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Traditional Sweet Chestnut and Hybrid Varieties: Chemical Composition, Morphometric and Qualitative Nut Characteristics

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Abstract: The chemical composition, morphometric and qualitative nut characteristics were studied in four traditional sweet chestnut and hybrid varieties produced on private estates in the Lovran surroundings, on the eastern slopes of Mount Učka, under the specific conditions of the sub-Mediterranean climate. Seven morphological characteristics were measured, and 12 nut and kernel qualitative characteristics were estimated using standardized descriptors. In addition, the samples were analyzed for proximate constituents (moisture, crude fat, crude protein, ash and total carbohydrates) and macro- and micro-nutrients (K, Mg, Ca, Na, Mn, Fe, Zn and Cu). Significant differences between traditional sweet chestnut and hybrid varieties were found in almost all of the studied morphometric and chemical nut characteristics. In general, chestnuts of hybrid varieties were characterized by larger fruits with higher moisture, crude protein, potassium, magnesium, sodium, iron and copper contents than in traditional sweet chestnut varieties. On the other hand, nuts of traditional sweet chestnut varieties were richer in total carbohydrates and crude fat. In addition, the presence of raised stripes, a small hilar scar and transversally ellipsoid nut shape were found to be typical for the traditional sweet chestnut varieties. Overall, our results suggest that the traditional varieties of the sweet chestnut can be easily differentiated from the new modern hybrid varieties by both morphological and chemical characteristics of the nut, and because of these differences, these two groups of chestnut varieties can have different practical uses.

Keywords: traditional sweet chestnut varieties; hybrid varieties; morphometric characteristics; standardized descriptors; chemical composition

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1. Introduction

Chestnut (*Castanea* Mill.) is a monoecious and deciduous genus from the family Fagaceae native to the temperate forest zone of the northern hemisphere [1]. It includes eight economically and ecologically important tree and shrub species of which the Japanese (*C. crenata* Siebold & Zucc.), Chinese (*C. mollissima* Blume.) and European chestnut (*C. sativa* Mill.) have been cultivated for centuries for their economic importance as nut producing trees [2]. The current global chestnut production is estimated at 2.3 million tons, distributed across 612 thousand hectares [3]. China is the leading producer of chestnuts, followed by Turkey, Italy, South Korea, Greece, Portugal, Japan, Spain and North Korea.

Chestnut fruits are highly desirable and widely consumed throughout the world, and generally have several beneficial nutritional characteristics [4–6]. Chestnuts contain high levels of carbohydrates and appreciable levels of dietary fiber, but low amounts of crude protein and, unlike typical nuts (walnuts, almonds, hazelnuts), low levels of crude fat [4,7–12]. Starch accounts for up to 50% of chestnut kernel mass, which has led to the chestnut being referred to as the "bread tree" by some authors [12–14]. In addition, chestnut fruits are a good source of important macro- (K, P, Mg, Ca, Na) and micro-nutrients (Mn, Fe, Zn and Cu) and some vitamins [4,8,9,15].

Almost all the countries that traditionally cultivate sweet chestnut have their own local varieties. Nowadays, more than 300 chestnut varieties have been selected in Italy [16–18]. Similarly, in France [19,20], Spain [21–25], Portugal [26] and other Mediterranean regions, as well as in some Central European countries [27–30], several hundred sweet chestnut varieties have been recorded. Nevertheless, of all the sweet chestnut varieties, marrons are the most recognizable [31]. They are generally characterized by the best nut quality traits, i.e., desirable organoleptic qualities and large one-seeded nuts that are more easily peeled than the wild sweet chestnuts. Traditional sweet chestnut varieties are usually described and classified according to geographical origin and/or morphological and phenological descriptors [28,32,33]. In addition, chestnut varieties can be divided into several groups, depending on the main use of the nuts they produce. Some varieties produce nuts ideal for fresh marketing and candying ("marrons glacés"), while others produce nuts suitable for drying and flour production [17,33,34]. However, the growing of traditional varieties is decreasing, and they are gradually being replaced by new, hybrid varieties [24,35].

Hybrids between *Castanea sativa* and other chestnut species have been developed to improve nut production [36], as well as to increase resistance to the chestnut blight and ink disease [37,38]. Sometimes, these hybrids are also less susceptible to the chestnut gall wasp. In recent years, Japanese and European crosses are becoming increasingly popular in European commercial orchards [28,35,39,40]. The most common hybrid is 'Bouche de Bétizac', characterized by early fructification, and large to very large fruits.

In Croatia, in the bay of Kvarner, the indigenous variety 'Lovran Marron' is grown in unique forest-orchard systems under the specific conditions of the sub-Mediterranean climate [35]. The export of chestnuts from this region to other countries is reported to have been established already in the 17th century, which places marron fruits in the category of farming products with long-standing importance, together with olives, grapevine and sweet cherries [41]. However, from the 20th century onwards, changes in the local population's way of life and the appearance of the chestnut blight resulted in partial neglect of the plantations and their stagnation, all of which was consequently reflected in decreased nut production [42,43]. Nevertheless, in the last ten years, many of the old chestnut orchards have been pruned for renewal, and the damage caused by the chestnut blight has been reduced. It should also be highlighted that this indigenous sweet chestnut variety has great socioeconomic significance and helps promote the local identity of the entire Lovran area [35]. Thus, for example, the symbol of the Učka Nature Park includes chestnuts, and every October since 1973, Lovran and the nearby villages of Liganj and Dobreć have been the venue of the traditional gastronomic tourism event Marunada, in which various local chestnut products have the central role.

In the last few decades, different molecular markers and isozymes have been used to characterize and distinguish sweet chestnut varieties [32,33,39,44–52]. Nevertheless, morphological characteristics [49,53–57] and/or chemical composition [6–10,12,15,34,48,58–60] have been widely used for descriptive purposes and are also commonly used to distinguish sweet chestnut varieties. In our previous study, we used microsatellites [42,43,61] and morphological and chemical markers [35] to characterize the traditional Croatian variety of the sweet chestnut known as the 'Lovran Marron'. We found that the marrons from Lovran mostly belonged to a single clone, with no variations between individuals. In addition, our results confirm the existence of natural hybrids of marrons and wild sweet chestnut trees in those areas.

Although many studies have already addressed the chemical composition and morphology of traditional sweet chestnut varieties, similar data for hybrid varieties are still missing. Accordingly, this study was focused on the analysis of chestnut fruits from traditional varieties and Euro-Japanese hybrids, including: (1) morphometry of chestnuts; (2) standardized descriptors; and (3) chemical composition of kernels.

2. Materials and Methods

Samples of chestnuts for morphometric and chemical research were obtained from private groves in the wider area encompassing Liganj, Dobreć, Lovranska Draga, Mošćenička Draga and the vicinity of the Učka Tunnel, located in the bay of Kvarner, near the city of Rijeka (Croatia), in October 2018, during the seasonal harvest. In total, fruit samples were collected from 40 trees: 25 trees of the traditional Croatian variety of the sweet chestnut 'Lovran Marron'; five trees of the Italian traditional sweet chestnut variety 'Marradi'; five trees of the Euro-Japanese hybrid variety 'Bouche de Bétizac'; and five trees of the Euro-Japanese hybrid variety 'Marsol'. Fruit samples from the indigenous sweet chestnut variety 'Lovran Marron' were collected from previously genotyped trees [42,43,61].

The morphometric analysis included 30 nuts per tree, located laterally in cupules. Seven nut traits and eight ratios were examined to assess the variations between traditional sweet chestnut and hybrid varieties. Nut mass was determined first, and then a digital caliper was used to measure the following traits [35,62]: nut height, width and thickness, hilum length and width, and length of the longest intrusion of the seed coat into the kernel. From the measured traits the following ratios were calculated: nut height/nut width, nut thickness/nut height, nut thickness/nut width, hilum length/nut width, hilum width/nut thickness, hilum width/hilum length, length of the longest intrusion of the seed coat into the kernel/nut thickness.

In addition, the official descriptor list and guidelines of the International Union for the Protection of New Varieties of Plants [63] were used to characterize the traditional sweet chestnut and hybrid varieties. In total, ten nut and kernel qualitative characteristics were estimated using standardized descriptors: time of beginning of fruit ripening; nut size, shape, color and glossiness; hilum size; number of kernels; penetration of seed coat into the kernel; degree of penetration of seed coat into the kernel; and kernel color. Furthermore, the presence of longitudinal raised stripes on the nut [35,64] and seed coat peeling were evaluated.

Immediately after collection, the nuts were peeled and prepared for chemical analysis as described in Poljak et al. [35]. Chestnut samples were analyzed for proximate composition (moisture, crude protein, crude fat and ash) using the AOAC (Association of Official Analytical Chemists) procedures [65–68], and each sample was analyzed in triplicates. The moisture content in the samples was determined by a physical, indirect method, in which a sample of known mass was dried in an air dryer (Instrumentaria, Zagreb, Croatia) at 105 °C until constant weight was achieved (5 h). Ash content analysis was carried out by a process known as dry ashing, i.e., the nut samples were burned in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 525 °C, again until constant weight was achieved. The Kjeldahl method was employed to determine the total nitrogen content, in combination with a copper catalyst using the block digestion system Foss Tecator 6—1007 Digestor (Foss Tecator, Höganäs, Sweden) and the Foss Kjeltec™ 8100 Auto Distillation unit (Foss Tecator, Höganäs, Sweden). Crude protein content was obtained by multiplying total nitrogen by a conversion factor of 5.30 [67]. The total crude fat extraction was performed by the Soxhlet apparatus (Inkolab d.o.o., Zagreb, Croatia); diethyl ether was used for extraction during 16 h. Carbohydrate content was determined using the following formula [6,10,35,69,70]:

$$W\text{ (carbohydrate)} = 100 - (w\text{ (moisture)} + w\text{ (crude protein)} + w\text{ (crude fat)} + w\text{ (ash)}) \quad (1)$$

Macro- and micronutrients (K, Ca, Mg, Na, Cu, Fe, Mn and Zn) were determined by atomic absorption spectrometry using a Varian SpectrAA 220 spectrophotometer (Varian, Mulgrave, Victoria, Australia).

For all of the studied variables, standard descriptive statistical parameters were calculated: arithmetic mean (M), standard deviation (SD) and coefficient of variation (CV/%). To assess the possibility of conducting multivariate statistical analyses and parametric tests, the symmetry, unimodality and homoscedasticity of data were verified [71]. Statistically significant differences between studied the sweet chestnut and hybrid varieties were tested using the analysis of variance (ANOVA). The above statistical analyses for the chemical and morphological data were performed using the software package STATISTICA (TIBCO Software Inc., Palo Alto, CA, USA) [72].

The differentiation of studied chestnut varieties was investigated through principal component (PC) analysis and discriminant analysis, using the software packages SAS (SAS Institute Inc., Cary, NC, USA) [73] and R (R Foundation for Statistical Computing, Vienna, Austria) [74] following the manual of Koutecký [75]. Prior to PC and discriminant analysis, Pearson's correlation coefficient was used to identify interactions between nut and chemical traits and to detect potential redundant variables, i.e., highly correlated variables were excluded from the analyses. In total, eight out of 13 morphological traits, as well as all of the studied chemical variables, were included in the multivariate statistical analysis. Principal component analysis was performed using PROC PRINCOMP in SAS, and the PC biplot was constructed by two principal components showing analyzed individuals and traits. In addition, a stepwise discriminant analysis was performed to evaluate the utility and importance of eight morphological traits by determining which were most useful in maximally discriminating chestnut varieties. After the stepwise discriminant analysis was performed, a subset of three of the most important morphological traits contributing to the differentiation of studied groups was chosen to calculate the proportion of correctly classified individuals into studied groups of chestnut varieties. The input data in multivariate statistical methods were previously standardized, i.e., standardization of characters to zero mean and unit standard deviation was performed prior to each multivariate analysis.

3. Results and Discussion

All of the measures of chestnut fruits varied significantly among varieties. Nut morphological features clearly displayed bimodal distributions across the collection, with samples from hybrid varieties having larger nuts than those from traditional varieties (Table 1). The average nut mass for the variety 'Bouche de Bétizac' was 27.8 g, and for the variety 'Marsol' 22.2 g. In addition, we noted that trees of the Italian variety 'Marradi' produce smaller nuts than those of the traditional Croatian variety 'Lovran Marron'. The average nut mass for the traditional sweet chestnut variety 'Marradi' was 9.6 g, and for the variety 'Lovran Marron' 12.9 g. Besides in the size of the nuts, traditional and hybrid varieties also differed in the derived variables. Thus hybrid varieties typically had higher values for the nut height to width ratio, hilum length to nut width ratio, and hilum width to nut thickness ratio. On the other hand, traditional sweet chestnut varieties were characterized by higher values of the nut thickness to height ratio. Observed mean values for measured nut traits are comparable with those published previously for the studied traditional sweet chestnut and hybrid varieties [34,35,76].

Statistically significant differences between the studied groups of chestnut varieties (traditional and hybrid) were observed in all morphological nut traits (Table 1). The studied hybrid varieties differed at the significance level of 0.01 in nut mass, nut and hilum width, number of seeds per nut, nut height to width ratio, nut thickness to width ratio, hilum length to nut width ratio and hilum width to nut thickness ratio, while at the level of 0.05 this difference was significant for nut thickness to height ratio and hilum length to width ratio. Statistically significant differences at the level of 0.01 between the studied traditional sweet chestnut varieties were found for eight out of 16 morphometric traits.

Coefficients of variation for the measured nut traits were low to medium except for the length of the longest intrusion of the seed coat into the kernel. Low morphological variability can be attributed to the low within-variety variation [42,43,61]. In contrast, the wild sweet chestnut trees from naturally growing populations were found to be very diverse both genetically [43,61,77–80] and morphologically [62,81–83].

Appreciable qualities of chestnuts are: large nut size (for fresh market and candying, i.e., marron glacé), easy peeling (for fresh market and processing), low degree of penetration of the seed coat into kernel and monoembryony (for fresh market) [35,84]. In addition, the sweet chestnut fruit characteristics important to the consumers are also the nut shape, color and shine, hilum size and color, and the marked longitudinal, darker and slightly protruding stripes on the fruit [35,82,84].

The 'Bouche de Bétizac' nuts are characterized by their very large size (less than 60 nuts per kilogram), transversally broad ellipsoid shape and light reddish-brown to dark brown color (Table 2). The pericarp is easily removed from the episperm and the color of the kernel in fresh state is yellowish white. In addition, nuts of this hybrid variety contain multiple embryos, which is why 'Bouche de Bétizac' is not desirable for industrial processing. According to Bounous [84], to make industrially processed candied marrons or marrons glacés, the essential requirements are that chestnuts have a low percentage of polyembryonic nuts, and that they are suitable for mechanical peeling. In addition to having very large nuts, 'Bouche de Bétizac' is characterized by early ripening, being often the first variety to enter the market. This makes it an ideal variety to grow alongside the traditional, late ripening varieties, as it fills the gap in the market and produces nuts suitable for roasting, a cooking method favored by consumers in autumn [84–86].

Euro-Japanese hybrid variety 'Marsol' is characterized by broad ovoid and shiny reddish-brown colored nuts (Table 2). The nuts of this variety are very large (45 nuts per kilogram) with a less than 3% occurrence of double embryos. According to Tonelli and Gallouin [85], they can be consumed fresh and used for processing. The fruits, however, are described as having a poorer flavor. This hybrid is usually used as rootstock because of its good graft compatibility with many other varieties [20,86]. In addition to being used as rootstock, 'Marsol' is a common pollinator tree, planted in orchards alongside auto-sterile hybrid varieties, most notably 'Bouche de Bétizac'.

One kilogram of 'Lovran Marron' fruits requires 77 nuts, which classifies them as large (Table 2). Nut size, shape uniformity, shine, reddish brown color with longitudinal, darker and slightly protruding stripes, easy pellicle removal, and a sweet and tasty flavor are all valuable traits of this traditional variety for the fresh market. In addition, the absence of double or divided kernels makes this variety desirable for industrial production of candied marrons (marrons glacés). Nevertheless, nowadays most of the nuts of this traditional Croatian marron variety are sold for the fresh market as there is no established production of processed products (flour, candy). This variety is also an important part of the tourist offer, being the focal point of many gastronomic events such as Marunada and numerous fairs in autumn.

It is well known that in Italy chestnut germplasm is very diverse [16–18,86] and the cultivated forms include hundreds of varieties selected for candying, roasting, drying and flour production. In recent years, 'Marradi', named after the village of Marradi in Upper Mugello, Tuscany, together with some other Italian marron varieties, has been cultivated in Croatia. Since 1996 it has been defined and protected by PGI (Protected Geographical Indication) under the name 'Marrone del Mugello' [34]. Its nuts are of small to medium size, bright and lightly reddish-brown in color, with darker stripes (Table 2). They are mostly used roasted and in confectionery for candying [86], and since they are classified as small to medium sized nuts, they are also suitable for the production of dried chestnuts and for making flour. A few traditional sweets and dishes are made with this variety, such as *castagnaccio* bread, *marroni* cake and *tortelli di marroni*.

The presence of raised stripes, a small hilar scar and transversally ellipsoid nut shape were found to be typical of the traditional sweet chestnut varieties. On the other hand, Euro-Japanese hybrids were characterized by a larger hilar scar, often with cracks, making them less appealing to consumers [86]. Moreover, the nuts of hybrid varieties did not have dark protruding stripes which are a desirable trait for selling them fresh.

The long history of selection and cultivation of traditional sweet chestnut varieties resulted in specific varieties suitable for fresh consumption, drying for flour production, or candying [84]. However, the importance of local varieties extends beyond nut production and is connected to the history of the local communities, which have planted and looked after the orchards for centuries [13,18,41,42]. High-quality wood, tannins, charcoal, honey and providing an ideal environment for the growth of edible wild mushrooms, are some of the uses and roles chestnut trees have played throughout their very long history of cultivation [4,35,64,84]. Both chestnut fruits and the chestnut landscapes in which traditional, local varieties are the building blocks, have played a significant role in the culture and history of many Mediterranean countries [8,13,15]. Moreover, traditional varieties continue to be favored by consumers over the hybrid ones, partially also due to this cultural and historical connection people have with them, as well as due to the gastronomic heritage [84].

Table 1. Morphometric characteristics—descriptive statistical parameters and levels of significance.

Trait	Traditional Sweet Chestnut Varieties				Hybrid Varieties				ANOVA		
	'Lovran Marron'		'Marradi'		'Bouche de Bétizac'		'Marsol'				
	Mean Value ± SD	**CV/%**	**Mean Value ± SD**	**CV/%**	**Mean Value ± SD**	**CV/%**	**Mean Value ± SD**	**CV/%**	**T/H**	**T**	**H**
Nut mass/g	12.9 ± 2.5	19.3	9.6 ± 1.4	14.6	27.8 ± 5.1	18.4	22.2 ± 3.7	16.8	>0.01	>0.01	>0.01
Nut height/mm	28.4 ± 1.8	6.3	27.1 ± 1.6	6.0	39.5 ± 1.5	3.7	39.7 ± 1.6	4.0	>0.01	>0.01	ns
Nut width/mm	35.0 ± 2.8	8.1	33.6 ± 2.4	7.1	45.5 ± 3.3	7.3	39.8 ± 2.7	6.8	>0.01	ns	>0.01
Nut thickness/mm	21.9 ± 2.6	12.0	19.0 ± 1.6	8.4	26.0 ± 2.9	11.0	25.2 ± 2.4	9.7	>0.01	>0.01	ns
Hilum length/mm	23.4 ± 2.8	11.9	24.2 ± 1.7	7.1	34.0 ± 3.2	9.5	34.8 ± 4.1	11.9	>0.01	ns	ns
Hilum width/mm	12,0 ± 1.7	13.8	11.8 ± 1.1	9.3	17.3 ± 2.0	11.3	18.6 ± 1.9	10.3	>0.01	ns	>0.01
Seeds per nut	1.0 ± 0.1	10.2	1.1 ± 0.2	20.1	1.6 ± 0.6	34.4	1.00 ± 0.0	0.0	>0.01	ns	>0.01
Number of intrusions	1.3 ± 1.3	95.1	0.9 ± 0.8	85.0	2.2 ± 1.3	58.5	1.8 ± 1.1	60.1	>0.01	ns	ns
Length of the longest intrusion of the seed coat into the kernel/mm	2.3 ± 1.7	72.9	2.1 ± 1.7	82.2	3.4 ± 1.7	49.1	3.4 ± 1.7	50.4	>0.01	ns	ns
Nut height/nut width	0.82 ± 0.06	7.24	0.81 ± 0.05	5.93	0.87 ± 0.05	5.65	1.00 ± 0.06	6.13	>0.01	ns	>0.01
Nut thickness/nut height	0.77 ± 0.09	11.63	0.70 ± 0.07	10.03	0.66 ± 0.06	9.68	0.64 ± 0.06	10.08	>0.01	>0.01	>0.05
Nut thickness/nut width	0.63 ± 0.08	12.58	0.57 ± 0.05	9.03	0.57 ± 0.04	6.92	0.63 ± 0.06	9.47	>0.01	>0.01	>0.01
Hilum length/nut width	0.67 ± 0.05	8.09	0.72 ± 0.04	5.57	0.75 ± 0.03	3.61	0.87 ± 0.07	7.84	>0.01	>0.01	>0.01
Hilum width/nut thickness	0.55 ± 0.06	11.25	0.62 ± 0.04	6.75	0.67 ± 0.04	5.84	0.74 ± 0.08	11.21	>0.01	>0.01	>0.01
Hilum width/hilum length	0.51 ± 0.06	12.25	0.49 ± 0.04	9.08	0.51 ± 0.03	6.60	0.54 ± 0.08	14.59	>0.01	>0.01	>0.05
Length of the longest intrusion of the seed coat into the kernel/nut thickness	0.10 ± 0.08	72.96	0.11 ± 0.10	93.42	0.13 ± 0.07	51.04	0.13 ± 0.07	50.44	>0.01	ns	ns

SD = standard deviation, CV = coefficient of variation, dm = dry matter, ns = non-significant value, T/H = differences between traditional sweet chestnut and hybrid varieties, T = differences between traditional sweet chestnut varieties 'Lovran Marron' and 'Marradi', H = differences between hybrid varieties 'Bouche de Bétizac' and 'Marsol'.

Table 2. Standardized descriptors.

Major Details	Name of Descriptor	Traditional Sweet Chestnut Varieties		Hybrid Varieties	
		'Lovran Marron'	**'Marradi'**	**'Bouche de Bétizac'**	**'Marsol'**
UPOV number 26	Time of beginning of fruit ripening	late	late	very early	early
UPOV number 27	Fruit: embryony	monoembryonyc	monoembryonyc	polyembryonyc	monoembryonyc
UPOV number 29	Fruit: penetration of seed coat into the kernel	present	present	present	present
UPOV number 30	Fruit: degree of penetration of seed coat into the kernel	low/medium	low/medium	low/medium	low/medium
UPOV number 31	Fruit: shape	transverse ellipsoid	transverse ellipsoid	transverse broad ellipsoid	broad ovoid
UPOV number 32	Fruit: size of hilum	small	small	medium	medium
UPOV number 34	Fruit: glossiness (immediately after opening of cupule)	present	present	present	present
UPOV number 35	Fruit: color	reddish brown	reddish brown	reddish brown, quickly turning dark brown	reddish brown
UPOV number 36	Fruit: size	big	small/medium	very big	very big
UPOV number 38	Kernel: color	cream	cream	cream	cream
Poljak et al. [35], Martin et al. [64]	Fruit: raised stripes	present	present	absent	absent
This study	Seed: seed coat peeling	easy	easy	easy	easy

UPOV—International Union for the Protection of New Varieties of Plants.

As far as the proximate composition and macro- and micro-nutrients of hybrid chestnut varieties are concerned, the data available in the literature are poor [12,87,88]. However, there are many more published research papers on the chemical composition of traditional sweet chestnut varieties. According to those studies, the chemical composition of chestnuts varies by genotype [6,8,10,89,90], ecotype [34,59], soil and climatic conditions [7,9,11], altitude [91], area of production [9] and production practices [92,93]. Given that the samples in this study were collected under the same climatic and soil conditions, the differences in the proximate composition (moisture, crude protein, crude fat, ash and total carbohydrates) and macro- and micro-nutrients can be interpreted as a result of genetic differences between the studied varieties. Differences in the chemical composition between traditional and hybrid sweet chestnut varieties have been statistically significant in almost all researched traits, except for the mass fraction of Ca and Zn (Table 3). In addition, the proximate composition significantly varied within traditional varieties, and macro- and micronutrients within hybrid varieties.

Due to high moisture content, chestnut fruits cannot be preserved for a long period. According to Breisch [36], the moisture content of chestnuts should be between 49 and 60% for adequate storage. The average moisture content of the traditional and hybrid varieties considered in this study ranged between 56.0% and 64.2%, with hybrids proving to have a very high-moisture content (Table 3). A similar result was obtained by Glushkova et al. [87] in a study on the nut quality assessment of chestnut varieties in Bulgaria: chestnuts of *C. sativa* × *C. crenata* variety 'Marigoule' were shown to have the highest moisture content among those tested. Similar results were obtained by Mert and Ertürk [12] for the hybrid variety 'Marigoule'. However, higher moisture content complicates storage due to mold development, thus making the fruit of hybrid chestnuts more suitable for the fresh market. In the literature, moisture content of fresh chestnuts ranges from 41 to 59% for Italian [34,59], Spanish [7–9] and Portuguese traditional varieties [6,10,15,94]. Fruits from both traditional sweet chestnut varieties in this study showed moisture content suitable for conservation.

Chestnuts stand out from other edible nuts as they are mainly composed of carbohydrates, primarily starch [4,6] and sucrose, which is one of the most important parameters for the assessment of their commercial quality [89]. In this study, total carbohydrates found in the traditional sweet chestnut varieties were somewhat higher than those in the hybrid varieties (Table 3). Similar average values were given by Barreira et al. [6,10] for Portuguese chestnut varieties and by Ertürk et al. [95] for chestnut varieties and European-Japanese hybrids. In addition, our data for 'Marradi' are somewhat higher than those reported by Bellini et al. [17].

Considering the total crude fat, data obtained in this research confirm the low-fat level in chestnuts compared to other nuts [96], such as almonds, walnuts and hazelnuts. The highest crude fat content was recorded for the 'Lovran Marron', while the lowest values were recorded for the hybrid varieties (Table 3). The results for the traditional sweet chestnut varieties from this study are in the 1.26–3.50 g per 100 g dm range reported by Borges et al. [15,96] and De La Montaña Míguelez et al. [7] for marron varieties from the Iberian Peninsula. On the other hand, somewhat higher average crude fat content was reported by Dinis et al. [11] and Pereira-Lorenzo et al. [8] for varieties from Portugal and Spain (2.78–3.0 g per 100 g dm), and Saccheti et al. [59] and Bellini et al. [17] for sweet chestnut varieties and ecotypes from Italy (3.0–4.64 g per 100 g dm). However, possible differences found in papers for the chestnut's crude fat may be explained by the differences in solvents and temperatures used during the crude fat extraction [93].

Crude protein content was medium, ranging from 3.7% to 5.6%, with the highest values for the studied hybrid varieties (Table 3). Results for the 'Lovran Marron' were basically similar to those we reported earlier for the same variety [35]. In general, this Croatian traditional sweet chestnut variety is characterized by lower crude protein content than other chestnut varieties from the Apennine [17,59,97] and Iberian Peninsula [6–8,10,15,94]. Our results confirm that the 'Lovran Marron' nuts have lower crude protein content than the Italian traditional sweet chestnut variety known as 'Marrone del Mugello'. In addition, according to Poljak et al. [35], nuts from wild sweet chestnut trees were richer in crude protein than the nuts from grafted cultivated 'Lovran Marron' trees. Although it was detected earlier that there is a relationship between the crude protein content of chestnut fruits and the type of soil in which they had grown [7,8], the results of this study indicate that crude protein content is also affected by the genetic makeup of the chestnut varieties.

According to the literature, the average ash content in sweet chestnut varieties ranges from 1.02 g to 3.22 g per 100 g dm [6–10,15,59,94,95]. Our results for the traditional and hybrid sweet chestnut varieties are within the values referred to by the authors mentioned above. In general, we revealed that nuts from the Euro-Japanese hybrid varieties have a higher content of potassium, magnesium, sodium, iron and copper compared to the nuts from traditional sweet chestnut varieties (Table 3).

As for the chemical composition of fruits, the lowest variability was recorded for the moisture and total carbohydrate contents (Table 3). Furthermore, lower variability was also recorded for ash content. Low to intermediate variability characterized the majority of macro- and micro-nutrients and crude protein and crude fat contents. Manganese was proven to be the most variable characteristic. In general, higher variability of certain chemical properties can be explained by microsite differences. For instance, certain pedo-physio-graphic parameters, such as particle size distribution, pH value of soil, total carbon and nitrogen contents, and trace elements contents can influence a higher degree of variability of individual chemical properties of sweet chestnut fruits. Similar coefficients of variation for proximate constitutes and macro- and micro-nutrients were also recorded by other authors [7,15,34,35] for Mediterranean traditional sweet chestnut variates. According to those authors, the coefficient of variation depended on the variety, the harvesting year, and their interaction.

Table 3. Chemical composition—descriptive statistical parameters and levels of significance.

Trait	Traditional Sweet Chestnut Varieties				Hybrid Varieties				ANOVA		
	'Lovran Marron'		'Marradi'		'Bouche de Bétizac'		'Marsol'				
	Mean Value ± SD	CV/%	Mean Value ± SD	CV/%	Mean Value ± SD	CV/%	Mean Value ± SD	CV/%	T/H	T	H
w(moisture)/(g/100 g)	55.8 ± 2.2	4.0	52.9 ± 1.5	2.9	62.5 ± 1.3	2.1	64.5 ± 1.2	1.8	>0.01	>0.01	>0.05
w(fat)/(g/100 g dm)	2.6 ± 0.5	19.3	1.7 ± 0.3	18.7	1.3 ± 0.2	10.9	1.4 ± 0.3	20.0	>0.01	>0.01	ns
w(protein)/(g/100 g dm)	3.7 ± 0.4	9.4	4.6 ± 0.3	5.6	5.6 ± 0.8	14.8	5.0 ± 0.7	14.2	>0.01	>0.01	ns
w(ash)/(g/100 g dm)	2.3 ± 0.3	11.3	2.6 ± 0.2	7.1	2.6 ± 0.2	7.4	2.8 ± 0.2	9.1	>0.01	>0.05	ns
w(total carbohydrate)/(g/100 g dm)	91.4 ± 0.5	0.5	91.1 ± 0.4	0.4	90.4 ± 0.8	0.9	91.0 ± 1.0	1.1	>0.01	ns	ns
w(K)/(mg/100 g dm)	1282.5 ± 194.3	15.2	1218.9 ± 205.1	16.8	1554.6 ± 48.5	3.1	1383.9 ± 58.7	4.2	>0.01	ns	>0.01
w(Mg)/(mg/100 g dm)	74.7 ± 5.1	6.8	80.3 ± 10.5	13.0	91.2 ± 5.2	5.7	97.9 ± 6.8	7.0	ns	ns	ns
w(Ca)/(mg/100 g dm)	79.1 ± 7.8	9.8	79.4 ± 16.5	20.7	56.9 ± 3.8	6.7	100.7 ± 5.8	5.7	ns	ns	>0.01
w(Na)/(mg/100 g dm)	28.1 ± 5.6	19.9	18.4 ± 2.6	14.0	31.8 ± 2.5	7.9	39.2 ± 0.4	1.1	>0.01	>0.01	>0.01
w(Mn)/(mg/100 g dm)	2.1 ± 1.0	49.3	4.8 ± 0.7	15.4	2.2 ± 0.6	27.3	1.0 ± 0.4	38.3	>0.01	>0.01	>0.01
w(Fe)/(mg/100 g dm)	1.4 ± 0.4	27.5	1.2 ± 0.4	31.3	1.6 ± 0.1	5.9	2.0 ± 0.1	6.3	>0.01	ns	>0.01
w(Zn)/(mg/100 g dm)	1.5 ± 0.2	12.7	1.3 ± 0.3	22.0	1.4 ± 0.04	3.3	1.5 ± 0.1	6.5	ns	ns	>0.05
w(Cu)/(mg/100 g dm)	0.9 ± 0.2	23.9	1.0 ± 0.2	17.0	1.3 ± 0.04	2.8	1.6 ± 0.1	11.5	>0.01	ns	ns

SD = standard deviation, CV = coefficient of variation, dm = dry matter, ns = non-significant value, T/H = differences between traditional sweet chestnut and hybrid varieties, T = differences between traditional sweet chestnut varieties 'Lovran Marron' and 'Marradi', H = differences between hybrid varieties 'Bouche de Bétizac' and 'Marsol'.

As expected, nut dimension variables were highly correlated (Figure 1), which was also previously reported by other authors for various species: cornelian cherry [98]; common medlar [99]; three species of *Ziziphus* Mill. genus [100]; edible fig [101]; white mulberry [102]; wild pomegranate [103] and for sweet, sour and duke cherry [104]. The highest correlations, above 0.85, were found between nut mass and nut height, width, and thickness, and between hilum length and width. In addition, strong correlations between chemical and morphological traits were observed as well. The dimensions of nuts in chestnut varieties were positively correlated with chemical variables, especially with moisture, ash and crude protein content. Analyses of other tree varieties have similarly revealed positive correlations between fruit dimensions and moisture, ash and crude protein content [105] and seed dimensions and crude protein content [106]. In contrast, negative correlations between morphometric traits and crude protein content were found for the Mediterranean buckthorn fruits [107]. Furthermore, crude fat and total carbohydrate contents were statistically negatively correlated with the nut and hilum measured traits. However, Pereira-Lorenzo et al. [8] reported that there was no negative correlation between nut size and total sugar content for Spanish traditional sweet chestnut varieties. On the other hand, Boublenza et al. [108] revealed that the largest pods of cultivated carob trees have the highest sugar content. Furthermore, our data show that there were some positive and negative correlations between chemical variables. Non-significant correlations were found between Ca and Zn content, and other chemical variables. Moisture content was in positive correlation with six chemical variables (crude protein, ash, K, Mg, Fe, and Cu), and in negative correlation with the crude fat and total carbohydrate content. Indeed, some authors discussed that some of the organic components in fruit depend on moisture content [107]. According to Izhaki et al. [107], this can be attributed to the differing solubility of organic compounds—carbohydrates are the most soluble in water, lipids are hydrophobic, and proteins may be soluble to some extent. In addition, strong positive and negative correlations were found between Mg and Cu and other chemical variables, and strong negative correlations were found between crude protein and crude fat, and total carbohydrate contents. Although correlations of morphological and chemical diversity in some cases can be explained by the specific edaphoclimatic conditions [109], our results are probably a consequence of genetic differences between the studied hybrid and marron varieties. Nevertheless, we cannot exclude the possibility that some of the interrelationships in the chemical composition of chestnuts may indicate synergistic and antagonistic interactions between nutrients [107,110].

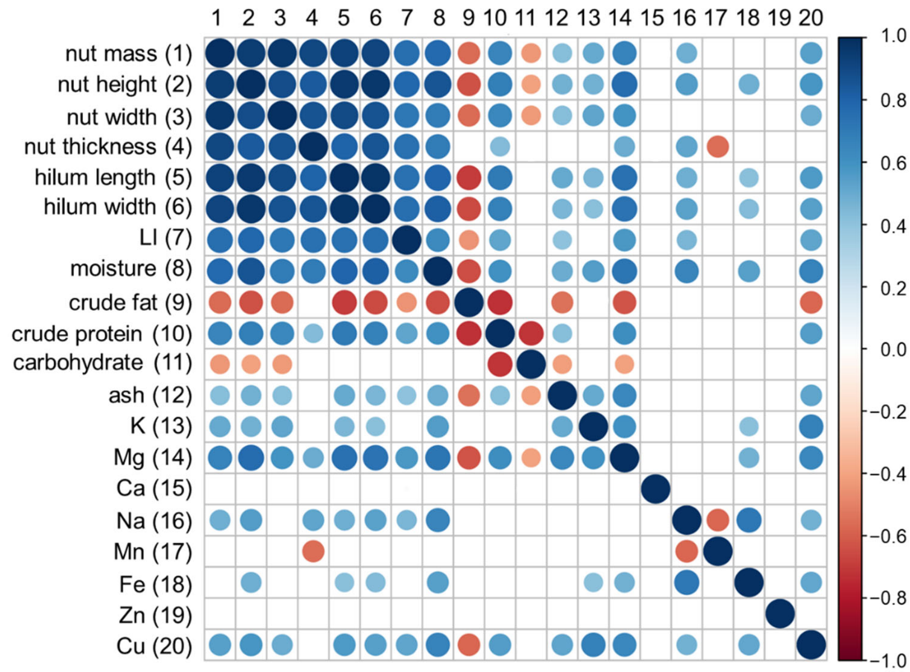

Figure 1. Correlations between morphometric and chemical variables in the studied traditional and hybrid chestnut varieties. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. Non-significant values, $p > 0.01$, are blank.

Principal component analysis based on eight morphological traits in four chestnut varieties revealed that the first two principal components had an eigenvalue >1 and accounted for 74.9% of the total variation (Table 4). The first principal component (PC1) was strongly positively correlated with the nut thickness to height ratio, while strong negative correlations were observed between the first principal component and nut mass, length of the longest intrusion of the seed coat into the kernel, nut height to width ratio, hilum length to nut width ratio, and length of the longest intrusion of the seed coat into the kernel to nut thickness ratio. Strong positive correlations were observed between the second principal component (PC2) and the nut thickness to width ratio and hilum width to length ratio. The biplot constructed by the first two principal components is presented in Figure 2.

Table 4. Pearson's correlation coefficients between eight morphological traits and scores of the first three principal components.

Trait	PC—Principal Component		
	PC1	**PC2**	**PC3**
Nut mass	−0.817	0.011	0.093
LI	−0.865	0.176	0.461
Nut height/width	−0.831	0.247	−0.407
Nut thickness/height	0.800	0.430	0.342
Nut thickness/width	0.150	0.929	0.010
Hilum length/Nut width	−0.851	−0.071	−0.299
Hilum width/length	−0.168	0.856	−0.215
LI/Nut thickness	−0.767	0.044	0.560
Eigenvalue	4.111	1.881	0.954
% of variance	51.39	23.51	11.93

LI—Length of the longest intrusion of the seed coat into the kernel.

The results of the principal component analysis (Table 5, Figure 3), which included the proximate composition and macro- and micro-nutrients, show cumulative variability of 68.3% in the first three PC axes. The first principal component, which accounts for 40.6% of variability, separates the samples with high moisture, ash, crude protein, Cu, Fe, Mg, K and Na content, which are highly negatively correlated with it, from the samples with high crude fat and total carbohydrate content, which is highly positively correlated with the same principal component. The second and third PC axes contribute substantially less to the overall variability, with 17.8 and 10.3%, respectively. The components highly correlated with the second PC axis are Mn (negatively) and Na (positively). The third PC axis is highly positively correlated with Mn and Ca.

Table 5. Pearson's correlation coefficients between 13 chemical traits and scores of the first three principal components.

Trait	Principal Component		
	PC1	**PC2**	**PC3**
Moisture	−0.885	0.139	−0.150
Ash	−0.679	−0.320	0.371
Crude fat	0.716	0.252	−0.241
Crude protein	−0.718	−0.503	−0.240
Total carbohydrate	0.462	0.523	0.408
K	−0.694	−0.025	0.059
Mg	−0.870	−0.046	0.133
Ca	−0.175	0.462	0.547
Na	−0.598	0.615	−0.325
Mn	0.186	−0.616	0.608
Fe	−0.646	0.531	0.036
Zn	−0.109	0.584	0.308
Cu	−0.835	−0.042	0.080
Eigenvalue	5.273	2.309	1.340
% of variance	40.56	17.76	10.31

Figure 2. Biplot of the principal component analysis based on eight morphological traits in studied traditional and hybrid chestnut varieties.

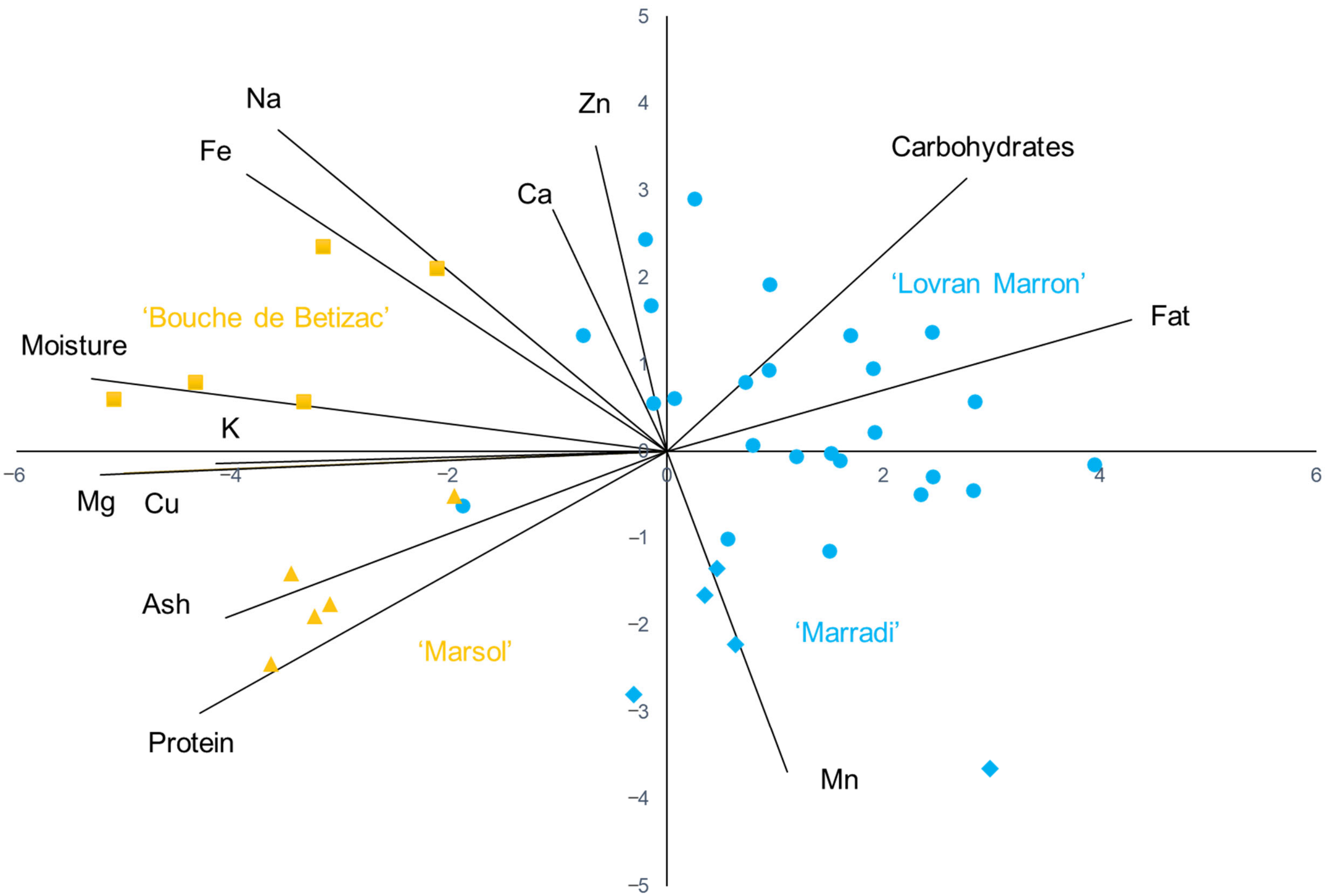

Figure 3. Biplot of the principal component analysis based on 13 chemical traits in studied traditional and hybrid chestnut varieties.

The overall results of the discriminant analysis suggest that the differentiation between the studied chestnut varieties is significant: Wilks' λ = 0.00073; F (24.84) = 39.082; p < 0.0001. Three out of eight morphological traits were determined by stepwise discriminant analysis to be the best differentiating factors between the studied chestnut varieties (Table 6). The results indicated that the hilum length to nut width ratio and chestnut mass were the most important factors contributing to the differentiation of chestnut varieties, followed by the nut height to width ratio. The overall classification success was 100%. In other words, within both studied groups of varieties, all individuals were included in the correct group.

Table 6. Results of the stepwise discriminant analyses. $p(\lambda)$, significance of Wilks' λ: *** significant at $p < 0.001$, ** significant at $0.001 < p < 0.01$, * significant at $0.01 < p < 0.05$, ns non-significant values ($p > 0.05$).

Trait	Wiks' Lambda	Partial Lambda	F-Remove	*p*-Value
Hilum length/Nut width	0.0026189	0.2781729	25.08390	***
Nut mass	0.0026156	0.2785233	25.04018	***
Nut height/width	0.0012205	0.5968889	6.528419	**
Nut thickness/height	0.0009738	0.7480803	3.255298	*
Nut thickness/width	0.0009544	0.7632529	2.998424	*
Hilum width/length	0.0009509	0.7661024	2.951315	*
LI	0.0007851	0.9278293	0.751916	ns
LI/Nut thickness	0.0007623	0.9556403	0.448716	ns

LI—Length of the longest intrusion of the seed coat into the kernel.

The results of the descriptive statistics and analysis of variance were confirmed by multivariate statistical methods, suggesting the existence of a clear divergence between the studied traditional sweet chestnut and hybrid varieties. In general, results of the PC analysis revealed that chestnuts of hybrid varieties can easily be distinguished from the traditional varieties by larger nuts with higher moisture, crude protein, potassium, magnesium, sodium, iron and copper content, and lower total carbohydrate and crude fat content. In both PC analyses, individuals of all four variates were found to group with others of the same variety. Similar results were also obtained by other authors. Thus, for instance, Pereira-Lorenzo et al. [8] successfully distinguish Spanish sweet chestnut cultivars using proximate constituents and macro- and micro-nutrients. In addition, Peña-Méndez et al. [9] stated that the combination of physicochemical characterization and the multivariate analysis allows for a differentiation of chestnuts according to the variety and the area of production. According to Peña-Méndez et al. [9], the area of production has a higher influence on the physicochemical variables than the variety, particularly on the mineral composition. Similar results were presented in the paper published by Dinis et al. [11]. The mentioned authors [11] revealed that the morphological and chemical heterogeneity within the same variety can be affected by the area of production. Since the samples in our study were collected under the same climatic and soil conditions, the differences in the fruit morphology and kernel chemical composition can be interpreted as a result of genetic differences between the studied varieties.

4. Conclusions

The traditional sweet chestnut and hybrid varieties included in this research can easily be distinguished morphologically and by their chemical composition. Overall results suggest that due to the aforementioned differences, these two groups of chestnut varieties have different practical applications. The analysis of Euro-Japanese hybrids showed that they have some disadvantages although they are characterized by early production and very large nuts. They have a very high moisture content, which makes storage and conservation challenging. In general, chestnuts from hybrid varieties are not very sweet and some of them produce polyembryonic nuts. In view of all this, fruits of those varieties are not suitable for the production of candied marrons. In contrast, chestnuts of traditional marron varieties are sweeter and, owing to the lower moisture content, can be stored for a longer period. Large nuts, small hilar scar, presence of raised stripes, easy pellicle removal, absence of double or divided kernels and a sweet and tasty flavor are all valuable traits of the traditional sweet chestnut varieties. These characteristics make traditional varieties desirable for both the fresh market and industrial processing, e.g., to make candied marrons or marrons glacés. Although these traditional varieties are better adapted to the local environmental conditions, they are quite sensitive to diseases such as the chestnut blight and ink disease. Unfortunately, this is one of the reasons why the growing of traditional varieties is decreasing and why they are gradually being replaced by newer, hybrid varieties. Nevertheless, for the sake of genetic heritage preservation, we should promote and conserve local and traditional sweet chestnut varieties which have specific nut characteristics preferred by consumers.

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