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HORSERADISH, A RESERVOIR OF USEFUL BIOACTIVE COMPOUNDS

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Abstract

Horseradish (Armoracia rusticana) is a plant of the Brassicaceae family, known for its strongly aromatic and spicy root. Horseradish, like many other root vegetables, is rich in biologically active compounds with antibacterial, anti-inflammatory, antioxidant and anticarcinogenic properties. The composition of the biologically active compounds of horseradish varies depending on the species of horseradish and the environmental conditions in which it is grown. The aim of this work was to determine the composition of some bioactive compounds from horseradish roots and leaves from the Romania and central part of Serbia. The elemental composition was determined by the XRF method, it being known that many mineral elements and trace elements are essential for the normal functioning of human bodies, reducing the risk of chronic diseases. The compounds with antioxidant activity from plant matrices are known as protectors of cells against oxidative stress, with a role in supporting the immune system. Consequently, the total content of polyphenols and antioxidant activity were determined from horseradish extracts by spectrophotometric methods. The results obtained recommend the tested horseradish as a beneficial food for health.

***Key words**: horseradish, bioactive compounds, elemental composition, antioxidant capacity.*

INTRODUCTION

Horseradish (*Armoracia rusticana*) is a plant of the *Brassicaceae* family, rich in biologically active compounds with antioxidant properties. The composition of the biologically active compounds of horseradish varies depending on the species of horseradish and the environmental conditions in which it is grown.

Polyphenols are chemical compounds with antioxidant properties that are found in large quantities in vegetables. The content of polyphenols and compounds with antioxidant properties differs from one plant to another, but it is also influenced by a number of factors related to the cultivation conditions (soil type), the growth and development stage of the plant, or the processing of it. Among the polyphenols identified in horseradish, the most important are glucosinolates and flavonoids. The roots and leaves of horseradish also contain numerous organic acids, vanillin, amino alcohols, tannins, amino acids, vitamins, minerals, protein and enzymes, carbohydrates [Negro et al., 2022, Knez et al., 2022].

Glucosinolates are sulfur compounds, the most widespread in horseradish being gluconasturtin, sinigrin and glucobrassicin [Prieto et al., 2019]. Through the enzymatic hydrolysis of glucosinolates, mainly isothiocyanates and thiocyanates, nitriles are formed. [Popović et al., 2020].

Mineral elements such as potassium, calcium, magnesium and phosphorus contained by horseradish are essential for the general health and functioning of the plant but also of the human body, following its consumption. But horseradish can also contain transition metals (zinc, copper, iron, manganese etc.), and their excessive content can create toxicity and have adverse effects on human health.

All the biologically active compounds contained in horseradish made it to be used since ancient times for pharmaceutical purposes and for food improvement and preservation [Agneta et al., 2013, Knez et al., 2022, Petrović et al., 2017].

Determining the content of minerals, polyphenols and antioxidant capacity of horseradish is important for evaluating its quality as food, understanding its potential for health and protection against chronic diseases and the relationship between nutrition and health.

The aim of this work was to evaluate the elemental composition, the polyphenols content and antioxidant capacity of horseradish roots and leaves from western Romania and central part of Serbia.

MATERIALS AND METHODS

Folin-Ciocalteu's phenol reagent, gallic acid (GAE), Trolox, neocupreine, bovine serum albumin (BSA), copper chloride, sodium carbonate and ethanol were purchased from Merck. All the other chemicals were obtained from local suppliers or were commercially available reagent grade products and were used without further purification.

Mature horseradish collected from 2 locations, one from Romania and the other one from Serbia, were harvested in October 2023. The vegetables (roots and leaves) were cleaned and washed with distilled water. The leaves were removed from the roots. The roots were ground, the bark being collected separately from the inner part. The vegetable parts (leaves, root's inner part and root's bark) were lyophilised and stored until use at 4^0C, in closed vials. The freeze drying was carried out until a constant mass of vegetable samples.

The horseradish extracts for the polyphenols, antioxidant capacity and protein analyses were obtained in an alcoholic medium (ethanol 50%) by mixing lyophilised vegetable parts with the extraction medium in a ratio of 1:10. The extraction time was 30 minutes, under magnetic stirring, 22^0C.

The total phenolic content was assayed by Folin-Ciocalteu method by using galic acid as standard [Folin et al., 1927, Singleton et al., 1999]. The Folin–Ciocalteu reagent was reduced by phenolic compounds contained by horseradish alcoholic extract in an alkaline environment. The absorbance of the reduced product containing a blue chromophore was measured at 750 nm. The total phenolic content was expressed as mg GAE/g dry weight of horseradish.

The total antioxidant capacity of the lyophilised horseradish extract was measured by CUPRAC method by using Trolox as standard [Apak et al., 2004]. In CUPRAC reaction the ligand copper (II)-neocuproine is reduced to a complex containing Cu^+. While the redox reaction takes place the colour changed from light blue to yellow-orange. The absorbance of the reduced CUPRAC product was measured at 450 nm. The total antioxidant capacity was expressed as μmol Tolox/g dry weight of horseradish.

The protein content was assayed according to the Lowry method, using the Folin-Ciocalteu's phenol reagent and bovine serum albumin (BSA) as standard [Lowry et al., 1951]. The protein content was expressed as mg_{BSA}/g dry weight of horseradish.

The elemental analysis of the horseradish was assayed by X-ray fluorescence (XRF). The vegetable parts (leaves, root's inner part and roots bark) used for XRF analysis was dried by lyophilization and milled in a mortar to a powder in order to minimize the particle size effects.

Statistical analysis was completed using PAST Version 2.17c software (Hammer et al, 2001).

RESULTS AND DISCUSSIONS

The lyophilised horseradish from Romania and Serbia was analysed to determine the total antioxidant activity, polyphenol and protein content, minerals and heavy metals. The analyses were carried out both for the internal part of the horseradish roots, obtained after removing the bark, as well as for the bark and leaves.

The horseradish samples had different content of water, the dry substance ranging from 19 to 46%. Our results are consistent with those obtained by Biller at al., 2018 and Agneta et al., 2014, they measured values for dry substances in the range 13-30% and 25-36%, respectively.

In plants there is a great diversity of compounds with antioxidant activity which, together with enzymes, have the role of neutralizing free radicals in cells. The individual quantitative analysis of antioxidants is very difficult because it involves their separation and then their qualitative analysis. Considering that the molecules with antioxidant activity act synergistically, the quantification of each one separately would not lead to significant results. So that the total antioxidant capacity is more proper in evaluating the positive effects on the human health.

In this work the antioxidant activity was assayed by using cupric reducing anti - oxidant capacity method (CUPRAC). The CUPRAC assay is based on the oxidoreduction reaction between the CUPRAC reagent ($Cu(NeoCu)_2^{2+}$) and the antioxidants (AOX_S)from the horseradish extract, forming the reduced form blue $Cu(NeCup)_2^{1+}$ and the oxidized form of antioxidants $oxAOX_s$

$$Cu(NeCup)_2^{2+} + AOX_S \leftrightarrow Cu(NeCup)_2^{1+} + H^+ + oxAOX_s$$

The results can be found in the Figure 1.

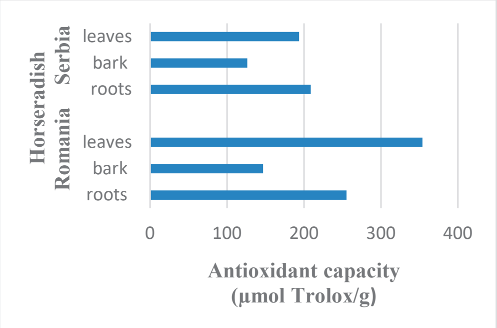

Figure 1. The antioxidant capacity of tested horseradish parts

It can be seen that the antioxidant capacity measured in the horseradish harvested in Romania is higher than that from Serbia, for all three parts of horseradish tested. The highest oxidizing activity was in the leaves of horseradish harvested in Romania and is 1.4 times higher than that in the internal part of roots.

The results obtained by us agree with those obtained in other studies and which show that the antioxidant properties can be influenced by the area where the horseradish is grown and the genotype of plants [Biller et al., 2018].

The total phenolic content was measured in the parts of horseradish plants by using the Folin-Ciocalteu method (Figure 2), the most used method for the quantitative analysis of plant extracts. The phosphomolybdic /phosphotungstic acid complex from the Folin-Ciocalteu reagent was reduced by the polyphenols from horseradish and a blue chromophore was formed.

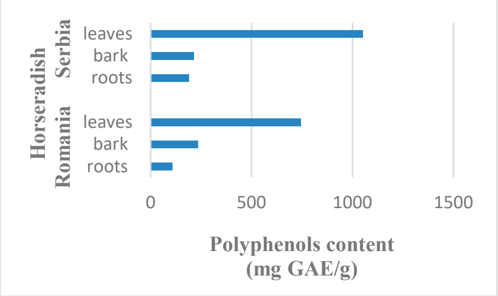

Figure 2. The polyphenol content of tested horseradish parts

The highest content of polyphenols was assayed in horseradish harvested in Serbia. Internal part of the horseradish roots from Serbia contains polyphenols in an amount 1.8 times higher than the same part of horseradish collected in Romania.

The polyphenol content of leaves is 5-7 times higher than that in internal part of roots of the same plant. Our results are in accordance with the results of Tomsone et al., 2020 and prove that the polyphenols are in different amounts in different parts of the same plant.

The differences measured for the biochemical parameters analysed in the tested horseradish could be due to the differences between the culture environments where the plants were grown and their potentially different varieties. According to studies of Biller et al., in many countries, the horseradish plants grew semi-wild and their varieties are not known [Biller et al., 2018].

The protein content was measured in leaves, root's internal part and roots bark extract. The horseradish collected from Serbia had a higher protein content measured in leaves and roots, but the values are quite closed.

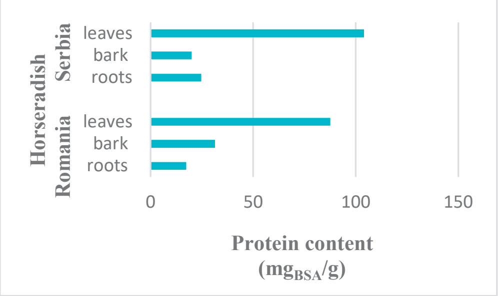

Figure 3. The protein content of tested horseradish parts

The elemental analysis of three different parts of horseradish was investigated by XRF method (Figure 4). The X-ray fluorescence method is a very used technique for evaluation of elements in plants materials, being an economical and less consuming time procedure.

This study followed two main directions: (1) determining the content of essential minerals for the growth and health of plants, minerals that can be beneficial to the human body after consuming horseradish; (2) establishing the potential toxicity due to the presence of heavy metals.

Cluster Analysis was performed (Figure 4) based on Paired Group Algorithm using Euclidean distance as Similarity measure shows a high correlation regarding mineral content of investigated samples (Cophenetic correlation coefficient= 0.991).

Principal Component Analysis (PCA) was performed using Variation-Covariation Matrix with the role to reduce the number of data while preserving the most important trends or patterns (Figure 5).

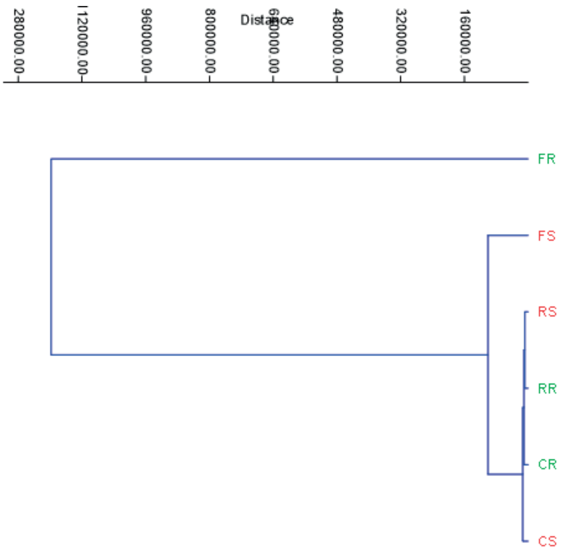

Figure 4. Cluster Analysis representation based on Euclidian Distances (RR-horseradish roots harvested in Romania, CR- horseradish bark harvested in Romania, FR- horseradish leaves harvested in Romania, RS- horseradish roots harvested in Serbia, CS- horseradish bark harvested in Serbia, FS- horseradish leaves harvested in Serbia)

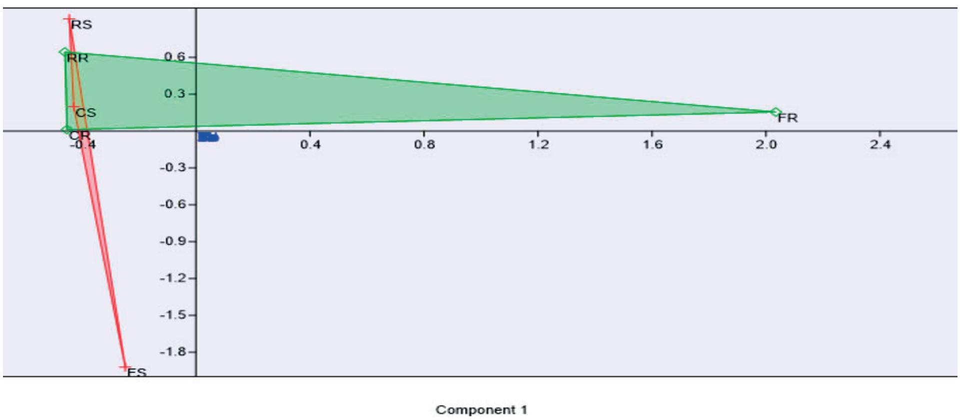

Figure 5. PCA Scatter Diagram of mineral composition data

Same like Cluster Analysis PCA shows also that the elemental data corresponding to the horseradish samples of Serbian origin are similar to the horseradish samples of Romanian origin, but the variation of data is much higher in the samples originated from Romania.

The trace elements with essential roles in physiological processes have been measured in all horseradish samples, in all tested parts of the plants. The content of K, Ca, Fe, Zn, Mn, Mg, Cu was found in concentrations similar to those from the scientific literature data, for all tested samples [Jabeen et al., 2010].

Roots have the ability to absorb heavy metals from the soil, such as Cd, Pb, Hg, As, etc. These metals, depending on their concentration, may have toxic effects as a result of consuming of the plants. None of these metals were in detectable doses in the horseradish tested samples.

CONCLUSIONS

The results of our research showed that the total antioxidant capacity and the content of polyphenols were affected by the environment where the plants grown and the variety of plants. All parts of horseradish had an important content of antioxidants, especially the leaves.

The horseradish grown in Romania has a higher antioxidant activity than the one in Serbia.

No toxic doses of heavy metals were detected in any of the tested samples.

The results obtained recommend the tested horseradish as a beneficial food for health.

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