

## Article

# Evaluation of Proximate Composition, Mineral Elements and Bioactive Compounds in Skin and Flesh of Beetroot Grown in Lithuania

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**Abstract:** In the world, red beetroot is regarded as one of the most important vegetables due to its valuable nutritional features; however, the industrial processing of beetroot produces large amounts of waste, such as skin, which could be a relevant source of bioactive compounds, minerals, fiber, and so on. In this study, the variations in the proximate composition, mineral element amounts, and some antioxidants in the skin and flesh of beetroot genotypes grown in Lithuania were appraised. Proximate compositions (total soluble solids, dry matter, fiber, protein, ash, and total sugars), amounts of some minerals (N, P, Ca, Mg, K, Fe, Zn, Cu, B, and Mn), total phenolics, and total anthocyanins and betalains were determined. The results revealed that proximate composition, minerals, total phenolics, and total anthocyanins and betalains depends on the root part and genotype of the beetroot. All investigated beetroot skin samples have significantly greater amounts of protein, dry matter ash, fiber, total sugars, minerals (except K), total phenolics, and total anthocyanins and betalains than the flesh. ‘Alto F1’ skins had the highest amounts of protein, fiber, ash, Na, Mg, and Zn. ‘Kosak’ skins contained the greatest amounts of dry matter, total sugars, K, P, Ca, Fe, Mn, and total anthocyanins and betalains. It can be concluded that the tested beetroot skins (especially ‘Alto F1’ and ‘Kosak’), due to their valuable nutritional compositions, can be used as a source of natural supplements that can enrich the quality of various food products or be used for the manufacture of functional food.

**Keywords:** anthocyanins; beetroot skin; betalains; fiber; phenolics; potassium; iron; sugars



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## 1. Introduction

Red beetroot (*Beta vulgaris* L.) is a traditional and popular vegetable that is consumed worldwide due to its high amounts of bioactive compounds (polyphenols, betalains, inorganic nitrates, folates), mineral elements, and vitamins [1]. This vegetable is widely grown in Central and Eastern Europe, Asia, and America [2]. Today, beetroot is used predominantly for food (fresh, after fermentation, or after thermal processing) [3,4]; moreover, in the food industry, beetroot is used as an additive or food colorant as it improves the red color of various food products [5]. This crop is among the ten vegetables with the highest antioxidant activity, largely due to its betalains and phenolic compound content [6]. Betalains are water-soluble pigments that contain nitrogen. They are separated into two structural groups: yellow–orange pigments (betaxanthins) and red–violet pigments (betacyanins). The ratio of betacyanins to betaxanthins determines the intensity of beetroot colour [7,8]. Betalains are antioxidant and antiradical agents that can protect against disorders caused by oxidative stress. The functions of these compounds relate to the reduction of homocysteine levels, which regulate vascular homeostasis, vascular tone, and thrombotic activity while maintaining platelet function [9]. Other compounds present in this vegetable are fiber, proteins, and sugars with low energetic values [10]. Additionally, red beetroot includes crucial minerals that are beneficial to human health, including iron, zinc, phosphorus, potassium, calcium, magnesium, manganese, and copper [5]. The chemical

composition and nutritional value of red beetroot differs depending on the genotype, the growth conditions, and the anatomical part of the plant (stem, leaf, root, flesh, skin) [11]. The industrial processing of beetroot produces a large amount of waste, which causes nutritional and economic losses, as well as negative environmental impacts [12]. Usually, beetroot waste includes skin, parings, and stalks [13]; however, this waste is a relevant source of natural pigments, biologically active substitutes, mineral elements, and fiber that can be used to develop pharmaceutical products, functional and novel food products, as well as other beneficial compounds for industries [14,15]. It is crucial to continue using beetroot byproducts to cut down on made waste. Some studies have indicated that beetroot waste, such as skins, could be used as a source of natural supplements that can enrich the quality of various food products, such as mayonnaise [16], fish [17], and meat [18]. According to the literature, the chemical composition of beetroot skin is poorly covered in the literature [19]. Some reports have shown that the skin of the beetroot contains more betalains and phenolic compounds than the flesh [20]; however, limited studies were performed to establish the distribution of proximate composition and mineral elements in different parts of beetroot. Therefore, the aim of the study was to establish the mineral and proximate compositions and investigate the bioactive compounds in the skin and flesh of four red beetroot genotypes grown in Lithuania.

## 2. Materials and Methods

### 2.1. Materials

The following red beetroot (*Beta vulgaris* L.) varieties and hybrids were selected for the investigations: 'Kosak', 'Alto F1', 'Tanus F1', and 'Pablo F1'. All these beetroot genotypes are later maturing. Using standard agronomic practices, red beetroots were cultivated in 2021 at a farm in the Širvintų district (Lithuania, latitude, 55.038° N; longitude, 24.715° E). The beetroot seeds were sown in the middle of May. They were harvested at maturity during the first week of October.

### 2.2. Total Soluble Solids and Dry Matter Analysis

Dry matter and total soluble solids were analysed in fresh beetroot flesh and skin samples. Dry matter was determined by drying samples at 105 °C to a constant weight [21]. The amount of total soluble solids was measured using the refractometry method with a digital pocket refractometer (Atago, Kobe, Japan) [22]. The results were expressed as Brix.

### 2.3. Sample Preparation for Fiber, Ash, Proteins, Total Sugars, Macro- and Micro-Elements Determination

The fresh beetroots were washed and carefully peeled manually (0.5–1 mm thick) with a standard hand-held vegetable peeler. The beetroot flesh was also separated and cut into slices with a thickness of approximately 1 cm. The beetroot flesh and skins were oven-dried at a temperature of 60 °C for 12 h and then pulverized in a Grindomix GM 200 laboratory mill (Retsch GmbH, Haan, Germany). The obtained powder was packed in plastic bags and kept in the dark until analyses were carried out.

#### 2.3.1. Fiber, Ash, Proteins and Totals Sugars Analysis

The amount of fiber from dried beetroot flesh and skin was determined by the Association of Official Agricultural Chemists (AOAC) official methods, the amount of ash was determined by combustion at 550 °C [23], and the amount of proteins was determined by the Kjeldahl method using KJELDATHERM (Gerhardt, Königswinter, Germany). The total sugar content was established based on the Luff Schoorl method [23]. The results of the total sugar content were recalculated in fresh matter (FM).

#### 2.3.2. Macro- and Microelement Analysis

The elements of calcium (Ca), potassium (K), phosphorus (P), iron (Fe), magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), and boron (B) were measured in dried

beetroot flesh and skin. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to identify these elements [24]. Beetroot skin and flesh samples were treated with hydrogen peroxide and nitric acid at 100 °C until digestion was complete in order to mineralize them before ICP-AES testing. A total of 1 g of beetroot sample was mixed with known quantities of nitric acid and hydrogen peroxide and then heated. After digestion, the resulting solution was examined using an Optima 2100 DV atomic-emission spectrometer (Perkin Elmer, Waltham, MA, USA). The appropriate standards for each mineral were made within the concentration range of the mineral elements in the beetroot (skin and flesh) samples. Standard blank solutions were prepared in the same conditions as the samples. The amount of N (nitrogen) was determined using the Kjeldahl method with a KJELDATHERM (Gerhardt, Königswinter, Germany). All chemical analyses were performed in triplicate. The results for N, Ca, K, P, and Mg were expressed in mg 100 g<sup>-1</sup> DM, while the results for Fe, Zn, B, Mn, and Cu were expressed in mg kg<sup>-1</sup> DM.

#### 2.4. Sample Preparation for Bioactive Compounds Determination

The fresh beetroots were washed extensively with water to remove all dirt and sand. After that, they were carefully peeled (0.5–1 mm thick) with a common hand-held vegetable peeler. Additionally, the beetroot flesh was separated and divided into slices with a thickness of about 1 cm. The flesh and skin were frozen at –20 °C until lyophilisation. Then, they were lyophilized using the freeze-dryer Sublimator (ZIRBUS Technology GmbH, Bad Grund, Germany). Using a lab mill, the Grindomix GM 200 (Retsch GmbH, Haan, Germany), the freeze-dried beetroot flesh and skin samples were ground into a fine powder. The obtained powder was vacuum-packed and kept until analyses were carried out.

##### 2.4.1. Total Phenolic Amount Analysis

By using the Folin–Ciocalteu spectrophotometric method, as reported by Tamilselvi et al. [25], the total phenolic amount of freeze-dried beetroot flesh and skin was determined. A total of 0.1 g of freeze-dried beetroot sample was mixed with 10 mL of 70% ethanol and extracted for 30 min in an ultrasonic laboratory bath AU-65 (Argolab, Torino, Italy). After that, the extract was centrifuged at 3000 rpm for 30 min. The obtained extract was then combined with 1 mL of sodium carbonate (20%), 0.2 mL of the Folin–Ciocalteu reagent, and 5 mL of pure water. A spectrophotometer (Labomed Inc., Los Angeles, CA, USA) was used to measure the absorbance at 760 nm after 30 min of incubation at 20 °C in the dark. The total phenolic content was calculated with the calibration curve using gallic acid equivalent standards. The results for the total phenolic content were expressed as mg of gallic acid equivalent (GAE) per g of DM.

##### 2.4.2. Total Anthocyanins Amount Analysis

The total anthocyanin amount of freeze-dried beetroot flesh and skin samples was determined using the pH differential method with a spectrophotometer (Labomed Inc., Los Angeles, CA, USA) [26]. A total of 0.1 g of beetroot sample was dissolved in 10 mL of solvent (85 % ethanol + 015% 1 M HCl). The obtained extract was extracted in an ultrasonic laboratory bath AU-65 (Argo lab, Torino, Italy) for 10 min, then centrifuged at 3500 rpm for 10 min. After that, 9 mL of pH 1.0 buffer solution were combined with 1 mL of the filtered extract; the same steps were repeated by dilution with 9 mL of pH 4.5 buffer solution. The absorbance at 510 nm and 700 nm was measured after 30 min of incubation at room temperature. For quantification of total anthocyanins, the molecular weights (449 g/mol) and molar extinction coefficients (26,900 M<sup>-1</sup> cm<sup>-1</sup>) were applied. The results were given in mg of cyanidin-3-glucoside per kg of dry matter.

##### 2.4.3. Betalains Analyses

The amount of betalains in freeze-dried beetroot flesh and skin was identified by spectrophotometric method, as outlined by Ravichandran et al. [27]—with minor modifications. A total of 0.1 g of freeze-dried sample was dissolved in 10 mL of ethanol (50% *v/v*)

and agitated for 10 s. The extract was then centrifuged at 6000 rpm for 10 min. After centrifugation, the extract was collected and the same procedure was repeated twice more to assure maximum extraction of betalains. The betalains in this extract were measured using a spectrophotometer (Labomed Inc., Los Angeles, CA, USA). The absorbance of betacyanins was measured at 480 nm, and for betaxanthins it was measured at 538 nm. For quantification of betacyanins and betaxanthins, the molecular weights (550 g/mol and 308 g/mol, respectively) and molar extinction coefficients (60,000 L/mol cm in H<sub>2</sub>O and 48,000 L/mol cm in H<sub>2</sub>O, respectively) were applied. The results for betalains were given in mg per g of dry matter.

### 2.5. Statistical and Multivariate Analysis

A two-way analysis of variance (ANOVA) method was used to statistically process all data. Fisher's LSD (least significant difference) test ( $p < 0.05$ ) was performed to determine the statistical significance of the differences between the means. The software program, STATISTIKA (Statistica 10; StatSoft, Inc., Tulsa, OK, USA), was employed to analyse the data. The arithmetic means and standard deviations were used to express the data. The beetroot skin and flesh samples were classified using principal components analysis utilizing Software XLSTAT (XLSTAT, 2018, New York, NY, USA)—based on their proximate and mineral compositions, as well as their bioactive substances.

## 3. Results

### 3.1. Proximate Composition

The proximate composition results for the skin and flesh of four red beetroot genotypes are reported in Table 1.

**Table 1.** Proximate composition in skin and flesh of different red beetroot genotypes.

Beetroot Genotype	Root Part	Dry Matter (%)	TSS (°Brix FM)	Protein (% DM)	Fiber (% DM)	Ash (% DM)	Total Sugars (% FM)
'Alto F1'	flesh	8.80 ± 0.06 d *	8.40 ± 0.01 b	10.8 ± 0.0 f	6.27 ± 0.03 d	8.66 ± 0.04 c	4.90 ± 0.03 d
	skin	13.3 ± 0.4 b	7.20 ± 0.14 d	15.8 ± 0.1 a	12.4 ± 0.4 a	11.4 ± 0.1 a	7.26 ± 0.04 b
'Kosak'	flesh	11.0 ± 0.1 c	9.90 ± 0.01 a	12.5 ± 0.0 d	5.41 ± 0.20 ef	8.05 ± 0.07 cd	6.06 ± 0.05 c
	skin	15.0 ± 0.7 a	7.85 ± 0.07 c	15.5 ± 0.1 b	9.44 ± 0.56 c	10.2 ± 0.0 b	8.23 ± 0.05 a
'Pablo F1'	flesh	10.4 ± 0.1 c	8.35 ± 0.07 b	11.7 ± 0.1 e	5.14 ± 0.06 f	7.65 ± 0.03 d	2.65 ± 0.03 g
	skin	13.8 ± 0.1 b	6.45 ± 0.07 e	13.1 ± 0.1 c	10.6 ± 0.0 b	10.1 ± 0.0 b	4.00 ± 0.04 e
'Taunus F1'	flesh	9.44 ± 0.17 d	8.53 ± 0.11 b	11.6 ± 0.2 e	6.04 ± 0.39 de	8.86 ± 0.04 c	2.62 ± 0.03 g
	skin	14.0 ± 0.3 b	7.50 ± 0.42 dc	13.2 ± 0.1 c	10.9 ± 0.3 b	10.2 ± 0.0 b	3.60 ± 0.04 e
<i>p</i> -Value:							
Genotype		<0.001	<0.001	<0.001	<0.001	0.011	<0.001
Root part		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interactions of genotype × root part		NS	0.006	<0.001	0.009	NS	<0.001
Average	flesh	10 ± 1 b	8.8 ± 0.7 a	11.6 ± 0.7 b	5.72 ± 0.53 b	8.31 ± 0.56 b	4.06 ± 1.71 a
	skin	14.0 ± 0.7 a	7.25 ± 0.60 b	14.4 ± 1.0 a	10.8 ± 1.2 a	10.5 ± 0.6 a	5.77 ± 2.32 a

\* Averages in the same column marked with different letters differ significantly at  $p < 0.05$ . FM—fresh matter; DM—dry matter. NS—no significant differences.

One of the most relevant components of raw vegetables is dry matter. Its amount varies depending on genotype, cultivation method and year, as well as the part of the plant [28]. The findings of the present study suggest that genotype differences and root part significantly influenced the dry matter amount of the tested samples. The values of this component in the skin of the investigated red beetroot ranged from 13.3–15.0%, which are significantly higher than those in the flesh. The skin of 'Kosak' showed the highest amounts of dry matter. On the contrary, red beetroot flesh showed significantly higher amounts of total soluble solids (TSS) than skin. The 'Kosak' flesh had the highest amounts of this component (9.90 °Brix) among genotypes. According to the literature, the amounts of dry matter and TSS for the whole beetroot varied widely from 11.0–18.1% [28] and from 6.40–10.8 °Brix [29], respectively.

The amounts of fiber, protein, ash, and total sugars can be affected by various factors. A statistical analysis of the data from this study established the significant impact of beetroot genotype, root part, and the interactions of beetroot genotype and root part on the amount of fiber, protein, and total sugars. Only red beetroot genotype differences and root part differences significantly influenced the ash amount. The current research showed that the skin tissue had, on average, 23.5% higher amounts of protein, 89.2% higher amounts of fiber, 26.0% higher amounts of ash, and 42.1% higher amounts of total sugars compared to the flesh. Among genotypes, 'Alto F1' skin had the highest amounts of fiber, protein, and ash; this suggests that this skin genotype can be considered a source of previously mentioned components. The highest total sugar content is contained in 'Kosak' skin.

The obtained results for fiber and protein in beetroot skin in this study were higher than the results reported by Shuaibu et al. [30]. These researchers stated that beetroot skins contained 6.98% fiber and 4.1% protein; however, they also reported a similar amount of ash for beetroot skin (10.6%). Šeremet et al. [19] investigated red beetroot skin and reported higher amounts of crude protein (18.3%), crude mineral (12.1%), dietary fiber (36.6%), and soluble sugars (12.5%). These differences in the proximate composition of beetroot skin can be caused by many factors, such as genotype, growing location and conditions, as well as different extraction procedures and different determination methods.

### 3.2. Mineral Elements

Mineral elements in plants and humans play a relevant role in many physicochemical processes and can affect overall health. Humans require not only proteins, lipids, carbohydrates, and vitamins, but also require some essential nutrients, such as minerals. Therefore, the nutritive value of vegetables such as beetroot is based on the amount of minerals, which are necessary for a healthy body [31,32]; however, there is lack of information in the literature on the mineral element amounts in beetroot flesh and skin. According to the literature, the amount of minerals in beetroot is affected by the genotype, weather conditions, soil nutrient amount, fertilizer use, and harvest maturity state [33,34].

The amount of macroelements in the skin and flesh of four red beetroot genotypes is shown in Table 2. A two-way ANOVA indicated significant variations in the amounts of macroelements such as K, Mg, Ca, and N, depending on the beetroot genotype, root part, and their interaction. The amount of P was significantly affected by beetroot genotype and root part only.

The results, as supplied in Table 2, indicate that all investigated samples contained higher amounts of K followed by N, Mg, P, and Ca. Furthermore, 'Kosak' skin contained the highest amount of K. According to EU Regulation No. 1169/2011 [35], the recommended daily allowance (RDA) of K for adults is 2000 mg/day. Therefore, the consumption of 55.5 g per day of 'Kosak' skin powders supplies 100% of the RDA for K. In the case of flesh, 'Alto F1' and 'Kosak' exhibited the maximum values of K (3331 and 3283 mg 100 g<sup>-1</sup> DM, respectively). The intake of approximately 60 g per day of 'Alto F1' or 'Kosak' flesh powder supplies 100% of the RDA for K. 'Taunus F1' showed the lowest values of K in the flesh and skin with 2901 mg 100 g<sup>-1</sup> DM and 2803 mg 100 g<sup>-1</sup> DM, respectively.

Beetroots are also a valuable source of Mg. The flesh samples demonstrated 1.65–2.68 times lower values of Mg. In the skin samples, the highest amount of this mineral was observed for 'Alto F1' (831 mg 100 g<sup>-1</sup>). In the flesh samples, the greatest amount of Mg was found for 'Kosak' (401 mg 100 g<sup>-1</sup>). The RDA for Mg is 375 mg per day [35]. Therefore, the consumption of 50 g per day of 'Alto F1' skin powders supplies 111% of the RDA for Mg, while 100 g of 'Kosak' flesh supplies only 53.5%.

Our study indicated that an average N amount in the skin was 2366 mg 100 g<sup>-1</sup>, which is significantly higher than that of the flesh. 'Alto F1' skin showed the highest N amount.

A comparison of the amount of P between the skin and flesh of red beetroot indicated that the amount of this mineral was significantly higher, from 41.4–80.5%, in the skin than in the flesh. 'Kosak' and 'Pablo F1' skin samples had the greatest amounts of this element (472 mg 100 g<sup>-1</sup> DM and 456 mg 100 g<sup>-1</sup> DM, respectively). The RDA of P is

700 mg/day [32]. The consumption of 100 g per day of the investigated beetroot skin powders supplies between 54.3% and 67.5% of the RDA for P, while 100 g of flesh powders supplies between 30.1% and 46.1% of the RDA for P.

**Table 2.** Macro- and microelement amounts in skin and flesh of different beetroot genotypes.

Beetroot Genotype	Root Part	Macroelements (mg 100 g <sup>-1</sup> DM)				
		Potassium (K)	Nitrogen (N)	Phosphorus (P)	Calcium (Ca)	Magnesium (Mg)
'Alto F1'	flesh	3331 ± 30 b *	1720 ± 57 e	211 ± 14 d	151 ± 9 c	310 ± 11 g
	skin	3200 ± 78 c	2720 ± 49 a	380 ± 7 b	400 ± 21 b	831 ± 13 a
'Kosak'	flesh	3283 ± 24 b	1901 ± 13 d	310 ± 13 c	140 ± 4 cd	401 ± 16 e
	skin	3602 ± 11 a	2462 ± 54 b	472 ± 18 a	450 ± 14 a	661 ± 11 d
'Pablo F1'	flesh	3000 ± 28 d	1772 ± 139 de	323 ± 32 c	120 ± 4 d	292 ± 11 g
	skin	3151 ± 12 c	2150 ± 57 c	456 ± 5 a	381 ± 16 b	701 ± 13 c
'Taurus F1'	flesh	2901 ± 16 e	1504 ± 9 f	221 ± 12 d	125 ± 4 cd	370 ± 14 f
	skin	2803 ± 10 f	2131 ± 41 c	390 ± 9 b	390 ± 6 b	762 ± 17 b
<i>p</i> -Value:						
Genotype		<0.001	<0.001	<0.001	0.002	<0.001
Root part		0.007	<0.001	<0.001	<0.001	<0.001
Interactions of beetroot genotype × root part		<0.001	<0.001	NS	0.025	<0.001
Average	flesh	3129 ± 210 a	1724 ± 166 b	266 ± 58 b	134 ± 14 b	344 ± 51 b
	skin	3189 ± 33 a	2366 ± 281 a	425 ± 46 a	406 ± 31 a	739 ± 74 a
Beetroot genotype	Root part	Microelements (mg kg <sup>-1</sup> DM)				
		Iron (Fe)	Zinc (Zn)	Boron (B)	Manganese (Mn)	Copper (Cu)
'Alto F1'	flesh	45.8 ± 1.0 d	23.9 ± 1.3 d	20.74 ± 0.9 d	15.3 ± 0.4 d	10.4 ± 0.4 c
	skin	564 ± 14 c	78.8 ± 0.3 a	27.4 ± 0.0 b	47.9 ± 1.3 c	18.7 ± 0.5 b
'Kosak'	flesh	53.0 ± 0.2 d	25.7 ± 1.0 d	19.3 ± 0.4 e	17.3 ± 0.1 d	10.4 ± 0.4 c
	skin	759 ± 20 a	64.5 ± 1.0 b	27.3 ± 0.6 b	52.3 ± 1.9 a	17.7 ± 0.3 b
'Pablo F1'	flesh	49.3 ± 0.3 d	21.7 ± 1.0 e	19.3 ± 0.7 e	11.9 ± 0.4 e	8.58 ± 0.82 d
	skin	646 ± 14 b	79.0 ± 1.4 a	29.0 ± 0.5 a	49.6 ± 0.9 cb	17.5 ± 0.1 b
'Taurus F1'	flesh	35.6 ± 0.8 e	24.6 ± 0.5 d	19.5 ± 0.7 de	15.9 ± 0.4 d	10.4 ± 0.4 c
	skin	785 ± 16 a	56.3 ± 0.4 c	25.5 ± 0.4 c	51.3 ± 1.6 ab	20.4 ± 1.3 a
<i>p</i> -Value:						
Genotype		<0.001	<0.001	0.013	0.002	0.004
Root part		<0.001	<0.001	<0.001	<0.001	<0.001
Interactions of beetroot genotype × root part		<0.001	<0.001	0.010	NS	NS
Average	flesh	45.9 ± 7.5 b	24.0 ± 1.7 b	19.7 ± 0.7 b	15.1 ± 2.3 b	9.98 ± 0.94 b
	skin	689 ± 103 a	69.7 ± 11.2 a	27.3 ± 1.5 a	50.3 ± 2.0 a	18.6 ± 1.3 a

\* Averages in the same column marked with different letters differ significantly at  $p < 0.05$ . DM-dry matter. NS—no significant differences.

Beetroot skin also had a 2.66–3.21 times greater amount of Ca than beetroot flesh; this shows that skin removal may cause significant losses of this mineral. 'Kosak' had a higher amount of Ca in the skin. 'Kosak', 'Pablo F1', and 'Taurus F1' flesh had the lowest amount of Ca with no significant difference between them. The RDA of K is 800 mg per day [35]. The intake of 100 g per day of the investigated beetroot skin powders supplies between 47.6% and 56.3% of the RDA for Ca, while 100 g of the flesh powders supplies only 15.0–18.8% of the RDA for Ca.

Research by Šeremet et al. [19] also indicates that the main macroelement in red beetroot skin was K (4185 mg 100 g<sup>-1</sup> DM), followed by a lower quantity of P (781 mg 100 g<sup>-1</sup> DM), Mg (657 mg 100 g<sup>-1</sup> DM), Na (438 mg 100 g<sup>-1</sup> DM), and Ca (285 mg 100 g<sup>-1</sup> DM). According to the findings of Shuaibu et al. [30], the macroelement composition of beetroot skin was supplied as follows: potassium, phosphorus, and magnesium, while calcium was not detected.

The amount of microelements in the skin and flesh of beetroot is summarized in Table 2. The statistical analysis revealed significant variations in the amounts of Fe, Zn, and B, depending on the beetroot genotype, root part, and the interactions between the

genotype and root part; however, only the beetroot genotype and root part significantly influenced the amounts of Mn and Cu.

The dominant microelement in both the flesh and skin of the investigated beetroot was Fe. The amount of this element varied widely in the beetroot tissue. The skin contained significantly higher amounts of Fe—by 12.3–22.0 times compared with the flesh. Whereas the removal of skin highly decreases the nutritional value of some beetroot genotypes (especially the amounts of Fe). The skins of 'Kosak' and 'Taurus F1' had significantly greater amounts of Fe (759 and 785 mg kg<sup>-1</sup> DM, respectively). The results of this study showed that beetroot skin (especially the skins of 'Kosak' and 'Taurus F1') could be used as an alternative source of Fe, which could increase the amount of this element in food products. The RDA for Fe is set at 14 mg per day for adults [35]. Therefore, 10 g of 'Kosak' or 'Taurus F1' skins can provide approximately 55% of the RDA of Fe.

An analysis of the Zn amount in the individual parts of the beetroot showed that the average amount of this mineral in the skin reached 69.7 mg kg<sup>-1</sup> DM, and in flesh it was lower—by over 2.9 times. The skins of 'Alto F<sub>1</sub>' and 'Pablo F<sub>1</sub>' showed the greatest amount of Zn at 78.8 and 79.0 mg kg<sup>-1</sup> DM, respectively. The results from the current study indicate that the values of B were significantly higher in the skin than the flesh. 'Pablo F<sub>1</sub>' skin had the highest amount of B, while the flesh of this hybrid had the lowest amount of this mineral. The amount of Mn in the skin of beetroot was significantly higher compared to the flesh. Our findings suggest that the removal of this part of the beetroot greatly reduced the amount of Mn by 3.02–4.15 times. Consequently, the highest amount of Mn was accumulated in the skin of 'Kosak' (52.3 mg kg<sup>-1</sup> DM). This study also revealed that the amount of Cu was significantly higher, from 68.2–103.9%, in the skin compared to the flesh of beetroot. The 'Taurus F<sub>1</sub>' genotype contained the greatest Cu amount (20.4 mg 100 g<sup>-1</sup> DM) in the skin. The RDAs are 10 mg/day for Zn, 2 mg/day for Mn, and 1 mg/day for Cu in adults [35]. Consequently, the consumption of 100 g of the investigated beetroot skin powders supplies between 56.3% and 79.0% of the RDA for Zn, between 240% and 262% of the RDA for Mn, and between 175% and 204% of the RDA for Cu. Meanwhile, 100 g per day of the flesh powder supplies between 21.7% and 25.7% of the RDA for Zn, between 59.5% and 86.5% of the RDA for Mn, and between 86% and 105% of the RDA for Cu.

A study by Szekely et al. [36] also showed that the skin of beetroot was characterized by a large amount of Fe. These researchers also found that higher amounts of Ca, Mn, and Mg were present in the skin and the top part of the tested beetroots compared to its end and middle parts. Šeremet et al. [19] reported that beetroot skin has an Fe content of 159 mg kg<sup>-1</sup> DM, Zn content of 49 µg kg<sup>-1</sup> DM, Cu content of 19 µg kg<sup>-1</sup> DM, and Mn content of 52 µg kg<sup>-1</sup> DM. According to Shuaibu et al. [30], fresh beetroot skins contain sodium (41.7 mg 100 g<sup>-1</sup>), iron (264.6 mg 100 g<sup>-1</sup>), and copper (2.1 mg 100 g<sup>-1</sup>)—while zinc was below the detectable limit.

### 3.3. Bioactive Compounds

Phenolic compounds are the secondary metabolites of plants and contribute to the plant's growth, pigmentation, and reproduction, as well as affecting the sensorial attributes (flavour, taste, and colour) and functional properties of plant food products [37,38]. According to the literature, the highest amount of these compounds is located in the skin (50%) of fruits and vegetables, followed by the crown (37%) and the flesh (13%) [20].

The total phenolic, total anthocyanins, total betalains and individual betalains contents in the skin and flesh of four red beetroot genotypes are presented in Table 3.

A statistical analysis showed a significant impact of beetroot genotype, root part, and the interactions between the genotype and root part on the amount of investigated bioactive compounds. The skin of beetroot contained 1.27–2.37 times more total phenolic compounds compared with the flesh samples. Among the skin samples, the highest amount was found in 'Kosak' (35.5 mg g<sup>-1</sup> DM) and 'Pablo F1' (36.1 mg g<sup>-1</sup> DM). Among the flesh samples, 'Alto F1' had the greatest total phenolic content (19.5 mg g<sup>-1</sup> DM). This was in line with a

study by Zin et al. [39], which reported that the total phenolic content values in the skin extract and flesh extract were 34.5 mg GAE g<sup>-1</sup> DM and 12.7 mg GAE g<sup>-1</sup> DM, respectively. Kujala et al. [40] evaluated red beetroot skin extracts prepared by different extraction methods and solvents and found that the total phenolic content in them was lower and varied from 17.4 to 24.1 mg GAE g<sup>-1</sup> DM. Carrillo et al. [41] detected that the total phenolic content for the whole beetroot grown under conventional and organic conditions ranged from 5.6–19.1 mg GAE g<sup>-1</sup> DM. Data collected in the scientific literature show that the total phenolic content in red beetroot is quite variable and may be influenced by many factors. The quantities of phenolic compounds vary even among plants of the same species due to the differences in various aspects of the plants, such as genotype, growing conditions, developmental stage of the plant, maturity, and analysed plant part, as well as pre-harvest factors [42,43].

**Table 3.** Bioactive compound amounts in skin and flesh of different beetroot genotypes.

Beetroot Genotype	Root Part	Total Phenolics (mg g <sup>-1</sup> DM)	Total Anthocyanins (mg kg <sup>-1</sup> DM)	Betacyanins	Betaxanthins	Total Betalains
				(mg g <sup>-1</sup> DM)		
'Alto F1'	flesh	19.5 ± 0.9 d *	41.7 ± 1.0 d	8.31 ± 0.01 d	4.26 ± 0.03 e	12.6 ± 0.0 e
	skin	24.9 ± 1.1 c	58.7 ± 3.0 c	8.51 ± 0.03 c	4.92 ± 0.01 b	13.4 ± 0.0 c
'Kosak'	flesh	17.9 ± 0.1 e	28.3 ± 4.2 e	8.10 ± 0.02 e	4.47 ± 0.04 d	12.6 ± 0.0 e
	skin	35.5 ± 0.2 a	108 ± 2.8 a	9.04 ± 0.01 a	5.09 ± 0.01 a	14.1 ± 0.0 a
'Pablo F1'	flesh	15.2 ± 0.0 f	42.6 ± 2.5 d	7.90 ± 0.01 f	4.24 ± 0.01 e	12.2 ± 0.0 f
	skin	36.1 ± 1.1 a	63.9 ± 3.6 c	8.84 ± 0.01 b	4.93 ± 0.01 b	13.8 ± 0.0 b
'Taurus F1'	flesh	14.4 ± 0.1 f	27.8 ± 3.3 e	8.12 ± 0.02 e	4.41 ± 0.03 d	12.5 ± 0.0 e
	skin	27.5 ± 0.0 b	78.0 ± 1.7 b	8.55 ± 0.05 c	4.73 ± 0.01 c	13.3 ± 0.1 d
<i>p</i> -Value:						
Genotype		<0.001	0.002	<0.001	<0.001	<0.001
Root part		<0.001	<0.001	<0.001	<0.001	<0.001
Interactions of beetroot genotype × root part		<0.001	<0.001	<0.001	<0.001	<0.001
Average	flesh	16.8 ± 2.2 b	35.1 ± 9.9 b	8.11 ± 0.15 b	4.34 ± 0.10 b	12.4 ± 0.2 b
	skin	31.0 ± 5.2 a	77.2 ± 20.9 a	8.74 ± 0.23 a	4.91 ± 0.13 a	13.6 ± 0.3 a

\* Averages in the same column marked with different letters differ significantly at  $p < 0.05$ . DM-dry matter.

Anthocyanins are natural plant pigments belonging to the class of polyphenolics [44]. According to the literature, the amount of total anthocyanins in beetroot is very low compared with betalains [45].

The skin showed, on average, 2.20 times higher values of total anthocyanins. Consequently, the highest amount of total anthocyanins was recorded in the skin of 'Kosak' (108 mg kg<sup>-1</sup>). The anthocyanins of different parts of beetroot are not well covered in the scientific literature, so it is not possible to make comparisons. Kovarovič et al. [45] evaluated the content of total anthocyanins in four cultivars ('Kahira', 'Cylindra', 'Crosby Egyptian', and 'Chioggia') of red beetroot at fresh weights. They detected that the amount of this pigment in investigated beetroot samples ranged from 14.5–84.5 mg kg<sup>-1</sup>. Guiné et al. [46] reported that the total anthocyanins amount, which was determined using the SO<sub>2</sub> bleaching method and expressed as malvidin equivalents, varied from 230–770 mg kg<sup>-1</sup> in fresh beetroot. The different anthocyanins extraction and analytical methods could be the causes of the differences in these investigations.

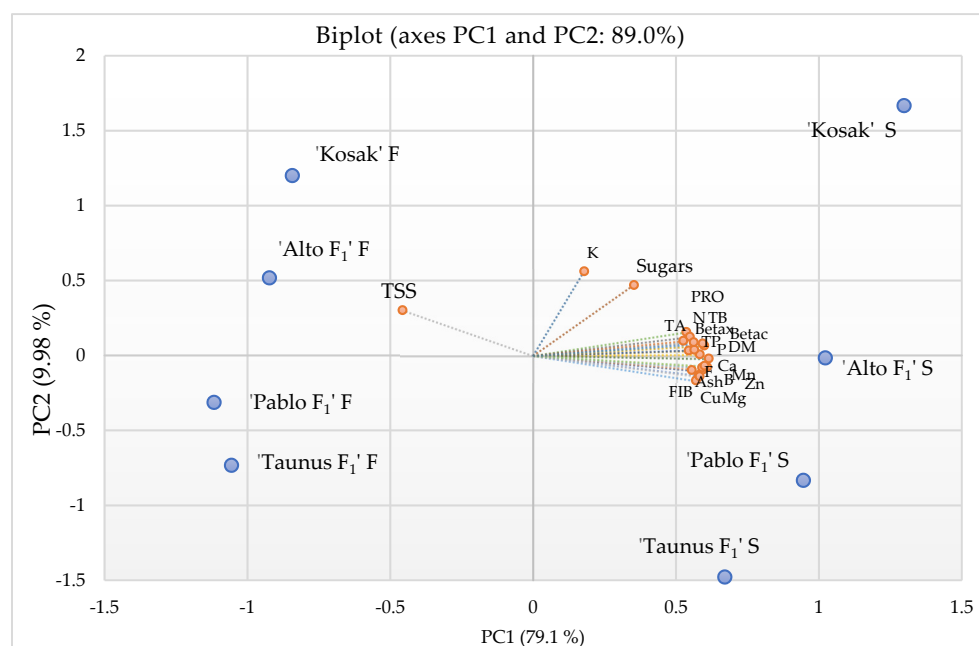
Betalains are water-soluble plant pigments that are divided into two groups: red–violet betacyanins and yellow–orange betaxanthins. These pigments provide beet roots with their typical color [47]. Our results showed that the amount of total betalains in the skin were about 10.0% significantly higher than those in the flesh. The predominant betalains in beetroot were betacyanins, which accounted for 41.0–66.1% of the total amount of betalains. The highest amount of both pigments, betacyanins and betaxanthins, were found in the skin compared to the flesh. The skin of 'Kosak' showed the highest amounts of total betalains and individual betalains. These results agree with previously received results. Kujala et al. [20] and Slatnar et al. [48] announced that there are different betalains distributions between the different parts of red beetroot and these pigments have higher concentrations in beetroot skin



than in the flesh. Sawicki et al. [11] also reported that the total amount of betalains varied significantly across genotypes ( $10.3\text{--}17.2\text{ mg g}^{-1}\text{ DM}$ ) and root parts ( $7.20\text{--}17.2\text{ mg g}^{-1}\text{ DM}$ ). In the case of root parts, the skin was noted for having the greatest amount of betalains. These authors found betacyanins from  $4.44$  (ring 6)– $12.8$  (skin)  $\text{mg g}^{-1}\text{ DM}$  and betaxanthins from  $2.75$  (ring 2)– $4.46$  (skin)  $\text{mg g}^{-1}\text{ DM}$  in seven parts of the root, the skin, and the six inner rings. In Czech Republic, Bárta et al. [49] investigated the betalains for different beetroot cultivars and the amount of these pigments in freeze-dried, peeled beetroot powders, which ranged from  $10.9\text{--}18.1\text{ mg g}^{-1}\text{ DM}$ . Betaxanthins and betacyanins concentrations in beetroot powders of red root cultivars varied from  $4.29\text{--}6.95\text{ mg g}^{-1}\text{ DM}$  and from  $6.63\text{--}11.1\text{ mg g}^{-1}\text{ DM}$ , respectively.

### 3.4. Principal Component Analysis (PCA)

A PCA was used to estimate the relationships between the different sample types and variables shown in Tables 1–3. According to the PCA results, the first two axes explained 89.0% of the total variance: the first principle component (PC1) explained 79.1% and the second principal component (PC2) accounted for 9.98% of the variation (Figure 1). The eigenvalues of PC1 and PC2 were greater than one (16.1 and 2.09, respectively). The dry matter, fiber, protein, ash, investigated mineral elements (except K), and bioactive compounds were highly positively associated with PC1, whereas the total soluble solids content was negatively associated with PC1; sugars and potassium (K) were positively associated with the second factor (PC2). As shown in Figure 1, PC1 separated the skin samples from the flesh samples. The skin samples are defined by high amounts of dry matter, protein, ash, fiber, investigated mineral elements (except K), and bioactive compounds, while the flesh samples are particularly characterized by high amounts of total soluble solids.



**Figure 1.** PCA results for the relationship between variables (contents of dry matter (DM), total soluble solids (TSS), protein (PRO), ash (Ash), fibre (FIB), N (nitrogen), potassium (K), magnesium (Mg), phosphorus (P), calcium (Ca), iron (Fe), boron (B), zinc (Zn), copper (Cu), manganese (Mn), total phenolics (TP), total anthocyanins (TA), betacyanins (betac), betaxanthins (betax), total betalains (TB), and beetroot samples ('Alto F<sub>1</sub>' skin ('Alto F<sub>1</sub>' S), 'Kosak' skin ('Kosak' S), 'Pablo F<sub>1</sub>' skin ('Pablo F<sub>1</sub>' S), 'Taurus F<sub>1</sub>' skin ('Taurus F<sub>1</sub>' S), ('Alto F<sub>1</sub>' flesh ('Alto F<sub>1</sub>' F), 'Kosak' flesh ('Kosak' F), 'Pablo F<sub>1</sub>' flesh ('Pablo F<sub>1</sub>' F), 'Taurus F<sub>1</sub>' flesh ('Taurus F<sub>1</sub>' F)).

#### 4. Conclusions

The proximate and mineral compositions, bioactive compounds (total phenolics, total anthocyanins, betalains) in the skin and flesh tissues of different genotypes of beetroot grown in Lithuania were compared. The findings of this investigation allow for the conclusion that beetroot is a valuable source of macro- and micronutrients. The amounts of these nutrients in the skin of most of the investigated beetroot far exceed their quantity in the flesh. Therefore, special attention should be paid to the potential use of skin as an additive for improving the quality of food products, for the development of new functional products, or for use in the pharmaceutical industry.

Among all investigated beetroot samples, the skins of ‘Alto F1’ and ‘Kosak’ were the most valuable. The highest amounts of protein, fiber, ash, N, Mg, and Zn were found in ‘Alto F1’ skin, while the skin of ‘Kosak’ contained the greatest amounts of dry matter, total sugars, K, P, Ca, Fe, Mn, total anthocyanins, and betalains. The skins of ‘Kosak’ and ‘Pablo F1’ had a maximum total phenolic content.

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