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Fat and protein content in Turkish hazelnut (*Corylus colurna* L.) in Kastamonu province

Kastamonu yöresinde yetişen Türk fındığının (Corylus colurna L.) yağ ve protein içeriği

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Abstract
Turkish hazelnut (*Corylus colurna* L.), the mother land and natural spreading area of which is Anatolia, is one of the important hazelnut species in Turkey. Moreover, thanks to its adaptation capability to extreme climate and poor soils conditions, it is a forest tree species playing a key role in climate change scenarios. Turkish hazelnut, a Euro-Siberian flora element in Turkey, is a taxon present in the ''*Low Risk*'' category according to the IUCN Red List. Turkish hazelnut which spreads disorderly-partially and in an isolated way in small stands, groups, clusters as individuals, has the most intense spreading in the Northwestern Anatolian forests in Anatolia. The fruits of the Turkish hazelnuts are being utilized as a valuable traditional medicinal herbal product in different regions of the world. This paper aims to identify and to compare the fat and protein composition of Turkish hazelnut kernels among and within four populations (Ağlı-Tunuslar, Ağlı-Müsellimler, Araç-Güzlük and Tosya-Küçüksekiler) in Kastamonu region. According to the results of the study; the mean values of fat and protein content were found to be 62.78% and 16.32% respectively. In terms of protein values, significant differences were identified among the populations. However, there were no significant differences among the populations regarding the fat values. The highest median value of the protein was found in Ağlı-Tunuslar with a rate of 18.43%. There is no variation within populations as to fat and protein values. The study suggests that similar studies be continued in detail based on different ecological conditions and genotypes.

Özet
Ana yurdu ve doğal yayılış alanı Anadolu olan Türk fındığı (*Corylus colurna* L.), Türkiye için önemli fındı türlerinden biridir. Üstelik, ekstrem iklim ve fakir toprak koşullarına adaptasyon kabiliyeti sayesinde iklim değişikliği senaryolarına karşı anahtar bir orman ağacı türü rolündedir. Türkiye'nin Avrupa-Sibiry flora elementi olan Türk fındığı, IUCN Kırmızı Listesine göre "*Düşük Risk*" kategorisinde bir taksondu Türkiye'de dağınık-parçalı, izole halde küçük meşcere, grup, küme ve yer yer münferit bireyler halind yayılış gösteren Türk fındığı, Anadolu'daki en yoğun yayılışını Kuzeybatı Anadolu ormanlarınd gerçekleştirmektedir. Türk fındığının meyveleri, Dünya'nın farklı bölgelerinde geleneksel tıbbi değer bir ilaç olarak kullanılmaktadır. Bu çalışmada, Kastamonu yöresindeki dört Türk fındığı popülasyonu (Ağlı-Tunuslar, Ağlı-Müsellimler, Araç-Güzlük ve Tosya-Küçüksekiler) meyvelerinin yağ ve protei içerikleri bakımından popülasyon içi ve arası mukayesesi amaçlanmıştır. Çalışmanın sonuçlarına göre sırasıyla yağ ve protein değerleri %62.78 ve %16.32'dur. Protein değerleri bakımından popülasyonla arasında anlamlı farklar tespit edilmiştir. Hâlbuki yağ değerleri bakımından popülasyonlar arasınd anlamlı fark tespit edilmemiştir. Protein değeri bakımından en yüksek medyan değeri, %18.43 ile Ağl Tunuslar popülasyonunda bulunmuştur. Protein ve yağ değerleri bakımından popülasyonlar içerisind varyasyon tespit edilmemiştir. Farklı ekolojik şartlar ve genotipler üzerinde benzer detay ve ile çalışmalar sürdürülmelidir.

INTRODUCTION

Hazelnut *(Corylus* spp.), the mother land and natural spreading area of which is Anatolia, is one of the important nut species for Turkey's economy. *Corylus avellana* L.*, Corylus maxima* Miller*, Corylus colurna* L. are important hazelnut species. Turkish hazelnut (*Corylus colurna* L.) is one of these species and is uncultivated. Thanks to its adaptation capability to extreme climate and poor soils conditions, it is a forest tree species playing a key role in climate change scenarios. It is known by the names of "*tree hazelnut*, "*rock hazelnut*", "*Balkan hazelnut*", "*bear hazelnut*" and "*Turkish hazelnut*" in the literature (Ayan et al. 2016a). Turkish hazelnut, which is a Euro-Siberian flora element in Turkey, is a taxon present in the ''*Low Risk*'' category according to the IUCN Red List

(Shaw et al. 2014). Turkish hazelnut which spreads disorderly-partially and in an isolated way in small stands, groups, clusters as individuals, has the most intense spreading in the Northwestern Anatolian forests (Ayan et al. 2016a).

Hazelnut is one of the most important raw materials for the pastry and chocolate industry due to their organoleptic characteristics. In addition, hazelnut adds flavor and texture to bakery, confectionery, cereal, salad, entrée, sauce dairy, and dessert formulation (Alasalvar et al. 2003; Kaleoğlu et al. 2004; Oliveira et al. 2008; Ozdemir and Akıncı 2004; Ayan et al. 2016b). Besides, hazelnuts play a major role in human nutrition and health because of their special composition of fat, protein, carbohydrate, vitamins, minerals and nutrients antioxidant (Alasalvar et al. 2009; Garcia et al. 1994; Köksal et al. 2006). There is gradual revival of interest in the use and research of medicinal plants throughout the world owing to the fact that herbal drugs are reported to be safe and free from side effects, which are generally associated with synthetics and antibiotics. The fruits of the Turkish hazelnut are being utilized as a valuable traditional medicinal herbal product in different regions of the world (Akhtar et al. 2010).

The fruit possesses varied medicinal properties and therapeutic uses. It is used as a brain and intestinal tonic, aphrodisiac and expectorant and is prescribed in weakness of brain and liver, gonorrhea, and palpitation. It is mixed with honey and given as expectorant in cough and asthma (Chopra et al. 1956; Kirtikar and Basu 1975). People who consumed nuts five or more times a week had a 50% reduced risk of coronary heart disease relative to those who never consumed nuts (Fraser et al. 1992; Erdoğan and Aygun 2005). Similar results about the effect of hazelnuts on human health were also reported in different studies (Ebrahem et al. 1994; Koyuncu et al. 1997; Savage and McNeil 1998). This positive effect of hazelnuts depends on their fatty acid composition, especially unsaturated fatty acid (Garcia et al. 1994). The data shows that the majority of the fatty acids in *C. colurna* kernels is unsaturated fatty acids (92.23%) on average, while saturated fatty acids comprised only 7.76% (Erdoğan and Aygun 2005). Polyunsaturated fatty acids have a great importance for human nutrition and health. It is necessary to take about 1 g of fatty acids, which are also regarded as vitamin F, on a daily basis. This can be obtained from 8 hazelnut kernels (Agar et al. 1995; Erdoğan and Aygun 2005).

This study aims to identify and compare the fat and protein composition of Turkish hazelnut kernels among and within four populations (Ağlı-Tunuslar, Ağlı-Müsellimler, Araç-Güzlük and Tosya-Küçüksekiler) in Kastamonu province of Northwestern Black Sea Region where Turkish hazelnut shows its natural spreading in the most intense way.

MATERIAL AND METHODS

Four populations (Ağlı-Tunuslar, Ağlı-Müsellimler, Araç-Güzlük and Tosya-Küçüksekiler) of Turkish hazelnut in Kastamonu province of Northwestern Black Sea Region identified by Ayan et al. (2016a) were analyzed in this study. The study locations of Kastamonu populations are shown on GoogleEarth below (Figure 1). Detailed information on populations is given in Table 1.

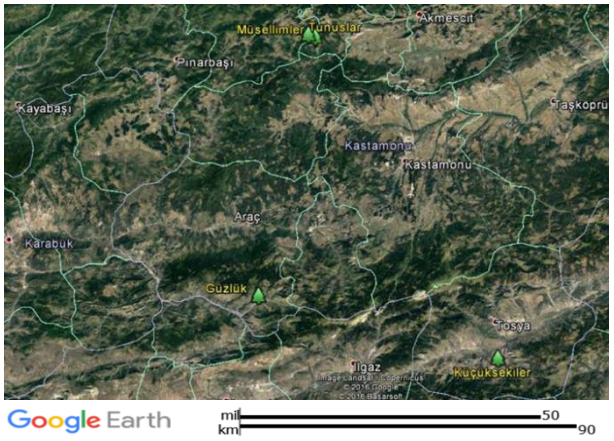

Figure1. Locations of Turkish hazelnut populations in Kastamonu.

Araç-Güzlük, Tosya-Küçüksekiler and Ağlı-Müsellimler populations were represented with five individuals and Ağlı-Tunuslar population was exemplified by two individuals to collect data for the study. All sample trees were selected randomly from four populations studied. From each individual, approximately 1 kg nut were collected at maturation period between September 10-30, 2014. After the numbering, nut samples were air-dried for a month.

Table 1. Introductory information on the populations used in the research.

No	Population/Location	Management Unit	Climate type as to EAI (Index)*	Climate type as to TCC (Index)*	Coordinates N-S	Altitude range (m)	Aspect
1	Ağlı-Müselimler Location	Kastamonu-Daday	Humid (52,76)	Semi-humid (3.32)	41°38' 05.93" – 33°29' 41.56" 41°38'14.62" – 33°30' 46. 81"	1151-1326	S
	Ağlı-Müsellimler population is characterized shallow soil structure and the soil is form forest land features. On the other hand; it has been seen that some of the individuals have remained in agricultural parcels. The soil structure in these areas carries deep soil characteristics. The population is generally south-facing and gravelly.						
2	Ağlı-Tunuslar Location	Kastamonu-Daday	Humid (52,76)	Semi-humid (3.32)	41°37' 46.08" – 33°31' 10.65" 41°37' 46.93" – 33°30' 53.85"	1290-1340	S - N
	The Ağlı-Tunuslar population is located at the highest elevation (1320 m) in the other populations. The soil structure is usually on the surface or close to the surface of the mainland, the soil thickness is very shallow and very stony. It is different from the other populations in that it is entirely within the boundaries of the forest.						
3	Tosya-Küçüksekiler Location	Kastamonu-Tosya	Semi-humid (33,48)	Semi-arid (-20,82)	40°54' 33.33" – 34°02' 54.69" 40°54' 46.13" – 34°02' 37.87"	940-980	N-NW-Flat
	The population of Tosya-Küçüksekiler remained within the agricultural area. Because of this, the soil structure carries more alluvial agricultural land properties. In addition, in sandy-tin permeable structure, the yield is high and the slope is close to zero.						
4	Araç-Güzlük Location	Kastamonu-Araç	Semi-humid (37,65)	Semi-humid (14,02)	41°03' 08.22" – 33°21' 10.78" 41°02' 56.38" – 33°21' 00.35"	980-1140	N
	The Araç-Güzlük population is located; 15-20° more sloping, somewhat stony and shallow in soil structure, and in the form of forest land.						

EAI=Erinç aridity index, TCC= Thornthwaite's Climate Classification

* The information was taken by Temel et.al. (2017) except Araç-Güzlük population.

Having been separated from their husks, the nuts were ground and prepared for fat and protein analyses. Two subsamples were taken from each sample tree in order to determine fat and protein values. Fat extractions of nut samples taken from four different Turkish hazelnut populations under different local ecological conditions were performed as described in AOAC (1995) with "Soxhlet" apparatus using hexane as solvent. The protein amounts of sample nuts were determined by Kjeldahl analysis (Kadaster 1960; Kacar 1984) (Figure 2, 3, 4). Following the chemical analysis, the equations (1) and (2) were used to determine the amounts of fat and protein. The fat and protein contents of samples were expressed as percentages of the sample weights.

$$\text{Fat (\%)} = [(E - R) / L] \times 100 \quad (1)$$

In this equation; E: Extraction beaker + Fat (g), R: Extraction beaker (g), L: Sample weight (g).

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25 \quad (2)$$

Nitrogen (%) in equation 2 is calculated as;

$$\text{Nitrogen (\%)} = [(T-B) \times N \times 1.4] / S \quad (3)$$

In this equation; T is the standard amount of acid (ml) reacting with ammonium during sample distillation, B is the amount of standard acid (ml) reacting with ammonium during witness distillation, N is the exact normality of standard acid and S is the amount of sample used in the analysis.

a b

Figure 2. Kjeldahl digestion (a), Kjeldahl distillation and titration (b)

Figure 3. Fat content determination

Figure 4. Soxhlet extraction (a), working of soxhlet extraction (b), fat extraction (c)

Variations among populations for fat and protein amounts were analyzed by the Kruskal Wallis test because of small sample numbers per population (n<30). To determine variations within populations, Kruskal Wallis test was also used to determine variations within three populations (Araç-Güzlük, Tosya-Küçüksekiler and Ağlı-Müsellimler) and Mann Whitney U test was used for Ağlı-Tunuslar population.

RESULTS

The results of fat and protein analyses on *Corylus colurna* fruits from different populations are given in Table 2. General basic statistics of fat and protein values are shown in Table 3.

According to Kruskal Wallis test results, significant differences were found in terms of protein content ($p < 0.05$), while there were no significant differences among the populations in terms of fat content ($p > 0.05$). The results obtained are given in Table 4; the Mann Whitney U test was used to determine the differences among the groups for the protein. Three homogenous groups were formed according to protein values. Araç-Güzlük and Tosya-Küçüksekiler populations were included in the first group. And in the last group, Ağlı-Müselimler and Ağlı-Tunuslar populations were included.

Among the trees of all populations (within population), there were no significant variations in the fat and protein contents ($p>0.05$). The results of these analyses are given in Table 5.

Table 2. Fat and protein values of Turkish hazelnut populations

Population	Sample No	Fat values (%)	Protein values (%)
Araç/Güzlük	1-1	61.12	13.24
	1-2	63.08	13.57
	2-1	64.92	16.48
	2-2	63.86	16.81
	3-1	61.74	14.22
	3-2	64.28	14.22
	4-1	63.52	16.64
	4-2	65.42	16.00
	5-1	64.64	11.79
	5-2	62.34	15.03
Tosya/Küçüksekiler	1-1	64.86	13.41
	1-2	68.54	13.73
	2-1	59.56	14.54
	2-2	57.32	15.19
	3-1	59.50	17.94
	3-2	63.04	17.13
	4-1	67.28	15.03
	4-2	69.62	14.54
	5-1	62.84	16.48
	5-2	64.68	16.00
Ağlı/Müsellimler	1-1	62.86	17.62
	1-2	61.84	18.43
	2-1	68.26	18.26
	2-2	65.72	18.10
	3-1	68.84	14.22
	3-2	66.22	15.03
	4-1	65.04	16.64
	4-2	61.86	16.32
	5-1	59.34	16.00
	5-2	60.74	16.64
Ağlı/Tunuslar	1-1	63.32	18.75
	1-2	90.64	18.91
	2-1	56.60	17.62
	2-2	58.82	18.10

Table 3. Basic statistics of fat and protein values

	Population	n	Mean	Std. Deviation	Min.	Max.	Coefficient of Variation (%)
Fat (%)	Araç-Güzlük	10	63.49	1.42	61.12	65.42	2.24
	Tosya-Küçüksekiler	10	63.72	4.09	57.32	69.62	6.42
	Ağlı-Müsellimler	10	64.07	3.22	59.34	68.84	5.03
	Ağlı-Tunuslar	4	59.85	2.85	56.60	63.32	4.76
	Total	34	62.78	3.22	56.60	69.62	5.09
Protein (%)	Araç-Güzlük	10	14.80	1.68	11.79	16.81	11.35
	Tosya-Küçüksekiler	10	15.40	1.47	13.41	17.94	9.55
	Ağlı-Müsellimler	10	16.73	1.41	14.22	18.43	8.43
	Ağlı-Tunuslar	4	18.34	0.60	17.62	18.91	3.27
	Total	34	16.32	1.82	11.79	18.91	11.40

Table 4. Kruskal Wallis and Mann Whitney U test results for variations among population

Population	n	Fat (%)		Protein (%)		
		Median	***P***	Median	***P***	Homogenous Groups
Araç-Güzlük	10	63.69	*0.178*	14.63	*0.003*	a
Tosya-Küçüksekiler	10	63.86		15.11		ab
Ağlı-Müsellimler	10	63.95		16.64		bc
Ağlı-Tunuslar	4	59.73		18.43		c

Table 5. Kruskal Wallis and Mann Whitney U test results for variations within populations

	Araç-Güzlük		Tosya-Küçüksekiler		Ağlı-Müsellimler		Ağlı-Tunuslar	
	X^2	*P*	X^2	*P*	X^2	*P*	*Mann Whitney U*	*P*
Fat	1.418	0.841	3.055	0.549	6.764	0.149	2.000	1.000
Protein	3.979	0.409	4.143	0.387	2.881	0.578	1.000	0.439

DISCUSSION AND CONCLUSION

According to the results of the present study, significant differences were found among the populations in terms of protein content, while there were no significant differences in terms of fat content. The fat content of the Turkish hazelnut populations ranged from 59.85% to 64.07% and protein content from 14.80% to 18.34% at population-based as mean. In addition, on the genotype basis, the fat content ranged from 56.60% (in Ağlı-Tunuslar pop.) to 90.64% (in Ağlı-Tunuslar pop.) and protein content ranged from 11.79% (in Araç-Güzlük pop.) to 18.91% (in Ağlı-Tunuslar pop.). There were no significant differences within the populations for fat and protein values (p>0.05). However, in their studies carried out in 41 genotypes of *Corylus colurna* in Kashmir, Kumar Srivastava et. al. (2010) noted the fat and protein values to be 48.49% and 16.37%, respectively. In addition, Kumar Srivastava et. al. (2010) reported that in the case of *Corylus colurna*, genotypes originating from the same locality were grouped in separate clusters, which indicates a wide diversity among genotypes originating from the same place. Murty and Arunanchalam (1966) stated that the genetic diversity among genotypes could be due to various factors such as genetic structure of the populations, developmental traits and heterogeneity.

When the fat and protein content results of *C. colurna* were compared with the results of previous studies on *C. avellana* varieties, great differences were found in the contents of these analyzed compounds (Köksal et al. 2006; Oliveira et al. 2008; Ozdemir and Akıncı 2004). Köksal et al. (2006) determined that some varieties of *C. avellana* such as 'Tombul' and 'Sivri' contain ash content 1.87-2.72 g/ 100 g and protein 11.7-20.8 g/100 g. In another study on four common varieties of *C. avellana* in

Iran; the varieties showed fat content in a range from 53.36% to 63.5%; protein, 16.03-23.26% (Rezaei et. al. 2014).

52.29% of the total fat content in *Corylus avellana* "Tombul" and 48.24% in *Corylus avellana* "Palaz" at the date of harvest is completed at the last period of the fruit development (Koyuncu et. al. 1997). Ebrahem et. al. (1994) found that fat content increased during the fruit development period in hazelnuts. Protein and fat contents in *Corylus avellana* 'Uzunmusa' were 15.6-18.5% and 64.7-67.5%, respectively (İslam 2003). Protein contents were determined as 13-16% (İslam, 2000), 14.42% (Turan and İslam 2016) and 16.3% (Baş et al. 1986). Fat content in *Corylus avellana* was recorded to be 64.7% (Çetiner 1976), 63% in Spanish 'Negret' cultivar (Romero et al. 1997), and 51.93% in 'Çakıldak' cultivar (Turan and İslam 2016).

Many studies have so far reported that the nut compositions of hazelnut are affected by variety, harvest year, soil, climate and method of cultivation (Köksal et al. 2006; Oliveira et al. 2008; Alasalvar et al. 2009). Overall, it seems that further studies are required to resolve the roles of environmental factors in quality of hazelnut. By comparing essential substances in different Turkish hazelnut population and genotypes, researchers in their future studies can introduce the genotypes of high quality.

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