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Research article

Potential of a new beetroot cultivar 'Śnieżna kula' (*Beta vulgaris* L. ssp.)

Katarzyna Gościnna*, Elżbieta Wszelaczyńska and Jarosław Poberężny

Institute of Food Technology, Faculty of Agriculture and Biotechnology, University of Science and Technology, 85-225 Bydgoszcz, Poland

*** Correspondence**: Email: katarzyna.goscinna@utp.edu.pl; Tel: +48523749388.

Abstract: Beetroot is a vegetable known since antiquity, valued due to its high pro-health potential resulting from the high content of betalaine pigments, mainly betanin. Due to the allergenic nature of this compound, there may be some of the restrictions concerning red beetroots consumption. This study analysed the physicochemical characteristics of a new white beetroot cultivar 'Śnieżna Kula' ('*Snowball*'), which can become an alternative to red beetroot. The effects of the size and particular parts of the roots were assessed on betalain pigment, reducing sugar, total sugar, phenolic compounds, nitrate (III) and nitrate (V) contents, and on the antioxidant capacity (expressed as a FRAP and ABTS value). The mass of the beetroots of the 'Śnieżna Kula' cultivar varied and ranged from 279 to 1118 g. The analysed beetroot is devoid of red betalain pigments. The total sugar content was the highest for group B (401–600 g) with an average of 33.5 mg kg^{-1} FW. The roots with a less weight, i.e. those of groups A contained their greater amount phenolic compounds and chlorogenic acid (415 mg kg^{-1} FW i 279 mg kg^{-1} FW, respectively). A the same time, less roots contained more nitrates (V), ranging from 3 301 to 3 534 mg kg^{-1} FW. The inner parts of the root contained more nitrates (III) and (V), while the outer parts of the roots were richer in polyphenols and chlorogenic acid. The antioxidant capacity was correlated with polyphenol and chlorogenic acid contents. The roots for group A (<400 g) the bottom under leaf part and the bottom under leaf part proven antioxidant capacity respectively 17.5; 16.3 mmol Fe^{2+} kg^{-1} FW (FRAP) and 8.50; 8.40 mmol Trolox kg^{-1} (ABTS).

Keywords: Beet; white root; betalain pigments; nitrate (V); antioxidant capacity

1. Introduction

The beetroot (*Beta vulgaris* L. ssp.) is a vegetable that is particularly popular in the central and eastern parts of Europe, which is due to its palatability and high levels of health-promoting

compounds with antioxidant capabilities [1,2]. The highest activity in free radical capture is attributed to betanin [3–5]. Red beetroot betalain extract, consisting mostly of betanin (E162), which is widely used as a natural colorant in many dairy products, beverages, candies and cattle products. However, this compound can cause allergies and for this reason some of the restrictions concerning consumption by children have been introduced. Following a request from the European Commission, the European Food Safety Authority (EFSA) has submitted an opinion regarding the safety usage of beetroot red/betanin (E 162) in Foods for Special Medical Purposes (FSMP) for young children aged 1–3 years [6]. According to EFSA the safe content of betanin in FSMP is 20 mg L^{-1} of the final product. In the root vegetable group, the beetroot is among those exhibiting a high tendency to accumulate nitrates (V) [7]. Nitrates associate negatively due to N-nitrosamines, which are formed indirectly from nitrates (V) in the acidic environment of the stomach. However, they can perform important and beneficial functions in the human body, associated with the formation of nitric oxide (NO) [4]. This compound can reduce blood vessel tone, inhibit platelet aggregation, and improve the physical performance of the organism [8,9]. Therefore, it is reasonable to look for an alternative to red beetroot. A possible solution could be the use of beetroot in food processing, free of allergenic betanin, and containing lower amounts of harmful compounds. Two beetroot cultivars with a distinctive white root colour are known: 'Albina Vereduna' originating from the Netherlands, and 'Śnieżna Kula' bred at Torseed SA in Toruń, Poland. The Polish cultivar 'Śnieżna Kula' is the world's first non-GMO beetroot cultivar registered in the European Union. A characteristic feature of the beetroot with white-coloured flesh is the lack of betalain pigments found in the flesh of purple and red beetroot [10,11]. Beetroot is by far the best vegetable in terms of antioxidant compounds [12–15]. Moreover, the beetroot is a very good source of carbohydrates, protein, dietary fibre, organic acids (citric, oxalic, malic, and tartaric), folic acid and many minerals [1,14,16,17].

It is known that the most important tool in shaping the properties and quality of a food product is the appropriate selection of raw material. In food processing, the whole raw material is usually used, however, to increase the health-promoting value of the final product, one should consider the use of its parts. Therefore, conducting research in this area is fully justified and needed.

The aim of the study was to determine the physicochemical characteristics of the white beetroot cultivar 'Śnieżna Kula' in terms of selected nutrient and harmful component contents and its antioxidant capacity that determine its pro-health value. The characteristics were determined, depending on the size of the root and its parts.

2. Materials and methods

2.1. Materials

The beetroots were obtained from a cultivation carried out at a farm in Pędzewo near Bydgoszcz, and sown mechanically on 29 May 2016 on soil of the 3rd soil valuation class. Mineral fertilisation was applied (Korn-kali at 400 kg/ha, ammonium phosphate at 150 kg ha^{-1}, and urea at 230 kg ha^{-1}) as well as two herbicidal treatments: Betanal Maxx Pro 209OD (desmedifam 47 g L^{-1}, etofumesat 75 g L^{-1}, lenacyl 27 g L^{-1}, fenmedifam 60 g L^{-1}; Bayer SAS, France) at 1.2 l ha^{-1} and Goltix S 700 SC (metamitron 700 g L^{-1}; ADAMA Sp. z o.o., Poland) at 1.0 l ha^{-1}. After the harvest, representative samples were placed in a modern storage chamber with a constant temperature (+1 °C) and 98% relative air humidity.

2.2. Chemicals

Urea, acetic acid, sodium nitrate, sodium hydroxide and phosphate buffer were purchased from Merck KGaA, Darmstadt, Germany, and CA from Sigma–Aldrich Co., LLC, USA.

2.3. Preparation of samples

Washed raw material was divided into four size groups: *A*—roots weighing < 400 g (an average of 279 g); *B*—roots weighing 401–600 g (an average of 488 g); *C*—roots weighing 601–900 g (an average of 737 g); and *D*—roots weighing > 901 g (an average of 1118 g). Within each group, the roots were cut transversely to the axis into four parts: the head with the upper hypocotyl part (*G*) and three hypocotyl parts—the upper (*GCP*), middle (*ŚCP*), and lower (*DCP*) (Figure 1) [18]. The *G* part accounted for 11.4% of the entire root, *GCP* for 39.9%, *ŚCP* for 39.4%, and *DCP* for 9.3%. The separated parts of the beetroots, as well as the entire roots, were then used to extract juice using a Zelmer ZJE 1200G (power—500W, speed—1200 rpm) juice extractor (ZELMER, Poland).

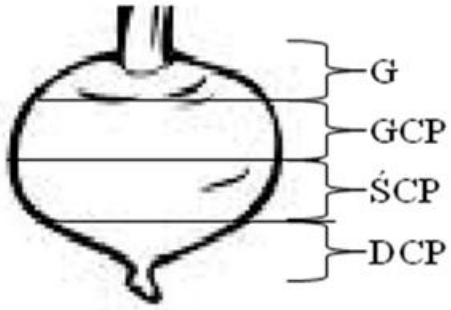


Figure 1. The allocation of red beet root into parts: G—head with the upper under leaf part, GCP—upper under leaf part, SCP—the central under leaf part, DCP—the bottom under leaf part [18].

2.4 Laboratory analysis procedure

The plant material was purified manually (using for a knife) and foreign substances (which included soil and dust particles) were then removed under running cold water and plant tissue samples were reduced to a 0.5 to 1.0 cm size to ensure uniformity. Raw roots were then cut into 1-cm-thick slices, frozen in a Whirlpool AFG 6402 E-B freezer (Italy) to −22 ℃ and freeze-dried (CHRIST ALPHA 1–4 LSC, Germany) in order to achieve a permanent weight. The final moisture content of the material was below 2%. Operational parameters of the lyophiliser: Condenser temperature of −55 ℃, vacuum 4 kPa at 20 ℃. The drying was carried out for 24 hours. Freeze-dried samples were then ground into flour using an electric grinder (power-800W, speed-24000 rpm, time-30 s) (CHEMLAND, Type FW 177, Poland) and were then used for a chemical analysis. The obtained flour samples were then stored in sealed plastic bags at −20 ℃ before analysis.

2.5. Total soluble solid

The determination of the total soluble solid content was performed using a PAL-1 digital camera (Atago, Japan) refractometer at 20 ℃.

2.6. Betalain pigment content

Betalain pigments were determined by the spectrophotometric method using a Shimadzu UV-1800 spectrophotometer (UV Spectrophotometer System, Japan) according to Nillson, which involved the determination of purple and yellow [19]. The purple pigment content was expressed as betanine, and the yellow pigment content as vulgaxanthin. Prior to the absorbance measurement, the sample was diluted with a phosphate buffer (0.2 N) with pH of 6.5 so that the absorbance value fell within the range of 0.2–0.8. The blank test was a phosphate buffer. Absorbance was measured at the layer thickness of 1 cm and at wavelengths of 476, 538, and 600 nm.

2.7. Phenolic compounds content

The assay was carried out using the method developed by Fang et al. [20]. The method involves the measurement of absorbance of the complex formed from the reaction between polyphenols and the tungsten and molybdenum reagent (Folin-Ciocalteu reagent). The assay was conducted as follows: 200 μL of a suitably diluted sample was transferred to a test tube and 800 μL of water was added. After thorough mixing, 5 mL of 0.2 N Folin-Ciocalteu reagent was added to the test tube and mixed again. After 3 minutes, 4 mL of sodium carbonate was added (75 g L^{-1}). The prepared samples were then incubated in a dark room for two hours. Absorbance was measured at the layer thickness of 1 cm and at a wavelength of 735 nm. The polyphenol content was calculated based on the standard curve prepared for chlorogenic acid.

2.8. Chlorogenic Acid content

The CA—chlorogenic acid content was determined colorimetrically by the method of Griffiths et al. [21]. Briefly, the diluted extract was vortexed with 2 mL of urea (0.17 M) and acetic acid (0.10 M). To this, 1 mL of sodium nitrite (0.14 M) was added, followed by 1 mL of sodium hydroxide (0.5 M) after incubation at room temperature for 2 min. The suspension was then centrifuged (Hettina Zentrifugen, Rotina 420 R, Germany) at 2250 g for 10 min. An aliquot of the supernatant was taken and the absorbance of the cherry red complex formed was read at 510 nm (UV-1800, UV Spectrophotometer System, Japan). A standard curve was prepared using different concentrations of CA and the results were expressed as mg of CA kg^{-1} of fresh beetroot.

2.9. Sugar content

Reducing sugars were assayed using the Talburt and Smith [22] method. 2 g of freeze-dried sample was mixed with 150 mL of distilled water and shaken vigorously for 60 minutes. The flask was then made up with distilled water to 250 mL and mixed for 3 minutes. The mixture was then filtered through Whatman No.1 filter paper. 1 mL of the filtrate and 3 mL of the DNP reagent were

transferred to a test tube and heated in a water bath at 95 ℃ for 6 minutes. After the heating, the samples were immediately cooled to room temperature. The absorbance of the mixture was measured at a wavelength of 600 nm using a Shimadzu UV-1800 (UV Spectrophotometer System, Japan). Reducing sugar content was then estimated using a glucose standard curve.

The total soluble carbohydrate content was determined following sugar hydrolysis. 40 mL of the filtrate was transferred to an Erlenmeyer flask and a few drops of concentrated HCl were added. The samples were heated for 30 minutes in a water bath, then cooled and neutralised with a few drops of concentrated NaOH. 1 mL of the filtrate was mixed with 3 mL of the DNP reagent, and the procedure for determining reducing sugar content was then followed [22].

2.10. Nitrate (III) and (V) content

Nitrate and nitrite contents were determined using the ion-selective method [23]. For the assay, a multi-functional ELMETRON CX-721 device equipped with a nitrate electrode, a double junction reference electrode (fill outer chamber with 0.02 M $(NH_4)_2SO_4$ solution; Merck, Germany), a specific ion meter and a pH millivolt^{-1} (mV) meter with a 0.1 mV readability was used. Nitrates (V) were extracted using the $KAl_2(SO_4)_3$ solution (Merck, Germany) and determined potentiometrically using the ion-selective electrode. The determination limit was established at 30 mg kg^{-1}, and the measurement error was at a level of 15% (k = 2, norm.), depending on the sample matrix that was measured.

2.11. Assessment of the antioxidant capacity (FRAP)

The determination of the antioxidant capacity by the FRAP method was conducted using the method developed by Benzie and Strein [24]. Immediately prior to the assay, a FRAP working solution was prepared. 250 mL of acetate buffer with pH of 3.6, 25 mL of the TPTZ solution (2,4,6-Tri(2-pyridyl)-s-triazine (10 millimoles in 40 mmol HCl), and 25 mL of an iron(III) chloride hexahydrate solution (20 mmol) were mixed. The solution was incubated at 37 ℃ and assays were then performed. 6 mL of the FRAP solution was taken, and 200 μL of the sample and 600 μL of H_2O were added to it. After 4 minutes from the addition of the sample, absorbance was measured at a wavelength of 593 nm. Based on the conducted measurements, a curve of dependence of the absorbance value on the juice concentration was plotted. Based on the curve, the absorbance value was determined at a concentration equal to the mean of the dilutions used, and the antioxidant capacity was calculated at the same absorbance value based in the standard curve determined for Fe^{2+} iron ions. In order to remove solid parts, the samples prior to the assays were centrifuged for 5 minutes on a Rotina 420R centrifuge (Hettich, Germany) at 3,000 revs min^{-1}. All assays were carried out in three laboratory replications.

2.12. Assessment of the antioxidant capacity (ABTS)

To determine the antioxidant capacity, the $ABTS^{+\bullet}$ cation radical [2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate] method was employed according to Re et al. [25]. A stock solution of the $ABTS^{+\bullet}$ radical cationwas generated chemically by mixing 7 mM ABTS solution (Sigma Aldrich) and 2.45 mmol $K_2S_2O_8$ solution (POCH Gliwice), mixed in a ratio of 1:0.5. This

mixture was allowed to stand for 12 h in the dark. On the day of analysis 0.5 mL of $ABTS^{+\bullet}$ radical cation stock solution was mixed with 2 mL of phosphate buffer (pH 7.4) in a cuvette and the absorbance at 734 nm wasmeasured. For the measurement, four different sample dilutions were prepared so that the reduction in cation radical absorbance fell within the range of 20–80%. The measurement was performed as follows: 50 μL of the sample was added to 5 mL of diluted cation radical, shaken and incubated in a water bath at 30 ℃ for 6 minutes. After that time, the absorbance was measured using a Shimadzu UV-1800 (UV Spectrophotometer System, Japan), at a wavelength of 734 nm, against phosphate buffer. The decrease in absorbance caused by theaddition of Trolox as the standard was measured by the same pro-cedure for each concentration of Trolox (1–15 mmol kg^{-1}) and thecalibration curve for the decrease in absorbance. Trolox concentration was constructed by linearregression. The results were provided expressed as mmol Trolox kg^{-1}, having taken into account the dilution of samples.

2.13.Statistical analysis

The results were statistically processed using STATISTICA 13.0 software. An analysis of statistical differences was carried out using ANOVA variance analysis followed by Tukey's test, at a significance level $\alpha = 0.05$. The linear correlation coefficient between the beetroots quality characteristics was investigated at $P < 0.01$ and $P < 0.05$. The results were presented as arithmetic means with standard deviations (SD).

3. Results and discussion

The total soluble solid in the examined white beetroot cultivar ranged from 158 g kg^{-1} for *DCP* to 161 g kg^{-1} fresh weight (FW) for *G*, and was significantly dependent on the size group (Table 1). The lowest total soluble solid was noted for the roots of group *D*, and the highest for the roots of group *A*. This indicates the occurrence of a relationship between the total soluble solid and the size of the root. With an increase in the weight of the roots, the extract content decreased (Table 1). The differences between the values were statistically significant. Similar total soluble solids are noted for the red beetroot. As reported by Biegańska-Marecik et al. [26] the total soluble solid in red beetroot varies, and may range from 120 to 180 g kg^{-1} FW. For food processing, a higher extract content in the root is preferable, as it affects the density of beet juice.

Table 1. Content of total extract in the white beetroot cv. 'Śnieżna Kula' depending on the size and weight of beetroots.

Weight group	Average root weight [g]	Total soluble solid [mg kg^{-1} FW]	Betalain pigments [mg kg^{-1} FW]
A	279 ± 42^{a}	161.1 ± 0.2^{D}	0.0
B	488 ± 65^{b}	160.1 ± 0.2^{C}	0.0
C	737 ± 92^{c}	159.2 ± 0.3^{B}	0.0
D	1118 ± 56^{d}	158.1 ± 0.1^{A}	0.0

Note: Means sharing the same letter in column are not significantly different from each other (Tukey's significant difference test, $P < 0.05$). Data are the averages ($n = 16$). Means sharing the same letter in column are not significantly different from each other ($P \leq 0.05$): a, b…—within the root portion; A, B...—within the mean size for a given group.

The results of the current study demonstrated that the roots of white-coloured beetroot contain no pigment, unlike the red beetroot which contains both purple and yellow pigments (Table 1). According Bean et al. [27] white beets make betalains but accumulate very low levels of betalain pigmentation compared to red beets. Their amounts vary greatly from 400 to 2100 mg kg^{-1} FW for purple pigments, and from 200 to 1400 mg kg^{-1} FW for yellow pigments [19], and is significantly determined by the size, shape, and part of the root [28–31].

The total sugar and reducing sugar contents in the white beetroot averaged 32.3 and 1.7 mg kg^{-1} FW, respectively. In the red beetroot, the contents of these compounds are found at a significantly higher level, and are mainly determined by the cultivar. For the conventionally cultivated 'Czerwona Kula' cultivar, the total sugar content amounted to 40.0 mg kg^{-1} FW, reducing sugar content to 19.7 mg kg^{-1} FW, and for the 'Regulski' cultivar, it was 49.3 and 16.8 mg kg^{-1} FW, respectively [14]. Jabłońska-Ceglarek and Rosa [30] determined the total sugar and reducing sugar contents to be 164 and 7.4 mg kg^{-1} FW, respectively, for the 'Opolski' cultivar. On the other hand, Wruss et al. [31], having tested seven cultivars, obtained a total sugar content ranging from 62.0 mg kg^{-1} FW for the 'Egyptische Plattrunde' cultivar to 92.0 mg kg^{-1} FW for the 'Forono' cultivar and reducing sugar content from 1.64 mg kg^{-1} FW for the 'Redval' cultivar to 5.89 mg kg^{-1} FW for the 'Egyptische Plattrunde' cultivar. The sugar content of the beet roots is also determined by the cultivation system [32]. The authors obtained a total sugar content ranging from 126 mg kg^{-1} FW in conventional cultivation to 143 mg kg^{-1} FW in organic cultivation. As it results from the difference in the content of total sugars and monosaccharides (Table 1), the dominant compound in all 4 parts of the beet root is sucrose. Glucose and fructose were present in smaller amounts, because theroot is the storage organ of plants and energy in beetroots is storedin the form of sucrose [33]. Sucrose is delivered by the phloem to the mostdistant root tips and, en route to the tip, is used by the different root tissues formetabolism and storage [34]. The study demonstrated a relationship between the total sugar and simple sugar contents and the size of the root (Table 2). For total sugars, significant differences were demonstrated between the size groups *B* and *D*, and their content ranged from 30.7 mg kg^{-1} FW for group *D* to 33.5 mg kg^{-1} FW for group *B*. On the other hand, reducing sugar content was the lowest for group *B* with an average of 1.2 mg kg^{-1}, and the highest for group *A*, (2.3 mg kg^{-1}). No effect of the part of the root on the total sugar content was proven in any of the size groups (Table 1). On the other hand, for reducing sugars, significantly higher contents were noted for the parts *G* and *DCP* in each size group (Figure 2a), earmarking them to be used in the production of special-purpose food.

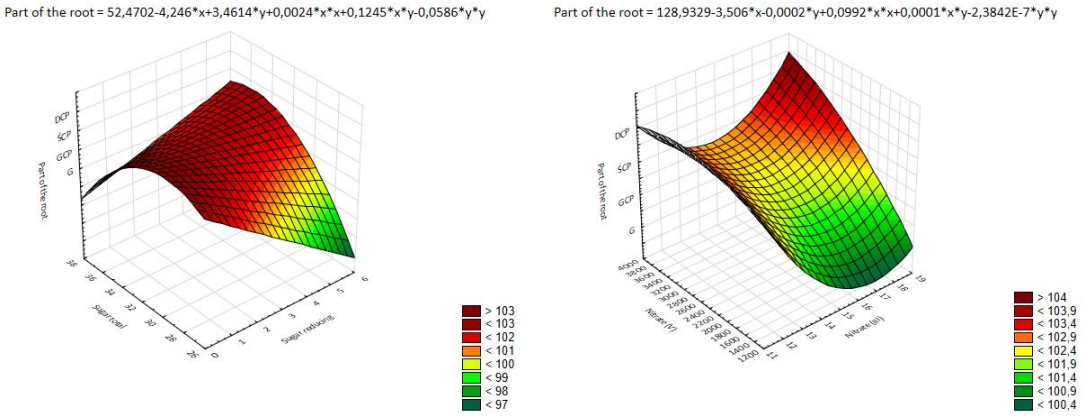
The nitrate (V) content of the beetroots of the 'Śnieżna Kula' cultivar varied and ranged from 2.29 mg kg^{-1} FW of the juice from the roots of the size group C to 3.53 mg kg^{-1} FW of the juice from the roots of size group A (Table 2).

Vasconcellos et al. [35] obtained nitrate (V) content of up to 12253 mg kg^{-1} in red beet juice. Other studies found a large variation in the nitrate (III) and (V) contents for the same vegetable species. The nitrate (V) content in beetroots originating from Estonia was 1446 mg kg^{-1} [36], 3.046 mg kg^{-1} in beetroots from Iran [37] and 4900 mg kg^{-1} [38] and 1306 mg kg^{-1} for beetroots from Poland [39].

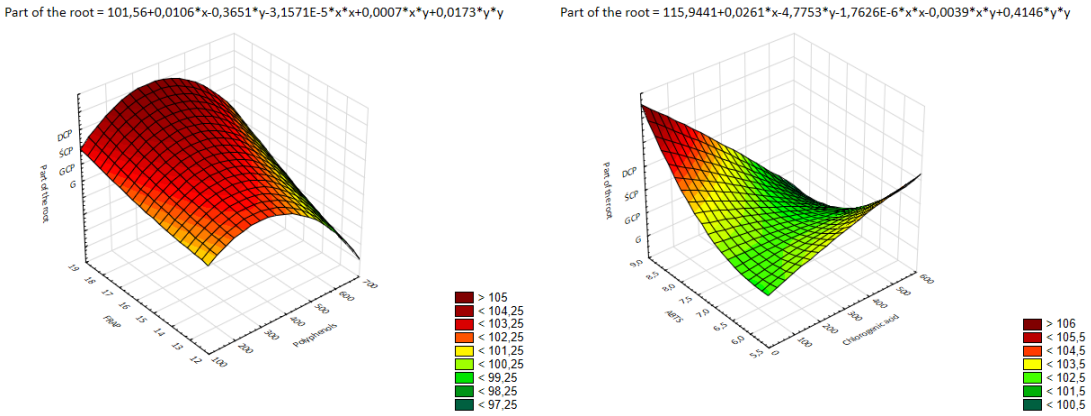
Table 2. Content of sugars and nitrogen compounds in root the white beetroot cv. 'Śnieżna Kula' depending on the size and part of the roots.

Weight group [g]	Part of the root	Reducing sugar [mg kg^{-1} FW]	Total sugar [mg kg^{-1} FW]	Nitrate (III) [mg kg^{-1} FW]	Nitrate (V) [mg kg^{-1} FW]
A < 400	*G*	4.66 ±0.90^{hi}	32.4 ±3.6^{bcd}	13.8 ±0.9^{abcd}	2375 ±36^{e}
	GCP	0.80 ±0.60^{abc}	34.1 ±6.6^{ad}	15.3 ±0.4^{bcdef}	3777 ±29^{k}
	ŚCP	0.60 ±0.36^{ab}	29.5 ±6.3^{ab}	14.6 ±1.0^{abcdef}	3692 ±33^{k}
	DCP	5.02 ±0.42^{i}	33.7 ±2.4^{cd}	13.4 ±1.2^{abc}	3244 ±25^{hi}
	Total root*	2.25 ±0.59^{B}	32.2 ±2.3^{AB}	14.7 ±0.6^{A}	3534 ±24^{D}
B 401–600	*G*	4.01 ±0.54^{h}	33.5 ±3.3^{cd}	12.6 ±0.8^{ab}	1825 ±40^{b}
	GCP	0.60 ±0.60^{ab}	33.8 ±1.8^{cd}	13.6 ±0.4^{abcd}	3476 ±25^{j}
	ŚCP	0.44 ±0.42^{a}	33.5 ±2.4^{cd}	16.8 ±0.7^{ef}	3550 ±19^{j}
	DCP	3.05 ±1.35^{fg}	32.1 ±3.0^{bcd}	12.5 ±1.2^{a}	3308 ±21^{i}
	Total root	1.15 ±0.52^{A}	33.5 ±1.5^{B}	14.6 ±0.8^{A}	3301 ±31^{C}
C 601–900	*G*	3.17 ±0.99^{g}	32.7 ±3.3^{bcd}	14.4 ±1.0^{abcde}	1746 ±14^{b}
	GCP	0.79 ±0.87^{abc}	33.4 ±1.5^{cd}	16.9 ±1.7^{ef}	2328 ±18de
	ŚCP	1.39 ±0.72^{cd}	32.9 ±2.1^{bcd}	17.1 ±0.6^{ef}	2605 ±23^{f}
	DCP	1.78 ±0.93^{de}	30.5 ±1.2^{abc}	12.6 ±0.7^{ab}	1460 ±35^{a}
	Total root	1.39 ±0.83^{A}	32.9 ±1.9^{AB}	16.3 ±1.1^{AB}	2290 ±21^{A}
D > 901	*G*	2.37 ±0.51^{ef}	33.2 ±3.9^{cd}	16.2 ±0.6^{def}	2027 ±30^{C}
	GCP	1.30 ±0.60^{bcd}	28.3 ±3.0^{a}	16.0 ±0.5^{cdef}	3188 ±34^{h}
	ŚCP	1.81 ±0.42^{de}	32.0 ±1.8^{bcd}	17.2 ±0.9^{f}	2845 ±37^{g}
	DCP	1.97 ±0.48^{de}	33.0 ±2.1^{cd}	13.3 ±0.8^{abc}	2245 ±33^{d}
	Total root	1.69 ±0.51^{A}	30.7 ±1.2^{A}	16.2 ±0.7^{B}	2833 ±35^{B}

Note: The results for the total root were presented as a weighted average, taking into account the average percentage share of the individual parts in the total root. Means sharing the same letter in column are not significantly different from each other ($P \leq 0.05$): a, b…—within the root portion; A, B...—within the mean size for a given group. Means sharing the same letter in column are not significantly different from each other (Tukey's significant difference test, $P < 0.05$). Data are the averages (n = 16). *G*—head with the upper under leaf part, *GCP*—upper under leaf part, *SCP*—the central under leaf part, *DCP*—the bottom under leaf part (Figure 1).



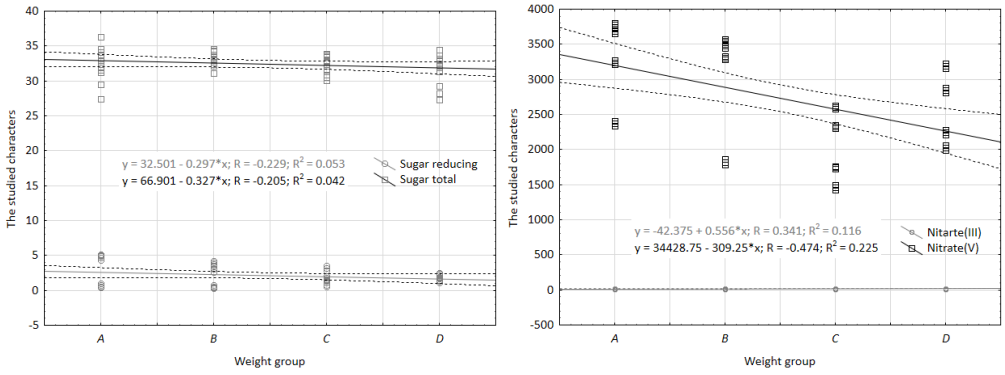
a) b)



c) d)

Figure 2. The significant relationship between the date of a) sugar reducing, sugar total, b) nitrate (V), nitrate (III), c) polyphenols, FRAP d) chlorogenic acid, ABTS and part of root.

Ziarati et al. [37] observed a reversed relationship between the vegetable size and the nitrate (V) content. The authors demonstrated higher nitrate (V) contents in smaller roots of the beetroot, which is in line with the authors' own study results R = −0.474 (Figure 3b).



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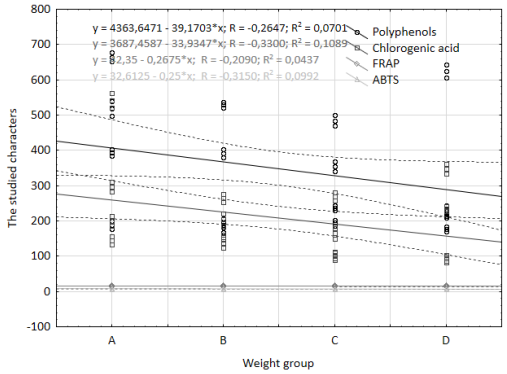

c)

Figure 3. The significant relationship between the date of a) sugar reducing, sugar total, b) nitrate (V), nitrate (III), c) polyphenols, chlorogenic acid, FRAP, ABTS and weight group.

For the nitrate (III) content, a reverse tendency was observed, as the roots with a greater weight, i.e. those of groups *C* and *D* contained their greater amount (16.3 and 16.2 mg kg^{-1}, respectively). Ziarati et al. [37] observed a reversed relationship between the vegetable size and the nitrate (III) content. Different tendencies were observed for nitrates (III), for which higher values were noted for the roots with a higher weight, i.e. for the size groups *C* and *D* (16.3 and 16.2 mg kg^{-1}, respectively) (Table 2).

Nitrate (V) and nitrate (III) values were higher for the inner parts of the roots (*GCP* and *ŚCP*) than for the outer parts (*G* and *DCP*) (Figure 2b). This sugests that the outer parts of the root are more useful for processing health-promoting food. Similar results were obtained by Czapski et al. [28] for the red beetroot. This is due to the fact that the vascular tissue contains more NO_3 than the parenchymal tissue, since NO_3^- in the form of mineral salts are transported from the soil with water via the vascular tissue [36]. A low ($P \leq 0.05$) but significant correlation was observed between reducing sugars and total polyphenols and nitrates (III) and (V) (Table 4).

The total polyphenol content varied greatly (from 178 to 665 mg kg^{-1} FW) and was determined by the size and part of the root (Table 3). The high total polyphenol content was also determined by Vasconcellos et al. [35] in red beet juice. On the other hand, no effect of the increase in the root weight on the content of these compounds was noted (Figure 3c). In size groups *A* and *D*, the highest content was noted for the part *G* (665 and 625 mg kg^{-1} FW) and in the size group *C* for the part *DCP* (485 mg kg^{-1} FW) (Figure 2c).

As regards chlorogenic acid, which is classified as a phenolic compound, the average content for the white beetroot amounted to 188 mg kg^{-1} FW. In studies by Kazimierczak et al. [14], chlorogenic acid content in the beetroots amounted, on average for the cultivars, to 60 mg kg^{-1} FW, and on average for various cultivation systems, to 52 mg kg^{-1} FW. Therefore, the chlorogenic acid content in the analysed beetroot of the 'Śnieżna Kula' cultivar was three times higher than in the red beetroots. Carrillo et al. [40] claim that the chlorogenic acid content of red beets ranges from 27.9 to 279.8 mg kg^{-1} FW. It was also demonstrated that the roots belonging to group A had the highest chlorogenic acid content. In each size group, chlorogenic acid was present in the greatest amounts in the outer parts of the roots (*G* and *DCP*) (Figure 2d). The literature on the subject shows that in the roots of the red beetroot, the highest polyphenol content is found in the skin and the part adjacent to the skin; the deeper into the root, the lower the polyphenol content [15]. Own study results confirm this relationship, as the skin-to-flesh ratio is the highest in the head and the lower hypocotyl part.

For the white beetroot, the antioxidant capacity averages 14.4 mmol Fe^{2+} kg^{-1} FW (FRAP) i 6.7 mmol Trolox kg^{-1} FW (ABTS) (Table 3). As demonstrated by Czapski et al. [3] and Wruss et al. [31], the antioxidant capacity is determined by the purple and yellow pigment contents of the roots. For the 'Śnieżna Kula' cultivar, the absence of betalain pigments resulted in significantly lower FRAP values, which were determined by the content of phenolic compounds ($R = 0.809$, $P \leq 0.05$), including chlorogenic acid ($R = 0.728$, $P \leq 0.05$). At the same time, a highly positive correlation was demonstrated between the total phenolic compound content and the chlorogenic acid content (0.791) (Table 4). As reported by Czapski et al. [37] and Carrillo et al. [40], the antioxidant capacity of the red beetroot is cultivar-specific. In those studies, the antioxidant capacity, measured using the FRAP and ABTS methods, averaged on the level from 63.7 to 276 mmol Fe^{2+} L^{-1} and from 21.1 to 146.0 mmol Trolox L^{-1} juice, respectively.

Table 3. Antioxidant capacity and polyphenols and chlorogenic acid content in root the white beetroot cv. 'Śnieżna Kula' depending on the size and part of the roots.

Weight group [g]	Part of the root	Polyphenols [mg kg^{-1} FW]	Chlorogenic acid [mg kg^{-1} FW]	FRAP[mmol Fe^{2+} kg^{-1} FW]	ABTS [mmol Trolox kg^{-1} FW]
A < 400	*G*	665 ± 13^{f}	542 ± 20^{i}	17.5 ± 1.2^{d}	8.50 ± 0.33^{e}
	GCP	189 ± 11^{a}	145 ± 12^{c}	13.7 ± 0.6^{ab}	6.55 ± 0.16^{ab}
	ŚCP	394 ± 10^{d}	200 ± 14de	14.5 ± 0.8^{abc}	6.70 ± 0.27^{ab}
	DCP	519 ± 20^{e}	297 ± 14^{g}	16.3 ± 1.3^{bcd}	8.40 ± 0.16^{de}
	Total root	415 ± 16^{B}	279 ± 13^{C}	14.7 ± 0.8^{B}	7.00 ± 0.22^{A}
B 401–600	*G*	529 ± 17^{g}	204 ± 9^{e}	15.3 ± 0.0^{abcd}	7.14 ± 0.37^{abc}
	GCP	188 ± 20^{a}	148 ± 12^{c}	14.6 ± 0.6^{abc}	6.76 ± 0.29^{ab}
	ŚCP	183 ± 14^{a}	139 ± 14^{bc}	13.6 ± 1.1^{ab}	6.44 ± 0.48^{ab}
	DCP	391 ± 11^{d}	265 ± 12^{fg}	15.8 ± 1.0^{abcd}	7.25 ± 0.36^{bcd}
	Total root	243 ± 12^{A}	199 ± 14^{B}	14.4 ± 0.2^{B}	6.68 ± 0.38^{AB}
C 601–900	*G*	355 ± 12^{d}	269 ± 14^{fg}	14.6 ± 1.2^{abc}	6.45 ± 0.58^{ab}
	GCP	195 ± 8^{ab}	103 ± 9^{ab}	13.3 ± 0.4^{a}	6.22 ± 0.48^{ab}
	ŚCP	238 ± 8^{c}	100 ± 10^{a}	14.4 ± 0.5^{abc}	6.39 ± 0.37^{ab}
	DCP	485 ± 14^{e}	165 ± 14^{cd}	16.8 ± 1.3^{cd}	8.33 ± 0.25^{de}
	Total root	257 ± 9^{A}	136 ± 10^{A}	14.2 ± 0.6^{A}	6.51 ± 0.43^{A}
D > 901	*G*	625 ± 19^{e}	348 ± 13^{h}	16.0 ± 1.4^{abcd}	8.05 ± 0.74^{cde}
	GCP	178 ± 7^{a}	94 ± 10^{a}	13.6 ± 1.1^{ab}	6.34 ± 0.17^{ab}
	ŚCP	214 ± 5^{abc}	94 ± 6^{a}	14.0 ± 0.4^{abc}	6.28 ± 0.36^{ab}
	DCP	235 ± 11^{bc}	234 ± 7^{ef}	14.9 ± 0.8^{abcd}	6.11 ± 0.25^{a}
	Total root	248 ± 8^{A}	136 ± 9^{A}	14.2 ± 0.8^{A}	6.53 ± 0.32^{A}

Note: The results for the total root were presented as a weighted average, taking into account the average percentage share of the individual parts in the total root. Means sharing the same letter in column are not significantly different from each other ($P \leq 0.05$): a, b…—within the root portion; A, B...—within the mean size for a given group. Means sharing the same letter in column are not significantly different from each other (Tukey's significant difference test, $P < 0.05$). Data are the averages (n = 16). *G*—head with the upper under leaf part, *GCP*—upper under leaf part, *SCP*—the central under leaf part, *DCP*—the bottom under leaf part (Figure 1).

The conducted study demonstrated that the FRAP and ABTS ranged respectively from 14.2 mmol Fe^{2+} kg^{-1} and 6.5 mmol Trolox kg^{-1} for the juice extracted from the size groups *C* and *D* to 14.7 mmol Fe^{2+} kg^{-1} and 7.0 mmol Trolox kg^{-1} for the juice extracted from the size group *A* (Table 3). However, based on statistical analysis, it was found that the antioxidant capacity was not significantly correlated with the root size $R = -0.209$ (FRAP) $R = -0.315$ (ABTS) (Figure 3c). Reverse results were obtained for the red beetroot of the 'Wodan' cultivar, for which the antioxidant capacity significantly decreased with an increase in the weight of the root; it ranged from 226 mmol Fe^{2+} kg^{-1} for the roots with an average weight of 220 g to 314 mmol Fe^{2+} kg^{-1} of the juice from the roots with an average weight of 170 g [28]. It should be noted, however, that red beetroot cultivars have a considerably lower average weight of the roots than the white beetroot, and the values provided by Czapski et al. [28] concern the root weight included in group *A* for the white beetroot.

Table 4. The correlation coefficients (R) between the studied characters beetroot cv. 'Śnieżna Kula'.

	Sugar reducing	Sugar total	Nitrate (III)	Nitrate (V)	Polyphenols	Chlorogenic acid	FRAP	ABTS
Sugar reducing		0.119	**−0.397**	**−0.506**	**0.648**	**0.726**	**0.697**	**0.459**
Sugar total			0.211	0.095	0.034	0.221	0.219	−0.218
Nitrate (III)				0.234	**−0.389**	**−0.480**	**−0.384**	**−0.590**
Nitrate (V)					**−0.461**	−0.243	−0.321	−0.214
Polyphenols						**0.791**	**0.809**	**0.662**
Chlorogenic acid							**0.728**	**0.555**

Note: Bold indicates that the correlation is significant at the 0.01 probability level.

4. Conclusions

The beetroot of the 'Śnieżna Kula" cultivar is characterised by high contents of phenolic compounds, including chlorogenic acid. It also exhibits a high antioxidant capacity (FRAP). It was found that the roots of the white beetroot with a lower weight have significantly higher sugar and total polyphenol (including chlorogenic acid) contents. At the same time, as for the red beetroot, attention should be paid to sustainable cultivation, including the application of nitrogen fertilisation to prevent excessively high nitrate (III) and (V) contents. Based on the conducted study, it was found that the beetroot of the 'Śnieżna Kula' cultivar, despite the absence of betalain pigments typical of red beetroots, is a vegetable that should be recommended for direct consumption and food processing.

Conflict of Interest

All authors declare no conflict of interest in this paper.

References

1. Szaefer H, Krajka-Kuźniak V, Ignatowicz, et al. (2014) Evaluation of the effect of beetroot juice on DMBA-induced damage in liver and mammary gland of female Sprague-dawley rats. *Phytother Res* 28: 55–61.
2. Clifford T, Howatson G, West D, et al. (2015) The potential benefits of red beetroot supplementation in health and disease. *Nutrients* 7: 2801–2822.
3. Czapski J, Mikołajczyk K. Kaczmarek M (2009) Relationship between antioxidant capacity of red beet juice and contents of its betalain pigments. *Pol J Food and Nutr Sci* 59: 119–122.
4. Betancourt C, Cejudo-Bastante MJ, Heredia FJ, et al. (2017) Pigment composition and antioxidant capacity of betacyanins and betaxanthins fractions of Opuntia dillenii (Ker Gawl) Haw cactus fruit. *Food Res Inter* 101: 173–179.

5. Indumathi D, Sujithra K, Srinivasan S, et al. (2017) Protective effect of betanin against streptozotocin-nicotinamide induced liver, kidney and pancreas damage by attenuating lipid byproducts and improving renal biomarkers in Wistar rats. *Int J Adv Res Biol Sci* 4: 160–170.
6. EFSA (2016) Journal Safety of the proposed extension of use of beetroot red (E 162) in foods for special medical purposes in young children. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *EFSA Journal* 14: 4487.
7. Cui Y, Li X, Xu L, et al. (2017) Nitrite contents in fresh vegetables of different families and genus. In: IOP Conference Series Materials Science and Engineering 275: 012015.
8. Bryan NS, Alexander DD, Coughlin JR, et al. (2012) Ingested nitrate and nitrite and stomach cancer risk: An updated review. *Food Chem Toxicol* 50: 3646–3665.
9. Lundberg JO, Weitzberg E (2013) Biology of nitrogen oxides in the gastrointestinal tract. *Gut* 62: 616–629.
10. Nemzer B, Pietrzkowski Z, Spórna A, et al. (2011) Betalainic and nutritional profiles of pigment–enriched red beet root (Beta vulgaris L.) dried extracts. Original Research Article. *Food Chem* 127: 42–53.
11. Mereddy R, Chan A, Fanning K, et al. (2017) Betalain rich functional extract with reduced salts and nitrate content from red beetroot (Beta vulgaris L.) using membrane separation technology. *Food Chem* 215: 311–317.
12. Guine RP, Goncalves F, Lerat C, et al. (2018) Extraction of Phenolic Compounds with Antioxidant Activity from Beetroot (Beta vulgaris L.). *Curr Nutr Food Sci* 14: 350–357.
13. Sentkowska A, Pyrzyńska K (2018) Zwitterionic hydrophilic interaction liquid chromatography coupled to mass spectrometry for analysis of beetroot juice and antioxidant interactions between its bioactive compounds. *LWT* 93: 641–648.
14. Kazimierczak R, Hallmann E, Treščinska V, et al. (2011) Estimation of the nutritive value of two red beet (Beta vulgaris L.) varieties from organic and conventional cultivation. *J Res Appl Agric Eng* 56: 206–210.
15. Kujala TS, Loponen JM, Klika KD, et al. (2000) Phenolics and betacyanins in red beetroot (Beta vulgaris) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. *J Agric Food Chem* 48: 5338–5342.
16. Wootton-Beard PC, Ryan L (2011) A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. *J Funct Foods* 3: 329–334.
17. Latorre ME, De Escalada Plá MF, Rojas AM, et al. (2013) Blanching of red beet (Beta vulgaris L. var. conditiva) root. Effect of hot water or microwave radiation on cell wall characteristics. *Food Sci Techno* 50: 193–203.
18. Gościnna K, Czapski J (2017) Effect on the size and parts of beetroot roots on health promoting compounds and antioxidant capacity juice. *Zeszyty Problemowe Postępów Nauk Rolniczych* 588: 149–157. (in Polish)
19. Nilsson T (1970) Studies into the pigments in beetroot (Beta vulgaris L. ssp. vulgaris var rubra L). *Lantbrukshogskolans Annaler* 36: 179–197.
20. Fang Z, Zhang M, Su Y, et al. (2006) How to improve bayberry (Myrica rubra Sieb et Zucc) juice color quality: Effect of juice processing on bayberry anthocyanins and polyphenolics. *J Agricul Food Chem* 54: 99–106.

21. Griffiths DW, Bain H, Dale MFB (1992) Development of rapid colorimetric method for the determination of chlorogenic acid in freeze-dried potato tubers. *J Sci Food Agricul* 58: 41–48.
22. Talburt W, Smith O (1987) *Potato processing* 1987, (No 6648 T3 1987) New York: Van Nostrand Reinhold, 1–796.
23. Baker WH, Thompson TL (1992) Determination of nitrate-nitrogen in plant samples by selective ion electrode. Southern cooperative series bulletin #368. Plant analysis reference procedures for the southern region of the United States 5: 23–26.
24. Benzie IF, Strain JJ (1996) The Ferric Reducing Ability of Plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* 239: 70–76.
25. Re R, Pellegrini N, Proteggente A, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26: 1231–1237.
26. Biegańska-Marecik R, Czapski J, Błaszczyk P (2007) Determination of cultivar and technological process effect on the occurrence of bitter taste in red beet. *Food Sci Technol Qual* 14: 62–70. (in Polish)
27. Bean A, Sunnadeniya R, Akhavan N, et al. (2018) Gain-of-function mutations in beet DODA 2 identify key residues for betalain pigment evolution. *New Phytologist* 219: 287–296.
28. Czapski J, Gościnna K, Kidoń M, et al. (2011) Beetroot juice. Influence of the weight and part of the beet root on the juice characteristics. *Food Industry* 65: 50–52. (in Polish)
29. Gościnna K, Czapski J, Mikołajczyk-Bator K, et al. (2012) Content betalain pigments, nitrates and antioxidant capacity of beetroot juices depending on cultivars and the size of beetroot roots. *Aparatura Badawcza i Dydaktyczna* 17: 85–90. (in Polish)
30. Jabłońska-Ceglarek R, Rosa R (2003) Influence of green manures on the quantity and quality of the yield of red beet. *Acta Sci Pol Hortorum Cultus* 2: 21–30. (in Polish)
31. Wruss J, Waldenberger G, Huemer S, et al. (2015) Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper. *Austria J Food Comp Anal* 42: 46–55.
32. Kazimierczak R, Hallmann E, Lipowski J, et al. (2014) Beetroot (Beta vulgaris L.) and naturally fermented beetroot juices from organic and conventional production: metabolomics, antioxidant levels and anticancer activity. *J Sci Food Agricul* 94: 2618–2629.
33. Bach V, Mikkelsen L, Kidmose U, et al. (2015) Culinary preparation of beetroot (Beta vulgaris L.): The impact on sensory quality and appropriateness. *J Sci Food Agr* 95: 1852–1859.
34. Van Die J (1962) The distribution of carbohydrates. *Acta Botanica Neerlandica* 11: 418–424.
35. Vasconcellos J, Conte-Junior C, Silva D, et al. (2016) Comparison of total antioxidant potential, and total phenolic, nitrate, sugar, and organic acid contents in beetroot juice, chips, powder, and cooked beetroot. *Food Sci Biotechnol* 25: 79–84.
36. Tamme T, Reinik M, Roasto M, et al. (2006) Nitrates and nitrites in vegetables and vegetable-based products and their intakes by the Estonian population. *Food Addit Contam* 23: 355–361.
37. Ziarati P, Arbabi-Bidgoli S (2014) Investigation of cooking method on nitrate and nitrite contents in crops and vegetables and assess the associatied health risk *J Plant Anim Environ Sci* 4: 46–50.

38. Bahadoran Z, Mirmiran P, Ghasemi A, et al. (2015) Is dietary nitrate/nitrite exposure a risk factor for development of thyroid abnormality? A systematic review and meta-analysis. *Nitric Oxide* 47: 65–76.
39. Raczuk J, Wadas W, Głozak K (2014) Nitrates and nitrites in selected vegetables purchased at supermarkets in Siedlce. *Poland Annals Nat Inst Hyg* 65: 15–20.
40. Carrillo C, Wilches-Pérez D, Hallmann E, et al. (2019) Organic versus conventional beetroot. Bioactive compounds and antioxidant properties. *LWT* 116: 108–552.

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