in terms of proline content were statistically insignificant ($p > 0.05$) (Table 3). In general, a decreasing longitude caused an increase in sucrose, moisture, FAA, GB, N (%), and total C (%) contents across the study sites. Moreover, the interaction effects between latitude and mean temperature of the study sites had positive effects on sucrose, FAA, GB, N (%), and total C (%) contents ($p < 0.05$) (Table 3). The latitude and mean temperature of the study sites have a statistically significant influence on GB as well ($p < 0.05$).

In this study, the concentration of AA ranged from 0.326 to 0.512 mg g^{-1} across the study sites, with an average AA of 0.41 mg g^{-1}. The mean TP changed between 0.488 and 0.944 mg g^{-1} with an average of 0.71 mg g^{-1} (Table 4). The mean concentration of AA and TP of the chestnut samples significantly changed among the study sites ($p < 0.05$) (Table 5). A decreasing longitude generally increased AA and TP contents across the study sites. Moreover, precipitation and the interaction effects between latitude and mean annual rainfall of the study sites had positive impacts on AA content ($p < 0.05$) (Table 5).

Table 4. The Antioxidant Content of the Chestnut Samples by Location

Location	Ascorbic Acid (mg g^{-1})	Total Polyphenol (mg g^{-1})
Bartin	0.462	0.944
Ulus	0.344	0.766
Kurucasile	0.475	0.877
Cide	0.455	0.876
Doganyurt	0.388	0.722

Inebolu	0.397	0.633
Evrenye	0.382	0.588
Bozkurt	0.344	0.488
Abana	0.512	0.877
Catalzeytin	0.458	0.833
Boyabat	0.377	0.544
Derekoy	0.388	0.577
Ayancik	0.326	0.533
Erfelek	0.367	0.633

Table 5. Multiple Regression Analyses on the Concentrations of Ascorbic Acid (AA) and Total Polyphenol (TP) of the Chestnut Samples by Location. LAT and LONG refer to latitude and longitude, respectively

Model	p-value	AIC
Ascorbic Acid = 3.71 – (0.09 LONG) – (0.014 PREC) + (0.001 PREC:LAT)	< 0.0001	-274.89
Total Polyphenol = 5.0004 – (0.127 LONG)	< 0.0001	-259.01

Table 6. Mineral Content (ppm) of the Chestnut Samples by Location

Location	Mg	P	S	K	Ca	Na	Mn	Fe	Ni	Cu	Zn
Bartin	10970	264	477	978	1156	1144	105.3	48.8	7.44	14.7	11.0
Ulus	11227	273	466	993	1122	1115	106.4	52.6	6.34	14.8	11.9
Kurucasile	13610	501	1622	1772	1120	1256	127.6	48.9	6.23	17.4	12.6
Cide	13610	488	1514	1657	1194	1246	125.5	115	14.6	16.4	10.6
Doganyurt	13610	476	1538	1824	1244	1286	124.6	118	16.4	18.4	11.8
Inebolu	11211	676	760	1572	989	1010	94.4	122	15.5	16.8	10.5
Evrenye	13610	501	1553	1647	1120	1260	127.6	67.8	13.5	13.5	8.86
Bozkurt	14130	572	1045	1926	1079	1090	58.8	77.2	30.6	14.6	9.42
Abana	11390	434	921	1404	1044	1050	244	155	12.8	13.1	15.1
Catalzeytin	11455	455	915	1433	996	985	265	162	13.4	12.4	14.5
Boyabat	15790	574	847	1750	1069	1590	222	132	33.2	17.7	12.8
Derekoy	10972	274	459	1417	1115	1106	106.2	52.3	6.12	15.2	11.9
Ayancik	14840	461	490	1687	988	1530	136.1	104	5.63	12.4	10.7
Erfelek	14554	476	433	1566	866	1589	144.2	88.8	6.35	15.9	11.5

Table 6 exhibits the changes in elements (ppm) of chestnut fruits collected in different sites. In general, concentrations of macronutrients (Mg, K, P, S, and Ca) were mainly higher than other elements, while the concentrations of Mn, Fe, Ni, Cu, and Zn were relatively lower (Tablo 6). The micronutrients (Mg, Ca, Na, Mn, Cu, and Zn) did not significantly differ from each other across the study sites ($p > 0.05$).

DISCUSSION

Many fruits have antioxidant molecules, classified as enzymatic and non-enzymatic, to protect themselves against oxidative damage caused by free radicals (Yang *et al.* 2015). Non-enzymatic compounds mainly consist of flavonoids, total polyphenols, anthocyanins, soluble free amino acids, pigments (carotene, lycopene), soluble sugars (glucose and sucrose), and vitamins (C, E) (Mert and Ertürk 2017; Martínez *et al.* 2022).

Previous studies pointed out that the amount of all phytochemicals in chestnut fruits may vary depending on the physical and ontogeny characteristics of the trees as well as climatic factors (Ertürk *et al.* 2006; Akbulut *et al.* 2017).

Chestnut interior content usually dries out faster than other nuts (Bounous and Marinoni 2005). Thus, the moisture content of chestnuts is crucial, especially for their shelf-life. Previous studies have reported that the moisture content of chestnuts range from 41 to 59% (Míguelez *et al.* 2004; USDA 2013). In a similar study, Yang *et al*. (2015) monitored the moisture content of chestnut fruits collected from different regions in China, and found that the moisture ranged from 45 to 54%. Compared to previous research, the current findings regarding the moisture content seem to fall between the suggested ranges. In addition to the local environmental differences (Mert and Ertürk 2017), the soil genesis of the studied areas may influence the moisture content of the chestnut samples (Borges *et al.* 2008).

Reducing sugars, such as glucose, fructose, and sucrose, which are deposited in the storage organs of plants, are sources of carbon and ATP required for embryo development, as well as natural energy sources for people (Yang *et al.* 2015). The primary sugar in chestnut fruit is sucrose, which may influence the sweetness of chestnut fruits (Mert and Ertürk 2017). Ertürk *et al*. (2006) examined the sucrose content of varying chestnut cultivars and found a relatively higher amount of sucrose than that observed in this study. It should be noted that their samples were collected in the Marmara district, northwestern Türkiye, substantiating the fact that decreasing longitude can increase the sucrose content of chestnut fruits. Because of the high carbohydrate and starch content in their chemical composition, the chestnut tree is named as the "bread tree" (Poljak *et al.* 2022).

As an organic nitrogen resource, amino acids are essential constituents of plants that participate in the structure of proteins and protein-specific molecules, phytohormones, and numerous secondary products and play a role as signaling molecules (Fernandez-Agullo *et al.* 2014). The primary protein-derived amino acids in chestnut fruits are aspartic acid and glutamic acid. However, the other amino acids, such as arginine, proline, glycine, and γ-aminobutyric acid (GABA), were found in a lower concentration (Vasconcelos *et al.* 2010). Total amino acid values obtained from chestnut samples agreed with the literature reporting that they range between 2.3 mg 100 g^{-1} and 8.7 g 100 g^{-1} (dos Santos Rosa *et al.* 2019). Similarly, the proline and GB ratio of the samples were within the range given in the literature studies (Vasconcelos *et al.* 2010).

Chestnut trees are spread across the northern hemisphere (Gonçalves *et al.* 2010). Previous studies have recommended that the chemical composition of chestnut fruits may differ depending on the ecological locations (Yang *et al.* 2015), as observed in this study. The differences in chestnut regions regarding the chemical components of the fruits are likely due to the local climatic and soil conditions. This can also be associated with the fact that the genetic diversity of sweet chestnut populations can be influenced by environmental characteristics (Míguez-Soto *et al.* 2019). Ertan (2006) observed the fruit composition of chestnuts in western Türkiye and found that genotype influenced total sugar, starch, carbohydrate, and protein content. The influences of latitude, longitude, and temperature observed in this study support this assertion. Peña-Méndez *et al*. (2008) examined the characterization of various chestnut cultivars. They found that the region of chestnut production had a more significant impact on the physicochemical characteristics of the fruits than the variety. Wu *et al*. (2020) also pointed out that climate zones, precipitation, and temperature can influence the chemical content and most minerals of the fruits.

The range of ascorbic acid content of chestnuts observed in this study was consistent with those examined in Italy and China (Neri *et al.* 2010; Yang *et al.* 2015). Antioxidants of all kinds were plentiful in all locations. Despite the minor differences among the locations, they are a great source of bioactive components for the diet. The differences in antioxidant properties of the chestnuts are likely due to the climatic variances among the locations and the harvesting time of nuts from the trees (Borges *et al.* 2008). Relevantly, Dinis *et al*. (2012) examined the antioxidant properties of different ecotypes of chestnuts and stated that climatic conditions may be a limiting factor for the production of phenolic compounds and, consequently, the antioxidant properties of chestnut fruits.

Ascorbic acid, a water-soluble vitamin, is an antioxidant that protects against oxidative stress damage in plants (Gallie 2013). Fruits and vegetables constitute the primary source of vitamin C for human nutrition, thereby determining the amount of ascorbic acid in fruits, which has attracted much attention in recent years. It has a vital role in repairing cartilage, bones, and teeth and in the easy relief of wounds (Pazianas *et al.* 2005). In contrast, polyphenols, which occur naturally in vegetative organs, such as leaves, flowers, and fruits, are secondary metabolites that play a direct role in the response of plants to different types of stress (Martínez *et al.* 2022). In addition, they may contribute to tartness, pungency, color, flavor, aroma, and oxidative stability (De Vasconcelos *et al.* 2007). Numerous epidemiological studies have revealed an inverse association between the risk of chronic human diseases and the use of polyphenolic-rich foods in diet (Alasalvar and Shahidi 2008).

The chestnut Cu and Zn content shows no substantial difference between locations. The differences in the mineral contents among the locations are likely due to the different nature of soil and/or different genotypes (Neri *et al.* 2010). The current findings regarding the mineral content of the chestnut samples are consistent with findings found by Şengül and İlgün (2017), who examined the trace elements of chestnut fruits collected in Giresun city in northeastern Türkiye. The author's findings substantiate the fact that chestnuts contain higher levels of carbohydrates than other nuts (*i.e.*, walnuts, almonds, and hazelnuts), which are consistent with former research (Neri *et al.* 2010; Poljak *et al.* 2021). Poljak *et al*. (2022) pointed out that the differences in the chemical composition of chestnut fruits may be due to the soil depth. Moreover, the chemical composition of chestnuts can be attributed to the different types of parent rock material and soil because they can influence the root zone aeration and the tree water potential (Borges *et al.* 2008).

Hard-shelled seeds, such as walnuts, almonds, hazelnuts, and chestnuts are an excellent source of macro-elements, such as Ca, P, K, Mg, and S, but also of trace elements (Fe, Cu, Zn, and Mn) (Vasconcelos *et al.* 2010). The mentioned minerals not only contribute to the control of growth and development in chestnuts but also to their adaptation or coping with the negativities occurring in the habitat in which they grow (Borges *et al.* 2008; Akbulut *et al.* 2017). In contrast, these elements have numerous benefits for human health. For example, Ca is involved in bone development and muscle contraction (Pazianas *et al.* 2005); K, together with Na, is used in the control of hypertension, transmission of nerve impulses, and muscle functioning (Willcox *et al.* 2004); Mg prevents aging, muscle contraction; and the P plays a role in ATP synthesis and phosphorylation of proteins and sugars (Sabaté *et al.* 2002). As Hasan *et al*. (2011) report, some minerals mentioned above are well-known co-factors for anti-oxidative enzymes found in fruits, such as iron, zinc, and copper. Martínez *et al*. (2022) highlighted that the antioxidant capacity of chestnut fruits can increase with decreasing temperature of the growing environment. Although

temperature itself did not have a significant influence on the antioxidant capacity of chestnuts in this study, it should be noted that the average temperature of chestnut collection sites generally decreases with decreasing longitude, which had a significant influence on the antioxidant capacity of the fruits.

In summary, the results of sucrose, polyphenol, amino acids, and polyphenol of chestnut fruits seemed to be rich. Therefore, consuming chestnuts and chestnut-derived products in the daily diet can contribute to protecting and strengthening human health. The values obtained from the parameters examined in the study are consistent with the dosages that are recommended for the daily diet of adults (Martin *et al.* 2005; Pazianas *et al.* 2005; Vasconcelos *et al.* 2010; Hasan *et al.* 2011; Landete 2013; dos Santos Rosa *et al.* 2019).

CONCLUSIONS

1. Analysis of the chemical compositions of Anatolian chestnut fruits collected at fourteen different locations showed major variations related to geographic locations. Latitude, longitude, TMA, and PMA were correlated with the mean concentrations of sucrose, free amino acid, glycine betaine, N (%), and total C (%) of the chestnut samples.
2. The moisture content of the fruits was affected by longitude. The antioxidant and mineral content of the chestnut samples varied by location as well.
3. The findings of the study may provide a range of applications of different kinds of Anatolian chestnuts, which may potentially help chestnut processing companies to choose specific chestnuts for the market.
4. Findings of the study may be helpful, especially in site selection, production, and conservation of chestnut cultivars in northern Türkiye.
5. Anatolian chestnut has a wider distribution range than the study area, thus, further research in different locations within the distribution range is recommended.

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