

## Effect of Boiling on Colour, Contents of Betalains and Total Phenolics and on Antioxidant Activity of Colourful Powder Derived from Six Different Beetroot (*Beta vulgaris* L. var. *conditiva*) Cultivars

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Powders prepared from freeze-dried roots of six beetroot (*Beta vulgaris* L.) cultivars with red (cv. D'Egypte, Karkulka, Betina, Alexis), yellow (cv. Burpee's Golden) or red-white (cv. Chioggia) colour of root flesh were evaluated before and after boiling treatment (20 min, 100°C). Water and fat holding capacity (WHC and FHC), colour, contents of betalains and total phenolics (TPCs), and antioxidant activity were all significantly affected by cultivar as well as powder thermal treatment. WHC increased after boiling of the powder and varied between cultivars from 2.14 g/g (cv. D'Egypte) to 2.59 g/g (cv. Chioggia). The highest FHC of 1.22 g/g was observed in cv. Betina. Colour stability was optimal in the cultivars with red root flesh; with their lightness decrease between 6 and 10%. This corresponded with betalain content that was the highest in red cv. Betina; for which the values before and after boiling were 18.10 and 15.04 mg/g DM, respectively. Cultivar Betina significantly exceeded the other cultivars also in TPCs and antioxidant activity. The TPCs values of this cultivar were 3.73 and 3.32 mg gallic acid equivalents/g DM in the unboiled and boiled variant, respectively. Antioxidant activity of Betina powder before and after boiling was 11.13 and 11.69 mg ascorbic acid equivalents/g DM, respectively. The results indicated the significance of beetroot cultivar selection for thermal processing. Beetroots with red flesh significantly exceeded yellow and red-white cultivars in all the evaluated characteristics.

### INTRODUCTION

Beetroot (*Beta vulgaris* L. *conditiva*) is a traditional and important vegetable all over the world that is usually consumed in traditional western cuisine [Nistor *et al.*, 2017; Sawicki *et al.*, 2016; Vasconcellos *et al.*, 2016]. It contains over 80% of water, while such components as saccharides, dietary fiber, nitrogenous substances, and ash prevail in its dry matter (DM) [Vasconcellos *et al.*, 2016; Wruss *et al.*, 2015]. However, consumer interest in beetroot has arisen due to the content of minor compounds implicated in numerous health benefits. These compounds include particularly betalains, phenolic acids, flavonoids, and ascorbic acid, which may increase the total antioxidant capacity of beetroot [Chhikara *et al.*, 2019; Ravichandran *et al.*, 2012; Vasconcellos *et al.*, 2016; Wruss *et al.*, 2015].

Attractiveness of beetroot for consumers is mainly due to the colour of root flesh, which as a cultivar-specific trait,

offers different shades of red and red-violet. Beetroot cultivars with yellow and red-white (alternate red and white rings in cross-section) root flesh colour are well-known (Plant Variety Database – European Commission). Root flesh colour is derived from betalains, being water-soluble nitrogenous pigments having a core structure known as betalamic acid [Ravichandran *et al.*, 2013]. Average content of betalains in beetroot is 0.1 g/100 g of fresh root matter but it can be up to 2 g/kg of fresh matter in some cultivars [Velíšek, 2014]. Betalains consist of two main groups, the red-violet betacyanins (major pigment is betanin) and the yellow betaxanthins (major pigment is vulgaxanthin) [Celli & Brooks, 2017; Sawicki *et al.*, 2016]. They are free radical scavengers and prevent oxygen-induced and free radical-mediated oxidation of biological molecules [Sawicki & Wiczowski, 2018]. However, betalains exhibit also anticarcinogenic [Chhikara *et al.*, 2019; Gengatharan *et al.*, 2015] and antimicrobial activities [Celli & Brooks, 2017]. Stability of betalain pigments is influenced by betalain structure and composition, pH value, water activity, oxygen, light, metal and antioxidant

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presence, and temperature that represents the most important factor [Güneşer 2016].

Beetroot can be consumed as fresh vegetable, beetroot juice, after heat cooking or as a component of, e.g., food products [Ranawana *et al.*, 2016]. Powder derived from red beetroot or beetroot pomace [Porto Dalla Costa *et al.*, 2017] can be applied in bakery [Kohajdová *et al.*, 2018], or meat products [Sucu *et al.*, 2018]. Functional properties, including water and fat holding (absorption) capacity, gelling ability, emulsifying activity and others are important for these types of beetroot application. Changes in these functional characteristics occurring during heat treatment are crucial for the use of beetroot in food applications. The attractiveness of beetroot powder is due to its red colour, pigment content, and total antioxidant activity, all of which can be influenced by heat treatment [Nistor *et al.*, 2017; Ravichandran *et al.*, 2013; Sawicki & Wiczowski, 2018].

The aim of this study was to evaluate the impact of boiling on colour, antioxidant activity, and betalain pigment and phenolic contents in different colourful powders prepared from several beetroot cultivars.

## MATERIAL AND METHODS

### Plant material and beetroot powder processing

Plants of six beetroot (*Beta vulgaris* var. *conditiva*) varieties (Table 1) were cultivated in south-bohemian region (GPS N 49° 39' 56", E 14° 88' 68") under organic crop management in 2018. No mineral fertilizers, pesticides or other chemical preparations were applied during the growing season. Roots of ten average beet plants were used to prepare a freeze-dried powder. Roots were thoroughly washed, dried, and weighed. Afterwards, they were cut into 2-mm thin slices, frozen at -20°C, and subsequently freeze-dried using a freeze-drier (ALPHA 1-4 LSD, Martin Christ, Germany) under a temperature of -50°C, pressure of ≤0.420 mbar, and time period of 72 h. Freeze-dried samples were grinded using a planetary mono mill (Pulverisette 6, Fritsch, Germany) under 450 rpm for 2 min to obtain a beetroot powder.

Colour, functional properties, and antioxidant characteristics were analysed in the beetroot powders before and after powder heat treatment. Two g of the prepared beetroot powders were mixed with 20 mL of deionised water and carefully shaken. These powder mixtures were placed in a water bath with a temperature of 100°C for 20 min (time was measured after reaching the temperature of 100°C in sample interior). After centrifugation (15 min, 3,600×g, and 20°C) and supernatant removal, the beetroot powders were cooled, freeze-dried to constant weight, and pulverised by using a planetary mill (Pulverisette 6) under 450 rpm for 2 min to obtain a boiled variant of the beetroot powder.

### Water solubility and water holding capacity

Water solubility (S) and water holding capacity (WHC) of unprocessed beetroot powders were analysed by weighing 300 mg of powder and mixing with 5 mL of deionised water. The tubes with the mixture were allowed to stand at room temperature for 30 min under continuous shaking. Samples were centrifuged (15 min, 3,600×g, and 20°C). The superna-

tant was discarded and tubes with pellet were weighed, freeze-dried (-50°C, <0.420 mbar, 48 h), and afterwards weighed again. Solubility and water holding capacity were computed from the obtained weight data according to the following formulas (Equations 1–3).

$$S (\%) = 100 * (m_s - m_{lp}) / m_s \quad (1)$$

$$WHC_{\text{powder}} (\text{g/g}) = (m_{wp} - m_{lp}) / m_s \quad (2)$$

$$WHC_{\text{non-soluble part}} (\text{g/g}) = (m_{wp} - m_{lp}) / m_{lp} \quad (3)$$

where: S – a water solubility of beetroot powder DM (%),  $m_s$  – weight of the sample (g),  $m_{lp}$  – weight of the freeze-dried pellet (g),  $WHC_{\text{powder}}$  – water holding capacity of beetroot powder (g of water per g of powder DM),  $m_{wp}$  – weight of the wet pellet (g), and  $WHC_{\text{non-soluble part}}$  – water holding capacity of DM of beetroot powder non-soluble part (g of water per g of non-soluble part DM).

Water solubility and water holding capacity of the heat-treated beetroot powder were determined by the same analytical procedure as described for the unprocessed powders and computed with Equation 1 and Equation 4, respectively.

$$WHC_{\text{non-soluble part after boiling}} (\text{g/g}) = (m_{wpab} - m_{lpab}) / m_{lpab} \quad (4)$$

where:  $WHC_{\text{non-soluble part after boiling}}$  – water holding capacity of non-soluble part of powder after boiling (g of water per g of non-soluble part DM),  $m_{wpab}$  – weight of the wet pellet after boiling (g), and  $m_{lpab}$  – weight of the freeze-dried pellet after boiling (g).

### Fat holding capacity

Fat holding capacity (FHC) was determined analogously as WHC with the following differences: 5 mL of rapeseed oil were used instead of 5 mL of water. Supernatant (oil phase) was thoroughly discarded after centrifugation and tubes with pellet were weighed. The obtained weight of fatted pellet data allowed computing the FHC values (Eq. 5).

$$FHC (\text{g/g}) = (m_{fs} - m_s) / m_s \quad (5)$$

where: FHC – fat holding capacity (g of oil per g of powder DM),  $m_{fs}$  – weight of the fatted pellet (g), and  $m_s$  – weight of the sample (g).

### Colour analysis

Colour of beetroot powder was measured for all evaluated cultivars before and after boiling, using a colorimeter Color-Eye XTH (X-Rite, Grand Rapids, MI, USA) which is based on CIE (Commission Internationale de l'Eclairage) system and provides  $L^*$   $a^*$   $b^*$  parameters ( $L^*$  lightness, 0% – black, 100 % – white;  $a^*$  red – green;  $b^*$  yellow – blue).

### Betalain content

The betalain content was determined spectrophotometrically. Beetroot powder (100 mg) was extracted with 1 mL of 50% ethanol for 30 min. Supernatants were collected after centrifugation (20°C, 3,600×g, 15 min). The extraction pro-

cedure was repeated twice and the final total extract volume was 3 mL. The absorbance of the obtained and diluted (1:50) extracts was read (BioMate 5 spectrophotometer, ThermoFisher Scientific, Waltham, MA, USA) at  $\lambda=540$  nm for betacyanins (BCs) and at  $\lambda=480$  nm for betaxanthins (BXs). The absorbance was used to calculate the betalain, betacyanin, and betaxanthin contents for each sample (Eq. 6).

$$\text{BC or BX (mg/g)} = A_{540/480} \times \text{DF} \times \text{MW} \times V / \varepsilon \times L \times \text{Wd} \quad (6)$$

where: BC or BX – content of betacyanins (BCs) or betaxanthins (BXs) (mg/g),  $A_{540/480}$  – absorbance at absorption maximum for BCs ( $\lambda=540$  nm) or BXs ( $\lambda=480$  nm), DF – dilution factor, MW – molecular weight: 550 g/mol for BCs and 308 g/mol for BXs, V – volume of sample solution (mL),  $\varepsilon$  – the molar extinction coefficient 60,000 1/(M $\times$ cm) for BCs and 48,000 1/(M $\times$ cm) for BXs, L – path length of the cuvette (1 cm), and Wd – weight of the dried sample (g).

### Total phenolic content

The total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu's reagent after previous extraction of phenolics from freeze-dried matter using 80% ethanol with a solid-to-solvent ratio of 1:20 (w/v). The extraction was performed for 24 h under room temperature, and the mixtures were then centrifuged at 3,600 $\times$ g for 10 min, filtered, and subsequently refrigerated at 4°C until analysed. The reaction mixture contained 20  $\mu$ L of the beetroot extract, 1980  $\mu$ L of distilled water, 100  $\mu$ L of Folin-Ciocalteu's reagent, and 300  $\mu$ L of 20% (w/v) sodium carbonate. Absorbance was read at  $\lambda=734$  nm (BioMate 5 spectrophotometer) after 2 h. Gallic acid was used as a standard and the results obtained were expressed as mg of gallic acid equivalent (GAE) per g of sample DM [Hamouz *et al.*, 2006].

### Antioxidant activity

The beetroot powder extracts for determination of the antioxidant activity (AOA) were prepared as described for TPC determination. Antioxidant activity was determined using ABTS and DPPH methods according to Šulc *et al.* [2007] with modifications. Ascorbic acid was used as the standard for both of the methods and results were expressed as mg of ascorbic acid equivalent (AAE) per g of sample DM.

(2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was dissolved in the amount

of 54.9 mg in 20 mL of a 5 mM phosphate buffer (pH 7.4), and BTS radical cations were activated by 1 g MnO<sub>2</sub>. The filtered solution (PTFE 0.25  $\mu$ m) was diluted in a 5 mM phosphate buffer to the absorbance of 0.800 $\pm$ 0.01 at  $\lambda=734$  nm. Absorbance of the reaction mixture (1 mL ABTS<sup>++</sup> solution and 100  $\mu$ L of beetroot extract) was measured at  $\lambda=734$  nm.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical in the amount of 0.025 g was dissolved in 100 mL of methanol to obtain a stock solution. The reaction mixture was prepared by mixing 975  $\mu$ L of 10% (v/v) DPPH<sup>•</sup> stock solution and 25  $\mu$ L of the beetroot extract. Absorbance of the reaction mixture was measured at  $\lambda=515$  nm after 30-min incubation under room temperature.

### Statistical analysis

Data obtained were statistically examined by the one-way (water and fat holding capacity) or two-way (content of betalain pigments, TPCs, antioxidant activity) analysis of variance (ANOVA), and significant differences were determined using the Fisher LSD test at a significance level of  $p<0.05$ . Relations among selected parameters were tested using the correlation analysis. Statistical evaluation was performed with the STATISTICA software, version 12 (StatSoft Inc., USA). Three independent replicates were performed for each evaluated parameter.

## RESULTS AND DISCUSSION

### Functional properties of beetroot powder

Our study was carried out with the group of six beetroot cultivars with different colour of root flesh. The list of the analysed cultivars with their basic characteristics (root flesh colour, weight, and DM content) are shown in Table 1. Average weight of the roots ranged between 202 (cv. Alexis) and 404 g (Burpee's golden), while differences between cultivars in DM content were found to be in a narrow range from 13.3 (cv. Burpee's golden) to 15.0 g/100 g (cv. D'Egypte). Similar DM of beetroots were also found by Szopińska & Gaweda [2013] and Kazimierczak *et al.* [2014].

High WHC values were determined in powders from cultivars Chioggia and Betina (Table 2), *i.e.* 1.88 and 1.81 g/g powder, respectively. However, WHC of these cultivars did not differ significantly from WHC of cultivars Burpee's golden and Karkulka. In contrast, the lowest WHC was determined in the powder from beetroot of cv. D'Egypte. The content

TABLE 1. List of beetroot cultivars and description of root characteristics.

Beetroot cultivar	Country of origin	Root shape	Root flesh colour	Average root weight (g)	Dry matter content (g/100 g)
Alexis	CZ	cylindrical	deep red	202	13.40
Betina	CZ	spherical	deep red	269	13.52
Burpee's Golden	UK	spherical	yellow (golden)	404	13.29
Chioggia	IT	spherical	rings of red and white	379	14.91
D'Egypte	PL (CZ, FR, ES, IT)	flattened	deep red	323	15.03
Karkulka	CZ	cylindrical	deep red	390	14.69

TABLE 2. Functional properties of beetroot powders.

Beetroot cultivars	Water solubility (%)	Water holding capacity (g/g)		Water holding capacity after boiling (g/g)		Fat holding capacity (g/g)
		Powder	Non-soluble part	Powder	Non-soluble part	
Alexis	68.53±2.65 <sup>a</sup>	1.65±0.05 <sup>bc</sup>	4.71±0.09 <sup>c</sup>	2.35±0.10 <sup>bc</sup>	6.21±0.07 <sup>c</sup>	1.09±0.04 <sup>b</sup>
Betina	65.45±0.95 <sup>ab</sup>	1.81±0.01 <sup>ab</sup>	5.24±0.12 <sup>a</sup>	2.59±0.05 <sup>a</sup>	6.91±0.12 <sup>a</sup>	1.22±0.06 <sup>a</sup>
Burpee's Golden	66.75±2.30 <sup>ab</sup>	1.69±0.05 <sup>ab</sup>	5.07±0.06 <sup>ab</sup>	2.23±0.08 <sup>c</sup>	6.41±0.06 <sup>bc</sup>	1.15±0.03 <sup>ab</sup>
Chioggia	64.48±2.36 <sup>b</sup>	1.88±0.13 <sup>a</sup>	5.28±0.28 <sup>a</sup>	2.56±0.18 <sup>ab</sup>	6.60±0.15 <sup>b</sup>	1.13±0.05 <sup>b</sup>
D'Egypte	68.27±1.54 <sup>a</sup>	1.53±0.19 <sup>c</sup>	4.78±0.32 <sup>bc</sup>	2.14±0.07 <sup>c</sup>	6.22±0.16 <sup>c</sup>	1.14±0.05 <sup>ab</sup>
Karkulka	65.76±1.90 <sup>ab</sup>	1.72±0.06 <sup>ab</sup>	5.02±0.10 <sup>abc</sup>	2.29±0.18 <sup>c</sup>	6.24±0.20 <sup>c</sup>	1.14±0.07 <sup>ab</sup>

Means with different subscript letters for the water and fat holding capacity indicate significant differences according to the one-way ANOVA (factor cultivar), Fisher LSD test ( $p < 0.05$ ). Results are shown for the dry matter of powder/non-soluble part of powder.

of water-soluble components varied from 64.48 to 68.53%, depending on cultivar. Increasing the proportion of water non-soluble components resulted in WHC increase before and after powder boiling. The boiling process increased WHC of the powders made of all of cultivars tested. After boiling, the highest WHC values were determined for the powders made of cultivars Betina and Chioggia and reached 2.59 and 2.56 g/g powder, respectively. Differences in WHC after boiling between these two cultivars were not statistically significant ( $p > 0.05$ ).

Similarly, high FHC value was determined in the powder of cv. Betina with a low water solubility. However, the determined FHC values did not differ significantly from these assayed in powders from beetroots of cultivars Burpee's golden, D'Egypte, and Karkulka. The FHC of Betina powder reached 1.22 g/g; the variability between cultivars was from 1.09 (Alexis) to 1.22 g/g (Betina). The WHC and FHC values of wheat powder have been usually determined at 1.1 and 2.5 g/g, respectively [Menon *et al.*, 2015]. Data obtained in our study show beetroot powder to be a material with high values of WHC and FHC; thus suitable as a component of powder mixtures usable in products expected to absorb water and/or fat. Porto Dalla Costa *et al.* [2017] have presented high WHC (mean 10.14 g water/g powder) and FHC (mean 3.3 g/g powder) for beetroot powder prepared from waste pomace. Such a high WHC was probably due to the high contents of insoluble fiber and protein in this type of beetroot material. In agreement with this assumption, we found the highest content of total dietary fiber in powder from cv. Chioggia (11.6% of DM) (not published data of authors). This explains the highest value of WHC and the lowest value of water solubility found in the powder from this beetroot cultivar. The two components, fibers and proteins, are considered to be predominant in the powder ability to retain oil and water [Menon *et al.*, 2015; Porto Dalla Costa *et al.*, 2017], and responsible for increasing viscosity, preventing syneresis, and modifying product's texture [Porto Dalla Costa *et al.*, 2017].

In this context, beetroot powder is a promising additive in food systems that can replace, at least partly, wheat and soybean powders as functional components in products expected to hold up water and/or oils [Kohajdová *et al.*, 2018] or in reduced-energy products [Chau *et al.*, 2004].

### Colour of beetroot powder

The colour of beetroot powder differed significantly among the cultivars ( $p < 0.05$ ) and the colour parameters were significantly influenced by the boiling process (Figure 1). Four tested cultivars had different shades of deep red colour, roots of one cultivar had red-white strips and another one yellow (golden) colour (Table 1). The  $L^*$  parameter values of beetroot powder of the non-boiled samples were (Figure 1) in the range from 46.86 (cv. Alexis) to 84.87 (cv. Burpee's Golden). The values of  $b^*$  colour parameter ranged from 0.40 (cv. Karkulka) to 47.50 (cv. Burpee's golden). Deep red colour was observed in beetroot powder from cultivars D'Egypte, Karkulka, Betina, and Alexis. The beetroot powder made of all cultivars tested had a more intensive colour before than after boiling. Colour parameters of beetroot samples changed after boiling and the  $L^*$  parameter values were from 40.16 (cv. Betina) to 57.78 (cv. Burpee's Golden). The greatest decreases of lightness caused by boiling were found in the powders prepared from Burpee's Golden (decrease by 27%) and Chioggia (decrease by 18.6%) roots. The decrease of  $L^*$  value noted for the root powder from the red-coloured cultivar was only between 6% and 10%. Beetroot characteristic coloration and colour stability during thermal treatment depends on the content and stability of highly active pigments, especially betalains [Guldiken *et al.*, 2016] and carotenoids [Ninfali & Donato, 2013]. Betalains (betacyanins and betaxanthins) may be degraded during heat processing mainly by isomerisation, hydrolysis, decarboxylation, and dehydrogenation to brown and yellow constituents [Bach *et al.*, 2015; Herbach *et al.*, 2004]. The main beetroot betaxanthin, *i.e.* vulgaxanthin I, is more degradable by heat than the main betacyanin such as betanin and isolbetanin [Herbach *et al.*, 2004].

The effect of temperature on the visually-assessed colour of beetroot powder was reported by Bach *et al.* [2015] and Chandran *et al.* [2014]. Chandran *et al.* [2014] observed that temperature had no significant effect on  $L^*$  value, but caused modification in  $a^*$  and  $b^*$  values. With an increase of temperature,  $a^*$  value decreased with a corresponding increase in  $b^*$  values. Similar trends were observed in our experiment with the exception of cv. Burpee's Golden with yellow colour of the original beetroot powder. However, data pre-

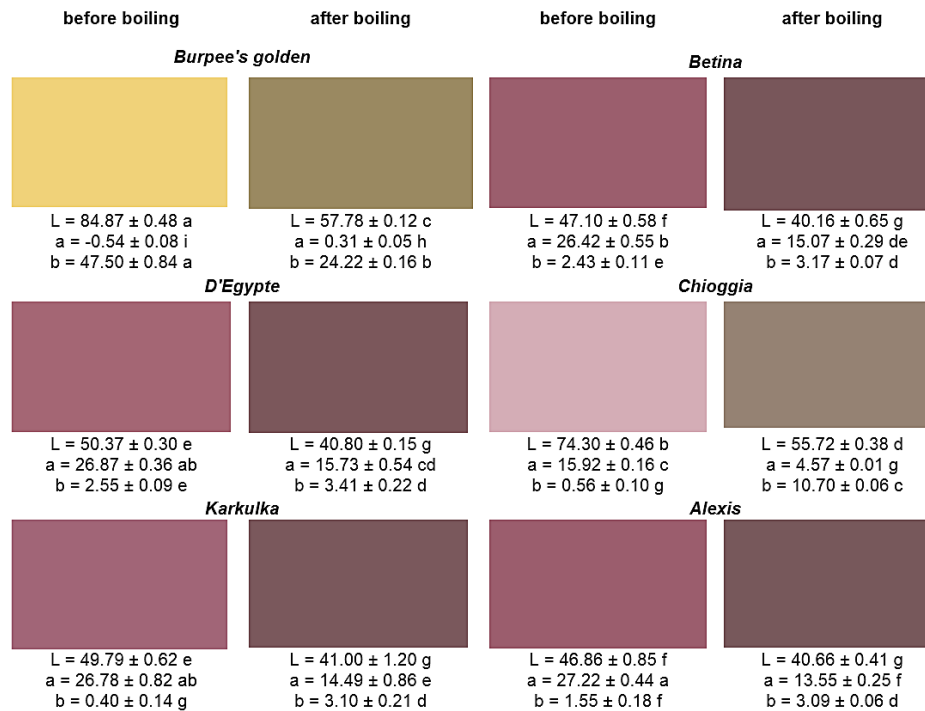


FIGURE 1. Colour of the powder from different beetroot cultivars before and after boiling treatment.

Means with different subscript letters for each colour parameter indicate significant differences according to the two-way ANOVA (evaluated factors: cultivar and sample treatment), Fisher LSD test ( $p < 0.05$ ).

sented by Chandran *et al.* [2014] were based on the analysis of one unknown deep purple cultivar. In turn, Bach *et al.* [2015] evaluated the effect of beetroot cultivar and culinary preparation on colour changes. The decrease of colour intensity was not so extensive in red beetroot cultivars (Taunus, Rosket a Pablo) as in the red-white beetroot cultivar Chioggia. In cv. Burpee's Golden, the authors did not observe such a great decrease of colour intensity as we found in our experiment.

#### Contents of betacyanins, betaxanthin, and betalains

As presented above, betalain content, composition, and thermal stability affect beetroot powder colour before and after boiling. Table 3 collates contents of betacyanins and betaxanthins, the two categories of betalains, in dependence on beetroot cultivar and thermal treatment of beetroot powder. Before boiling, the content of betalains (BLs) in powder of red beetroot cultivars ranged from 10.92 (cv. D'Egypte) to 18.10 (cv. Betina) mg/g DM. Powders of yellow beetroot cultivar Burpee's Golden and red-white cultivar Chioggia contained only 2.07 and 1.38 mg/g DM of BLs, respectively. A relative decrease in betalain content caused by boiling the beetroot powder ranged from 9% to 29%, depending on cultivar.

The content of red-violet pigments – betacyanins (BCs), in the tested powders followed the similar trend as it was found for BLs. The BCs content in beetroot powders of red root cultivars ranged from 6.63 (cv. D'Egypte) to 11.14 mg/g DM (cv. Betina). Red-white cultivar Chioggia and yellow root cultivar Burpee's Golden contained 0.90 and 0.54 mg/g DM of BCs, respectively. Boiling treatment led to a decrease in BCs content in the range from 3 to 28%.

The content of yellow-orange pigments – betaxanthins (BXs), was higher in the powder from the red root cultivars (interval within cultivars was found between 4.29 and 6.95 mg/g DM) than in the powder from the yellow cv. Burpee's Golden (1.53 mg/g DM) and red-white cv. Chioggia (0.48 mg/g DM). The lowest BCs/BXs ratio was demonstrated for the yellow cultivar in both types of powder before and after boiling. The highest ratio indicated the strong supremacy of betacyanins in roots of this cultivar. The BXs level was also reduced by the boiling treatment, the reduction varied between 8% and 36%. The content of BXs in the boiled powder samples varied between 0.44 (cv. Chioggia) and 5.31 mg/g DM (cv. Betina). Data of BCs/BXs indicate the lower thermal stability of BXs in comparison with BCs.

The BLs, BCs, and BXs contents found in our experiment were generally higher than literature data. The total content of BLs was mostly reported within the broad range of 4–17 mg/g DM, depending on the cultivar [Sawicki *et al.*, 2016; Slatnar *et al.*, 2015]. Sawicki *et al.* [2016] described, in a set of 13 red beetroot cultivars, the content of total BLs in the range from 10.26 to 17.15 mg/g DM, the contents of BCs and their derivatives from 7.18 to 13.50 mg/g DM, and BXs content within the range of 2.71–4.25 mg/g DM. The lowest content of betalains was found in red beetroot varieties from Finland (4.43–9.60 mg/g DM), as well as in those grown in the United States (0.65–0.80 mg/g FM) [Lee *et al.*, 2014]. Unfortunately, betalains are highly sensitive to light, heat, and oxygen [Kowalski *et al.*, 2014; Nistor *et al.*, 2017]. The colour and flavour of dried red beetroots are considered to be the most important quality attributes affecting their acceptability by consumers [Nistor *et al.*, 2017; Sorour

TABLE 3. Content of betalain pigments in beetroot powders before and after boiling treatment.

Beetroot cultivars	BC content (mg/g DM)		BX content (mg/g DM)		Betalains (mg/g DM)		BCs/BXs ratio	
	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling
Alexis	10.63±0.39 <sup>a</sup>	8.69±0.07 <sup>c</sup>	6.15±0.23 <sup>b</sup>	4.55±0.03 <sup>d</sup>	16.78±0.62 <sup>b</sup>	13.23±0.10 <sup>d</sup>	1.73	1.91
Betina	11.14±0.32 <sup>a</sup>	9.73±0.51 <sup>b</sup>	6.95±0.21 <sup>a</sup>	5.31±0.28 <sup>c</sup>	18.10±0.52 <sup>a</sup>	15.04±0.79 <sup>c</sup>	1.60	1.83
Burpee's Golden	0.54±0.01 <sup>e</sup>	0.50±0.50 <sup>e</sup>	1.53±0.05 <sup>f</sup>	0.98±0.12 <sup>e</sup>	2.07±0.05 <sup>i</sup>	1.48±0.12 <sup>i</sup>	0.35	0.51
Chioggia	0.90±0.02 <sup>e</sup>	0.72±0.72 <sup>e</sup>	0.48±0.01 <sup>h</sup>	0.44±0.05 <sup>h</sup>	1.38±0.02 <sup>j</sup>	1.16±0.13 <sup>j</sup>	1.88	1.64
D'Egypte	6.63±0.44 <sup>c</sup>	6.42±0.62 <sup>c</sup>	4.29±0.22 <sup>d</sup>	3.51±0.34 <sup>c</sup>	10.92±0.63 <sup>f</sup>	9.93±0.96 <sup>e</sup>	1.55	1.83
Karkulka	7.72±0.17 <sup>d</sup>	5.58±0.66 <sup>f</sup>	4.45±0.10 <sup>d</sup>	3.17±0.39 <sup>e</sup>	12.17±0.27 <sup>e</sup>	8.75±1.05 <sup>h</sup>	1.73	1.76

Means with different subscript letters for contents of betacyanins (BC), betaxanthins (BX), and betalains indicate significant differences according to the two-way ANOVA (evaluated factors: cultivar and sample treatment), Fisher LSD test ( $p < 0.05$ ); DM – dry matter.

TABLE 4. Total phenolic content and antioxidant activity of beetroot powder before and after boiling treatment.

Beetroot cultivar	TPC (mg GAE/g DM)		Antioxidant activity (mg of AAE/g DM)			
			ABTS method		DPPH method	
	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling
Alexis	3.25±0.21 <sup>cb</sup>	3.49±0.13 <sup>ab</sup>	10.58±2.64 <sup>a</sup>	10.7±0.69 <sup>a</sup>	6.77±1.30 <sup>def</sup>	16.90±0.31 <sup>ab</sup>
Betina	3.73±0.43 <sup>a</sup>	3.32±0.21 <sup>cb</sup>	11.13±1.19 <sup>a</sup>	11.69±0.09 <sup>a</sup>	8.68±0.90 <sup>d</sup>	17.59±0.29 <sup>a</sup>
Burpee's Golden	1.47±0.14 <sup>f</sup>	0.93±0.05 <sup>e</sup>	5.65±0.23 <sup>c</sup>	2.75±0.07 <sup>d</sup>	4.90±1.13 <sup>fe</sup>	13.45±0.47 <sup>c</sup>
Chioggia	1.39±0.14 <sup>f</sup>	0.64±0.11 <sup>e</sup>	5.73±0.23 <sup>c</sup>	2.03±0.03 <sup>d</sup>	4.29±0.85 <sup>e</sup>	8.36±2.77 <sup>d</sup>
D'Egypte	2.58±0.93 <sup>d</sup>	2.22±0.10 <sup>e</sup>	7.67±0.63 <sup>b</sup>	7.71±0.24 <sup>b</sup>	6.11±1.06 <sup>efg</sup>	16.44±0.65 <sup>ab</sup>
Karkulka	3.09±0.29 <sup>c</sup>	2.46±0.18 <sup>cd</sup>	8.47±0.66 <sup>b</sup>	7.88±0.18 <sup>b</sup>	7.96±0.52 <sup>de</sup>	15.22±1.25 <sup>bc</sup>

Means with different subscript letters for total phenolic content (TPC) and antioxidant activity indicate significant differences according to the two-way ANOVA (evaluated factors: cultivar and sample treatment), Fisher LSD test ( $p < 0.05$ ); GAE – gallic acid equivalent; AAE – ascorbic acid equivalent.

*et al.*, 2014]. However, the effect of cultivar on the betalain pigments and colour stability has not been described widely. Herbach *et al.* [2004] described novel yellow neobetanin structures and two orange-red betanin degradation products in thermally-treated (85°C) red beet juice explaining the yellow-orange shift of thermally-treated red beet powder. Nistor *et al.* [2017] similarly found a decreasing level of betacyanins and betaxanthins with increasing drying temperatures in beetroot material of cv. *Cylindria* and also confirmed betalains heat sensitivity.

#### Total phenolic content and antioxidant activity

Antioxidant activity was evaluated by the ability of beetroot powder extracts to scavenge ABTS<sup>•+</sup> and DPPH<sup>•</sup> and was expressed as the equivalent of ascorbic acid. The extracts of all the cultivars demonstrated a strong potential to scavenge both radicals (Table 4). These results indicated that DPPH radicals could be strongly scavenged by degradation products released in beetroot powder during boiling (substances modification, fragmentation, *etc.*). This conclusion was confirmed by high correlation coefficients determined using DPPH and betalains characteristics in the boiled variant (Table 5). Both, ABTS<sup>•+</sup> and DPPH<sup>•</sup> are suitable for evaluating the antioxidant activity

in individual items of fruit, vegetable and beverages; however, the DPPH method was presented as more suitable for the determination of the antioxidant activity of hydrophobic compounds [Kedare & Singh, 2011]. The results indicated that the shift of hydrophobicity of beetroot components after thermal degradation probably resulted in increased solubility of degradation products and their enhanced reactivity with DPPH radicals. Sawicki & Wiczowski [2018] did not find a significant decrease of the antioxidant activity in red beet samples after boiling when the ABTS method was employed, however, if they used the DPPH method, the level of antioxidant activity was significantly lower. Nevertheless, the study of Sawicki & Wiczowski [2018] was made with only one cultivar (*Czerwona Kula*), while our results indicate a significant effect of cultivar on betalain content, stability, and antioxidant activity. According to data presented by Sawicki & Wiczowski [2018], boiling and fermentation of beetroot material increased the content of betanidin (aglycon of betanin), that showed activity in both aqueous and lipid bilayers [Slimen *et al.*, 2017]. This fact could be responsible for the observed increase in the antioxidant activity of the boiled beetroot samples determined with the DPPH method.

TABLE 5. Correlation coefficients between betalain content or TPC and antioxidant activity of beetroot powders before and after boiling treatment.

	Before boiling	After boiling
BC content vs. AOA ABTS method	0.869***	0.986***
BC content vs. AOA DPPH method	0.794***	0.802***
BX content vs. AOA ABTS method	0.864***	0.987***
BX content vs. AOA DPPH method	0.806***	0.841***
BL content vs. AOA ABTS method	0.871***	0.988***
BL content vs. AOA DPPH method	0.801***	0.816***
TPC vs. AOA ABTS method	0.812***	0.982***
TPC vs. AOA DPPH method	0.831***	0.818***

\*\*\* correlation is significant at  $p < 0.001$ ; BC – betacyanins; BX – betaxanthins; BL – betalains; TPC – total phenolic content; AOA – antioxidant activity.

The antioxidant activity determined with ABTS<sup>•+</sup> and DPPH<sup>•</sup> before boiling varied between cultivars from 5.65 (cv. Burpee's Golden) to 11.13 mg AAE/g DM (cv. Betina) and from 4.29 (cv. Chioggia) to 8.68 mg AAE/g DM (cv. Betina), respectively. In turn, after boiling, the cultivar variability of antiradical activity against ABTS<sup>•+</sup> and DPPH<sup>•</sup> was between 2.03 (cv. Chioggia) and 11.69 mg AAE/g DM (cv. Betina) and between 8.36 (cv. Chioggia) and 17.59 mg AAE/g DM (Betina), respectively. The lowest values of the antioxidant activity were observed for the yellow and red-white cultivars Burpee's Golden and Chioggia in all evaluated variants. This indicates the significant effect of betalain content on the total antioxidant activity of both raw beetroot powders and beetroot products after thermal treatment. The highest content of betalains was determined in cv. Betina (Table 3). This cultivar had the highest antioxidant activity in both the treatment variants of the experiment. In general, the antioxidant activity of beetroot products is mostly defined as the same or the higher for thermally-processed variants in comparison with the original samples by most of the other authors [Gokhale & Lele, 2011; Ravichandran *et al.*, 2012, 2013]. On the contrary, Vasconcellos *et al.* [2016] detected with the HPLC method about 10% reduction in the antioxidant activity of the samples of boiled red beetroot as compared with the freeze-dried ones. In the study of Gokhale & Lele [2011], the increased antioxidant activity found after beetroot thermal treatment was ascribed to a higher extractability of betaxanthin and total phenolic compounds. This conclusion was not confirmed in our study. The increase in the antioxidant activity found with the DPPH method is rather caused by degradation products because no increase was observed in BLs and TPCs after boiling. Our results concerning the significant effect of the cultivar on components exhibiting the antioxidant activity confirm data of Sawicki *et al.* [2016] who observed a similarly broad range of antioxidant activities with both ABTS and DPPH methods for the extracts of 13 red beetroot cultivars. These authors also

observed a positive correlation between the antioxidant activity determined with ABTS and DPPH methods and total betalain and betacyanin content, which corresponds with our results presented in Table 5. The correlations between the antioxidant activity and contents of BCs, BXs, BLs, and TPCs were statistically significant and very similar in all of the comparisons. The highest correlation coefficients were found between antioxidant compound contents and results determined with the ABTS method for beetroot powders after boiling. Close correlations were also found between the content of total phenolics and antioxidant activity determined with ABTS and DPPH methods. Phenolic compounds belong to important beetroot antioxidant components [Chhikara *et al.*, 2019; Guldiken *et al.*, 2016], concentrated mainly in the peel and the crown [Kujala *et al.*, 2002]. In our study, TPCs ranged from 2.58 to 3.73 mg GAE/g DM and below 1.5 mg GAE/g DM in the powder from red root cultivars and in the powder prepared from roots with yellow and red-white colour of root flesh, respectively. The highest content was found in the cultivar Betina, while the lowest in the red-white cultivars Chioggia and Burpee's Golden. This corresponded again with the content of betalains and total antioxidant activity of beetroot powder. The TPCs was significantly lower ( $p < 0.05$ ) after boiling in all the cultivars except for cultivar Alexis. The most intensive decrease of TPC was observed in cultivars with non-red colour of root flesh. Similarly, Guldiken *et al.* [2016] reported TPC values of 2.55 and 2.38 mg GAE/g FM for raw and boiled beetroot variants, respectively. However, the analyses were given for only one cultivar and on fresh weight basis. On the contrary, Vasconcellos *et al.* [2016] found TPC at 0.75 and 2.79 mg GAE/g DM for freeze-dried slices of red beetroot and for the boiled variant (100°C, 40 min), respectively. In generally, the content of total phenolics of the vegetables is significantly affected by cooking style. Results obtained by other authors [Naveena *et al.*, 2016; Wen *et al.*, 2010] indicated that phenolic compounds were sensitive to heat treatment, but Wen *et al.* [2010] and Raikos *et al.* [2018] reported that different types of the cooking process differently affected various vegetables and some combinations of vegetable species/cultivars and heat processing increased their phenolic contents while others decreased them. This phenomenon could be explained by direct interactions between phenolics and some food components or food matrix such as fibre, fat, proteins or carbohydrates, affecting their phenolic content and bioaccessibility [Şengül *et al.*, 2013].

## CONCLUSIONS

The obtained data indicated a significant effect of cultivar on betalain content of beetroot powders that varied for boiled and raw variant from 1.38 to 18.10 and from 1.16 to 15.04 mg/g DM, respectively. Interestingly, the content of betaxanthins was significantly lower in the powders from the red-white cultivar Chioggia and yellow cultivar Burpee's Golden compared to the powders from the red-coloured beetroot cultivars. The thermal treatment of beetroot powder resulted in a decrease of both betacyanin and betaxanthin contents, but also total phenolic content and antioxidant activity (except for the antioxidant activity of cv. Alexis) when assessed

with the ABTS method. Interestingly, the use of the DPPH method resulted in a higher antioxidant activity determined in the boiled variant of beetroot powder in comparison with the non-boiled one. This indicates higher sensitivity of DPPH radical to degradation products exhibiting the antioxidant activity. Degradation of beetroot pigments during boiling treatment resulted in the shift of beetroot powder colour that was obvious especially for the yellow cultivar Burpee's Golden and red-white cultivar Chioggia. Beetroot powder before boiling was more intensive in all the evaluated cultivars. A decrease in  $L^*$  parameter (lightness) was lower for cultivars Betina and Alexis. Especially the cultivar Betina with a high antioxidant activity, total phenolic and betalain contents, but also water and fat absorption and colour stability was found promising for thermal processing. The obtained data confirmed the importance of beetroot cultivar not only for their chemical composition but also for their suitability to thermal processing and subsequent use.

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### CONFLICT OF INTEREST

Authors declare no conflict of interest.

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