Sweet chestnut shells (*Castanea sativa* Mill.) as a valuable resource: antioxidant activities, polyphenol content, and mineral composition

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Summary
Sweet chestnut (*Castanea sativa* Mill.) is a key tree species in Europe, with its shells serving as a nutrient-rich byproduct. The aim of this research is to analyse the shells of sweet chestnuts from various locations in Slovakia to find out how important they are in terms of reuse. Using spectrophotometric methods, the total content of polyphenols, antioxidant activity, and mineral substances were determined. Total content of polyphenols (expressed as gallic acid equivalents) ranged from 6.17 g·kg^{-1} dry weight (DW) in Modrý Kameň location to 27.94 g·kg^{-1} DW in Rovňany location. Antioxidant activity was determined using three assays based on free radical scavenging capacity, the ability to neutralise synthetic radicals, and reduction of ferric ions. The results (expressed as Trolox equivalents) ranged from 3.44 mmol·kg^{-1} to 4.58 mmol·kg^{-1} DW, 0.083 mmol·kg^{-1} to 0.237 mmol·kg^{-1} DW, and 70.43 mmol·kg^{-1} to 544.50 mmol·kg^{-1} DW, respectively. The highest antioxidant activity and total polyphenol content were found in shells from the location Rovňany. The order of content of the main elements, from highest to lowest, was calcium > potassium > magnesium > phosphorus. Consequently, the results indicate a significant potential for repurposing the waste from the production of sweet chestnuts.

Keywords
sweet chestnut shells; polyphenols; antioxidant activity; mineral substances

In several European countries, sweet chestnut (*Castanea sativa* Mill.) trees are very valuable. The nutritionally rich nuts of this tree are especially valued. There are three main regions in which chestnuts are found geographically: Europe (*Castanea sativa* Mill.), Asia (Japanese chestnut – *Castanea creanata*, Chinese chestnut – *Castanea mollissima*), and North America (*Castanea dentata* Borkh). While *Castanea sativa* Mill. is the most widely grown species in Europe, some hybrids resulting from genetic crossing (rootstock) with other species of chestnut have recently been developed for improved nut production and increased resistance to chestnut tree cancer and ink disease. The largest producers in Europe include Italy, Portugal, Spain, and France. Slovakia's orchards produced 8 hectares of sweet chestnuts in 2020 [1, 2].

Sweet chestnuts contain many different nutrients (starch, sugars, proteins, lipids/fats), vitamins, and minerals that positively affect human health. They consist mainly of carbohydrates, especially starch. The main properties of starch are swelling ability, thermal stability, and retrogradation. These properties are relevant in food science, cooking, and industrial applications, such as baking, thickening agents, and biodegradable materials. The maximum values of chestnut starch content range from 162.8 g·kg^{-1} to 190.4 g·kg^{-1}, which is higher than that of potato starch. Saccharose can make up to one-third of the total sugars. Mono- and disaccharides (glucose, fructose, and maltose) are also represented, which are very important for evaluating the commercial quality of chestnuts [3–5]. Another important component is lipids. Consumers show an increased interest in the composition of food, especially regarding the content of fatty acids. Essential fatty acids play a crucial role in various physiological processes that support overall health and normal bodily functions [6]. Free amino acids, including the essential ones (Leu, Lys, Val, Phe, and Thr), can be found in chestnuts [2]. They are also a good source of minerals (Ca, P, K, Mg, and Fe), vita-

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mins C and E, phenolic compounds, and alkaloids. Their preventive impact against oxidative stress, which is widespread in degenerative illnesses, may be explained by the natural dietary antioxidants found in them [7–9].

Not only sweet chestnut nuts have high potential, but also leaves, flowers, wood, bark, and shells. Chestnut flower honey is highly valued. It contains significant amounts of phenols, flavonoids, and quinoline alkaloids and has positive antibacterial properties for chronic wounds, burns, or skin ulcers. The primary sources of tannins used in leather processing and as cleaning substances come from the bark and wood of chestnuts [1, 10, 11].

About 30 % of residues with valuable bioactive substances and exceptional health-promoting qualities are produced by agro-industries. Chestnut shells, which are produced as a byproduct during processing, are an important source of many nutrients (tannins, phenolic acids, polyphenols), flavonoids (quercetin, catechin, epicatechin, apigenin, and rutin), and vitamin E. They have a significant content of proteins (28–31 $g \cdot kg^{-1}$ DW), a small content of fat (2–5 $g \cdot kg^{-1}$ DW), and high levels of carbohydrates (565–741 $g \cdot kg^{-1}$ DW). Significant amounts of dietary fibre, primarily cellulose, hemicellulose, and lignin, have been connected to the high carbohydrate content. The most commonly occurring monosaccharides have been determined to be glucose and xylose, while galactose, arabinose, and mannose were also detected. Chestnut shells account for approximately 20 % of the weight of nuts, with 9.0–13.5 % being outer shells and 6.3–5.1 % being inner shells. They are also widely used in many industries. The best-known uses are fuel and animal feed. In addition, they can be added to food (a natural source of antioxidants and prebiotics), allowing for greener ways of food production, such as recycling and reducing plant waste [1, 12–17]. Vázquez et al. [18] described the use of shells as bio-adsorbents of heavy metals, substitutes for wood adhesive formulations, and chromium alternatives for leather tanning. Morana et al. [19] and Vella et al. [20] report that shells contain approximately 36 % sugars, which can be used as raw materials to produce biofuels. This kind of waste may be used as a natural antioxidant source, depending on the amount of polyphenols. Their reported antioxidant activity has been related to the positive health effects of their polyphenolics, including cardioprotective, anticarcinogenic, and antimutagenic properties.

Polyphenolic compounds, which are widely recognised as essential components of human diets and bioactive substances, are among the many beneficial secondary metabolites that are produced by plants. Plants produce these chemicals as a defence mechanism to help them adapt and survive in harsh environmental conditions. Because of the numerous health advantages of polyphenolic compounds, there has been an increase in interest in and attention to them. Polyphenols have been shown to have a range of biological impacts, such as preventing heart disease, cancer, and neurological disorders. Furthermore, if added to food, polyphenols can impart organoleptic qualities like colour, bitterness, and astringency while also protecting against oxidative processes [8, 15]. As a result, current research is focusing on getting antioxidants from plants and other natural sources to replace the toxic synthetic antioxidants that cause a number of health issues [8, 18, 21]. The chemical composition of chestnut shells has been determined through earlier research. However, the genetic diversity of the cultivars, the location, and the method of treatment can all affect the composition [15]. As an environmentally friendly way of getting rid of this agricultural waste, composting has already been used, and chestnut shells are currently managed as a renewable resource [22]. Because they are widely available and reasonably priced, chestnut shells present an intriguing alternative that ought to be promoted. Reusing these underutilised industrial wastes is supported by scientific knowledge about their chemical composition, bioactivity, and safety [15, 16, 23]. Pinto et al. [15] summarised the possible use of this agro waste based on previous research in pharmaceuticals and food: antioxidant, antimicrobial, anti-inflammatory, anti-rancidity agent, prebiotic, enhancers of antibiotic action, adhesives, and food enrichment and stabiliser.

Based on antioxidant activity determined by methods of ferric reducing antioxidant power (*FRAP*), radical scavenging activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the total polyphenols content (*TPC*), and mineral substances (K, P, Mg, and Ca), the aim of this study is to evaluate the potential for the reuse of sweet chestnut shells from Slovak growing locations and so increase awareness and interest in *Castanea sativa* Mill.

MATERIALS AND METHODS

Sample collection and processing

Sweet chestnut samples were collected from ten sampling locations in Slovakia, including

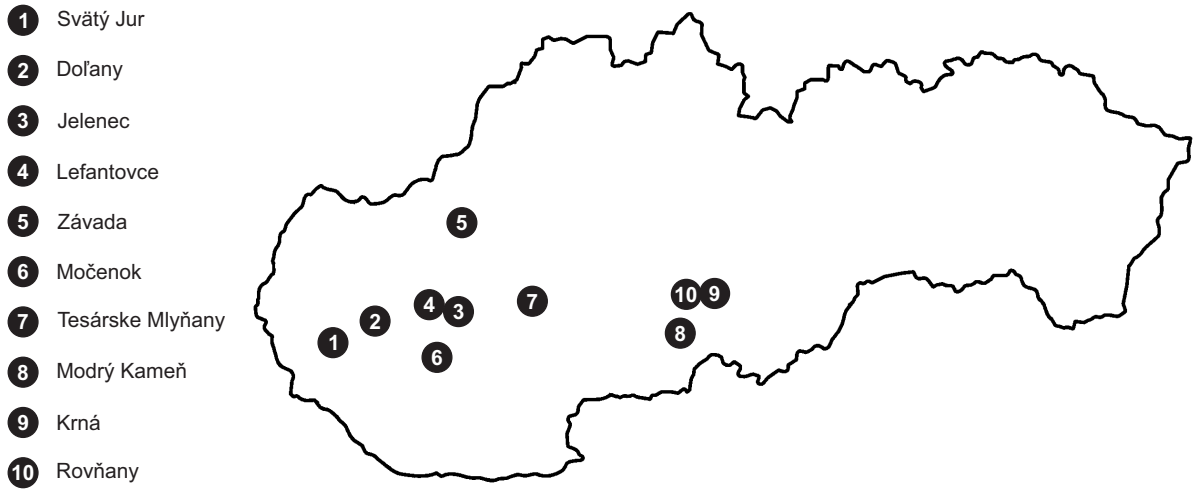

Fig. 1. Sampling locations in Slovakia.

Svätý Jur, Doľany, Jelenec, Lefantovce, Závada, Močenok, Tesárske Mlyňany, Modrý Kameň, Krná, and Rovňany (Fig. 1). Samples of chestnuts were collected in September and October 2023, when the autumn harvest began to take place. At each sampling location, nuts were gathered from two to three trees, depending on their availability and distribution. From each tree, an average of 50–70 nuts were collected, ensuring a representative sample. The total weight of the sample per location was approximately 1.5 kg. After collection, the samples were stored in a dry place to prevent microscopic deterioration and were analysed within 7 days. The samples were prepared, peeled, separated into seeds and shells (a mixture of the outer brown peel and the inner pellicle), homogenised using Grindomix GM 200 (Retsch, Haan, Germany) at 33.3 Hz for 30 s and extracted. The extraction was performed by mixing with 80% methanol (ratio 1 : 2) and shaking horizontally for 12 h using Promax 1020 (Heidolph, Schwabach, Germany) without heating. Afterwards, the suspensions were filtered using Munktell paper no. 392 (Munktell & Filtrac, Bärenstein, Germany) and the resulting liquid extracts were stored in vials at 4 °C for a maximum of 1 month until analyses. For the determination of mineral substances, part of the homogenised shells was dried at 40 °C using Memmert UN110 (Memmert, Büchenbach, Germany). After drying, the samples were finely homogenised and stored in resealable polyethylene bags for a maximum of 1 month. A moisture analyser KERN DLB 160–3A (KERN & SOHN, Balingen, Germany) was used to determine the dry matter content of the samples.

Total polyphenol content

TPC of the samples was determined using a UV-VIS spectrophotometer T92+ (PG Instruments, Leicester, United Kingdom) and Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), following the standard colorimetric method. In a volumetric flask, 0.1 ml of the sample extract was mixed with 5 ml of sodium carbonate (20%), 0.85 ml Folin-Ciocalteu's reagent, and adjusted with distilled water to the final volume of 50 ml. Gallic acid (Sigma Aldrich, St. Louis, Missouri, USA) was used as a standard for calculating *TPC*. After 2 h of stirring, a coloured complex (blue-coloured solutions) formed. The produced solutions' absorbance was measured at a wavelength of 765 nm. A total of four measurement runs were completed for each sample. Following conversion, the results were reported as grams of gallic acid equivalents (GAE) per kilogram DW.

Radical scavenging activity

The antioxidant activity was calculated using Trolox (Sigma Aldrich) as a standard. By dissolving 0.025 g of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich) in 100 ml of 99.8% methanol, a stock solution was formed that was stored at 4 °C. The DPPH stock solution and methanol (1 : 10) were combined to create the DPPH working solution prior to analysis. The 3.5 ml volume of DPPH working solution was pipetted into the empty vessel, and the absorbance value (A_0) was determined using a UV-VIS spectrophotometer T92+ at a wavelength of 515.6 nm. The reaction mixture was kept out of direct sunlight. Following the A_0 measurement, 0.1 ml of the sample extract was added and mixed well three times. When an anti-

oxidant chemical contained in the sample interacts with the DPPH radical, the DPPH is reduced and undergoes a colour shift. The absorbance was measured 10 min later (A_{10}). The measurement process was repeated four times for every sample. The Eq. 1 was used to obtain the percentage value of DPPH inhibition (I):

$$I = \frac{A_{10} - A_0}{A_0} \times 100 \quad (1)$$

where A_0 control is the absorbance of the blank at time 0 (DPPH solution), and A_{10} is the absorbance of the sample after 10 min.

Based on the calibration line (Trolox concentration vs DPPH inhibition percentage) and dry matter content, the results were recalculated as millimoles of Trolox equivalent (TE) per kilogram DW (marked as AA_{DPPH}).

The radical cation of 2,2‘-azinobis(3-ethylbenzthiazoline)-6-sulfone (ABTS•+) was prepared by the reaction of 2 ml ABTS stock solution (7.5 mmol·l^{-1}) (Sigma Aldrich) with 1 ml potassium persulfate stock solution (2.45 mmol·l^{-1}). After 5 min, 125 ml of 0.1 mol.l^{-1} sodium acetate buffer pH 4.3 was added. 3 ml of the prepared ABTS•+ solution was pipetted into the empty vessel, and then 50 μl of the methanol extract of the sample was added. The solutions were mixed, and after 20 min, the absorbance was measured on a UV-VIS spectrophotometer T92+ at a wavelength of 734 nm. Standard solutions (Trolox) were also prepared and measured using the same procedure. Results were recalculated based on a standard curve and dry matter content. Radical scavenging activity was expressed as millimoles TE per kilogram DW (marked as AA_{ABTS}).

Ferric reducing antioxidant power

2,4,6-tri(2-pyridyl)-s-triazine (TPTZ, Sigma Aldrich, 5 mmol·l^{-1} in 40 mmol·l^{-1} hydrochloric acid), ferric chloride solution (10 mmol·l^{-1}, Sigma Aldrich), and sodium acetate buffer (0.1 mol·l^{-1}, pH 3.6, Sigma Aldrich) were mixed in a ratio of 1 : 1 : 10, resulting in a master solution of the *FRAP* reagent. A mixture of 100 μl of the sample and 3.5 ml of the *FRAP* master solution was used. Trolox served as the standard. After 30 min of incubation at 37 °C, the absorbance was measured at a wavelength of 593 nm with a UV-VIS spectrophotometer T92+. The measurements were performed four times on each sample. The results were expressed in millimoles TE per kilogram DW.

Mineral substances

Mineral substances (K, P, Mg, and Ca) were measured in chestnuts as follows. 1 g of the previously homogenised sample, 10 ml nitric acid (65% w/w) and 5 ml perchloric acid (70% w/w) were mixed and processed using a closed microwave digestion system Mars X-Press 5 (CEM, Matthews, North Carolina, USA). (55 min., 800 W). To determine K, Mg and Ca content, 2 ml of the sample extract was diluted by adding redistilled water.

To evaluate phosphorus, the sample was diluted as follows. The reagent solution A was prepared by mixing 148 ml H_2SO_4 solution (98% w/w) with 15 g $(NH_4)_2MoO_4$ and 0.3 g $C_4H_4KO_7Sb$ (Sigma Aldrich). The reagent solution B was prepared by mixing 500 ml solution A with 2.64 g $C_6H_8O_6$ (Sigma Aldrich). 8 ml of solution B was mixed with 1 ml sample extract. The UV-VIS spectrophotometer T92+ was used to measure the concentration of phosphorus (666 nm).

The concentrations of K, Mg, and Ca were measured with an atomic absorption spectrometer VARIAN AA 240FS VIC (Varian Techtron, Mulgrave, Australia).

The contents of all measured elements are expressed in milligrams per kilogram DW.

Statistical analysis

XLSTAT software (Addinsoft, Paris, France) was used to study the relationships between different chestnut samples from ten sampling locations and the results from the analysis methods. The results are presented as a mean (n = 4) ± standard deviation. Kruskal-Wallis (non-parametric ANOVA) and multiple pairwise comparisons (Dunn‘s test) were used to determine statistical differences ($p < 0.05$) between the tested variables. The relationship between *TPC*, *FRAP*, radical scavenging activities and analysis of mineral substances was determined by Spearman‘s correlation coefficient.

RESULTS AND DISCUSSION

Total polyphenol content

The obtained results of *TPC* (expressed as GAE) in the samples of chestnut shells are shown in Tab. 1. The lowest content of *TPC*, 6.17 ± 0.07 g·kg^{-1}, was determined in the sample from the sampling location Modrý Kameň. The highest *TPC* content (27.94 ± 0.23 g·kg^{-1} DW) was determined in the sample from the sampling location Rovňany. Differences ($p \leq 0.05$) in *TPC* were observed between samples from different locations.

When using 80% methanol, i.e. the same solvent as in our study, Vázquez et al. [23]

Tab. 1. Total phenolic content and antioxidant activity of sweet chestnut shells.

Sampling location	*TPC* [g·kg^{-1}]	Antioxidant activity [mmol·kg^{-1}]		
		AA_{DPPH}	AA_{ABTS}	*FRAP*
Svätý Jur	17.64 ± 0.19 ^{abcd}	3.69 ± 0.01 ^{ab}	0.179 ± 0.003 ^{abc}	170.08 ± 0.96 ^{abcd}
Doľany	7.89 ± 0.15 ^{ab}	3.54 ± 0.02 ^{ab}	0.177 ± 0.002 ^{abc}	70.43 ± 0.31 ^{a}
Jelenec	26.81 ± 0.08 ^{cd}	3.96 ± 0.03 ^{abc}	0.088 ± 0.001 ^{ab}	85.10 ± 0.43 ^{ab}
Lefantovce	15.18 ± 0.30 ^{bcd}	3.79 ± 0.03 ^{abc}	0.083 ± 0.005 ^{a}	221.50 ± 0.72 ^{abcd}
Závada	26.21 ± 0.55 ^{abcd}	4.08 ± 0.01 ^{abc}	0.086 ± 0.002 ^{a}	223.25 ± 0.72 ^{abcd}
Močenok	9.47 ± 0.45 ^{abc}	3.73 ± 0.02 ^{abc}	0.189 ± 0.001 ^{abc}	106.67 ± 0.98 ^{abc}
Tesárske Mlyňany	17.74 ± 0.16 ^{abcd}	3.44 ± 0.05 ^{a}	0.161 ± 0.001 ^{abc}	194.85 ± 0.40 ^{abcd}
Modrý Kameň	6.17 ± 0.07 ^{a}	4.10 ± 0.01 ^{bc}	0.093 ± 0.001 ^{abc}	397.89 ± 0.60 ^{cd}
Krná	19.72 ± 0.48 ^{abcd}	4.06 ± 0.07 ^{bc}	0.200 ± 0.003 ^{bc}	377.09 ± 0.28 ^{bcd}
Rovňany	27.94 ± 0.23 ^{d}	4.58 ± 0.02 ^{c}	0.237 ± 0.002 ^{c}	544.50 ± 0.72 ^{d}

Values are expressed per kilogram of dry weight and represent mean ± standard deviation (n = 4). Antioxidant activity is expressed as millimoles of Trolox equivalents.
Different lowercase letters in superscript represent statistically significant differences between sampling locations for each analysis considered separately ($p \leq 0.05$).
TPC – total phenolic content (expressed as gram of gallic acid equivalents), AA_{DPPH} – antioxidant activity based on DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, AA_{ABTS} – antioxidant activity based on ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) assay, *FRAP* – method of ferric reducing antioxidant power.

obtained a *TPC* value of 36.0 g·kg^{-1} GAE per weight of oven-dried shells, while the optimal extraction with 2.5% Na_2SO_3 aqueous solution led to a higher value, 134 g.kg^{-1}. Other authors reported a *TPC* of 25.4 g·kg^{-1} in the outer skins and 102.6 g·kg^{-1} in the inner skins, based on the extraction with boiling water [12]. Fernández-Agulló et al. [24] measured a *TPC* of 21.52 g·kg^{-1} under extraction conditions with 50% methanol at 25 °C. Consequently, our determined *TPC* corresponds with the results by the authors mentioned.

Mustafa et al. [8] and Aires et al. [25] reported that sweet chestnut shells have a high content of gallic and ellagic acids compared to other natural sources. From natural sources like plants, both polyphenols have been recognised as potent antioxidants, anti-inflammatory, anti-cancer, and antibacterial agents [8, 26]. The process of producing gallic and ellagic acids for commercial use is costly and difficult. The levels of phenolic acids in chestnut shells are several times higher than the levels in nuts [8]. Our results, like those of the mentioned authors, showed that chestnut shells could be an interesting, innovative, and less expensive alternative.

Antioxidant activity

Three methods were used to assess the antioxidant activity, such as scavenging of synthetic free radicals (DPPH• and ABTS•$^+$) and reduction of ion complexes (iron-reducing antioxidant power, *FRAP*). The obtained results of antioxidant activity in samples of chestnut shells are presented in Tab. 1. The antioxidant activity (expressed as TE per kilogram DW) ranged from 3.44 mmol·kg^{-1} to 4.58 mmol·kg^{-1} (DPPH method), 0.083 mmol·kg^{-1} to 0.237 mmol·kg^{-1} (ABTS method), and 70.43 mmol·kg^{-1} to 544.50 mmol·kg^{-1} (*FRAP*). The highest antioxidant activity by all methods was determined in the sample from the Rovňany location (AA_{DPPH} 4.58 mmol·kg^{-1}; AA_{ABTS} 0.237 mmol·kg^{-1}; *FRAP* 544.50 mmol·kg^{-1}). Statistically significant differences ($p < 0.05$) were found between samples from many locations. In summary, there was a difference in the antioxidant activity of chestnut shells based on the method of extraction used. Furthermore, it appears that the antioxidant activity is influenced by geographic origins. Dinis et al. [27] pointed out the relationship between chestnut antioxidant activity and climatic conditions. Higher antioxidant activity values have been observed in chestnuts harvested in colder climates than in warmer environments. According to our results, the location Rovňany within the studied growing area of Slovakia is the most suitable for choosing chestnuts or chestnut shells as a source of antioxidants.

Barreira et al. [12] found that outer shells with the highest levels of polyphenols and flavonoids had a higher antioxidant activity based on erythrocyte haemolysis, inhibiting lipid peroxidation, and β-carotene bleaching inhibition. Additionally, compared to the flowers, nuts, and leaves of *Castanea sativa*, the shells showed higher anti-

oxidant activity. Because flavonoids and phenolic acids have been shown to be effective through two or more distinct oxidation processes, they really have similar antioxidant effects. VÁZQUEZ et al. [23] reported the antioxidant activity of *FRAP* 224 mmol·kg^{-1} expressed as ascorbic acid equivalent per DW chestnut shells, when 80% methanol, the same solvent as ours, was used in the extraction procedure. MUSTAFA et al. [8] reported that in raw chestnut, the inner shell showed the highest AA_{DPPH} (5.25 mmol·kg^{-1}), followed by the outer shell (5.04 mmol·kg^{-1}), expressed as TE per kilogram DW). YOUN et al. [28] state that the methanol extract of chestnut shells scavenged 34.2 % of DPPH and 78.8 % of ABTS radicals.

After additional analysis of our data, Spearman's correlation coefficient showed a relationships between the studied parameters. *TPC* (0.31) and *FRAP* (0.72) had the highest correlation with AA_{DPPH} (Tab. 2), reaching the levels of statistical significance.

Tab. 2. Spearman's correlation matrix.

	AA_{DPPH}	AA_{ABTS}	*FRAP*	*TPC*
AA_{DPPH}	–	0.11	0.72*	0.31*
AA_{ABTS}	0.11	–	0.19	0.10
FRAP	0.72*	0.19	–	0.26
TPC	0.31*	0.10	0.26	–

* – values are different from 0 with a significance level ($p \leq 0.05$).
TPC – total phenolic content, AA_{DPPH} – antioxidant activity based on DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, AA_{ABTS} – antioxidant activity based on ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) assay, *FRAP* – method of ferric reducing antioxidant power.

Tab. 3. Mineral content of sweet chestnut shells.

Sampling location	K	Ca	Mg	P
	[mg·kg^{-1}]			
Svätý Jur	1 869	1 867	973	529
Doľany	1 326	3 277	1 217	601
Jelenec	1 463	2 020	837	486
Lefantovce	1 531	3 577	939	558
Závada	1 795	1 886	831	815
Močenok	893	3 876	1 747	458
Tesárske Mlyňany	6 383	516	889	363
Modrý Kameň	1 176	3 143	763	343
Krná	1 379	1 799	765	601
Rovňany	1 204	2 173	1 011	501

Values are expressed per kilogram of dry weight.

Mineral composition

Research on the macro- and micronutrients found in chestnut shells is not abundant. It is known that they contain significant levels of K, Na, Ca, and Mg. Low content of minor and trace elements, such as As, Br, Cr, Ni, Pb, and Ti, have also been found [29].

The content of four mineral substances determined in chestnut shells from the ten studied locations (Tab. 3) was 893–6 383 mg·kg^{-1} (K), 516–3 876 mg·kg^{-1} (Ca), 763–1 747 mg·kg^{-1} (Mg), 343–815 mg·kg^{-1} (P). The sample from the Močenok location has the lowest K content, while the sample from the Modrý Kameň location has the lowest levels of Mg and P and Tesárske Mlyňany samples contain the lowest content of Ca. One of the elements that dominates chestnut shells is calcium. Its content was the largest in the sample from the Močenok location. For chestnut shells, the order of the average content of the main elements, from higher to lower, is Ca (2 413 ± 977 mg·kg^{-1}) > K (1456 ± 285 mg·kg^{-1}, excluding Tesárske Mlyňany sample as an outlier) > Mg (997 ± 281 mg·kg^{-1}) > P (526 ± 128 mg·kg^{-1}).

Similar mineral contents were determined in chestnut shells from Portugal as in our samples, (112–1 130 mg·kg^{-1} (P), 810–1 440 mg·kg^{-1} (K), 1 950–3 070 mg·kg^{-1} (Ca), 70–520 mg·kg^{-1} (Mg). The mineral substances in chestnut shells have many health benefits. Potassium is necessary in many physiological processes; it controls muscle contraction and the transmission of nerve impulses. Phosphorus is also involved in several mechanisms, such as the mineralisation of bones and teeth; it is part of cell structure. Calcium is necessary for the growth and formation of human bones and teeth, among other functions [30]. The content of mineral substances determined in sweet chestnuts from Slovakia was 3 770–7 890 mg·kg^{-1} (K), 269–1 030 mg·kg^{-1} (Ca), 587–1 010 mg·kg^{-1} (Mg), and 965–1 790 mg·kg^{-1} (P) [2].

The various levels of elements in shells compared to nuts could be explained by the distinct physiological processes involved in nuts growth, as well as their different roles in the plant, where the shells ought to protect the growing nuts. The content of elements depends on their variety or cultivar, locality of growth, climatic conditions, and fertilisers [30].

CONCLUSION

The results of antioxidant activity (*FRAP*, AA_{DPPH}, AA_{ABTS}), *TPC*, and mineral elements show a great potential for reusing the waste

of chestnut shell production. The highest antioxidant activity and *TPC* were found in chestnut shells from the location Rovňany (*TPC* 27.94 g·kg^{-1} DW, expressed as GAE; *FRAP* 544.5 mmol·kg^{-1}, AA_{DPPH} 4.58 mmol·kg^{-1}, AA_{ABTS} 0.237 mmol·kg^{-1}, expressed as TE per kilogram DW). The order of the average content of the main elements, from highest to lowest, was calcium > potassium > magnesium > phosphorus. These shells can be used to make food and medications that have pronounced positive effects on human health after being properly processed. Particularly, the food, cosmetic, pharmaceutical, and other industries might encounter new challenges in creating high-value products from these unused shells. In terms of sustainability, extracts from chestnut byproducts may soon offer chestnut processing companies a means of recycling, the development of cost-effective processing techniques, a reduction in the harmful effects of waste on the environment, and other financial benefits. To determine the possible industrial application of these byproducts, more research needs to be done, constantly keeping in mind the cost and toxicity of the extracts.

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