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INCREASING THE STABILITY OF POLYPHENOLS AND BETACYANINS IN BEETROOT CHIPS BY TREATMENT BEFORE DRYING

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ABSTRACT

An innovative trend in the production of snack products is the use of selected vegetables for the production of dried vegetable chips. Beetroot is a suitable vegetable for this type of product, as it is characterised by its high biological potential and its content of a wide range of phytonutrients with beneficial effects on human health. The aim of this work was to compare five varieties of beetroot in terms of the content of total polyphenols and betacyanins, to assess the effect of hot-air drying on these important components and to determine the appropriate method of treatment of beetroot slices before drying in terms of improving the stability of polyphenols and betacyanins during drying. In this work, 5 beetroot varieties (Betina, Renova, Cervena kulata, Pablo F1, Karkulka) were evaluated. The beetroot chips were dried at 70°C until a residual moisture content of 15±1% was reached. To treat the slices before drying a 0.1% solution of ascorbic acid, 0.01% solution of $CaCl_2$, 0.01% solution of $K_2S_2O_5$, 1% solution of NaCl was used followed by blanching-in a water bath at 96°C for 2 minutes. The highest content of total polyphenols and beta-cyanins was statistically significant ($p<0.05$) in the variety Pablo F1. During hot air drying, both polyphenols and betacyanins decreased significantly. The average decrease in betacyanins and total polyphenols was 50.97% and 48.70% respectively. To mitigate these changes, treatment with 0.1% solution of ascorbic acid and blanching appeared to be the most effective of the treatments tested. After treatment with ascorbic acid solution, the dried products retained on average 27.6% more betacyanins and 32.67% more total polyphenols than the control sample.

Keywords: beetroot chips, drying, total polyphenols, betacyanins

INTRODUCTION

The global trend in human nutrition is to focus on foods that, in addition to their nutritional function, have a positive effect on human health and quality of life. Many fruits and vegetables are included among such "functional foods" because they contain a wide range of phytochemicals with various effects on human health **(Leong *et al.*, 2018; Ceclu and Nistor, 2020)**. Beetroot (*Beta vulgaris* var. *conditiva* Alef) has recently gained much attention due to its nutritional value, health benefits and versatile use in food. The plant originates from the Mediterranean region from where it has spread worldwide **(Mirmiran *et al.*, 2020)**. Beetroot is one of the most valuable vegetables due to its high content of bioactive compounds, the most important of which are betalains, ascorbic acid, carotenoids, polyphenols, flavonoids and saponins **(Chhikara et al., 2019; Baiao et al., 2017)**. Other nutrients such as phytosterols, saturated and unsaturated fatty acids, vitamins A, B_1, B_2, B_3, B_5, B_6, B_9, C and mineral substances such as Na, Ca, Fe, P, K, Mg and Zn are also present **(Chhikara *et al.*, 2019, Bangar *et al.*, 2022)**. Current literature describes many health benefits of beetroot that depend on the content of bioactive compounds in the eyeball, which may vary depending on the variety, agroecological conditions, and growing and harvesting conditions **(Dhiman *et al.*, 2021)**. Consumption of beetroot has beneficial physiological effects and may contribute to improved clinical outcomes in a variety of diseases, including cardiovascular diseases **(Mirmiran *et al.*, 2020; Milton-Laskibar *et al.*, 2021)**; hypertension **(Bonilla Ocampo *et al.*, 2018)**; diabetes **(Aliahmadi *et al.*, 2021)**; cancer **(Lechner and Stoner, 2019; Tan and Hamid, 2021)**; steatosis and liver damage **(Al-Harbi *et al.*, 2021)**; biliary, nephrotic and urinary stones **(Alok *et al.*, 2013)**. Various epidemiological studies have found that betalain effectively removes oxidative and nitration stress, lowers low-density lipoprotein (LDL), prevents DNA damage, and suppresses cell proliferation **(Chen *et al.*, 2021)**.
Raw beetroot is used for salads, juices, soups or canned for various food products. Betalains, the colorants of beetroot, are also used as additives in the food industry for their natural colouring properties, good water solubility and health safety **(Koubaier *et al.*, 2014)**. Beetroot is considered to be the most important source of betalains. Betanin extract E162, approved as a red food colorant, is used as a colorant to modify the red color of jams, jellies, cereals, candies, soups, yogurts or meat products **(Chandran *et al.*, 2014; Neha *et al.*, 2018; Mukhtar *et al.*, 2023)**.
One of the uses of beetroot is to process it into beetroot chips. Processing vegetables by drying them into vegetable chips is a new alternative within the snack industry that brings good sensory properties and higher nutritional quality to innovative products **(Mesias *et al.*, 2019)**. The use of higher temperatures during production clearly has a beneficial effect on shelf-life extension and inactivation of microorganisms in the products, but also has a negative effect on bioactive compounds **(Dhiman *et al.*, 2021; Chaudhary, Kumar, 2020; Kathiravan *et al.*, 2014)**.
The aim of this work was to assess the effect of hot air drying on the content of selected bioactive compounds in beetroot chips as well as the effect of treatment of beetroot before drying to enhance the protection of these compounds from degradation during drying.

MATERIAL AND METHODS

In this work 5 varieties of beetroot were used, which were grown in the botanical garden of Slovak University of Agriculturae in Nitra. These varieties were represented by Betina (round), Renova (oblong), Cervena kulata (round), Pablo F1 (round), Karkulka (oblong) variety. The beetroot tubers were cleaned after harvesting, used fresh for analysis, and then dried. Drying of the samples was carried out in a laboratory drying oven MEMMERT UF 160 with the possibility of controlling the drying conditions. The drying temperature was 70°C and the drying time ranged from 8 to 10 hours until the samples reached a residual moisture content of 15±1%. The final moisture content was monitored on a KERN MRS 120-3 moisture analyser.
In addition to the basic hot-air drying without pre-drying treatment, the samples were treated in various antioxidant solutions or by heat treatment - blanching - prior to drying. The treatments used were: 0.1% ascorbic acid solution, 0.01% $CaCl_2$ solution, 0.01% $K_2S_2O_5$ solution, 1% NaCl solution. The treatment was carried out for 2 minutes. Blanching in a water bath was at 96°C for 2 minutes. Betina and Renova varieties were used for the treatment.
The content of total polyphenols in fresh and dried samples was determined spectrophotometrically using the Folin-Ciocalt reagent (**Lachman *et al.*, 2003**). Before analysis, fresh samples were homogenized on a HEIDOLPH, SILENT CRUSHER M disintegrator at a speed of 10 000 min^{-1} for 3 min. Homogenization of the dried samples was performed on a BOSH MKN 6003 grinder for 3 min. The samples were extracted in 80% ethanol on a HEIDOLPH GSL 3006 shaker at 150 rpm for 24 h. For the determination of total polyphenols alone, 50 µl of the extract was gradually added to a 50 ml flask with 2.5 ml of Folin-Ciocalteu reagent, 5 ml

of Na_2CO_3 (20% aqueous solution) and distilled water. The prepared mixture was allowed to stand for 2 hours at room temperature. During this time, the polyphenols present reacted with the Folin-Ciocalteu reagent to form a blue-colored complex of varying intensity. The intensity of the blue color was measured on a spectrophotometer at a wavelength of 765 nm. A double beam UV-VIS spectrophotometer (Jenway 6405 UV/VIS) was used for the measurement. The total content of polyphenols was calculated from the calibration curve of gallic acid with a concentration of 5 - 200 mg.L^{-1}. The blank was prepared in a similar way, but unlike the experimental samples, it did not contain the standard or beetroot extract.

Determination of the content of betacyanins was carried out spectrophotometrically according to the methodology of **Ravichandran *et al.* (2013)**. 5 g of homogenized fresh sample or 0.5 g of dried sample was repeatedly extracted in distilled water on a HEIDOLPH GSL 3006 shaker at 150 rpm for 30 minutes, and 50 ml of stock solution was made up successively to a volume of 50 ml. The obtained extracts were purified by filtration and centrifugation and suitably diluted with distilled water before analysis. The content of betacyanins converted to betanin as the dominant betacyanin content was calculated as follows BT = A x DF x MV x 1000/ε x l ; where A - absorption, DF - dilution factor, l - cuvette length (1 cm), MW - molecular weight (550 g.mol^{-1}, ε-molar absorption coefficient (60 000 L.mol^{-1}.cm^{-1})

The experiment was performed in three replicates. The experiment was conducted in three repetitions. Data were evaluated using one-way analysis of variance (ANOVA) followed by post hoc analysis of statistically significant differences between beetroot variants treated before drying using LSD multiple range test (Statistica 12, Statsoft), assuming that differences were statistically significant at α=0.05.

RESULTS AND DISCUSSION

Table 1 Evaluation of the betacyanins and total polyphenols content in beetroot

Variety	Dry matter (%)	Betacyanins (mg.kg^{-1} DM)	Total polyphenols (mg GAE.kg^{-1} DM)
Renova	11.08	199.64±6.45 a	1908.09±39.27 a
Cervena kulata	8.72	204.417±5.19 a	2102.58±37.13 c
Karkulka	9.49	205.443±22.84 a	2320.48±24.00 d
Betina	11.46	211.47±9.55 a	1987.04±43.65 b
Pablo F1	10.38	255.96±6.74 b	2756.18±32.30 e

a-e homogeneous group, ± standard deviation, different letters at mean represent statistically significant differences among treatments (p<0.05)

The betacyanine content of fresh beetroot eyeballs ranged from 199.64±6.45 to 255.96±6.74 mg.kg^{-1} DM (Table 1). By multiple comparisons of means by LSD test, the varieties were divided into 2 homogeneous groups. Statistically significant differences (p<0.05) as the highest betacyanine content were detected in Pablo F1 variety. No statistically significant difference (p>0.05) was detected among the remaining cultivars (Renova, Cervena kulata, Karkulka and Betina).

The content of total polyphenols in beetroot ranged from 1908.09±39.27 to 2756.18±32.30 mg GAE.kg^{-1} DM. On the basis of the LSD test results, the varieties were arranged into 5 homogeneous groups according to the total polyphenol content. Statistically significant differences (p<0.05) were found among all the tested varieties with each other. The highest content was detected in the variety Pablo F1 (2756.18±32.30 mg GAE.kg^{-1} DM) and the lowest in the variety Renova (1908.09±39.27 mg GAE.kg^{-1} DM).

Betacyanins, together with betaxanthines, are among the 2 main groups of colorants found in beetroot, which together form the betalain pigments of beetroot. Betacyanins are characterised by their raddish-purple colour and are condensation products of betalamic acid and cyclo-DOPA[cyclo-3-(3,4-dihydroxyphenylalanine)]. The major betacyanin in beetroot is betanin, which accounts for 75-95% of all betacyanins, followed by isobetanin and neobetanin **(Herbach *et al*., 2006; Nemzer *et al*., 2011; Clifford *et al*., 2015; Pauliuc *et al*., 2015; Chhikara *et al*., 2019)**.

The evaluation of the content of betacyanins and betaxanthins in 12 beetroot cultivars was reviewed by **Czapski *et al.* (2009)**. The authors evaluated the relationship between dye content and antioxidant activity of beetroot and found a statistically significant correlation between betacyanins content and antioxidant activity. They did not confirm a correlation between betaxanthine content and antioxidant activity. Concerning the content of betacyanins, the authors report contents ranging from 0.62 mg.mL^{-1} in the Red Round variety to 1.40 mg.mL^{-1} in the Nochowski variety.

The representation of betalains in beetroot was investigated by **Sawicki *et al.* (2016)**. Authors used 13 red beet varieties and analyses were performed in different parts of the beetroot. In their research, they found that the dominant betacyanins in beetroot are betanin and isobetanin, and the highest content of betalains is found in the superficial parts of the eyeball. In their work they evaluated 10 new cultivars and 3 varieties (Czarnota, Rywal and Crosby) from the Research Institute of Vegetable Crops in Skierniewice, Poland. The content of betacyanins in the samples studied ranged from 7.18 mg.g^{-1} DM (Czarnota) to 13.5 mg.g^{-1} DM (new cultivar NOE2).

Skalicky *et al.* (2020) evaluated the betacyanin content in red beetroot, fodder beet, sugar beet and chard. They clearly found the highest contents in red beet varieties, namely 9.69 mg.100 mL^{-1} in Monorubra and 8.42 mg.100 mL^{-1} in Libero cultivar. The betacyanin content was found 0.11 mg.100ml^{-1} in sugar beet, 0.15 mg.100 mL^{-1} in fodder beet and 0.09 mg.100 mL^{-1} in chard. In red beet the authors reported a predominance of betacyanins over betaxanthins, in the other species a predominance of betaxanthins over betacyanins.

Vaitkevičiene *et al.* (2020) performed a comprehensive analysis of the nutritional composition of selected beetroot varieties. They focused on the basic composition (sugars, fats, proteins, minerals), but also on the content of colorants and total polyphenols. The authors found that the content of betacyanins in Pablo F1, Alto F1, Taunus F1 and Kosak varieties ranged from 7.9 to 9.04 mg.g^{-1} DM. Authors detected total polyphenol content in the values of 14.4 – 36.1 mg.g^{-1} DM, which are comparable to our findings.

Kujala *et al.* (2001) and **Ceclu and Nistor (2020)** reported that beetroot is one of the richest vegetables in terms of polyphenol content. **Maraie *et al.* (2014)** reported that beetroot contains significant amounts of hydroxybenzoic and hydroxycinnamic acid derivatives which include several phenolics such as catechin, epicatechin, rutin, caffeic acid, p-coumaric acid, protocatechuic acid, ferulic acid, ferulic acid and syringic acid.

The content of total polyphenols in beetroot juice was also monitored by **Kazimierczak *et al.* (2016)**. Authors found an average total polyphenol content of 129.31 mg.100 g^{-1} FW, identifying gallic acid as the dominant one with an average content of 120.37 mg.100 g^{-1} FW.

The content of total polyphenols in beetroot cultivars with different flesh coloration was observed by **Šlosár *et al.* (2020**). In their work, they used beetroot cultivars with yellow, white, white-red and red flesh colour. The authors found statistically significant differences (p<0.05) in the content of total polyphenols between the tested varieties and also groups of varieties. The lowest content of total polyphenols was found in the white-fleshed variety White Detroit (717.27 mg.kg^{-1} DM) and the highest in the red-fleshed variety Pablo F1 (2731.0 mg.kg^{-1} DM), a value that is also consistent with our measurements. The highest TPC was found in red-fleshed cultivars (1537.64 – 2731.00 mg GAE.kg^{-1} DM), followed by beetroot cultivars with yellow (995.45 – 1183.72 mg GAE.kg^{-1}, DM), red-white (756.93 – 941.41 mg GAE.kg^{-1} DM) and white (717.27 – 933.17 mg GAE.kg^{-1} DM) colour of root flesh.

Koubaier *et al.* (2014) analysed beetroot grown in Tunisia and found an average beta-cyanin content of 53 mg.g^{-1}, while the betaxanthins content was only 11 mg.g^{-1}. The content of total polyphenols in the work of **Koubaier *et al.* (2014)** ranged from 6.6 – 10.4 mg.g^{-1}. The values of polyphenol content varied significantly with the extraction reagent used.

Salamatullah *et al.* (2021) analyzed the content of total polyphenols in beetroot after extraction with different solvents and found that after extraction with methanol diluted with water in a 1:1 ratio, the beetroot peel contained the highest content of chlorogenic acid (78.24 mg.100 g^{-1}), after extraction with 100% methanol, the authors found the highest content of 1,2 dihydroxybenzene.

Table 2 Evaluation of betacyanins and total polyphenols in beetroot chips

variety	dry matter (%)	betacyanins (mg.kg^{-1} DM)	loss on drying (%)	total polyphenols (mg GAE.kg^{-1} DM)	loss on drying (%)
Betina	85.62	106.49±3.38 c	49.64	1108.20±33.29 b	44.23
Karkulka	85.06	108.02±1.93 c	52.58	1150.99±33.93 b	50.40
Pablo F1	84.35	120.58±1.75 d	47.11	1369.39±37.01 c	50.32
Renova	84.76	100.44±3.46 b	49.69	1019.68±31.93 a	46.60
Cervená kulata	84.89	94.63±4.09 a	53.71	1009.39±31.77 a	51.99

a-d homogeneous group, ± standard deviation, different letters at mean represent statistically significant differences among treatments (p<0.05)

After drying, there was a decrease in both beta-cyanine and total polyphenol content. Statistically (p<0.05) the highest content of betacyanins after drying was shown by the variety Pablo F1 (120.58±1.75 mg.kg^{-1} DM), in which the content of betacyanins was reduced by 47.11% during drying, which is the lowest loss. The most significant decrease in betacyanin content was observed in the Cervena kulata (94.63±4.09 mg.kg^{-1} DM) variety. The average decrease in betacyanin content after drying for all varieties studied was 50.67%.

The average decrease in the content of total polyphenols after drying for the varieties studied was 48.7%. Statistically ($p<0.05$) the highest content of total polyphenols was retained after drying in the variety Pablo F1 (1369.39±37.01 mg GAE.kg^{-1} DM) . The best stability of total polyphenols was observed in the variety Betina (1108.20±33.29 mg GAE.kg^{-1} DM), where the content of total polyphenols decreased by 44.23% after drying. The highest decrease in the content of total polyphenols after drying was observed in the variety Cervena kulata (1009.39±31.77 mg GAE.kg^{-1} DM) .

In order to better protect the nutritionally important components, we treated the beet slices with antioxidant solutions and blanching before drying.

Antioxidant treatment of beetroot slices before drying with ascorbic acid, $CaCl_2$, NaCl and blanching showed statistically significant ($p<0.05$) positive result on the stability of betacyanin content (Figure 1). In the case of total polyphenols, all treatments prior to drying showed statistically significant ($p<0.05$) positive result on the content of total polyphenols (Figure 2). Ascorbic acid treatment and blanching appeared to be the most effective treatments in both parameters studied. In the case of betacyanins and total polyphenols content, we did not find a statistically significant difference ($p>0.05$) between the treatments (0.01% $CaCl_2$ solution, 0.01% $K_2S_2O_5$ solution, 1% NaCl solution). After the blanching treatment, an average of 29.47% more betacyanins and 36.26% more total polyphenols were retained in the studied cultivars after drying. After treatment with ascorbic acid solution, 27.6% more betacyanins and 32.67% more total polyphenols were retained in the dried products. The treatments with calcium chloride and sodium chloride solution were evaluated as less effective and no statistically significant difference was found between them ($p>0.05$).

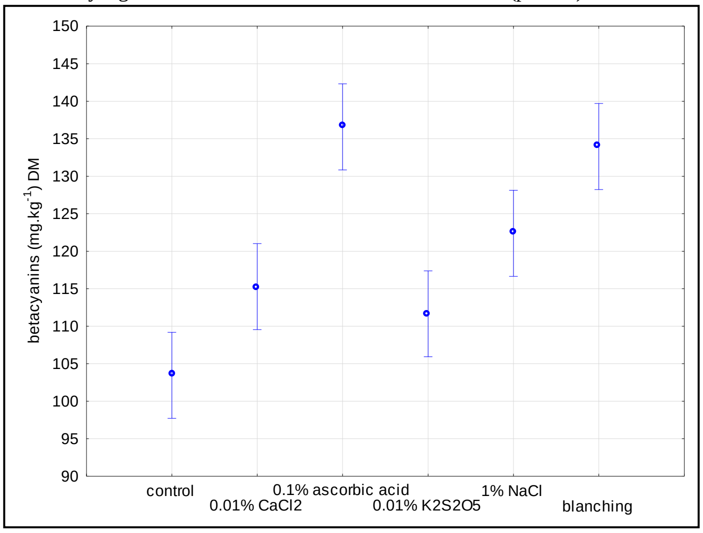

Figure 1 Marginal averages of betacyanins content in beetroot chips (mg.kg^{-1} DM) as a function of treatment method before drying

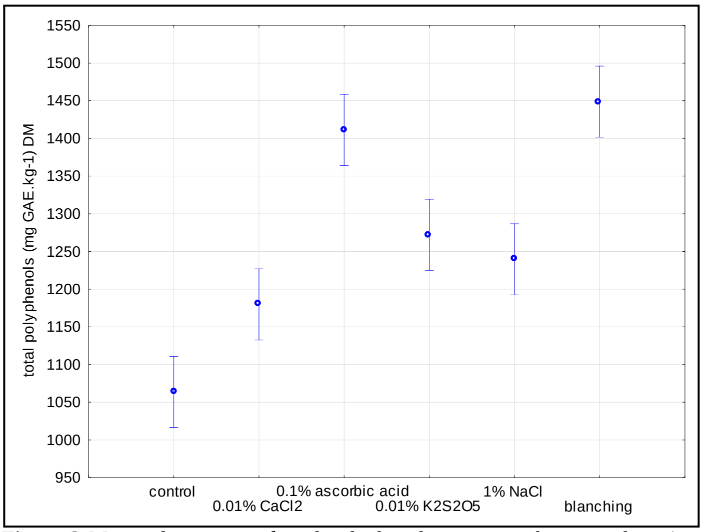

Figure 2 Marginal averages of total polyphenols content in beetroot chips (mg GAE.kg^{-1} DM) as a function of treatment method before drying

Nistor *et al.* (2017) compared the effect of hot-air drying at 50, 60 and 70°C for 510, 390 and 300 min and a combination of microwave heating at 315 W and hot-air drying at 50, 60 and 70°C for a total duration of 219, 159 and 129 min on the content of colorants and total polyphenols in beetroot. The authors found that the combined drying methods were preferable in terms of the balance of bioactive components. The best stability of total polyphenols was observed with the combined drying method of microwave heating and hot-air drying at 60°C. The combination of microwave heating and drying temperature of 70°C was more favourable in terms of stability of the colorants. Also the work of **Seremet *et al.* (2020)** indicates that microwave or ohmic heating combined with hot air drying is a suitable way to improve the balance of polyphenols, colorants and antioxidant activity of dried beetroot.

A study by **Dehghannya *et al.* (2023)** aimed to investigate the effect of pulse ratio and microwave power during drying of red beetroot using combined intermittent microwave - hot air at low temperature (40°C). A significant decrease in drying time was observed by increasing the microwave power from 360 to 900 W and decreasing the pulse ratio from 6 to 2. The authors found that by increasing the microwave power and decreasing the number of pulses, the beta-cyanin content increased and up to 79.47% of the original content was retained in the product. The authors further state that betalains are part of the cell structure, and microwaves applied to red beetroot samples at higher intensity 600 and 900 W can lead to the destruction of cell walls and, as a result, the release of pigments from the plant tissue to the external environment increases.

Hidangmayum *et al.* (2023) studied effect of high pressure (100-300 MPa for 5-15 min) pretreatments on the drying characteristics and microstructure of beetroot. The authors observed that high pressure pretreatment increased the drying rate and thus resulted in approximately 20% reduction in drying time for the 300 MPa modified sample to 4% for the 100 MPa modified sample. The color change of samples treated with high pressure was minimal.

Malakar *et al.* (2022) compared the drying kinetics, color changes, and changes in bioactive compounds of beetroot slices in a solar vented dryer with sun drying. Authors reported that by use of drying in solar vented dryer, a higher drying temperature (63.78°C) and a higher maximum drying rate are achieved than sun drying. They also report that drying in the oven preserves 63.98% more betalain in the products. The content of total polyphenols in the sun-drying process was 2.08 mg GAE.g^{-1} DM, whereas in the oven-drying process it was 3.02 mg GAE.g^{-1} DM, which is more than 45% higher.

Lisiecka and Wójtowicz (2021) used dried beetroot powder to flavour extruded rice starch snacks. The authors found that the final product had increased protein and soluble fiber content, along with enhanced total phenolics content and antioxidant activity. This varied positively with the increase in beetroot pulp content.

CONCLUSION

The aim of the work was to compare five varieties of beetrootin terms of total polyphenols and betacyanins content, to determine the suitable variety for the production of dried beetroot chips and to determine the appropriate treatment of beetroot slices before drying in order to increase the stability of the total polyphenols and betacyanins content during the hot-air drying process. Five beetroot varieties (Betina, Renova, Cervena kulata, Pablo F1, Karkulka) were used in this work. The drying of beetroot chips was carried out at 70°C until a residual moisture content of 15±1% was reached. A 0.1% ascorbic acid solution, 0.01% $CaCl_2$ solution, 0.01% $K_2S_2O_5$ solution, 1% NaCl solution were used to treat the slices before drying, and blanching in a water bath was conducted at 96 °C for 2 minutes. Pablo F1 appeared to be the most suitable of the beetroot varieties studied for the production of beetroot chips with respect to nutritional composition, achieving statistically significantly ($p<0.05$) the highest content of both total polyphenols (2756.18±32.30 mg GAE.kg^{-1} DM) and betacyanins (255.96±6.74 mg.kg^{-1} DM). In this cultivar, the lowest decrease in the content of betacyanins (47.11%) and medium decrease in the content of total polyphenols (50.32%) occurred during hot air drying. To increase the stability of these important parameters of the nutritional composition of beetroot chips, it is advisable to treat the beetroot slices with a 0.1% ascorbic acid solution or by blanching in a water bath at 96°C for about 2 minutes before drying.

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