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IMPACT OF HEAT TREATMENTS ON THE ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF SWEET CHESTNUTS (*Castanea sativa* MILL.)

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Abstract

Sweet chestnuts are highly regarded and widely consumed throughout Europe because of their nutritional composition and health benefits, which have become important in the human diet, for example, in gluten-free diets. This study aims to assess the impact of heat treatment using the UV-VIS spectrophotometry methods. TPC values (mg GAE/g DW - dried weight) ranged from 1.935 to 6.165 (raw flesh), 1.676 to 4.342 (boiled samples), 1.580 to 3.091 (roasted samples), 1.193 to 8.272 (microwaved samples), and 2.556 to 5.655 (steamed samples). The DPPH values (µmol TE/g DW) for raw flesh ranged from 4.232 to 5.094, for boiled samples from 3.396 to 5.147, for roasted samples from 3.185 to 4.726, for microwaved samples from 2.798 to 5.816, and for steamed samples from 4.441 to 5.171. The FRAP antioxidant activity (µmol TE/g DW) values ranged from 10.971 to 207.11 (raw flesh), 4.058 to 134.651 (boiled samples), 11.954 to 132.476 (roasted samples), 7.795 to 179.129 (microwaved samples), and 17.468 to 367.957 (steamed samples). Among the methods used, steaming and microwaving had the greatest impact on total polyphenol content and antioxidant activity (DPPH, FRAP).

Key words*: DPPH, FRAP, heat treatments, polyphenols, sweet chestnuts*

INTRODUCTION

The European chestnut (*Castanea sativa* Mill.) is part of the Fagaceae family. For many decades, chestnut nuts have been one of the major food sources in farm-based regions, and the tree is regarded as crucial in the agricultural and forestry economies. Nowadays, a significant amount of nuts is consumed fresh or processed to produce goods like flour, chestnut purée, and other items that are part of gluten-free diets (Squillaci et al., 2018).

Sweet chestnuts are rich in vitamins, minerals, proteins, lipids, free sugars, and starch - all of which are beneficial for the consumer's health. Various mono- and disaccharides, such as glucose, fructose, sucrose, and maltose, are components of carbohydrates and play a key role in determining the commercial quality of chestnut nuts. Starch made of polysaccharides is also heavily present in the entire mixture. Up to one-third of the entire amount of carbs can be attributed to the free sugar sucrose. The estimated average of total carbohydrates in 100 g of fresh chestnut fruit is 44.7 g (Ramadan, 2019; De Vasconcelos et al., 2010a; Pino-Hernández et al., 2021). Crude fat, which is high in unsaturated fatty acids and low in saturated fatty acids, is found in very small amounts in sweet chestnuts. Depending on the harvesting year and chestnut cultivar, the protein content ranges from 2 to 3%. The protein fraction from chestnuts includes 17 amino acids, but in relatively small amounts (Barreira et al., 2009; Barreira et al., 2012; Murthy et al., 2020).

Humans cannot synthesise vitamin C, also known as ascorbic acid, which is a necessary substance for good health. Vitamin C is an antioxidant in the human body's biochemistry and molecular composition. There is a correlation between vitamin E and a lower risk of cardiovascular disease and cancer. One hundred grams of chestnuts have 1.9 mg of vitamin E, or 12.7% of the recommended daily intake (RDI) for both genders. Similarly, one hundred grams of fresh chestnut fruits contain 15.6 mg of vitamin C, or 20.8% of the RDI for women and 17.3% for men (De Vasconcelos et al., 2010b).

Many factors affect the change in chemical composition and representation of individual bioactive substances in sweet chestnuts.

Significant differences are observed between species/cultivars and between chestnuts from different geographical areas. Furthermore, their quality and nutritional composition are affected by seasonal variability and climatic factors such as temperature, sunlight, amount of precipitation; environmental conditions, altitude of cultivation, methods of agriculture (soil, nutrients, minerals, cultivation, pests, diseases, and periods for storage) (Martinéz et al., 2022).
The redox effect caused by phenolic substances is responsible for chestnuts antioxidant ability. Important antioxidants and phenolic acids protect the human body from the damaging effects of free radicals (Šnirc et al., 2023). Chestnut's mineral and total polyphenolic content are best preserved by boiling. Cooked chestnuts contain a significant amount of polyphenols, hydrolysable and condensed tannins, gallic and ellagic acids, and organic acids and phenolics. Boiling and baking are the most popular heat-treatment methods. Boiling methods impact chestnuts' sensory and nutrient content, boosting their organoleptic properties, bioavailable nutrients, and shelf life (Braga et al., 2014).
This study aims to determine the ways heat treatments affect the polyphenol content and antioxidant activity (DPPH, FRAP) of sweet chestnuts from important Slovakian growing crop regions.

MATERIALS AND METHODS

Sample collection and processing

In this study, analysed samples of sweet chestnuts were collected at five sampling points in Slovakia: Rovňany, Močenok, Modrý Kameň, Jelenec, and Svätý Jur. Chestnut samples were gathered during the autumnal harvest (in September and October). Depending on the distance and amount of nuts, 1-3 trees were gathered at each sampling point. The sample's average weight was 1.5 kg. Samples of sweet chestnuts were kept at −18°C for a month following harvest. The samples were prepared, peeled, divided into seed and shell, and heat treated using four methods: boiling (30 min/100°C), roasting in the oven (20 min/180°C), microwaving (2 min/800 W), and steaming (30 min/100°C). Following heat treatment, the materials were homogenised (Grindomix GM2000 Retsch, 2000 rpm, 30 sec), extracted with 80% methanol (ratio 1:2), and conducted for 12 hours at a horizontal shaker (Heidolph Promax 1020, Heidolph Instruments GmbH, Schwabach, Germany). Following preparation, the samples were filtered using Muktell paper no. 392 (Munktell & Filtrac GmbH, Bärenstein, Germany) and kept in a vial tube at 4°C until they were subjected to TPC, DPPH, and FRAP analyses. Chemicals were acquired from Merck (Germany) and Sigma-Aldrich (Sigma Aldrich ChemieGmbH, Steiheim, Germany). The dry matter content of the samples was determined using a moisture analyzer (KERN DLB 160–3A, KERN & SOHN GmbH, Balingen, Germany).

Determination of Total Polyphenol Content (TPC) by Folin–Ciocalteu Assay

Total polyphenol content (TPC) was measured in the samples using a UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire, United Kingdom) and Folin-Ciocalteu reagent by the standard colorimetric method described by Lachman et al. (2006). 0.1 mL of sample extract was combined with 5 mL of sodium carbonate (20%), distilled water, and Folin-Ciocalteu's reagent in a volumetric flask. The calculation's standard for total polyphenol content was gallic acid. A colored complex (blue-colored solutions) forms after two hours of churning. At a wavelength of 765 nm, the absorbance of the created solutions was measured. Every sample completed a total of four runs of the measurement process. The results after conversion were expressed as mg GAE/g DW - milligram equivalents of gallic acid per gram of dry matter.

Determination of Antioxidant Activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity and the FRAP (ferric reducing ability of plasma) techniques were used to measure the antioxidant activity.
With minor modifications, the DPPH assay was performed using the methods of Brand-Williams et al. (1995) and Martínez et al. (2022). Trolox was used as a standard to calculate the antioxidant activity. A stock solution of 0.025 g of DPPH was prepared and

kept cold at 4°C by dissolving it in 99.8% methanol. Before analysis, the DPPH working solution was made by mixing the DPPH stock solution with methanol (1:10). The absorbance value (A0) of a 2,2-diphenyl-1-picryhydrazyl solution was measured using a UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire, United Kingdom) at a wavelength of 515.6 nm after the solution was pipetted (3.5 mL). The reaction mixture was stored in a dark environment. The DPPH radical is decreased and changes color when it interacts with an antioxidant substance. After measuring A_0, 0.1 mL of the extract was added and mixed three times. After ten minutes, the absorbance (A_{10}) was measured. The measurement of each sample was repeated four times. The percentage value of DPPH inhibition was calculated based on the following equation:

$$DPPH\ inhibbition(\%)=[(A_0-A_{10})/A_0]\times 100,$$

where A_0 control is the absorbance of the blank at time 0 and A_{10} is the absorbance of the sample after 10 minutes. The results were represented as micromole Trolox equivalent (TE) per gram in dry weight (μmol TE/g DW).

The FRAP was assessed according to Paulová et al. (2004). A master solution of the FRAP reagent was made by mixing 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) with ferric chloride hexahydrate ($FeCl_3.6H_2O$). Acetate buffer (pH = 3.6) was prepared by mixing acetic acid and sodium acetate. FRAP master solution was mixed with 100/50 μL of sample. Trolox was used as a standard. The absorbance was measured at a wavelength of 593 nm using a UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire, United Kingdom) during a 30-minute incubation period at 37°C. Every sample underwent four repetitions of the measurement process. Micromole Trolox equivalent (TE) per gram in dry weight (μmol TE/g DW) was used to represent the results.

Statistical Analysis

To study the relationships between different chestnut samples depending on steaming, cooking, microwaving, and roasting, the total polyphenol content and antioxidant activity were evaluated using XLSTAT software. The analyses were carried out four times, with the findings given as mean ± standard deviation (SD). The Kruskal-Wallis (nonparametric ANOVA) and Multiple pairwise comparisons - Dunn's tests were used to determine the statistical differences ($p < 0.05$) between the tested variables. TPC, DPPH, and FRAP have been determined to be correlated using Spearman's correlation coefficient.

RESULTS AND DISCUSSIONS

Chestnuts may have a preventive effect against oxidative stress, which is a typical occurrence in degenerative disorders, because of the antioxidants included in plant foods. Polyphenols are one of the most significant antioxidant classes found in chestnuts. For human neuroblastoma cells, they provide a neuroprotective function that helps to reduce neurological issues (Pandey & Rizvi, 2009; Barros et al., 2011; Brizi et al., 2019). Based on the Folin-Ciocalteu technique, chestnuts have a significant total polyphenol content (TPC). Compared to other varieties of nuts, which have 100 mg of gallic acid equivalents (GAE/100 g), sweet chestnuts, pistachios, and pecans contain more than 1000 mg (Mustafa et al., 2021).

The obtained results of TPC in raw and heat-treated sweet chestnut samples are presented in Table 1. Samples from sampling point Jelenec showed the highest TPC content (2.497–8.272 mg GAE/g DW). Samples from sampling point Močenok showed the lowest values (1.193–2.556 mg GAE/g DW).

Based on these results, climatic conditions were the main factor affecting the differences in polyphenol levels in individual sampling points. TPC increased variably in heat-treated samples. Of the thermal treatments, the values were highest after microwaving (1.193–8.272 mg GAE/g DW) and after steaming (2.556–5.655 mg GAE/g DW). The lowest values (1.580–3.091 mg GAE/g DW) were shown by most of the samples after roasting in the oven. The exception was the sampling points Močenok and Svätý Jur, where the lowest TPC value was determined in the microwaved sample (1.193; 1.371 mg GAE/g DW).

Table 1. Contents of total phenolic content, and antioxidant activity (DPPH, FRAP) of sweet chestnuts

Sampling point	Treatment	TPC (mg GAE/g DW)	DPPH (μmol TE/g DW)	FRAP (μmol TE/g DW)
Rovňany	raw	6.165±0.058^{ab}	4.841±0.013^{bc}	87.662±0.197^{a}
	boiled	3.876±0.543^{ab}	4.712±0.029^{abc}	36.097±0.253^{a}
	roasted	3.091±0.069^{a}	4.076±0.019^{a}	44.311±0.091^{a}
	microwaved	6.311±0.232^{ab}	4.996±0.022^{ab}	97.825±0.026^{a}
	steamed	4.540±0.244^{b}	4.984±0.074^{c}	59.534±0.041^{a}
Močenok	raw	2.342±0.029^{ab}	4.232±0.074^{bc}	18.398±0.113^{a}
	boiled	1.676±0.144^{ab}	3.396±0.259^{abc}	15.955±0.056^{a}
	roasted	1.580±0.077^{a}	3.185±0.054^{a}	16.313±0.160^{a}
	microwaved	1.193±0.126^{ab}	3.017±0.054^{ab}	7.996±0.209^{a}
	steamed	2.556±0.202^{b}	4.441±0.024^{c}	22.107±0.187^{a}
Modrý Kameň	raw	5.904±0.124^{ab}	5.094±0.010^{bc}	207.110±0.127^{a}
	boiled	4.342±0.209^{ab}	5.147±0.012^{abc}	134.651±0.396^{a}
	roasted	1.702±0.094^{a}	4.726±0.026^{a}	132.476±0.181^{a}
	microwaved	6.065±0.837^{ab}	5.816±0.019^{ab}	179.129±0.412^{a}
	steamed	5.655±0.215^{b}	5.171±0.063^{c}	367.957±0.213^{a}
Jelenec	raw	2.497±0.023^{ab}	4.676±0.018^{bc}	20.233±0.633^{a}
	boiled	3.034±0.135^{ab}	4.567±0.172^{abc}	15.148±0.642^{a}
	roasted	2.774±0.098^{a}	4.111±0.018^{a}	22.618±0.141^{a}
	microwaved	8.272±0.265^{ab}	3.484±0.075^{ab}	91.597±0.170^{a}
	steamed	3.800±0.139^{b}	4.989±0.045^{c}	30.348±0.231^{a}
Svätý Jur	raw	1.935±0.086^{ab}	4.270±0.047^{bc}	10.971±0.244^{a}
	boiled	1.682±0.148^{ab}	4.112±0.028^{abc}	4.058±0.166^{a}
	roasted	1.880±0.120^{a}	3.240±0.025^{a}	11.954±0.431^{a}
	microwaved	1.371±0.096^{ab}	2.798±0.037^{ab}	7.795±0.310^{a}
	steamed	2.615±0.143^{b}	4.819±0.020^{c}	17.468±0.326^{a}

Note: mean ± standard deviation (n = 4); a–c statistically significant differences between heat treatments for each analysis (TPC, DPPH, FRAP) considered separately, p-value 0.0167 (TPC); <0.0001 (DPPH); 0.2529 (FRAP)

The obtained results about the highest value of TPC in microwaved samples are compared to Wani et al. (2017). It states that the TPC value changes during heat treatment, the highest being obtained for microwaved chestnuts samples. The degradation of hydrolyzable tannins after heating into smaller phenolic compounds can be used as an explanation for the rise in the TPC of chestnuts. It is well known that heat treatment can affect the chemical composition of certain molecules, such as proteins associated with phenolic chemicals.

High TPC in plant food following heat treatment might result from an increase in their amount (Lemos et al., 2012). However, the TPC values obtained were not higher in all heat treatments, as the authors state (Mustafa et al., 2021; Neri et al., 2010). Therefore, a more detailed analysis of the effect of processing on sweet chestnuts is needed. Statistical differences were observed in TPC between steamed and roasted samples.

There is a clear correlation between the amount of total phenolics in extracts and plant capacity to function as antioxidants. The defence mechanism against hazardous oxidative damage is aided by phenolic chemicals, which help prevent disorders linked to oxidative stress (Braga et al., 2014). Using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) techniques, the

antioxidant activity of sweet chestnuts was determined. The obtained results of antioxidant activity (DPPH, FRAP) in samples of raw and heat-treated chestnuts are presented in Table 1. Samples from sampling point Modrý Kameň showed the highest antioxidant activity of DPPH and FRAP (4.771–5.816; 132.476–367.957 µmol TE/g DW). The lowest values were shown by the samples from sampling point Svätý Jur (2.798–4.819; 4.058–17.468 µmol TE/g DW). Antioxidant activity increased variably in heat-treated samples as well as in TPC. The DPPH values were highest in heat-treated samples after microwaving (2.798–5.816 µmol TE/g DW) and after steaming (4.441–5.171 µmol TE/g DW). The FRAP values of heat-treated samples increased in the order: steamed>microwaved>raw>baked>boiled. In other studies described in the literature, the antioxidant activity of different varieties of chestnuts from different locations was measured by several different methods, and it is difficult to compare the results. Differences in antioxidant activity values are significantly influenced by the use of two individual measurement methods. Most antioxidants are found in nut skins or shells. In addition, the antioxidant activity decreases considerably when the shell is removed from the nuts. After that, less than 10% is present in nuts (Mustafa et al., 2021). Blomhoff et al. (2006) report values of 7.55 µmol per 1 g for Italian chestnuts using the FRAP antioxidant activity method. The antioxidant capacity of different types of sweet chestnuts ranges from 0.564 to 1.046 µmol TE/g, according to Barros et al. (2011). This indicates that the antioxidant capacity is crop-specific. There were statistically significant differences in DPPH between the steamed/raw, roasted, and microwaved samples. Significant statistical differences were not observed in FRAP between monitored samples.

Physiological and reproductive cycles of species are influenced by environmental conditions, which also change fruit quality and yield. Differences in the environmental conditions of the same variety of chestnuts also affect their antioxidant activity. Increased levels of specific metabolites are among the defense mechanisms against the harmful effects of external factors (Yang, et al., 2018).

Spearman's correlation coefficient showed a strong correlation between monitored parameters (TPC, DPPH, FRAP). Phenolic substances are the most important contributors to the capacity to scavenge free radicals and, consequently, antioxidant activity (Franková et al, 2022). Antioxidant activity FRAP showed the strongest correlation with TPC ($r = 0.796$) in this study. Antioxidant activity DPPH showed 0.696 correlation. The connection between antioxidant capacity and TPC in sweet chestnuts is also confirmed by previous studies by the authors (Barreira et al., 2008; Vázquez et al., 2008; Neri et al., 2010; Mustafa et al., 2021).

CONCLUSIONS

These days, people are becoming more and more interested in foods' antioxidant qualities and how they improve consumers' health. More precise information is highly desired, not only regarding the effects of heat treatment on the bioactive elements of chestnuts but also regarding the influence of cultivars in the context of the nutritional and antioxidant qualities of chestnuts. Thermal treatments associated with common consumption of chestnuts – steaming, roasting, microwave oven and cooking – affect antioxidant activity and total content of polyphenols. Steamed and microwaved chestnuts show a positive increase in antioxidant activity and total polyphenol content. However, the results of our study show that the antioxidant activity and the total polyphenol content are most influenced by the geographical and climatic conditions of Slovakia. A more detailed analysis of the given parameters is therefore necessary to clarify the results and impacts.

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REFERENCES

Barreira, J.C.M., Casal S., Ferreira I.C.F.R., Oliveira M.B.P.P. & Pereira J.A. (2009). Nutritional, fatty acid and triacylglycerol profiles of *Castanea sativa* Mill cultivars: a compositional and chemometric approach. *Journal of Agricultural and Food Chemisty*, *57*, 2836–2842.

Barreira, J.C.M., Casal, S., Ferreira, I.C.F.R., Peres, A.M., Pereira, J.A., & Oliveira, M.B.P.P. (2012). Chemical characterization of chestnut cultivars from three consecutive years: chemometrics and contribution for authentication. *Food and Chemical Toxicoly*, *50*, 2311–2317.

Barreira, J.C.M., Ferreira, I.C., Oliveira, M.B.P., & Pereira, J.A. (2008). Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chemistry, 107,* 1106–1113.

Barros, A. I., Nunes, F. M., Gonçalves, B., Bennett, R. N., & Silva, A. P. (2011). Effect of cooking on total vitamin C contents and antioxidant activity of sweet chestnuts (*Castanea sativa* Mill.). *Food Chemistry*, *128*(1), 165-172.

Blomhoff, R., Carlsen, M. H., Andersen, L. F., & Jacobs, D. R. (2006). Health benefits of nuts: potential role of antioxidants. *British Journal of Nutrition, 96*(S2), S52-S60.

Braga, N., Rodrigues, F., & P.P. Oliveira, M. B. (2014). Castanea sativaby-products: a review on added value and sustainable application. *Natural Product Research, 29*(1), 1–18.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology, 28*(1), 25-30.

Brizi, C., Santulli, C., Micucci, M., Budriesi, R., Chiarini, A., Aldinucci, C., & Frosini, M. (2019). Neuroprotective effects of *Castanea sativa* Mill. bark extract in human neuroblastoma cells subjected to oxidative stress. *Journal of Cellular Biochemisty, 117*, 510–520.

De Vasconcelos, M.C., Bennett, R.N., Rosa, E.A., & Ferreira-Cardoso, J.V. (2010a). Composition of European chestnut (*Castanea sativa* Mill.) and association with health effects: fresh and processed products. *Journal of Sciience of Food and Agriculture, 90*(10), 1578-89.

De Vasconcelos, M. D. C. B., Nunes, F., Viguera, C. G., Bennett, R. N., Rosa, E. A., & Ferreira-Cardoso, J. V. (2010b). Industrial processing effects on chestnut fruits (*Castanea sativa* Mill.) 3. Minerals, free sugars, carotenoids and antioxidant vitamins. *International journal of food science & technology, 45*(3), 496-505.

Franková, H., Šnirc, M., Jančo, I., Čeryová, N., Ňorbová, M., Lidiková, J., & Musilová, J. (2022). Total polyphenols and antioxidant activity in sweet potatoes (*Ipomoea batatas* L.) after heat treatment. *Journal of microbiology, biotechnology and food sciences, e5356-e5356.*

Lachman, J., Hamouz, K., Čepl, J., Pivec, V., Šulc, M., & Dvořák, P. (2006). The effect of selected factors on polyphenol content and antioxidant activity in potato tubers. *Chemické listy, 100*(7).

Lemos, M. R. B., de Almeida Siqueira, E. M., Arruda, S. F., & Zambiazi, R. C. (2012). The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts (*Dipteryx alata* Vog.). *Food Research International, 48(*2), 592-597.

Martínez, S., Fuentes, C., & Carballo, J. (2022). Antioxidant activity, total phenolic content and total flavonoid content in sweet chestnut (*Castanea sativa* Mill.) cultivars grown in northwest spain under different environmental conditions. *Foods, 11*(21), 3519.

Murthy, H. N., & Bapat, V. A. (Eds.). (2020). Bioactive Compounds in Underutilized Fruits and Nuts. Reference Series in Phytochemistry. doi:10.1007/978-3-030-30182-8

Mustafa, A. M., Abouelenein, D., Acquaticci, L., Alessandroni, L., Abd-Allah, R. H., Borsetta, G., ... & Caprioli, G. (2021). Effect of roasting, boiling, and frying processing on 29 polyphenolics and antioxidant activity in seeds and shells of sweet chestnut (*Castanea sativa* Mill.). *Plants, 10*(10), 2192.

Neri, L., Dimitri, G., & Sacchetti, G. (2010). Chemical composition and antioxidant activity of cured chestnuts from three sweet chestnut (*Castanea sativa* Mill.) ecotypes from Italy. *Journal of Food Composition and Analysis, 23,* 23–29.

Pandey, K.B., & Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity, 2*, 270–278.

Paulova, H., Bochorakova, H., & Taborska, E. (2004). In vitro methods for estimation of the antioxidant activity of natural compounds. *Chemické listy, 98*(4).

Pino-Hernández, E., Pereira, R. N., Ballesteros, L. F., Vicente, A. A., Abrunhosa, L., & Teixeira, J. A. (2021). Effects of moderate electric fields on the post-harvest preservation of chestnuts. *Food and Bioprocess Technology, 14*(5), 920-934.

Ramadan, M. F. (Ed.). (2019). Fruit oils: chemistry and functionality (p. 911). Switzerland: Springer.

Squillaci, G., Apone, F., Sena, L. M., Carola, A., Tito, A., Bimonte, M. & Morana, A. (2018). Chestnut (*Castanea sativa* Mill.) industrial wastes as a valued bioresource for the production of active ingredients. *Process Biochemistry, 64*, 228-236.

Šnirc, M., Lidiková, J., Čeryová, N., Pintér, E., Ivanišová, E., Musilová, J., ... & Rybnikár, S. (2023). Mineral and phytochemical profile of selected garlic (*Allium sativum* L.) cultivars. *South African Journal of Botany, 158*, 319-325.

Vázquez, G., Fontenla, E., Santos, J., Freire, M. S., González-Álvarez, J., & Antorrena, G. (2008). Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. *Industrial crops and products, 28*(3), 279-285.

Wani, I.A., Hamid, H., Hamdani, A.M., Gani, A., & Ashwar, B.A. (2017). Physico-chemical, rheological and antioxidant properties of sweet chestnut (*Castanea sativa* Mill.) as affected by pan and microwave roasting. *Journal of Advanced Research. 8*, 399–405.

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules, 23*(4), 762.