

TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF RUTABAGA (*BRASSICA NAPUS* L. VAR. *NAPOBRASSICA*) ROOTS

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ABSTRACT

This study aims to determine the total content of polyphenols and the antioxidant activity by DPPH spectrophotometrically in rutabaga (*Brassica napus* L. var. *napobrassica*) samples, cultivated in the Trenčín region, in the Púchov district, at the border of the White Carpathian and Javorníky mountains, Slovakia. For analysis were used three types of rutabaga, named after the collection sites (Mestečko, Lúky 1, Lúky 2). The total polyphenol content in rutabaga was ranging from 2.41 (Mestečko) to 2.82 mg GAE.g⁻¹ dry weight (Lúky 2). It was established that that the studied rutabaga samples do not show a significant difference in the total content of polyphenols between the collection locations. In the antioxidant activity of rutabaga, differences were confirmed in AAs. A statistical difference was established between locations Lúky 2 and Mestečko. DPPH radical scavenging activity values were from 5,19 (Mestečko) to 7,49 μmol TE.g⁻¹ DW (Lúky 2). Spearman's test showed a positive relationship between both methods used. These results confirm the promising potential of rutabaga use in functional food preparation.

Keywords: rutabaga, polyphenols, antioxidant activity

INTRODUCTION

Rutabagas (*Brassica napus* L. var. *napobrassica*) are used as a winter cover crop, their leaves and roots can be eaten as leafy vegetables, and their roots can be fed to animals. In some regions of Slovakia this root vegetable was used in the past as a component of traditional meals. At present rutabaga belongs to almost forgotten vegetable species, but due to its interesting composition and several chemoprotective properties it has a potential to be recovered and used in novel or innovative foods with remarkable benefits for the human health. Rutabaga is one of the cold-weather root vegetables that can be stored for a long time. Rutabaga is a very nutrient-dense vegetable that is packed with important nutrients and a variety of phytochemicals. Rutabaga's phytochemical richness has a remarkable biological potential, particularly in terms of its anti-proliferative and antioxidant properties.

Since the 1950s and 1960s, rutabagas have been grown for commercial purposes in Canada (Fredau-agyeman *et al.*, 2020). It is low-cost, and the seeds can be used to make vegetable oil. The root is used extensively as food and spice in China, Japan, India, and northern Europe. The root's flesh ranges in colour from white to orange-yellow, and it tastes sweet and mildly spicy. It can be baked in an oven, cook or bake in foil under hot coal (Stefanucci *et al.*, 2020). Rutabaga is a root vegetable that is suitable for long -term storage in the cold season. Rutabaga has a shelf life of 4-6 months at 0 °C and 98-100 % relative humidity (Jakopic *et al.*, 2021). Rutabaga sprouts (*Brassica napus* L. var. *napobrassica*) have been evaluated as a potential new example of functional food with proapoptotic effects. Rutabaga roots are a potential source of polyphenols, glucosinolates and vitamin C (Paško *et al.*, 2020). Rutabaga can be recommended as a vegetable that can protect against various diseases, because of its interesting chemical composition, which includes sulphur and phenol compounds, vitamins, and fibre (Paško *et al.*, 2019).

Plant cultivar, soil conditions, growing season weather, fertiliser use, and plant maturity at harvest time are all known to be impacted by the presence of minerals and trace elements in the plant population. Calcium, magnesium, iron, phosphorus, sulphur, chlorine, potassium, copper, manganese, and sulphur are among the minerals found in rutabaga (Kapusta-Duch *et al.*, 2021; Stefanucci *et al.*, 2020). This vegetable contains high levels of vitamins (A, C, E), tocopherols (mainly α -tocopherol and γ -tocopherol) and folic acid. It also contains carotenoids, among them are the most represented lutein and β -carotene, which are able to prevent oxidative damage (Kapusta-Duch *et al.*, 2021; Stefanucci *et al.*, 2020).

Phytochemicals with sulphur found in the *Brassicaceae* family are called glucosinolates. Glucobrassicin, glucobrassicinapine, and neoglucobrassicin are the most well-known. Progoitrine, glucorapanin, glucualysine, glucoerucine, glucobetteroin, and glukonsturtin are additional glucosinolates that can be found in rutabaga. The primary glucosinolate found in rutabaga roots is glucoerucine, whereas progoitrine has only been found in footprints. In the seeds, progoitrine is the main constituent. These bioactive compounds, along with myrosinase, provide protection against plant pathogens and insects due to their cyano and sulphate groups. When a plant experiences tissue damage, myrosinase can react with glucosinolates and transform them into isothiocyanates and indoles, which are the breakdown products. They are chemopreventive against a variety of tumours, including liver, colon, and pancreatic tumours (Jakopic *et al.*, 2021; Paško *et al.*, 2019; Stefanucci *et al.*, 2020).

Rutabaga contains polyphenolic compounds, namely: 3-caffeoylquinic (chlorogenic), 4-caffeoylquinic (cryptochlorogenic), 5-caffeoylquinic (neochlorogenic), 5-feruloylquinic, 3-feruloylquinic, 3-p-coumaroylquinic, 4-p-coumaroylquinic, and sinapic acid. Apigenin, luteoline and myricetin are found in the root of rutabaga (Huang *et al.*, 2007; Jakopic *et al.*, 2021). Vegetables belonging to the *Brassicaceae* family contain plant polyphenols that have anti-inflammatory, antioxidant, cardioprotective, antimicrobial, and anticancer properties (Doniec *et al.*, 2022).

Although many plants are well-known for their health advantages, some still need more care. The goal of this study was to ascertain the total content of polyphenols and antioxidant activity in this nutrient-dense vegetable.

MATERIAL AND METHODS

Chemicals

The following materials were acquired from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany): methanol (99.8%), gallic acid (p.a.), DPPH (2,2'-diphenyl-1-picrylhydrazyl), Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid). Na₂CO₃ was purchased from CentralChem (Slovakia), and Folin-Ciocalteu reagent was acquired from Merck (Merck KGaA, Darmstadt, Germany).

Study areas and samples preparation

One variety of rutabaga (Dalibor) with dark yellow fruits (Fig. 1), grown in three different locations (Mestečko, Lúky 1, Lúky 2), which was harvested at full

maturity in autumn 2021, was used for the analysis. The collection sites are located in the Trenčín region, in the Púchov district, at the border of the White Carpathian and Javorníky mountains. The soil type in this area is cambism, with a neutral soil

reaction (pH/KCl 6.48 – 7.24) and humus content from 2.41 to 5.86%. Other soil characteristics are listed in Table 1. Climatic conditions are listed in Table 2.

Table 1 Properties of the soils of the locations of interest

Study area	K [mg/kg]	Ca [mg/kg]	Mg [mg/kg]	P [mg/kg]	C _{ox} [%]	N _{an} [mg/kg]
Mestečko	987.60	8183.20	767.70	291.10	1.54	21.31
Lúky 1	320.30	1999.90	102.10	103.60	1.40	16.28
Lúky 2	684.00	3352.80	305.30	392.70	3.40	34.01

Note: Results are presented as mean (n=4); C_{ox} – organic carbon content; N_{an} – inorganic nitrogen content.

Table 2 Climatic conditions in study area

Area	Altitude [m]	Average temperature [°C]	Average amount of rain [mm]
Mestečko	314	18.5	16.7
Lúky 1	339	18	16.9
Lúky 2	342	18	17.2

Note: Average values (temperature, amount of rain) from sowing to harvesting season (April, May, June, July, August); <https://www.meteoblue.com/>

In each location, a sample was taken from three different places of rutabaga growth. At each sampling point, 1.5 kg of rutabaga was collected, from which we prepared an average sample (500 g) for analysis. After taking the samples of the researched crops from the cultivation areas, the crops were cleaned in distilled water. Subsequently, the samples were mixed (Grindomix GM 200, Retsch, Haan, Germany; 30 sec) and homogenized. The extract was prepared from 25 g of homogenized sample poured with 50 mL of 80% methanol. The samples thus prepared were extracted for 12 hours on a horizontal shaker (Heidolph Promax 1020, Heidolph Instruments GmbH, Schwabach, Germany). Extracts were filtered through Muktel No 392 paper (Munktell & Filtrac GmbH, Bärenstein, Germany) and stored in sealed 50 mL centrifuge tubes at 4 °C in a refrigerator. The dry matter of the samples was determined on a moisture analyzer (KERN DLB 160 – 3A, KERN & SOHN GmbH, Balingen, Germany).



Figure 1 Rutabaga roots (URL 1)

Determination of total polyphenol content

Total polyphenol content (TPC) was determined by spectrophotometry (UV-VIS spectrophotometer T92+, PG Instruments, Leicestershire, United Kingdom) using a Folin-Ciocalteu agent in accordance with **Lachman et al. (2006)**. The Folin-Ciocalteu reagent was added to the volume of sample extract (0.1 mL) in the volumetric flask (50 mL). After three minutes, 5 mL of 20% potassium carbonate aqueous was added, and distilled water was added to the mark. The same process was used to prepare standard gallic acid solutions for the calibration curve. The prepared solutions were mixed and then allowed to stand at room temperature for two hours. Subsequently, the absorbance of the solutions at 765 nm was measured. The polyphenol concentration of each sample was expressed as milligrammes of gallic acid equivalent per gramme of dry weight (mg GAE.g⁻¹ DW).

Spectrophotometric determination of total antioxidant activity by the DPPH method

We followed the methodology of **Brand-Williams et al. (1995)**. The DPPH solution was prepared by dissolving 0.025 g of DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol (99.8%) in a 100 mL volumetric flask stored in a dark and cool place. A ten-fold diluted DPPH stock solution was used for analysis. 3.9 mL of DPPH was pipetted into 1 cm wide cuvettes, and then the absorbance value (A₀) was measured on a UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire, United Kingdom) at a wavelength of 515.6 nm. After measuring the initial absorbance, we added 0.1 mL of the extract and mixed it three times using a stirrer. We measured the absorbance (A₁₀) again after ten minutes. Based on the absorbance of the DPPH solution (A₀) and the absorbance at time t = 10

minutes (A₁₀) after the addition of the sample, we calculate the percentage of DPPH inhibition using the formula:

$$\%DPPH \text{ inhibition} = [(A_0 - A_{10}) / A_0] \times 100.$$

We express the result as Trolox equivalent (TE) (μmol TE.g⁻¹) in dry weight (DW).

Statistical analysis

All analyzes were performed in 4 replicates (n = 4). Results are expressed as arithmetic mean ± standard deviation (SD). First, the dataset was tested for normality. All tested variables were non-parametrically distributed. Non-parametric ANOVA test (Kruskal-Wallis) and Mann-Whitney test were used for comparison between the tested variables. Spearman's correlation coefficient was used to determine the relationship between the investigated parameters (TPC and DPPH). Calculations, including graphic presentations, were carried out using the RStudio (2020) software package.

RESULTS AND DISCUSSION

Total polyphenol content

The total content of polyphenols (TPC) was determined in the roots of rutabaga (*Brassica napus* L. var. *napobrassica*), which we obtained from three different locations (Mestečko, Lúky 1, Lúky 2). TPC values ranged from 2.41 (Mestečko) to 2.82 mg GAE.g⁻¹ DW (Lúky 2) (Table 3). A higher total content of polyphenols (10.76-11.57 mg GAE.g⁻¹ DW) was determined in rutabaga in the study by **Stefanucci et al. (2020)**. The higher content of polyphenols could have been influenced by the difference in the preparation of the extract, since the authors lyophilized the rutabaga and prepared the extract for analysis from the thus prepared sample. In another study (**Huang et al., 2009**) they also found a higher TPC content in rutabaga samples, namely 14.0 mg GAE.g⁻¹ DW. **Rydenheim (2008)** reports a TPC value in a fresh rutabaga sample (0.61 mg GAE.g⁻¹), which is also slightly higher than our results when converted to dry mass. Also **Pasko et al. (2013)** found the total content of polyphenols in rutabaga root to be 5.1 mg GAE.g⁻¹ DW. The variation of total phenolic content in vegetables can depend on many factors such as cultivar and harvest time. Compared to field cabbage (*Brassica rapa* var. *Rapifera*), whose TPC is 2.1 mg GAE.g⁻¹ DW (**El-Esawi, 2018**), the TPC in the roots of the investigated rutabaga is similar. Also in the study by **Xiao et al. (2019)**, TPC values in turnip (1.60 mg GAE.g⁻¹ DW) are similar to the values of our rutabaga samples.

Like other phytochemicals, species, growing circumstances, maturity at harvest, and postharvest storage conditions are just a few examples of the intrinsic and extrinsic factors that can impact phenolic content. The polarity and stability of phenolic compounds vary, so the technique used to prepare the samples can have an impact on the result (**Xiao et al., 2019**). As a result, comparing data presented by various researchers can occasionally be challenging. The composition of fruit can also be greatly influenced by variables like light, temperature, and soil nutrients (**Stojanović et al., 2017**).

Table 3 Total polyphenol content and antioxidant activity

Collection site of rutabaga	TPC [mg GAE.g ⁻¹ DW]	AA [μmol TE.g ⁻¹ DW]
Mestečko	2.41 ± 0.37	5.19 ± 0.08
Lúky 1	2.50 ± 0.21	5.38 ± 0.10
Lúky 2	2.82 ± 0.47	7.49 ± 0.08

Legend: GAE: gallic acid equivalent; TE: Trolox equivalent; results are presented as mean ± SD.

By statistical examination of the observed rutabaga species (*Brassica napus* L. var. *napobrassica*), we found that rutabaga species do not show a statistically significant difference in the total content of polyphenols between the collection locations ($p=0.39$) (Fig. 2).

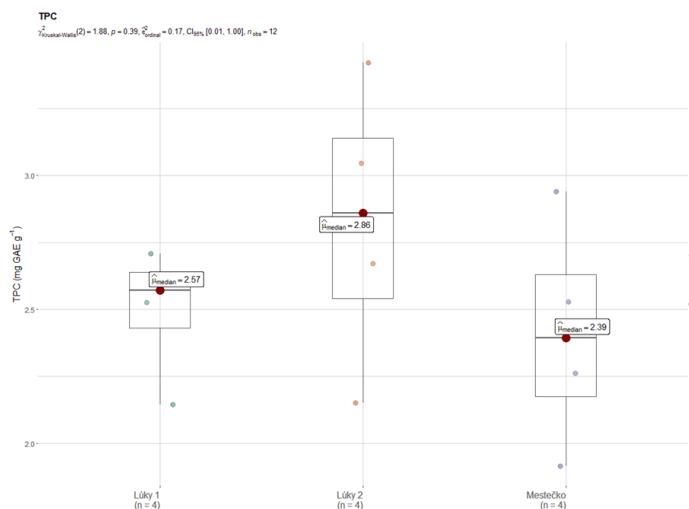


Figure 2 Statistical differences in TPC between rutabaga

Antioxidant activity

Total antioxidant activity (AA) by the DPPH method was investigated in rutabaga, where it ranged from 5.19 to 7.49 $\mu\text{mol TE} \cdot \text{g}^{-1} \text{DW}$. By statistical examination of the antioxidant activity of rutabaga, differences were found between AAs (Fig. 3). A significant difference was confirmed between locations Lúky 2 and Mestečko ($p_{\text{Holm-adj.}}=9.31 \cdot 10^{-3}$). **Stefanucci et al. (2020)** prepared rutabaga extracts in two ways, namely homogenizer-assisted extraction and ultrasound-assisted extraction, especially for the peel and pulp of rutabaga root. They then investigated the antioxidant activity of the extracts, where the peel showed AA up to 5220 $\mu\text{mol TE} \cdot \text{g}^{-1} \text{DW}$ (homogenizer) and 8110 $\mu\text{mol TE} \cdot \text{g}^{-1} \text{DW}$ (ultrasound), respectively, while extracts from the root pulp showed no AA. **Pasko et al. (2013)** reported AA in rutabaga root as 32.9 $\mu\text{mol TE} \cdot \text{g}^{-1} \text{DW}$. They report even higher AA values in rutabaga sprouts (644.1 $\mu\text{mol TE} \cdot \text{g}^{-1} \text{SH}$). **Xiao et al. (2019)** determined AA in different species of the *Brassicaceae* family, while in rutabaga the AA value was 3.14 $\mu\text{mol TE} \cdot \text{g}^{-1}$ of fresh mass, which after conversion is approximate to our measured results. For the comparison of AA in turnips, the study by **Xiao et al. (2019)** 1.83 $\mu\text{mol TE} \cdot \text{g}^{-1}$ fresh mass. **Huang et al. (2009)** investigating the antioxidant activity in rutabaga roots found that rutabaga has a relatively high antioxidant activity (DPPH), around 67%. The authors also examined AA using the ORAC method, which had a value of 18.2 $\mu\text{mol TE} \cdot \text{g}^{-1} \text{DW}$.

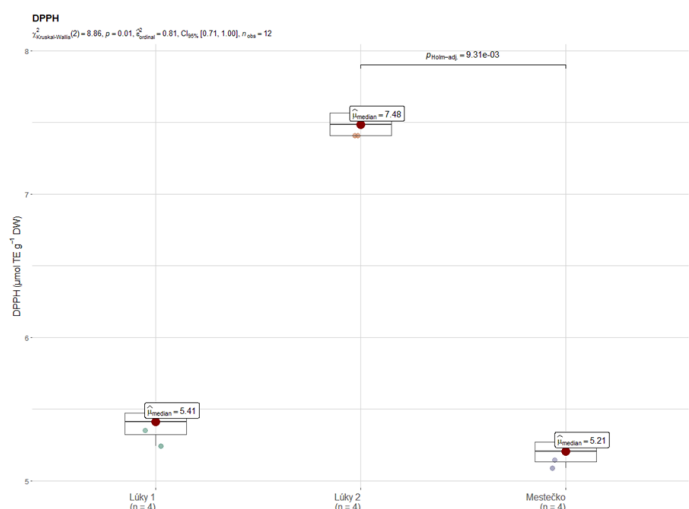


Figure 3 Statistical differences in AA of rutabaga

Spearman's correlation coefficient

Spearman's correlation coefficient was used to determine the relationships between the monitored parameters, total polyphenol content (TPC) and antioxidant activity (DPPH). The total content of polyphenols in the investigated

samples showed a strong positive relationship with the antioxidant activity of DPPH ($r=0.52$).

CONCLUSION

Present results established the significant impact of growing location on the antioxidant activity of rutabaga roots of studied samples. Due to the high antioxidant activity of rutabaga roots, they represent an interesting source of chemical compounds that can be a substitute for food additives. Rutabaga roots can enrich the diet with active ingredients such as essential vitamins, minerals and antioxidants. The content of substances, important for antioxidant activity of rutabaga samples could be influenced by many factors, such as species, growing conditions, maturity at harvest and post-harvest storage conditions. From the point of view of use and further recommendation not only for the food industry, but also for ordinary consumers, further evaluation and research of different rutabaga varieties as well as different factors which can influence the content of biologically valuable components of this almost forgotten vegetable is necessary.

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