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ORIGINAL PAPER

Chemical parameters profile analysis by liquid chromatography and antioxidative activity of the Saskatoon berry fruits and their components

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Abstract
The main goals of the study were to determine nutritional parameters including contents of organic acids, sugars, and bioactive compounds as well as the antioxidant activity of fruits and fruit components of seven selected Saskatoon berry (*Alnifolia alnifolia*) genotypes. Fruits of three Polish clones ('no 5/6', 'type N', and 'type S') and of four Canadian cultivars ('Martin', 'Pembina', 'Smoky', and 'Thiessen') and their components including peel, flesh and seeds were used in the study. The antioxidant potential was determined by the radical scavenging capacity assay, the profiles of organic acids and sugars were analyzed by high-pressure liquid chromatography and the bioactive compounds were quantified using ultra-high performance liquid chromatography. Fruits of the tested genotypes and their components differed significantly in their antioxidant activity and in their contents of sugars, organic acids and bioactive compounds. Fruits of the 'clone type S' showed the highest antioxidant activity, while those of cvs. 'Thiessen' and 'Smoky' had the highest amount of polyphenols and organic acids. The highest contents of organic acids and bioactive compounds were determined in the fruit peel of the cvs. 'Thiessen' and 'Smoky' and 'clone type S'. The total content of organic acids in fruits of the tested genotypes and their components ranged from 2.50 g/100 g dm (seeds) to 20.50 g/100 g dm (flesh). Malic, quinic, and tartaric acids were found to prevail in these fruits. In turn, the cluster analysis and principal components analysis helped determining the most important variables and identifying three groups of genotypes clustered depending on similarities in the content of individual compounds testes. Results indicated that fruits of the tested Saskatoon berry genotypes and their components were valuable sources of natural antioxidants, while fruit components such as peel and seeds could be used to develop new functional foods, and super foods with health-promoting properties, and also as medical, pharmaceutical or cosmetic components.

Keywords *Amelanchier alnifolia* · Genotypes · Polyphenols · Sugars · Organic acids · Bioactive compounds · Cultivars · Antioxidant potency · UPLC · PCA

Introduction

Plants like herbs, fruits or vegetables have long been used as excellent components of a healthy diet. As a result, scientific and consumer awareness about the important role of cultivated plants including fruit crops in human nutrition, health, and prevention against diseases has increased. Therefore, this work focuses on the chemical composition of raw material of different species, including the Saskatoon berry (*Alnifolia alnifolia*), which provides the health benefits. Because little information is available in scientific literature on the mineral composition profile of these fruits and their components, further research is necessary in this respect [1–3].

The Saskatoon berry is highly adaptable under subtropical, tropical, and temperate climate conditions of America, Asia, Africa and Europe [4, 5]. Its fruit seem to be valuable for food processing and also suitable for direct consumption due to good flavor qualities. In addition, their seeds have a strong almond-like flavor. Fruit of the Saskatoon berry are suitable for homemade and industrial processing into different products, such as: juices, jams, smoothie-type products, lickers or dried foods [6–8].

Furthermore, fruit of the Saskatoon berry contain various phytochemical compounds, which have anti-microbial,

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and anti-inflammatory properties. The best health-promoting properties are attributed to plant secondary metabolites including polyphenolic compounds and isoprenoids (irinoids, triterpenoids, tetraterpenoids). Polyphenols constitute a large group of phytochemical substances. Phenolic compounds show sensory properties and also physiological and biological functions beneficial for human health including antioxidant, anti-inflammatory, anti-allergenic, and anti-microbial activities [9, 10]. In turn, isoprenoids exhibit antioxidant properties that protect the body against free radicals. Other essential components of these fruit are the organic acids, which affect the proper course of chemical reactions in the human body. They are also intensively used in the pharmacy due to their strong antioxidant properties [11, 12]. In addition, the sugar-to-organic acid ratio is a very important criterion in the tested fruit flavor acceptance by consumers [13].

Many authors have determined contents of bioactive compounds of the Saskatoon berry genotypes grown in different countries of the world, such as Canada, Finland, Czech Republic, Poland, and Pakistan [2, 14–16]. They focused mainly on the content and profile of polyphenolic compounds, triterpenes, and tetraterpenes in fruit of different genotypes of this crop. While, detailed data regarding the profile and content of organic acids is scarce. In turn, contents of individual nutritional compounds in Saskatoon berry fruit and their components have been studied occasionally.

The main aim of this studies was to determine nutritional parameters including contents of organic acids, sugars, and bioactive compounds as well as the antioxidant activity of the selected Saskatoon berry genotypes, and to compare them in fruit and their components. Furthermore, the principal component analysis model was applied to all tested parameters to determine the variables factors that explained dependencies between fruit of the tested genotypes and their components.

Materials and methods

Reagent and standard

Acetonitrile, formic acid, methanol, ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), methanol acetic acid, and phloroglucinol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, di-caffeic quinic acid, procyanidin B2, *p*-coumaric acid, quercetrin-3-*O*-galactoside, caffeic acid, cyanidin-3-*O*-galactoside and cyanidin-3-*O*-glucoside were purchased from Extrasynthese (Lyon, France). Analytic standards of tartaric acid, citric acid, malic acid, lactic acid, succinic acid, fructose, glucose, sorbitol were purchased from Dr. Ehrenstrofer (Teddington, UK). Acetonitrile [high-pressure liquid chromatography (HPLC) gradient grade] and sulfuric acid (analytical grade) were purchased from POCH (Gliwice, Poland). Formic acid (LC–MS grade) was purchased from Fischer Scientific (Schwerte, Germany). Acetonitrile for ultra-performance liquid chromatography (UPLC; Gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany).

Fruit samples

Fruits of the Saskatoon berry (*A. alnifolia*) of three Polish breeding clones: 'no. 5/6', 'type N' and 'type S' as well as four Canadian cultivars (cvs.): 'Martin', 'Pembina', 'Smoky', 'Thiessen' and their components such as peel, flesh and seeds were used in the study. Fruit samples (~ 10.0 kg each) of tested genotypes were collected from bushes grown in the field trial established in 2011 at the Experimental Orchard at Dąbrowice, belonging to the Research Institute of Horticulture in Skierniewice, Central Poland (51°55′24″N, 020°5′58″E). Fruits were collected at the optimum ripening time, at the beginning of July in 2018. The raw material was directly frozen in liquid nitrogen and freeze-dried (24 h; Christ Alpha 1–4 LSC; Germany). The homogeneous dry material was obtained by crushing the dried tissues using a closed laboratory mill (IKA A.11, Germany). The powders were kept in a refrigerator (− 80 °C) until extract preparation.

Quantification of polyphenols

For the extraction and determination of phenolic compounds, the protocol described before by Lachowicz et al. [15] was applied in our studies. Analysis of polyphenols was carried out using an ACQUITY Ultra Performance LC system (UPLC) equipped with binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column (1.7 µm, 2.1 mm × 50 mm, Waters Corp., Milford, MA, USA) at 30 °C and a Q-T of micro mass spectrometer (Waters, Manchester, UK) with an ESI source operating in negative and positive modes The samples (10 µL) were injected, and the elution was completed in 15 min with a sequence of linear gradients and isocratic flow rates of 0.45 mL/min. The mobile phase consisting of solvent A (4.5% formic acid, v/v) and solvent B (100% acetonitrile) was used. The program began with isocratic elution with 99% solvent A (0–1 min), and then a linear gradient was used until 12 min, reducing solvent A to 0%; from 12.5 to 13.5 min, the gradient returned to the initial composition (99% A), and then, it was held constant to re-equilibrate

the column. The analysis was carried out using full-scan, data-dependent MS scanning from m/z 100 to 1500. Leucine enkephalin was used as the reference compound at a concentration of 500 pg/L, at a flow rate of 2 L/min, and the $[M-H]^-$ ion at 554.2615 Da was detected. The $[M-H]^-$ ion was detected during a 15-min analysis performed within ESI–MS accurate mass experiments, which were permanently introduced via the Lock-Spray channel using a Hamilton pump. The lock mass correction was ± 1.000 for the mass window. The mass spectrometer was operated in negative and positive ion mode, set to the base peak intensity (BPI) chromatograms and scaled to 12,400 counts per second (cps) (100%). The optimized MS conditions were as follows: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 100 °C, desolvation temperature of 300 °C and desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Characterization of the single components was carried out via the retention time and the accurate molecular masses. Each compound was optimized to its estimated molecular mass in the negative mode, before and after fragmentation. The data obtained from UPLC–MS were subsequently entered into the MassLynx 4.0ChromaLynx Application Manager software (Waters). On the basis of these data, the software is able to scan different samples for the characterized substances. The runs were monitored at the following wavelengths: phenolic acids at 320 nm, flavonols at 340 nm, anthocyanins at 520 nm, flavan-3-ols at 280 nm. The photodiode array detectors (PDA) spectra were measured over the wavelength range of 200–600 nm in steps of 2 nm. The results were expressed as g per 100 g of dm.

Determination of sugars and organic acids

The contents of organic acids was measured by HPLC–RID method as described Kapusta et al. [17]. An analysis of sugars content was performed by the HPLC–ELSD method according to the protocol described by Oszmiański and Lachowicz [18]. All determinations were done in triplicate and the results were expressed as g per 100 g dm.

Determination of carotenoids

For the extraction of carotenoids, the protocol similar to that described previously by Lin and Chen [19] was applied in our studies. All incubations were done in triplicate. The results were expressed as g per 100 g of dm.

Determination of antioxidant activity

The radical scavenging capacity assay (ABTS), also known as Trolox equivalent antioxidant capacity (TEAC) assay was determined according Re et al. [20]. Determinations performed by the ABTS method used the UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). The antioxidant capacity was expressed as mmol of Trolox per 100 g dm.

Statistical analysis

Statistical analysis of variance (ANOVA), principal components analysis (PCA) and cluster analysis (CA) were conducted using Statistica version 12.5 (StatSoft, Kraków, Poland). The PCA was used to identify the patterns of multitrait variation in the tested genotypes. The CA using the Ward's method as a measure of dissimilarity was carried out to classify the genotypes into homogenous groups. Significant differences ($p \leq 0.05$) between mean values were compered by *t* test Duncana.

Results and discussion

Organic acids and sugars

The sugars and organic acids determined in the selected Saskatoon berry genotypes were determined in this study. Results of determinations of their content and their profile in fruit and their components (peel, flesh, and seeds) differed significantly (Table 1). The total content of sugars in fruit ranged from 9.12 g/100 g dm in 'clone no 5/6' to 20.61 g/100 g dm in 'clone type S'. The average content of sugars was 1.4 times higher than that determined in the Saskatoon berry cultivars by Mazza [6], and similar to that published by other authors [15]. Moreover, the average content of sugars in fruit flesh was 20.59 g/100 g dm and was 1.3, 1.4, and 8.2 times higher compared with their content in the peel and whole fruits, respectively. The obtained results show that in these fruits sugars are located mainly in the flesh. In our study, glucose was prevailing in the analyzed material and accounted for 37–50% of the total sugar content. The other sugars identified in fruits of the tested genotypes were fructose, sorbitol, and glucose which accounted for 32–41%, 8–31%, and 3–4% of total sugar. A similar distribution of sugars was shown in chokeberry (*Aronia melanocarpa*) fruits [18]. The low content of sucrose in fruit of the Saskatoon berry genotypes and their components was probably due to enzymatic hydrolysis after translocation from morphological parts of plants, such as leaves. In addition, sorbitol was identified only in fruit of chokeberry (*A. melanocarpa*), eastern shadbush (*Amelanchier arborea*, syn. *A. canadensis*) and rowanberry (*Sorbus aucuparia*) and was mainly used as the taxonomic criterion in these species classification [21]. It was also proved the positive correlation between total sugars, polyphenols and carotenoids ($r^2 = 0.251$ and $r^2 = 0.466$, respectively).

Considering *A. alnifolia* fruit components, the highest content of sugars was determined in the flesh and it was on average 20.59 g/100 g for the tested genotypes (Table 1). It was 1.3 and 8.0 times higher compared to that determined in peel and seeds, respectively. According to Kolniak-Ostek [22], the content of individual sugars in seeds of pear was 6 and 2.5 times lower than in the analyzed flesh and peel. It was also proved that sugars were located mainly in the flesh of fruits, which was confirmed by other authors [22, 23]. As presented by Ahmed et al. [23], sucrose of these fruit was sweeter than sorbitol, glucose, and fructose (0.6, 0.8, and 1.7 times, respectively). In addition, the content of individual sugars was high in the raw material and its components. Moreover, the content of sugars in the raw material depended mainly on climate, crop load, seasonal variability, and environmental conditions [24, 25].

According to data from the literature, the profile of organic acids has not been published until now. In our study the total organic acids content amounted between 3.45 g/100 g dm in fruit of 'clone type N' and 8.68 g/100 g dm in cv. 'Smoky'. These results were 10 and 9 times higher, respectively, when compared to the titratable acidity in fruits of the Saskatoon berry genotypes [6, 15]. The total organic acid content identified in fruit of two cvs. 'Thiessen' and 'Smoky' grown in the Czech Republic presented by Jurikova et al. [24] was 5.2 times lower than results obtained for both cultivars in our study. The low content of organic acids in fruit of the Saskatoon berry was typical of this crop. It could be affected by different environmental conditions and extraction techniques of the material as well as by chemical analyses [14, 24]. In our study, the content of organic acids was on average 7.67 g/100 g dm and it was higher in the peel than in flesh and seeds (1.3 and 2.3 times, respectively). The organic acid content was inversely proportional to the content of identified sugars, which was the highest in fruit flesh. The similar distribution of sugars and acids in the anatomical parts of the cultivated European pear (*Pyrus communis*) fruit was obtained by Kolniak-Ostek [22]. In the fruit of the tested Saskatoon berry genotypes and their components, the malic, quinic, and tartaric acids were the major organic acids, they accounted for 40–47%, 19–24%, and 7–17% of total acids. The quinic acid was mainly located in the flesh and its content was from 0.89 to 2.70 g/100 g dm, while the other analyzed organic acids were found in the peel. It was probably related to the accumulation of quinic acid in fruit vacuoles which was considered to be material reserved for phenol biosynthesis in fruit tissues [25, 26]. The other acids, such as oxalic, citric, succinic, and isocitric were identified at very low contents. However, fruit of the tested genotypes contained trace amounts (0.3–2.0%) of shikimic and fumaric acids. According to Mikulic-Petkovsek et al. [21], shikimic and fumaric acids accounted for only 3% of total organic acids in fruits of 25 tested wild or cultivated berry species.

It is common knowledge that sugars and organic acids determine the overall taste and quality and offer potential health benefits. Besides, the content and ratio of sugars and organic acids in the fruit was an important factor indicative of their quality for consumption. These parameters affected the development of individual teste and degree of sweetness in fruits and their components. The intensity of sweetness perceprion after consumption the fruit of the Saskatoon berry depended on the total contents of organic acids and sugars and also on their profiles [27]. In addition, their detailed assessment allowed determining the best genotypes in terms of sensory attributes and distribution of individual compounds in the fruits.

Phytochemical compounds

The total content of phytochemical (bioactive) compounds, such as polyphenols, tetraterpenoids, and carotenoids was determined for the different Saskatoon berry genotypes. Significant differences were found in their contents between fruit and their components (Fig. 1). The average content of polyphenolic compounds in the tested genotypes was at around 3.37 g/100 g dm. The highest average content of polyphenols was determined in the fruit peel and it was 3.54 g/100 g dm, while the lowest one in the flesh (1.43 g/100 g dm) and seeds (1.55 g/100 g dm). Similar results were presented earlier by Lavola et al. [16] and Lachowicz et al. [15]. The average amount of carotenoids (tetraterpenoids) in the tested genotypes was 0.40 g/100 g dm. The highest average content of carotenoids was found in the peel (0.35 g/100 g dm), while the lowest one in the flesh and seeds (1.4 and 1.7 times, respectively). Similar results were obtained in Finland by Lavola et al. [16] and in Poland by Lachowicz et al. [15]. As it was reported by González-Molina et al. [27], the different contents of bioactive compounds in fruit of the Saskatoon berry were influenced by growing conditions, chemical composition of soil, genetic differences, the natural environment and stage of fruit maturity (ripening). It was found that the content of polyphenols and tetraterpenoids depended significantly on the tested genotype, fruit and fruit components.

Antioxidant activity

The fruit of the selected Saskatoon berry genotypes and fruit components were also analyzed for their antioxidant potential (with the ABTS). The antioxidant activity of the analyzed materials (fruit, peel, flesh and seeds) depended on the genotypes and fruit components (Fig. 2). The antioxidant potential of the analyzed fruit samples ranged from 19.63 mmol Trolox (TE)/100 g dm in "clone no 5/6" to 32.32 mmol TE/100 g dm in cv. 'Thiessen'. The results of our study were similar to those presented by other authors [14, 15]. According to Jurikova et al. [4],

Table 1 Content of organic acids and sugars in fruit of the seven Saskatoon berry cultivars and fruit components (mg/kg)[a], in 2018

Genotypes	Parts	Organic acids										Sugars				
		OA	TA	CA	ICA	MA	QA	SA	ShA	FA	TOA	Fru	Sor	Glu	Sac	TS
Thiessen	Fruit	0.32 ± 0.01	0.70 ± 0.01	0.32 ± 0.01	0.60 ± 0.01	3.46 ± 0.07	1.37 ± 0.03	0.30 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	7.13 ± 0.14	6.96 ± 0.13	2.33 ± 0.04	8.72 ± 0.17	0.63 ± 0.01	18.02 ± 0.34
	Flesh	0.23 ± 0.00	0.56 ± 0.01	0.26 ± 0.00	0.29 ± 0.01	3.37 ± 0.06	1.74 ± 0.03	0.32 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	6.84 ± 0.13	9.08 ± 0.17	2.83 ± 0.05	11.51 ± 0.22	0.91 ± 0.02	23.43 ± 0.45
	Peel	0.34 ± 0.01	1.02 ± 0.02	0.56 ± 0.01	0.80 ± 0.02	3.92 ± 0.07	1.65 ± 0.03	0.37 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	8.71 ± 0.17	7.34 ± 0.14	2.24 ± 0.04	8.74 ± 0.17	0.73 ± 0.01	18.33 ± 0.35
	Seeds	0.21 ± 0.00	0.66 ± 0.01	0.21 ± 0.00	0.42 ± 0.01	1.45 ± 0.03	0.54 ± 0.01	0.20 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	3.78 ± 0.07	1.68 ± 0.03	1.04 ± 0.02	2.23 ± 0.04	0.17 ± 0.00	4.94 ± 0.09
Smoky	Fruit	0.37 ± 0.01	0.72 ± 0.01	0.36 ± 0.01	1.08 ± 0.02	3.57 ± 0.07	2.12 ± 0.04	0.40 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	8.68 ± 0.16	5.91 ± 0.11	2.65 ± 0.05	7.34 ± 0.14	0.59 ± 0.01	15.90 ± 0.30
	Flesh	0.27 ± 0.01	0.58 ± 0.01	0.29 ± 0.01	0.52 ± 0.01	3.48 ± 0.07	2.70 ± 0.05	0.43 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	8.33 ± 0.16	7.27 ± 0.14	2.15 ± 0.04	8.76 ± 0.17	0.73 ± 0.01	18.18 ± 0.35
	Peel	0.39 ± 0.01	1.05 ± 0.02	0.63 ± 0.01	1.44 ± 0.03	4.04 ± 0.08	2.55 ± 0.05	0.49 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	10.65 ± 0.20	6.29 ± 0.12	2.12 ± 0.04	7.78 ± 0.15	0.63 ± 0.01	16.19 ± 0.31
	Seeds	0.24 ± 0.00	0.69 ± 0.01	0.23 ± 0.00	0.75 ± 0.01	1.50 ± 0.03	0.84 ± 0.02	0.27 ± 0.01	0.07 ± 0.00	0.02 ± 0.00	4.61 ± 0.09	1.15 ± 0.02	0.74 ± 0.01	1.58 ± 0.03	0.12 ± 0.00	3.47 ± 0.07
Martin	Fruit	0.16 ± 0.00	0.45 ± 0.01	0.23 ± 0.00	0.42 ± 0.01	2.90 ± 0.06	1.20 ± 0.02	0.22 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	5.64 ± 0.11	3.98 ± 0.08	2.25 ± 0.04	4.86 ± 0.09	0.40 ± 0.01	11.09 ± 0.21
	Flesh	0.12 ± 0.00	0.36 ± 0.01	0.19 ± 0.00	0.20 ± 0.00	2.82 ± 0.05	1.54 ± 0.03	0.24 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	5.52 ± 0.10	6.47 ± 0.12	3.70 ± 0.07	7.88 ± 0.15	0.65 ± 0.01	18.06 ± 0.34
	Peel	0.17 ± 0.00	0.65 ± 0.01	0.40 ± 0.01	0.56 ± 0.01	3.28 ± 0.06	1.45 ± 0.03	0.27 ± 0.01	0.02 ± 0.00	0.04 ± 0.00	6.84 ± 0.13	4.97 ± 0.09	2.60 ± 0.05	5.97 ± 0.11	0.50 ± 0.01	13.54 ± 0.26
	Seeds	0.11 ± 0.00	0.42 ± 0.01	0.15 ± 0.00	0.29 ± 0.01	1.21 ± 0.02	0.48 ± 0.01	0.15 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	2.89 ± 0.05	0.52 ± 0.01	0.28 ± 0.01	0.80 ± 0.02	0.05 ± 0.00	1.60 ± 0.03
Pembina	Fruit	0.33 ± 0.01	0.70 ± 0.01	0.35 ± 0.01	0.31 ± 0.01	2.42 ± 0.05	1.65 ± 0.03	0.34 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	6.18 ± 0.12	4.65 ± 0.09	3.06 ± 0.06	5.75 ± 0.11	0.46 ± 0.01	13.46 ± 0.26
	Flesh	0.24 ± 0.00	0.56 ± 0.01	0.29 ± 0.01	0.15 ± 0.00	2.36 ± 0.04	2.11 ± 0.04	0.36 ± 0.01	0.03 ± 0.00	0.05 ± 0.00	6.14 ± 0.12	7.56 ± 0.14	5.05 ± 0.10	9.32 ± 0.18	0.76 ± 0.01	21.92 ± 0.42
	Peel	0.35 ± 0.01	1.01 ± 0.02	0.62 ± 0.01	0.42 ± 0.01	2.74 ± 0.05	1.99 ± 0.04	0.41 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	7.62 ± 0.14	5.80 ± 0.11	3.55 ± 0.07	7.06 ± 0.13	0.58 ± 0.01	16.41 ± 0.31
	Seeds	0.22 ± 0.00	0.66 ± 0.01	0.23 ± 0.00	0.22 ± 0.00	1.01 ± 0.02	0.65 ± 0.01	0.23 ± 0.00	0.08 ± 0.00	0.03 ± 0.00	3.33 ± 0.06	0.40 ± 0.01	0.27 ± 0.01	0.78 ± 0.01	0.04 ± 0.00	1.46 ± 0.03
'clone no 5/6'	Fruit	0.29 ± 0.01	0.46 ± 0.01	0.39 ± 0.01	0.35 ± 0.01	3.03 ± 0.06	1.24 ± 0.02	0.30 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	6.15 ± 0.12	3.18 ± 0.06	2.24 ± 0.04	3.69 ± 0.07	0.32 ± 0.01	9.12 ± 0.17
	Flesh	0.21 ± 0.00	0.37 ± 0.01	0.32 ± 0.01	0.17 ± 0.00	2.95 ± 0.06	1.58 ± 0.03	0.32 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	6.01 ± 0.11	3.49 ± 0.07	4.06 ± 0.08	5.25 ± 0.10	0.35 ± 0.01	12.8 ± 0.24
	Peel	0.31 ± 0.01	0.67 ± 0.01	0.68 ± 0.01	0.46 ± 0.01	3.43 ± 0.07	1.49 ± 0.03	0.36 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	7.50 ± 0.14	3.18 ± 0.06	2.30 ± 0.04	4.72 ± 0.09	0.32 ± 0.01	10.20 ± 0.19
	Seeds	0.19 ± 0.00	0.44 ± 0.01	0.25 ± 0.00	0.24 ± 0.00	1.27 ± 0.02	0.49 ± 0.01	0.20 ± 0.00	0.12 ± 0.00	0.03 ± 0.00	3.24 ± 0.06	0.38 ± 0.01	0.35 ± 0.01	0.66 ± 0.01	0.04 ± 0.00	1.74 ± 0.03
'clone type S'	Fruit	0.25 ± 0.00	0.78 ± 0.01	0.21 ± 0.00	0.27 ± 0.01	3.63 ± 0.07	1.29 ± 0.02	0.24 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	6.72 ± 0.13	8.62 ± 0.16	1.65 ± 0.03	10.34 ± 0.2	0.86 ± 0.02	20.61 ± 0.39
	Flesh	0.18 ± 0.00	0.62 ± 0.01	0.17 ± 0.00	0.13 ± 0.00	3.53 ± 0.07	1.64 ± 0.03	0.26 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	6.59 ± 0.13	10.17 ± 0.19	1.81 ± 0.03	12.53 ± 0.24	1.02 ± 0.02	24.51 ± 0.47
	Peel	0.27 ± 0.01	1.14 ± 0.02	0.37 ± 0.01	0.35 ± 0.01	4.10 ± 0.08	1.55 ± 0.03	0.30 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	8.13 ± 0.15	7.83 ± 0.15	1.42 ± 0.03	9.44 ± 0.18	0.78 ± 0.01	18.7 ± 0.36
	Seeds	0.16 ± 0.00	0.74 ± 0.01	0.14 ± 0.00	0.18 ± 0.00	1.52 ± 0.03	0.51 ± 0.01	0.16 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	3.50 ± 0.07	0.63 ± 0.01	0.14 ± 0.00	1.05 ± 0.02	0.06 ± 0.00	1.82 ± 0.03
'clone type N'	Fruit	0.13 ± 0.00	0.38 ± 0.01	0.23 ± 0.00	0.17 ± 0.00	1.61 ± 0.03	0.70 ± 0.01	0.17 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	3.45 ± 0.07	5.92 ± 0.11	5.83 ± 0.11	6.79 ± 0.13	0.59 ± 0.01	18.54 ± 0.35
	Flesh	0.10 ± 0.00	0.31 ± 0.01	0.19 ± 0.00	0.08 ± 0.00	1.57 ± 0.03	0.89 ± 0.02	0.18 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	3.37 ± 0.06	8.03 ± 0.15	8.00 ± 0.15	9.18 ± 0.17	0.80 ± 0.02	25.21 ± 0.48
	Peel	0.14 ± 0.00	0.56 ± 0.01	0.41 ± 0.01	0.22 ± 0.00	1.82 ± 0.03	0.84 ± 0.02	0.20 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	4.25 ± 0.08	5.47 ± 0.10	4.10 ± 0.08	6.57 ± 0.12	0.55 ± 0.01	16.14 ± 0.31
	Seeds	0.09 ± 0.00	0.36 ± 0.01	0.15 ± 0.00	0.12 ± 0.00	0.67 ± 0.01	0.28 ± 0.01	0.11 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	1.86 ± 0.04	0.71 ± 0.01	0.77 ± 0.01	1.10 ± 0.02	0.07 ± 0.00	2.58 ± 0.05
Mean	Fruit	0.26 ± 0.09f	0.60 ± 0.16c	0.30 ± 0.07e	0.46 ± 0.31d	2.95 ± 0.73a	1.37 ± 0.44b	0.28 ± 0.08f	0.03 ± 0.01h	0.04 ± 0.01g	6.28 ± 1.59b′	5.60 ± 1.85B	2.86 ± 1.38C	6.78 ± 2.27A	0.55 ± 0.18D	15.25 ± 4.20A′
	Flesh	0.19 ± 0.06g	0.48 ± 0.13c	0.24 ± 0.06e	0.22 ± 0.15f	2.87 ± 0.71a	1.74 ± 0.56b	0.30 ± 0.08d	0.03 ± 0.01i	0.04 ± 0.01h	6.12 ± 1.50c′	7.44 ± 2.12B	3.94 ± 2.11C	9.21 ± 2.38A	0.74 ± 0.21D	20.59 ± 4.46B′
	Peel	0.28 ± 0.09g	0.87 ± 0.23c	0.52 ± 0.13e	0.61 ± 0.41d	3.33 ± 0.83a	1.65 ± 0.53b	0.34 ± 0.09f	0.03 ± 0.01h	0.03 ± 0.01h	7.67 ± 1.94a′	5.84 ± 1.55B	2.62 ± 0.91C	7.18 ± 1.62A	0.58 ± 0.16D	15.64 ± 2.94C′
	Seeds	0.17 ± 0.06g	0.57 ± 0.15b	0.20 ± 0.05e	0.32 ± 0.21d	1.23 ± 0.31a	0.54 ± 0.17c	0.19 ± 0.05f	0.07 ± 0.03h	0.02 ± 0.01i	3.31 ± 0.84d′	0.78 ± 0.47B	0.51 ± 0.33C	1.17 ± 0.56A	0.08 ± 0.05D	2.52 ± 1.28D′

[a]Values are mean ± standard deviation, $n = 3$

a–i, A–D, mean values between different acid, a′–d′ and A′–D′, mean values between fruit and their parts followed by different letters are statistically different at $p \leq 0.05$

OA oxalic acid, *TA* tartaric acid, *CA* citric acid, *ICA* isocitric acid, *MA* malic acid, *QA* quinic acid, *SA* succinic acid, *ShA* shikimic acid, *FA* fumaric acid, *TOA* total organic acid, *Fru* fructose, *Sor* sorbitol, *Glu* glucose, *Sac* saccharose, *TS* total sugar

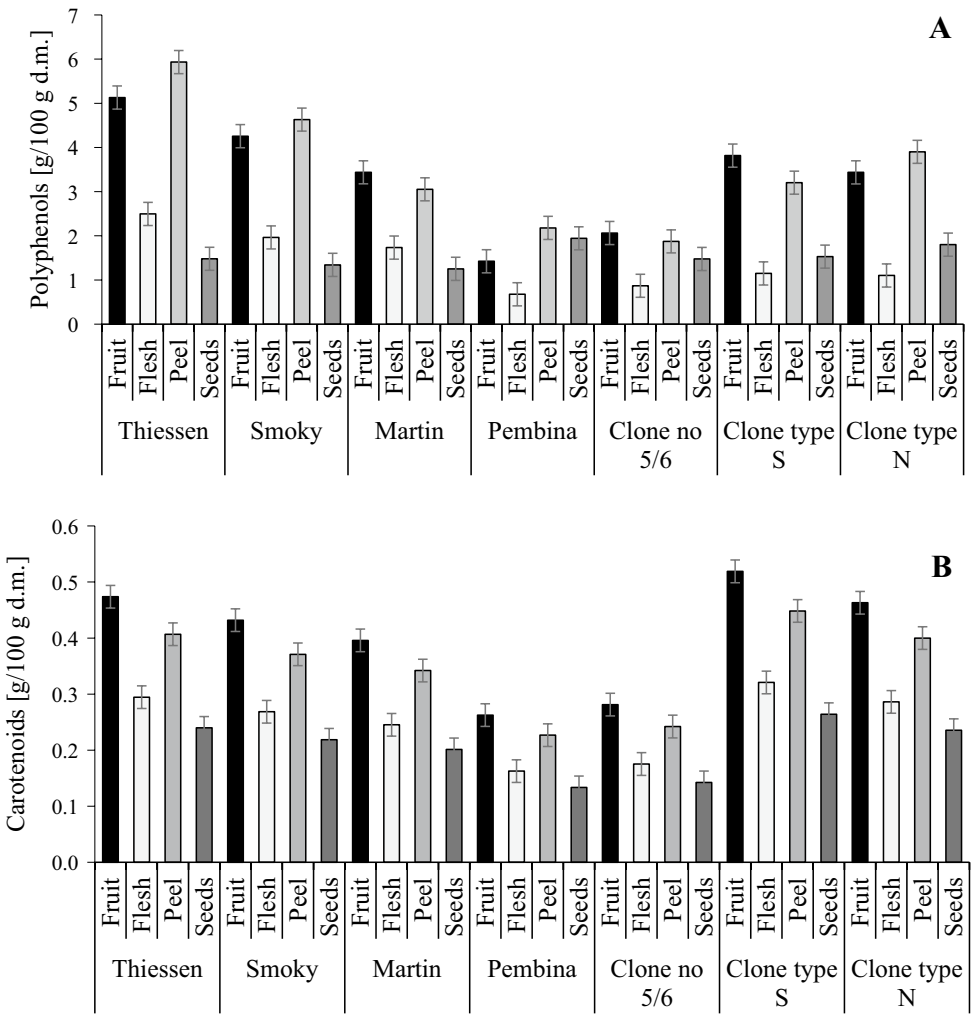

Fig. 1 Content of polyphenols (**a**) and carotenoids (**b**) in the fruit of the tested Saskatoon berry genotypes and fruit components (g/100 g dm), in 2018

the content of antioxidant compounds was 10 times lower than in cvs. 'Smoky' and 'Thiessen'. It was proved that the antioxidant potential was higher in the peel compared to the flesh and seeds. The highest antioxidant capacity was determined in fruits and their components (peel, flesh and seeds) of 'clones type S' and 'type N', and the lowest one in cvs. 'Martin' and 'Pembina' (Fig. 2). The antioxidant potential in the seeds was 2 and 10 times lower than in the peel and flesh, respectively. Differences in the antioxidant capacity of fruit of the tested Saskatoon berry genotypes and their components resulted mostly from their content of polyphenols. The antioxidant potential of fruit of the tested genotypes and their components depended mainly on their chemical composition [6, 28–30]. In addition, the antioxidant capacity was influenced by the structure, content and type of the bioactive compounds [16]. As it was reported by Hu et al. [14], the differences in content of antioxidant compounds might be affected by different climate and cultivation conditions as well as chemical analysis procedures. Results from our study showed that the content of antioxidant compounds in the Saskatoon berry fruit depended significantly on their genotypes and fruit components.

In our study, a strong and positive correlation ($r^2 = 0.918$) was determined between contents of tetraterpenoids and polyphenols. A similar relationship was also estimated between contents of organic acids and polyphenols and carotenoids and it was $r^2 = 0.704$ and $r^2 = 0.725$, respectively, while between acids and sugars it was $r^2 = 0.814$. Thus, the strongest positive correlation ($r^2 = 0.826$) was obtained between the antioxidant capacity and organic acids content. Also, a positive correlation ($r^2 = 0.607$) was calculated between contents of antioxidant compounds and antioxidant properties. The lowest correlation ($r^2 = 0.302$) was obtained between the content of sugars and antioxidants. Hence, the antioxidant potential of fruit of the Saskatoon berry genotypes and their components was correlated with the content of tetraterpenoids, polyphenols, organic acids, and sugars. Our results showed that the antioxidant activity depended on the content of phytochemicals, especially polyphenolic compounds. It was in agreement with results presented by Bakowska-Barczak and Kolodziejczyk [1].

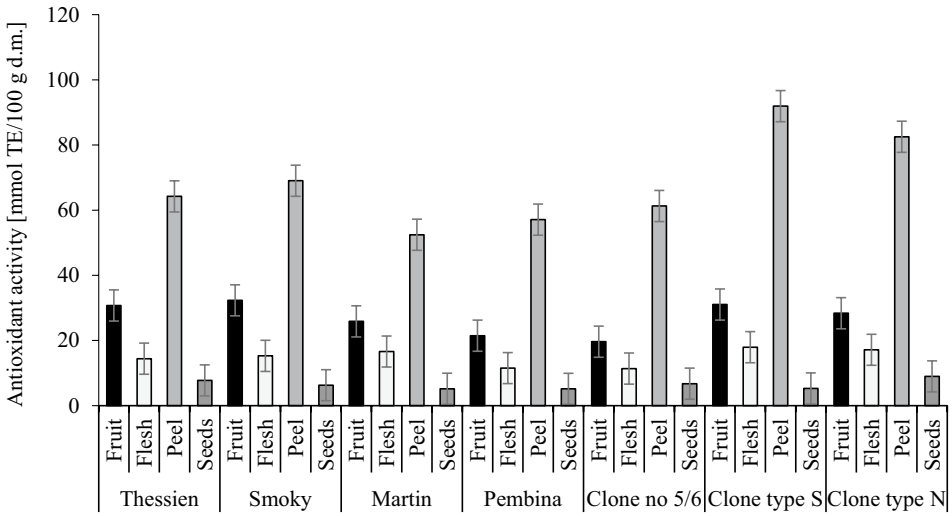

Fig. 2 Antioxidant activity of the tested Saskatoon berry genotypes in fruit and their components (mmol TE/100 g dm) in 2018

Our study showed that organic acids and their individual compounds also played an important role in the antioxidant potential. Organic acids with the high antioxidant capacity could be used in the risk suppression of degenerative diseases and scavenging of free radicals.

Principal component analysis (PCA) and cluster analysis (CA)

Two multivariate methods were used, i.e., principal component analysis (PCA) and CA using Ward's method. Results subjected to the statistical analysis for the PCA and cluster analysis (CA) are presented in Fig. 3a–d. The PCA showed differences between organic acids, sugars, bioactive compounds and antioxidants potential in fruit of the selected Saskatoon berry genotypes and fruit components. A clear grouping of tested genotypes and fruit components was presented, resulting from differences in sugars and organic acid profiles. Furthermore, the PCA for the fruit components (flesh, peel and seeds) of tested genotypes was illustrated (Fig. 3a–d). The principal components (PCs) extracted 69.10% of the total variance, while the first (PC1) and second (PC2) accounted for 46.25 and 22.87%, respectively of the variation. The PC1 factor was clearly related with the sugars and their individual components, polyphenols, tetraterpenoids, and fumaric as well as shikimic acids, while the PC2 was connected with other organic acids. The grouping of the fruit components (flesh, peel and seeds) was also explained (Fig. 3b–d). It was presented that the common factors for the fruit flesh of genotypes: 'Smoky', 'Thiessen' and 'clone type S' had the highest antioxidant activity, carotenoids, organic acids, sugars, and their individual compounds. However, the fruit peel of tested genotypes: 'Martin', 'Pembina', 'clone type N' and no 5/6 had the highest content of fumaric, citric, shikimic acids, and sorbitol. In turn, the seeds of tested Saskatoon berry cultivars: 'Smoky' and 'Thiessen' had the high antioxidant capacity and were richest in carotenoids, sugars and acids.

The classification of the tested Saskatoon berry genotypes was done based on the differences in the profile of chemical compounds between individual components. After grouping analysis, three groups of clusters among these genotypes were identified and presented on the dendrogram (Fig. 3e). The first group of cluster contained three genotypes 'Pembina', 'Martin' and 'clone no 5/6', which were characterized by a low content of bioactive compounds, sugars and organic acids. The second group of cluster included only 'clone type N', with a centered content of all analyzed components. The last group of cluster consisted of cvs. 'Smoky', 'Thiessen' and 'clone type S' and they contained the highest amount of polyphenolic compounds, carotenoids, sugars, organic acids, and their components. These genotypes were distinguished by the high content of malic, quinic, and tartaric acids, on average over 30% of total organic acids.

Conclusions

The study confirms significant differences in phytochemical, and antioxidant potential are confirmed in fruits and their components among seven selected Saskatoon berry genotypes grown in Central Poland. The richest in polyphenols, tetraterpenoids, organic acids, and antioxidant activity were cvs. 'Thiessen', 'Smoky' and 'clone type S'. Organic acids (malic, quinic, and tartaric) were the major acids and accounted for 78% of the total acids content. The highest content of organic acids and bioactive compounds was determined in the peel of fruit of the above-mentioned genotypes. It was proved that fruit of the Saskatoon berry genotypes and their components were valuable source of natural antioxidants. While such fruit components as peel and seeds seem to be suitable for developing new functional

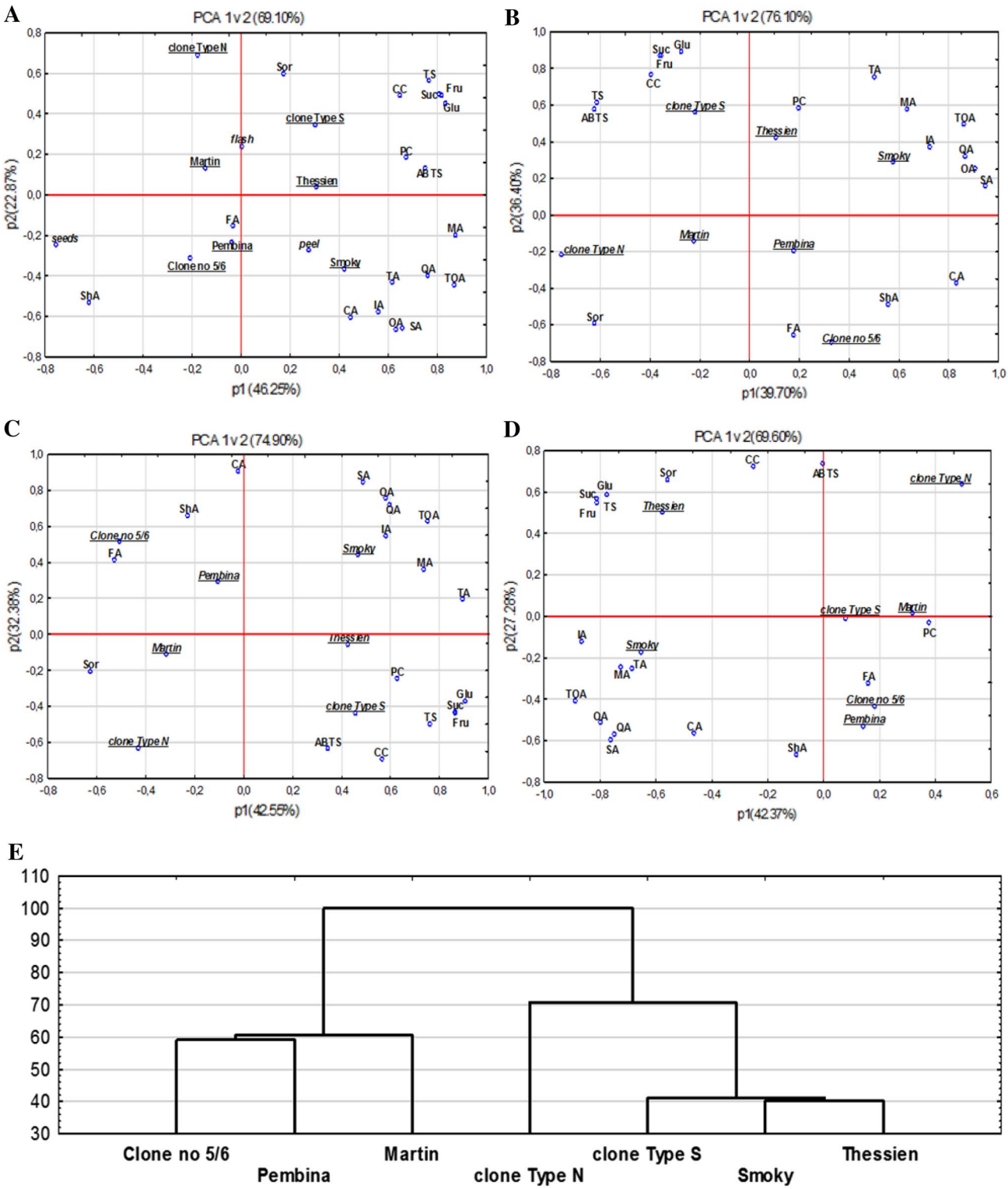

Fig. 3 PCA map showing the relationship among chemical composition and antioxidant activity in fruit and their parts (**a**), in separately for fruit (**b**), peel (**c**), flesh (**d**), and seeds (**e**) of the selected Saskatoon berry genotypes, 2018

food, super food with health-promoting properties as well as components for the medical, pharmaceutical or cosmetic industries. Results of the principal component analysis and cluster analysis showed the diversity of the tested Saskatoon berry genotypes. These findings provide an identification of

three groups, from among seven selected genotypes, with similar contents of specific parameters.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethical requirements This article does not contain any studied with human and animal subjects.

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