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Original Scientific Paper

## SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) AS A FUNCTIONAL FOOD: CHEMICAL COMPOSITION OF FRUITS FROM NATURAL POPULATIONS OF BOSNIA AND HERZEGOVINA

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**Abstract.** *The sweet chestnut fruit (*Castanea sativa* Mill.) represents an important functional food due to its high starch and dietary fiber content, low fat, and the presence of proteins, sugars, minerals, and bioactive components. In this study, peeled fruits from six natural populations in Bosnia and Herzegovina (Prijedor, Banja Luka, Kostajnica, Konjic, Bratunac, Bužim) were analyzed for water, ash, protein, fat, starch, and sugar content using standardized methods. The average composition across all populations was: moisture 41.6%, ash 1.1%, protein 3.4%, fat 2.4%, starch 33.2%, and sugars 6.9%. Notable variability was observed: Banja Luka and Prijedor samples showed elevated starch (39.0% and 37.6%), with Prijedor also high in sugars (8.5%); Kostajnica fruits had the highest fat (3.4%) and sugar (9.9%) content; Bratunac showed the highest protein level (3.7%); Konjic was distinguished by the highest moisture (48.1%) but lowest sugar and fat values. Bužim samples generally exhibited below-average values. These results confirm considerable nutritional diversity among indigenous chestnut populations, influenced by both genetic and ecological factors. The findings provide a foundation for further valorization of chestnut fruits, selection of populations with favorable traits, and their potential application in the food industry and genetic resource conservation.*

**Key words:** *sweet chestnut, starch, sugar, nutritional potential*

### Introduction

Sweet chestnut (*Castanea sativa* Mill.) is increasingly recognized as a significant nutritional resource and functional food. Its nutritional value stems from its high content of starch and dietary fiber, along with a lowfat content, and the presence of proteins and sugars. Unlike most nuts, which are rich in fats, chestnuts are characterized by a high proportion of starch and complex carbohydrates, making them more similar to foods like sweet potatoes, corn, or potatoes, and representing a significant energy source.

Due to these characteristics, sweet chestnut is increasingly used in human nutrition, and it is particularly important as a natural alternative to gluten-containing products. Since it is gluten-free, chestnut flour is an excellent alternative for people with celiac disease or gluten intolerance.

In addition to basic macronutrients, chestnuts also contain numerous minerals and bioactive compounds, which further enrich their functional value and contribute to the overall quality of the diet. Significant amounts of minerals include potassium, magnesium, phosphorus, iron, zinc, copper, and manganese, which support heart function, blood pressure, bone metabolism, and prevent anemia.

These characteristics make sweet chestnut a subject of growing interest in the food industry and nutritional research, with the aim of better utilizing its potential as a functional food. Understanding the chemical composition of sweet chestnut fruits is crucial for assessing their nutritional potential and further application in various food products, as well as for preserving the genetic resources of indigenous populations.

The chemical composition of chestnut fruits is an important factor influencing their nutritional value and usability in the food industry. Variations in the content of water, ash, proteins, fats, starch, and total sugars can reflect genetic differences between populations, as well as the influence of various ecological conditions. The aim of this analysis is to examine the chemical composition of chestnut fruits from different populations and identify possible differences that may be significant for the selection and improvement of the quality of this important forest fruit tree.

### Materials and Methods

This research encompassed maturing and mature chestnut stands, sampled from six localities within the natural distribution range of this species in Bosnia and Herzegovina. These specific localities, characterized by their ecological-vegetation peculiarities, include Banja Luka, Prijedor, Kostajnica, Bratunac, Bužim, and Konjic (Figure 1).

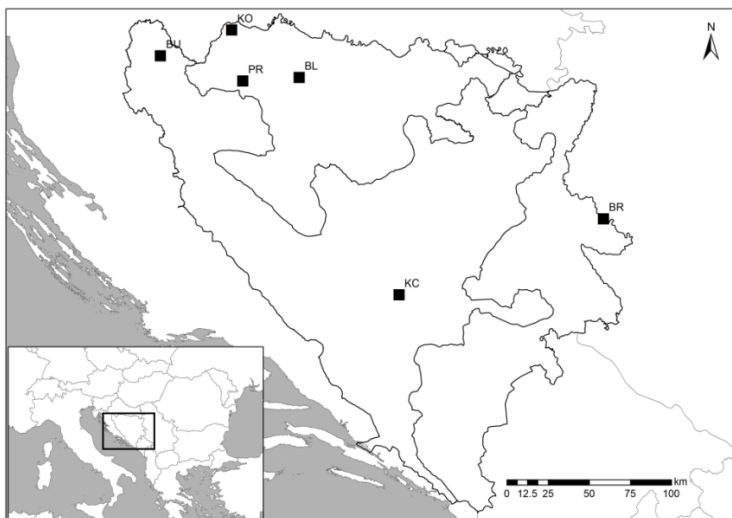


Figure 1. Geographical Location of the Studied Localities

BU-Bužim, KO-Kostajnica, BL-Banja Luka, PR-Prijedor, BR-Bratunac, and KC-Konjic  
At each of the six selected localities, we individually selected 15 superior trees based on their phenotypic characteristics, choosing those that stood out from other trees

within their respective populations. We collected fruits from these selected trees for analysis. Fruit collection involved shaking branches and gathering fallen fruits from the ground (1). Since chestnut fruits are susceptible to moisture loss (2, 3), we immediately packaged 30 healthy fruits from each tree into labeled paper bags to ensure identity preservation. Samples were transported to the laboratory within 24 hours and stored under controlled cool and dry conditions (4–6 °C, protected from direct light) until analysis. Prior to chemical analysis, both the outer shell (pericarp) and pellicle were removed, and the peeled fruits were finely ground. The nutritional value of the chestnut fruits was analyzed using standardized methods. Water content was determined by drying the samples at 105°C. Ash content was established through direct incineration at 550°C. Proteins were analyzed by determining nitrogen content according to standard JUS/ISO 937/1992, and fats were extracted using the Soxhlet method in accordance with JUS/ISO 1443/1992. Starch content was determined by the Evers method (BS EN ISO 10520:1998), while total sugars were analyzed using the Luff-Schoorl method. The analyses for water content, ash content, and total sugars were performed in accordance with the Rulebook on Methods of Sampling and Performing Chemical and Physical Analyses for Quality Control of Fruit and Vegetable Products. The analyses were conducted on peeled chestnut fruits. Below the outer shell (pericarp), the fruits are covered by a pellicle (4). During the preparation of samples for peeled fruit analysis, both the outer shell and the pellicle were removed. The thus prepared sample was finely ground in a mill.

## Results and Discussion

Table 1 presents the chemical composition data for peeled chestnut fruits from six different populations. This includes the content of water, ash, proteins, fats, starch, and total sugars, along with their statistical indicators of variability.

The water content in the peeled chestnut fruits ranged from approximately 37.4% (KO) to 48.1% (KC), with an average value across all populations of 41.57%. The coefficient of variation (Cv) was highest for KO (3.06) and lowest for KC (0.38). The average Cv for this property was 9.07%, indicating that water content is relatively stable with minimal dispersion in the results. Our research revealed an average moisture content of 41.57% in the peeled chestnut fruits from the examined populations. This is slightly lower compared to previous studies conducted in the Una-Sana Canton region of Bosnia and Herzegovina, where an average value of 47% was measured in the Bužim, Velika Kladuša, Cazin, and Bosanska Krupa populations (5). Furthermore, our obtained values are lower than data from other European countries. For instance, the average moisture content in chestnut fruits in Spain was 54% (6), with similar values reported by other researchers (7). In Croatia, the moisture content in the 'Lovran Maron' cultivar was significantly higher at 56.7% (8). Research across various European countries indicates that the moisture content in chestnut cultivars ranges from 40% to 60% (9-13). Compared to these figures, the average values obtained in our study are at the lower end of this range or even below the recommended values for preserving fruit quality. It's crucial that the moisture content in chestnut fruits does not fall below 49% to ensure adequate conservation and maintain fruit quality (14).

The ash content in the peeled chestnut fruits is relatively low, ranging from 0.90% (KC) to 1.20% (PR and BL), with an average of 1.08%. The highest coefficient of variation (Cv) was observed in BR (21.60%), indicating greater variability in ash content within that population. Overall, the ash content is quite consistent across the populations. According to research conducted in the Una-Sana Canton area, the average ash content in chestnut fruits from four populations was 1.1% (5). In comparison, the ash content in the Croatian cultivar 'Lovran Maron' was significantly higher, with an average value of 2.43 g per 100 g of fruit (8). Similar values were recorded for Turkish cultivars, where ash content ranged between 1.02 and 3.22 g per 100 g of fruit (12). Other authors report a wider range of values, from 0.83 to as high as 4.92 g per 100 g (15, 16, 17), suggesting significant variability in this trait among different genotypes and growing conditions. The ash content values obtained in our study are somewhat lower compared to some of the aforementioned studies. This could be due to specific ecological factors or the genetic structure of the local chestnut populations. Ash content indicates the mineral content of the fruit, so lower values may suggest a lower mineral content, which is important for the nutritional value of chestnuts. These data emphasize the need for further studies on the influence of ecological conditions and the selection of genotypes with a more favorable mineral composition, which could contribute to better quality and nutritional properties of chestnuts.

The protein content in the peeled chestnut fruits varied from 3.00% (BU) to 3.70% (BR), with an average value of 3.35%. The coefficient of variation (Cv) was highest in KC (17.86%), indicating greater variability in protein content within that population, while other populations exhibited relatively stable protein content. The average Cv for all studied populations was 10.11%. Our results align with values reported in a study by another research group (18), which found 2.9%, and also fall within the 3% to 6% range cited by a separate study (14). Conversely, Italian cultivars and ecotypes showed significantly higher protein contents, ranging from 4.18 g to 8.12 g per 100 g (13, 9), while Portuguese cultivars recorded values from 4.87 g to 12 g per 100 g (20). The protein content in chestnut fruits is notably lower when compared to other nuts, such as almonds and walnuts, which contain approximately 19% and 21% protein, respectively (6). These data highlight the specific nutritional role of chestnuts, which are primarily valued as a source of carbohydrates, with a relatively modest protein composition.

The fat content in the peeled chestnut fruits ranged from a low of 1.10% (KC) to a high of 3.40% (KO), with an average of 2.40% across all populations. The coefficient of variation (Cv) was highest for BR (11.41%), indicating greater variability within that specific population. While the fat content in chestnuts is generally lower compared to other nuts, it still represents an important nutritional component. Studies indicate that the fat content in chestnuts typically ranges from 1% to 7% per 100g of dry weight, with the predominant fatty acids being oleic, linoleic, and palmitic acids (19). The results obtained in this study are consistent with data previously published by other research groups (20, 21) for "Marron" type chestnut varieties originating from the Iberian Peninsula, as well as results from further studies (22, 23). The observed variability within the BR population (Cv=11.41%) further highlights the

heterogeneity within this group. This heterogeneity could be valuable for future breeding programs aimed at selecting genotypes with a desirable fat profile.

Starch is present in higher quantities, ranging from 29.50% (BU) to 39.00% (BL), with an average of 33.23%. The average coefficient of variation for all studied populations is 11.71%. The BL population showed the highest starch content. Research (19) confirms that starch is the dominant carbohydrate in fresh chestnuts, making up a large portion of their dry mass. Although specific figures for average starch content can vary among studies, the ranges cited for *Castanea sativa* typically fall between 30% and 60% of dry mass, which aligns with our results when considering water content. Their literature analysis shows that starch content varies significantly not only between different cultivars and geographical regions but also within the fruit itself during ripening and storage. The high starch content in our samples, particularly in the BL population, underscores the energy value of chestnuts and their potential applications in the food industry.

The total sugar content in the peeled chestnut fruits varied significantly, from 3.90% (KC) to 9.90% (KO), with an average of 6.92%. The highest variability in total sugars was observed in KC ( $C_v=19.97\%$ ) and BR ( $C_v=11.41\%$ ), while the average coefficient of variation for all studied populations was 31.37%. Sugars are crucial for the organoleptic properties of chestnuts, especially for their sweetness, which is a key factor in consumer acceptance (19). Research indicates that the total sugar content in chestnuts can vary considerably, typically ranging from 5% to 15% in fresh weight, which aligns with our observed range (3.90% to 9.90%). It's emphasized that sucrose is the primary sugar in chestnuts, with smaller amounts of glucose and fructose also present. The variability in sugar content, both in our study and in the literature, can be attributed to genetic differences, the stage of maturity at harvest, and specific climatic and soil conditions. The high coefficient of variation ( $C_v$ ) in populations KC and BR suggests heterogeneity within these groups regarding sugar metabolism, which could have implications for their perceived taste and potential applications. The KO population, with its highest sugar content, may be particularly interesting for selection programs aimed at improving chestnut flavor.

To determine the presence of statistically significant differences among the analyzed properties, an Analysis of Variance (ANOVA) was conducted, with the results presented in Table 2. The obtained data clearly indicate statistically significant differences between the examined populations for water content, fat content, total sugars, and starch content. To further investigate the differences between individual populations, a post-hoc analysis was performed using Duncan's test, and these results are presented in Table 3. The distribution of homogeneous groups, as determined by Duncan's test, allows for the identification of statistically significant differences between populations for all analyzed properties. Specifically, five homogeneous groups were identified for fat content, while four homogeneous groups were formed for starch, water, and total sugar content.

Table 1. Chemical Composition of Peeled Chestnut Fruits

<b>Analyzed Elements</b>		<b>PR</b>	<b>BU</b>	<b>BR</b>	<b>KO</b>	<b>BL</b>	<b>KC</b>	<b>All populations</b>
<b>Water Content (%)</b>	As	37.50	42.70	42.70	37.40	41.00	48.10	<b>41.57</b>
	Sd	0.91	0.18	0.18	1.14	0.91	0.18	<b>3.77</b>
	Cv	2.43	0.43	0.43	3.06	2.23	0.38	<b>9.07</b>
<b>Ash Content (%)</b>	As	1.20	1.10	1.00	1.10	1.20	0.90	<b>1.08</b>
	Sd	0.12	0.18	0.22	0.12	0.18	0.12	<b>0.18</b>
	Cv	9.62	16.60	21.60	10.50	15.21	12.83	<b>16.48</b>
<b>Proteins (%)</b>	As	3.40	3.00	3.70	3.40	3.40	3.20	<b>3.35</b>
	Sd	0.18	0.18	0.18	0.18	0.23	0.57	<b>0.34</b>
	Cv	5.37	6.09	4.93	5.37	6.79	17.86	<b>10.11</b>
<b>Fats (%)</b>	As	3.00	2.30	1.60	3.40	3.00	1.10	<b>2.40</b>
	Sd	0.18	0.26	0.18	0.18	0.18	0.12	<b>0.86</b>
	Cv	6.09	11.23	11.41	5.37	6.09	10.50	<b>35.70</b>
<b>Starch Content (%)</b>	As	37.60	29.50	31.40	32.00	39.00	29.90	<b>33.23</b>
	Sd	0.45	0.91	1.42	0.91	0.91	1.29	<b>3.89</b>
	Cv	1.21	3.09	4.51	2.85	2.34	4.33	<b>11.71</b>
<b>Total Sugars (%)</b>	As	8.50	5.50	8.00	9.90	5.70	3.90	<b>6.92</b>
	Sd	0.91	0.44	0.91	0.18	0.18	0.78	<b>2.17</b>
	Cv	10.74	7.99	11.41	1.84	3.20	19.97	<b>31.37</b>

Notes: As – arithmetic mean; Sd – standard deviation; Cv – coefficient of variation (%).  
 “All populations” – average values across all studied populations.

A cluster analysis was performed to classify the populations into groups, determining the closeness or distance between the analyzed populations. The dendrogram from the cluster analysis (Figure 2) shows that based on the investigated properties, two clusters with complete linkage were formed. The first cluster comprises the populations of Prijedor and Banja Luka, which are further linked with the Kostajnica population. The second cluster includes the populations of Bužim, Konjic, and Bratunac. This grouping may be a result of similar ecological conditions and genetic proximity within each cluster. The differences between the two clusters indicate the influence of various local factors on the fruit's chemical composition. Understanding these distinctions is crucial for guiding the selection and cultivation of chestnut, especially for preserving autochthonous traits and improving fruit quality according to the specific characteristics of each group.

Table 2. Analysis of Variance

<b>Analyzed Elements</b>		<b>Source of Variation</b>	<b>Degrees of Freedom</b>	<b>Sum of Squares</b>	<b>F</b>	<b>p</b>	
<b>Water Content</b>	Population		5	317.9	63.6	124.1	0.0
	Error		18	9.2	0.5		
	Total		23	327.1			
<b>Ash Content</b>	Population		5	0.3	0.1	2.1	0.1
	Error		18	0.5	0.0		
	Total		23	0.7			
<b>Proteins</b>	Population		5	1.1	0.2	2.6	0.1
	Error		18	1.5	0.1		
	Total		23	2.6			
<b>Fats</b>	Population		5	16.24	3.248	91.35	0
	Error		18	0.64	0.0356		
	Total		23	16.88			
<b>Starch Content</b>	Population		5	329.01	65.8	61.82	0
	Error		18	19.16	1.06		
	Total		23	348.17			
<b>Total Sugars</b>	Population		5	100.673	20.135	47.687	0
	Error		18	7.6	0.422		
	Total		23	108.273			

Tabela 3. Duncan test za analiziranelemente

<b>Analyzed Elements</b>	<b>KO</b>	<b>PR</b>	<b>BL</b>	<b>BR</b>	<b>BU</b>	<b>KC</b>	<b>Homogeneous Groups</b>
<b>Water Content</b>	37.4 <sup>a</sup>	37.5 <sup>a</sup>	41 <sup>c</sup>	42.7 <sup>b</sup>	42.7 <sup>b</sup>	48.1 <sup>d</sup>	4
<b>Ash Content</b>	1.1 <sup>ab</sup>	1.2 <sup>a</sup>	1.2 <sup>a</sup>	1 <sup>ab</sup>	1.1 <sup>ab</sup>	0.9 <sup>b</sup>	2
<b>Proteins</b>	3.4 <sup>ab</sup>	3.4 <sup>ab</sup>	3.4 <sup>ab</sup>	3.7 <sup>b</sup>	3 <sup>a</sup>	3.2 <sup>a</sup>	2
<b>Fats</b>	3.4 <sup>e</sup>	3 <sup>a</sup>	3 <sup>a</sup>	1.6 <sup>c</sup>	2.3 <sup>d</sup>	1.1 <sup>b</sup>	5
<b>Starch Content</b>	32 <sup>c</sup>	37.6 <sup>d</sup>	39 <sup>d</sup>	31.4 <sup>bc</sup>	29.5 <sup>a</sup>	29.9 <sup>ab</sup>	4
<b>Total Sugars</b>	9.9 <sup>d</sup>	8.5 <sup>b</sup>	5.7 <sup>a</sup>	8 <sup>b</sup>	5.5 <sup>a</sup>	3.9 <sup>c</sup>	4

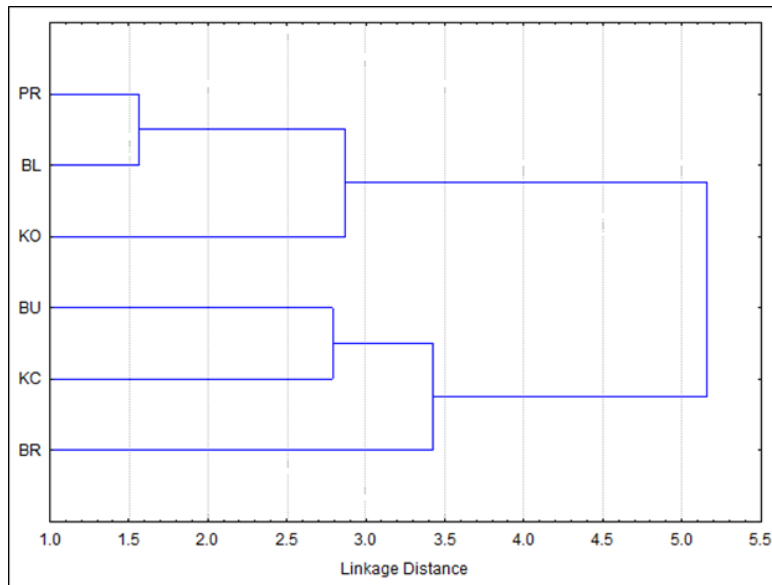


Figure 2. Cluster Analysis

### Conclusion

The analysis of the chemical composition of peeled sweet chestnut fruits from six natural populations in Bosnia and Herzegovina revealed significant variability in moisture, ash, protein, fat, starch, and total sugar content. The fruits were found to have somewhat lower moisture levels compared to certain European populations, which may positively influence preservation and storage. The ash and protein content indicated a moderate mineral and protein potential of chestnuts in this region. Statistical analyses (ANOVA and post-hoc tests) confirmed significant differences among populations, while cluster analysis distinguished two clear groups, likely reflecting the influence of local ecological conditions and genetic characteristics.

The observed chemical diversity has direct relevance for both the food industry and breeding programs. Populations with higher starch and sugar content are suitable for flour production and gluten-free bakery products, whereas those with elevated fat and protein levels may be particularly valuable for the development of nutritionally enriched and specialized products. These differences also provide an important resource for selection and quality improvement through breeding efforts.

The results highlight the importance of conserving the genetic diversity of indigenous chestnut populations in Bosnia and Herzegovina. Such efforts not only preserve natural heritage but also open opportunities for their further valorization through the development of functional foods and sustainable applications in the food industry.

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## **PITOMI KESTEN (*CASTANEA SATIVA* MILL.) KAO FUNKCIONALNA HRANA: HEMIJSKI SASTAV PLODOVA IZ PRIRODNIH POPULACIJA BOSNE I HERCEGOVINE**

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**Sažetak.** *Plod pitomog kestena (*Castanea sativa* Mill.) predstavlja značajnu funkcionalnu namirnicu zbog visokog sadržaja skroba i dijetnih vlakana, niskog udjela masti, kao i prisustva proteina, šećera, minerala i bioaktivnih komponenti. U ovom istraživanju analizirani su oljušteni plodovi iz šest prirodnih populacija u Bosni i Hercegovini (Prijedor, Banja Luka, Kostajnica, Konjic, Bratunac, Bužim) na sadržaj vode, pepela, proteina, masti, skroba i šećera primjenom standardizovanih metoda. Prosječan hemijski sastav svih populacija iznosio je: vlaga 41,6%, pepeo 1,1%, proteini 3,4%, masti 2,4%, skrob 33,2% i šećeri 6,9%. Uočena je značajna varijabilnost: uzorci iz Banje Luke i Prijedora imali su povišen sadržaj skroba (39,0% i 37,6%), pri čemu je Prijedor imao i viši nivo šećera (8,5%); populacija iz Kostajnice izdvajala se najvećim sadržajem masti (3,4%) i šećera (9,9%); Bratunac je imao najviši sadržaj proteina (3,7%); Konjic je pokazao najveći sadržaj vlage (48,1%) ali i najniže vrijednosti šećera i masti. Uzorci iz Bužima uglavnom su imali niže vrijednosti u odnosu na prosjek. Dobijeni rezultati potvrđuju postojanje značajne nutritivne raznovrsnosti među autohtonim populacijama kestena, uslovljene genetskim i ekološkim faktorima. Ova istraživanja predstavljaju osnovu za dalju valorizaciju plodova kestena, selekciju populacija sa povoljnim osobinama i njihovu potencijalnu primjenu u prehrambenoj industriji i programima očuvanja genetičkih resursa.*

**Ključne riječi:** *pitomi kesten, skrob, šećer, nutritivni potencijal*