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ORIGINAL PAPER

Composition of industrial seedless black currant pomace

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Abstract The aim of the work was to characterize the basic chemical composition including polyphenols of seedless industrial dried black currant pomace from two subsequent harvest seasons, 2006 and 2007. Pomace after removing seeds was separated into three fractions: with particles smaller than 0.8 mm, 2–5 mm and greater than 5 mm. The pomace was analyzed for dry substance, protein, fat, ash, total dietary fiber (TDF), soluble solids content, acidity, organic acids, saccharides and polyphenols. In addition, the antioxidant activity of the pomace was assayed by the DPPH method. All the fractions were characterized by a high content of total dietary fiber above 63% and a low content of saccharides below 3.15% and acidity below 3.2%. The composition of polyphenol compounds of all the fractions was qualitatively determined, yet it was shown that the fraction of the pomace of 2–5 mm is characterized by the greatest content of phytocompounds, of which anthocyanins constitute about 90% of the polyphenols determined. Moreover, the presence of myricetin and quercetin glycosides as well as considerable quantities of myricetin and quercetin aglycones was found in the pomace. The pomace had a high antioxidant activity ranging from 93.3 to 126.5 μM TEAC/g. It was also found that the content of most polyphenol compounds investigated, as well as protein, ash, TDF, acidity and saccharides, was statistically dependent on the type of fraction.

Keywords Black currant pomace · Black currant · TDF · Polyphenols

Introduction

Black currant fruit are an important raw material in the production of nectars and drinks [11, 30]. These products are characterized by good organoleptic properties, i.e. they have an intensive color, taste and aroma, for which they are a desirable market product [9, 27]. Black currant berries are known to be a good source of polyphenol compounds [1, 22].

Among the most important flavonoids occurring in black currant fruit are anthocyanins, the mean content of which in fresh fruit is about 250 mg/100 g, constituting approximately 80% of all flavonoids [1, 35]. Besides anthocyanins, black currants contain hydroxycinnamic acids, including derivatives of *p*-coumaric acids, and myricetin, quercetin, kaempferol glycosides as well as small amounts of isorhamnetin [1].

The flavonoids are known to work as free radical scavengers, enzyme inhibitors, hormones or antihormones, or inducers of gene expression [20, 23]. They also interfere with a large number of regulatory pathways including those of growth, energy metabolism, apoptosis, cell division and stress response [12, 20, 36]. Studies of Moyer et al. [24] indicate that phytocompounds present, among others, in black currant fruit have strong antioxidant properties measured as ORAC (oxygen radical absorbing capacity) and FRAP (ferric reducing antioxidant power). Studies of Ghosh et al. [14] show that polyphenol black currant extracts during in vitro studies demonstrated a protective

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effect on human cells and the DNA chain during the exposition to oxidizing factors.

Juice manufacturing can decrease the amount of phenolic compounds especially in the process of enzymatic depectinization of the fruit mash and pasteurization of juice [30]. Some commercial juice processing enzyme preparations are characterized by glycosidase side activities and can hydrolyze the anthocyanins glycosidic linkages and liberate unstable anthocyanidins [10, 33]. The progress in food production technology is improving the nutritional, sensorial quality of products and increasing the productivity of the process, but it is also changing the composition of by-products. The press cake is the main by-product of fruit juice production. By-products of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds, which may be used because of their favorable technological or nutritional properties [19, 34].

The studies of Kapasakalidis et al. [15] indicate that the extract of black currant pomace provides a good source of phenolic antioxidants, especially anthocyanins, the anthocyanins composition of the pomace being similar to the anthocyanins composition of fruit, where the four main anthocyanins are dephinidin-3-glucoside, dephinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside. Studies of Landbo and Meyer [18] show that seedless pomace contains a greater quantity of polyphenols than pomace with seeds; in addition, their in vitro studies also proved that black currant pomace extracts demonstrated an antioxidant activity against the oxidation of human LDL.

Despite many studies on black currant, the knowledge concerning the basic chemical composition and polyphenol composition of pomace and the properties of the extracts obtained from them is still unsatisfactory. This is partially caused by unhomogeneity of pomace linked to morphology of fruits, the manner of mash preparation, pressing of mash and drying conditions. The pomace can also be regarded as a polydisperse system containing small particles of flesh, hard seeds, ground skins and their agglomerates, stalks and small twigs. Seeds can be easily separated from dried pomace, while seedless material can be considered as a source of phytocomponents and dietary fiber preparations.

The aim of the work was to determine the composition and properties of the seedless fraction of dry industrial black currant pomace with different particle sizes, obtained from two successive harvest seasons. The particle size of three fractions was selected for possibly efficient seeds separation and for preparation of material to phytocomponents recovery by extraction.

Materials and methods

Plant material

Black currant pomace was taken from the concentrated juice production line of Alpex company in July 2006 and July 2007, in an amount of 500 kg each year. Fresh pomace was dried in an industrial vacuum dryer at a temperature below 70 °C in Polfarmex Company. After drying, the pomace was stored in hermetically closed PCV containers at a temperature of 4 °C; the storage time before analysis did not exceed 2 months. Then the black currant pomace was separated into a seedless fraction and a seed fraction by means of a sieve. The seedless fraction was additionally separated into three fractions, i.e. with a particle diameter smaller than 0.8 mm (F 0.8), of a diameter of 2–5 mm (F 2–5) and greater than 5 mm (F 5). The seed fraction was made up of 90% of seeds and 10% of seedless particles of a diameter of 0.8–2 mm.

From each seedless fraction, a mean laboratory sample was prepared, from which quantities of 100 g of material were taken and then ground in liquid nitrogen in an IKA A11 basic mill (IKA-Analytical Mill). The ground pomace was stored at a temperature of 4 °C before successive analyses.

Chemicals and standards

HPLC acetonitrile gradient-grade and formic acid were purchased from J.T. Baker (Witko, Poland). Ultrapure water (Millipore System) was used to prepare all solutions. Methanol for the extraction of polyphenols was purchased from J.T. Baker (Witko, Poland). Standards of cyanidyn-3-rutinoside, myricetin and kampferol-3-glucoside were purchased from Extrasyntese (Genay France); quercetin, kaempferol, quercetin-3-rutinoside and (−)epicatechin were purchased form Sigma-Aldrich. Organic acid standards of malic acid and citric acid, sugar standards as well as Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals to determine antioxidant activity were purchased from Sigma.

Soluble solids

Determination of soluble solids of the pomace was made according to AOAC official method 932.12 [4] using PR-32α Digital Refractometer (Atago, Tokyo, Japan). Two replicate measurements were done for each sample, and the results are given in percentage (%).

Protein

Double measurements of protein content in samples were made according to AOAC official method 920.152, Kjeldahl method [2]. The results are given as % of protein in the pomace.

Fat

Fat was determined using Soxhlet method with petroleum ether according to AOAC official method 930.09 [3]. All the pomace samples were analyzed twice. The results are expressed as % of fat per pomace mass.

Ash

Ash was determined according to AOAC official method 940.26 [5]. The results are expressed as % of ash per pomace mass.

Total dietary fiber

Total dietary fiber (TDF) was determined by the enzymatic weight method according to AOAC official method 985.29 [7]. The results are expressed as % per pomace mass.

Titratable acidity

Titratable acidity of samples was determined according to AOAC official method 942.15 [6]. The results are expressed as g/100 g of the pomace as a anhydrous citric acid.

Organic acids

Organic acids were determined according to the method described by Raffo et al. [28]; however, instead of extracting solution, hot water was used. Twenty-five milliliters of water was added to 2.5 g of the ground pomace. The suspension was heated up to 100 °C and kept at this temperature for 2 min. Then the solution was immediately cooled down to 20 °C, put into a 50 ml measuring flask, filled up with water and carefully stirred. After centrifuging at 5,000 rpm for 5 min, the extract was filtered and injected onto a HPLC column. The chromatographic column was a Gemini 5u Phenomenex (250 × 4.6 mm i.d., 5 μm), with a guard column Security Guard Gemini C18 (4 × 3.0 mm^2). The organic acids were eluted isocratically with 50 mM KH_2PO_4 (pH 2.5) at a flow rate of 1 ml/min, and the eluate was monitored at 214 nm. Quantification was achieved by means of calibration curves of malic and citric acid. All the samples were analyzed in duplicates. The results are expressed as grams of malic or citric acid per 100 g of pomace.

Saccharides

Saccharides were determined by the HPLC method by means of an Aminex calcium column. The extraction was carried as follows. Twenty-five milliliters of water and 1 g of $CaCO_3$ were added to 2.5 g of the ground pomace. The suspension was heated to a boiling point and maintained at this temperature for 5 min. Next, the solution was cooled, transferred quantitatively by means of water to a 50-ml volumetric flask, filled up to the graduation mark, kept at room temperature for 15 min and filtered. Then the filtrate was cleaned on SPE column containing cation exchanger resin Amberlite IR120 and anion exchanger resin Amberlite IRA420 at a volumetric ratio of 1:2. The samples cleaned were centrifuged for 5 min at 5,000 rpm and injected into HPLC column. The content of sugars in the samples was determined with the use of Aminex HPX87C column from Bio-Rad (0.78 × 30 cm^2, mobile phase: water, flow: 0.5 ml/min, at 85 °C). Standards of saccharose, glucose and fructose were used for the identification and quantification of the substances mentioned. RI detector (Knauer K-2301) and a Knauer integrating system were used. The results are expressed as grams of individual sugar per 100 g of pomace.

Extraction of polyphenols

Polyphenol compounds were extracted by a mixture solution of the composition of methanol:water:formic acid at a volumetric ratio of 50:48:2 v/v/v. Half gram of the ground pomace was placed in a 7-ml test tube, 5 ml of a solvent was poured on it, then it was mixed using a vortex and sonified for 5 min. Following the sonification, the solution was left in the dark for 15 min for extraction. The solution was centrifuged and poured into a flask. Above procedure was repeated for another four times; however, the third extraction was carried for 24 h. The combined extracts were poured into a 25-ml volumetric flask. Three extractions were made from each pomace type. The extracts thus obtained were stored at a temperature of −20 °C for 3 days at the maximum before further analysis.

Total phenolics

The total phenolics were measured by the method described by Singleton and Rossi [32] with some modification. Phenolic extract (0.25 ml) was placed in 25-ml flasks, and 0.25 ml of Folin–Ciocalteau agent and 2.5 ml of 20% Na_2CO_3 were added, filled up with water to the graduation mark and stirred. The incubation was carried at room temperature for 1 h. The absorbance of the solutions was measured at a wavelength of 720 nm. The results were expressed as milligrams of (−)epicatechin equivalent per

100 g of pomace. All the samples were analyzed in duplicate.

HPLC analysis of polyphenols

Before analysis, the extracts were centrifuged at 5,000 rpm. Anthocyanins and other phenolics were analyzed using KNAUER Smartline chromatograph (Germany) equipped with two pumps. The compounds in the phenolic extracts were separated on a 150 mm × 4.6 mm i.d., 5 μm Gemini 5u C18 110A column (Phenomenex, Torrance, CA) using gradient elution with 10% v/v formic acid in water (A) and 50:40:10 v/v/v acetonitrile:water:formic acid (B). The column temperature was set to 40 °C. The flow rate was 1 ml/min and the gradient program was as follows: 0–0.6 min, 12% B; 0.6–16 min, 12–30% B; 16–20.5 min 30–100% B; 20.5–22 min, 100% B; 22–25 min, 100–12% B, 25–35 min, 12% B. The injection volume was 20 μl. The data was collected by the Eurochrom 2000 program. Quercetin and myricetin compounds were assayed at a wavelength of 360 nm, while anthocyanins at were assayed at 520 nm.

Identification and quantification

To identify anthocyanins and the remaining flavonoids, standards purchased from Extrasyntese (Genay, France) and Sigma-Aldrich were used, and UV–vis spectra and the literature [1, 15] were employed.

The amounts of flavonoids in the samples were analyzed by HPLC. Standard curves made from cyanidine-3-rutinoside, rutin, quercetin, myricetin and kaempferol were used. To assay anthocyanins, cyanidin-3-rutinoside was used, whereas to assay quercetin and myricetin glycosides, rutin was used. Isorhamnetin was assayed on the basis of the quercetin standard.

DPPH radical-scavenging activity

DPPH scavenging activity was determined using the method of Dae-Ok Kim et al. [16]. Sixty-micromolar DPPH was dissolved in 80% aqueous methanol. The phenolics extract of 0.05 ml was added to 1.95 ml of a methanolic DPPH solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 515 nm for 30 min. The control consisted of 0.05 ml of 50% aqueous methanol and 1.95 ml of DPPH solution. A concentration-response curve for the absorbance at 515 nm after DPPH as a function of different Trolox concentrations was prepared. The DPPH radical-scavenging of black currant pomace phenolics extract was expressed as μM TEAC/g of pomace (TEAC—Trolox equivalent antioxidative capacity).

Statistics

The effect of particle size on the content of the particular components was determined using one-way analysis of variance, and significant differences between fractions were determined by Duncan's multiple range test. The differences were considered significant at $P \leq 0.05$.

Results and discussion

Granulometric and nutrient composition of black currant pomace

The granulometric composition of the dried pomace from two seasons is presented in Table 1. Black currant pomace from the industrial processing of fruit into juice contains seeds and seedless material in a proportion of 5.5:4.5. Seeds for which results are not reported here were isolated in a fraction of 0.8–2 mm. Fraction of a size of 2–5 mm constituted about 25% of seedless fraction, whereas the fraction of a size greater than 5 mm 12% and the material of a size under 0.8 mm 6%. About 4% of seedless fraction was removed with seeds.

The composition of the basic nutrient components of the black currant pomace seedless fraction is shown in Table 2. The protein content in each pomace fraction was similar and amounted to approximately 12%. Taking into consideration all the seedless fractions, the protein content in fraction F 5 was significantly different from the remaining fractions of the 2006 season. In the pomace of the 2007 season, no statistical differences in the protein content in the fraction was found. The fat content significantly depended on the kind of fraction in the 2006

Table 1 Mass proportions of the particular fractions in industrial black currant pomace

Fraction	Granulometric composition Mass proportion of particular fractions	
	Season 2006	Season 2007
Seedless fraction	**42.9**	**37.9**
$\varphi < 0.8$ mm (F 0.8)	6.1	4.8
2–5 mm (F 2–5)	23.2	25.1
$\varphi > 5$ mm (F 5)	13.7	7.9
Seed fraction (0.8–2.0 mm)	**57.1**	**62.1**
Seeds	53.4	57.3
Seedless particles	3.6	4.8

Table 2 Content of basic nutrient components in dried black currant pomace

Fraction	Dry substance ($n = 3$)	Protein ($n = 2$)	Fat ($n = 2$)	Ash ($n = 3$)	TDF ($n = 3$)	MC ($n = 2$)
2006 season						
F 0.8	95.7 ± 0.4a	11.8 ± 0.1a	2.5 ± 0.2a	3.4 ± 0.2c	67.1 ± 0.3c	10.8 ± 0.4b
F 2–5	94.0 ± 0.2b	12.0 ± 0.1a	3.6 ± 0.1c	2.3 ± 0.0a	67.6 ± 0.3c	8.5 ± 0.5b
F 5	97.1 ± 1.2a	12.6 ± 0.1c	4.1 ± 0.3d	2.4 ± 0.1a,b	64.6 ± 0.7a,b	13.3 ± 1,6a
2007 season						
F 0.8	96.8 ± 0.6a	12.1 ± 0.1a,b	2.6 ± 0.1a	4.1 ± 0.2c	63.3 ± 0.2a	14.9 ± 0.1a
F 2–5	96.3 ± 0.1a	12.1 ± 0.2a,b	2.5 ± 0.1a	2.5 ± 0.1a,b	65.2 ± 1.3b	14.0 ± 1.7a
F 5	96.3 ± 0.2a	12.3 ± 0.0b,c	3.2 ± 0.2b	2.6 ± 0.0b	64.4 ± 0.2a,b	13.8 ± 0.1a

Values are means ± standard deviation (SD) expressed in percentage

The results in the individual columns marked by the same letter do not differ statistically at $P < 0.05$

n number of measurements, *MC* metabolized carbohydrates

season, while in the 2007 season only fraction F 5 was significantly different from the remaining ones. Fat content in fractions F 5 in both seasons was the greatest and was statistically different from the remaining ones, which is probably connected with the greater proportion of seeds in large, conglomerated particles of this fraction. The ash content in the fractions studied also depends on the fraction. Its content ranges from 2.3 to 3.4% for the 2006 season and from 2.5 to 4.1% for the 2007 season. Statistically, the ash content in fraction F 0.8 is the greatest and is significantly different from the remaining ones. It has also been found that the content of metabolized carbohydrates in fractions F 0.8 and F 2–5 from the 2006 season was significantly different from the carbohydrate content in the greatest granulation fraction, F 5. The TDF content in the pomace was in the range from 64.6 to 67.6% for the 2006 season and from 63.3 to 65.2% for the 2007 season. Statistically, the TDF content was dependent on the particle size in the fractions studied; in 2006 fraction, F 5 was different from the remaining ones, whereas in the 2007 season, significant differences were found between fractions F 0.8 and F 2–5. Comparing the above results with the literature data [17] concerning the dry substance of fruit, it can be stated that the protein and fat content in the pomace is over twice as high as that in the dry substance of fruit. The studies of Nawirska and Kwaśniewska [25] indicate that the native dietary fiber of black currant pomace in which seeds were not separated was mainly composed of lignins and hemicelluloses, which constituted 74 and 15% of the dietary fiber fraction mass, respectively.

Depending on the fraction type and season, the soluble solids content of the pomace was characterized by a great variability in the range from 6.9 to 15.6% (Table 3). The fraction of the greatest particle size was the richest in water-soluble components. Statistically, the soluble solids content in fractions F 0.8 for both season differed significantly from the remaining ones.

Table 3 Content of soluble solids, saccharides and acids in dried black currant pomace

Fraction	Soluble solids ($n = 2$)	Acidity ($n = 3$)	Saccharides ($n = 3$)			Acids ($n = 2$)	
			S	G	F	M	C
2006 season							
F 0.8	6.9 ± 0.2c	1.3 ± 0.0c	0.34 ± 0.01a,b	0.04 ± 0.06b	0.13 ± 0.04b	0.04 ± 0.01c	1.69 ± 0.01a
F 2–5	11.2 ± 0.4a	1.8 ± 0.1a	0.55 ± 0.13a,b	0.29 ± 0.02a,b	0.50 ± 0.06a	0.10 ± 0.01a	1.83 ± 0.01a
F 5	11.8 ± 0.5a	1.7 ± 0.1a	0.59 ± 0.03a	0.53 ± 0.04a,c	0.82 ± 0.04c	0.09 ± 0.03a	1.53 ± 0.35a
2007 season							
F 0.8	10.4 ± 0.4d	2.9 ± 0.0b	0.30 ± 0.11b	0.33 ± 0.13a	0.56 ± 0.03a	0.09 ± 0.00a	2.69 ± 0.08b
F 2–5	14.9 ± 0.2b	3.2 ± 0.0d	0.38 ± 0.15a,b	0.79 ± 0.20c	1.21 ± 0.07d	0.15 ± 0.01b	2.72 ± 0.04b
F 5	15.6 ± 0.0b	3.0 ± 0.1b	0.59 ± 0.11a	1.13 ± 0.11d	1.43 ± 0.11e	0.15 ± 0.00b	2.47 ± 0.19b

Values are means ± standard deviation (SD) expressed in g/100 g

The results in the individual columns marked by the same letter do not differ statistically at $P < 0.05$

n number of measurements, *S* saccharose, *G* glucose, *F* fructose, *M* malic acid, *C* citric acid

In the case of saccharides content determined by the HPLC method, significant differences between the fractions were demonstrated by glucose and fructose. No statistical differences in the saccharose content were found between the fractions in 2006 season, while in 2007 season, statistical differences between F 0.8 and F 5 were found. The mean content of glucose in the pomace from both seasons was in the range of 0.04 g/100 g and 1.13 g/100 g, whereas the mean fructose content ranged from 0.13 g/100 g to 1.43 g/100 g. In both seasons, the glucose and fructose content was significantly dependent on the fraction type, except for glucose in fraction F 0.8 from the 2006 season. Because of the lack of literature data concerning the sugar content in black currant pomace, the results obtained by the authors were compared with the data for whole fruit. The studies of Boccorh et al. [8] showed that the saccharose content in fresh fruit calculated on dry mass ranged from 6.4 to 18.2%. Low values of sugars found in the pomace indicate that most of sugars, i.e. about 96%, pass to the juice during the production process. The acidity of the pomace from the 2006 season ranged from 1.3 to 1.8%, while from the 2007 season from 2.9 to 3.2%. Statistically, for both seasons, the acidity depended on the particle size. Acidity of fraction F 0.8 was smaller in both seasons than of the other fractions (about 25% in 2006 and 10% in 2007). According to the data given by Anttonen et al. [1] and Rubinskiene et al. and Siksnianas et al. [29, 31], the acidity of black currant fruit calculated on dry mass is in the range from 15.9 to 22.0%; authors' data confirm the fact that the majority of acids, approximately 92%, similarly to sugars, are extracted to the juice during production; extraction was particularly efficient from the material of small dimensions (F 0.8). Citric acid was the main organic acid occurring in black currant pomace; its content in the pomace from both seasons ranged from 1.53 to 2.72%. In the pomace, there was also malic acid, but its content in the particular fractions from both seasons did not exceed 0.16%. Statistically, the citric acid content did not depend on the fraction type, while the malic acid content depended on the fraction, the lowest content was found in fraction F 0.8.

Polyphenols and antioxidant activity

The total content of polyphenols was assayed by the Folin–Ciocalteu method. Statistically, significant differences between the fractions were demonstrated only in the pomace from the 2006 season. The total polyphenol content from the 2007 season was in the range from 2,189.6 mg/100 g to 2,285.6 mg/100 g (Table 4). Kapasakalidis et al. [15] obtained, in a seedless fraction of the pomace, the polyphenol content ranging from 940 mg/100 g to 7,300 mg/100 g, these values being dependent on the manner of preparation of a sample for analysis and on the manner the pomace was obtained.

Table 4 Total phenolics content and antioxidant activity of seedless black currant pomace

Fraction	Polyphenols (mg/100 g, $n = 4$)	Antioxidant activity (μM TEAC/g, $n = 4$)
2006 season		
F 0.8	2077.0 ± 44.4a	98.9 ± 2.4a,b
F 2–5	2072.6 ± 37.2a	101.0 ± 1.7a,b
F 5	1855.5 ± 22.3b	93.3 ± 2.1a
2007 season		
F 0.8	2241.6 ± 81.9a	115.4 ± 0.4a,b,c
F 2–5	2285.6 ± 117.9a	126.5 ± 23.8c
F 5	2189.6 ± 141.4a	119.3 ± 16.2b,c

Values are means ± standard deviation (SD) of four measurements

The results in the individual columns marked by the same letters do not differ statistically at $P < 0.05$

Polyphenols assayed by the HPLC method demonstrated greater differentiation (Table 5). The content of the particular polyphenols was substantially dependent on the fraction type. Anthocyanins were the main compounds in the pomace; they constituted approximately 90% of the polyphenols assayed by the HPLC method. The four main anthocyanins occurring in the pomace were delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside, which constituted about 96% of all the anthocyanins. With reference to pomace, a similar dependence was indicated by Kapasakalidis et al. [15], while Anttonen et al. and Matsumoto et al. [1, 21] showed that in fresh fruit the four anthocyanins constituted from 87 to 95% of the polyphenols assayed by the HPLC method. According to the studies of Anttonen et al. [1], there were also three myricetin glycosides (glucoside, rutinoside and malonyloglucoside) and three quercetin glycosides (glucoside, rutinoside and malonyloglucoside). In addition, there were trace amounts of kaempferol glucoside. The myricetin glycoside content in the pomace was in the range from 11.5 mg/100 g to 16.3 mg/100 g for 2006 and from 28.0 mg/100 g to 30.7 mg/100 g for 2007. Quercetin glycosides occurred in smaller quantities than those of myricetin, i.e., their content ranged from 5.0 mg/100 g to 10.4 mg/100 g for the 2006 season and from 14.4 mg/100 g to 15.9 mg/100 g for the 2007 season. A similar dependence was demonstrated by myricetin and quercetin aglycones; moreover, small amounts of kaempferol and isorhamnetin aglycones were found in the pomace.

Statistically, taking into account the four main anthocyanins, their content depended on the fraction type for both seasons. On average, in total, fraction F 2–5 was characterized by the greatest quantities of anthocyanins,

Table 5 Pomace polyphenols assayed by the HPLC method [mg/100 g]

Components	2006 season			2007 season		
	F 0.8	F 2–5	F 5	F 0.8	F 2–5	F-5
Myricetin glycosides[a]	11.5 ± 0.4c	15.2 ± 1.0a	16.3 ± 0.7a	28.0 ± 0.1d	30.5 ± 0.7b	30.7 ± 0.3b
Quercetin glycosides[a]	5.0 ± 0.0c	8.9 ± 1.9b	10.4 ± 0.2b	15.5 ± 0.8a	14.4 ± 0.3a	15.9 ± 0.1a
Myricetin	26.5 ± 0.1b	24.2 ± 0.4a	16.2 ± 0.4c	27.0 ± 0.9b	24.1 ± 0.4a	17.5 ± 0.1d
Quercetin	15.6 ± 1.2b	13.9 ± 2.0b	9.8 ± 0.5a	9.8 ± 0.1a	10.0 ± 0.4a	6.0 ± 0.1c
Kaempferol	3.8 ± 0.4a,b	3.4 ± 0.4a	2.4 ± 0.1c	4.2 ± 0.3a	1.9 ± 0.0c	3.8 ± 0.1a,b
Isorhamnetin[b]	1.0 ± 0.1b	1.2 ± 0.1b	1.1 ± 0.0b	0.6 ± 0.1a	0.4 ± 0.0a	0.7 ± 0.2a
Delphinidin-3-glucoside[c]	85.3 ± 1.2a	150.7 ± 8.6c	133.1 ± 5.9b	190.9 ± 0.3d	288.5 ± 8.1f	249.9 ± 7.6e
Delphinidin-3-rutinoside[c]	122.2 ± 1.7a	213.9 ± 12.9c	191.4 ± 13.2b	254.2 ± 3.4d	369.3 ± 2.6f	320.2 ± 1.3e
Cyanidin-3-glucoside[c]	27.1 ± 0.2a	45.9 ± 1.4c	39.5 ± 1.4b	68.9 ± 0.5d	101.5 ± 2.7f	87.1 ± 2.1e
Cyanidin-3-rutinoside[c]	84.1 ± 0.4a	135.1 ± 6.9c	122.3 ± 6.4b	185.5 ± 1.6d	256.8 ± 1.3f	226.1 ± 1.6e
Remaining anthocyanins[c]	26.0 ± 0.1a	31.6 ± 2.3b	27.5 ± 0.2a,b	30.3 ± 0.9a,b	30.1 ± 4.4a,b	28.5 ± 0.6a,b
Total aglycones	46.9 ± 1.6d	42,8 ± 3.0b	29,4 ± 0.3a	41,5 ± 1.2b	36,4 ± 0.6c	27.9 ± 0.6a
Total anthocyanins	344.6 ± 0.5a	577.1 ± 32.2c	513.7 ± 26.4b	729.7 ± 4.4d	1046.1 ± 10.4f	911.7 ± 13.1e
Total phytocompounds	407.9 ± 0.8a	644.0 ± 38.1c	569.9 ± 26.7b	814.5 ± 4.0d	1128.6 ± 9.8f	986.2 ± 13.2e

Values are means ± standard deviation (SD) of three measurements

The results in the individual lines marked by the same letters do not differ statistically at $P < 0.05$

[a] The content of glycosides calculated on quercetin-3-rutinoside

[b] The content of the substance calculated on quercetin

[c] The content of the substance calculated on cyanidin-3-rutinoside

while fraction F 0.8 by the smallest amount of anthocyanins.

The content of myricetin glycosides, myricetin, quercetin and kaempferol aglycones, similarly to that of anthocyanins, was also statistically dependent on the fraction type. Quercetin glycosides demonstrated such differentiation only in the 2006 season. In the case of isorhamnetin content, no statistical differences between the fractions for both seasons were found.

Taking the lack of myricetin and quercetin in fruit as the basis [1, 13], the degree of hydrolysis (SH) of their glycosides in the pomace was determined from the relationship:

$$\mathrm{SH} = \frac{\text{aglycone conc.}}{\text{aglycone conc.} + C_{\mathrm{AM}} \cdot \text{glycoside conc.}}, \quad \text{where } C_{\mathrm{AM}} = \frac{\text{aglyconemolarmass}}{\text{glycoside molar mass}}$$

The degree of hydrolysis of myricetin and quercetin glycosides is shown in Figs. 1 and 2. The degree of hydrolysis of both glycosides is in the range of 60–82% and it depends on the particle size, i.e., the fraction of the smallest particle size demonstrated a greater degree of hydrolysis of myricetin glycosides, on average by 20%, in relation to the fraction of the greatest particle size. The degree of hydrolysis of quercetin glycosides in the fraction of the lowest granulation was 23% higher, comparing to the

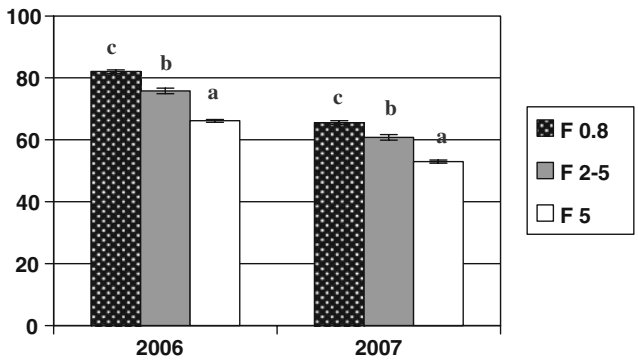

Fig. 1 Degree of hydrolysis of myricetin glycosides

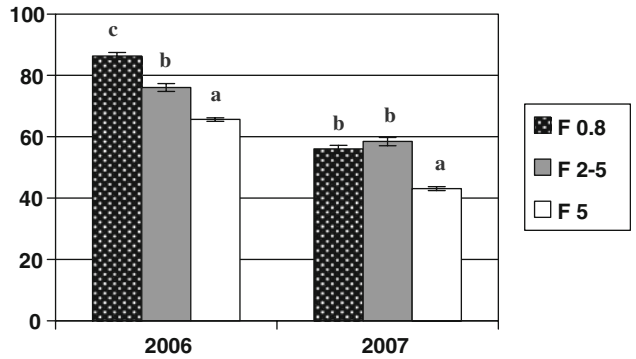

Fig. 2 Degree of hydrolysis of quercetin glycosides

fraction of the largest size. According to Landbo and Meyer [18], the high degree of hydrolysis is associated with the enzymation process, whereas the higher proportion of aglycones in finer particles of the pomace results rather from the greater solubility of glycosides and correspondingly lower solubility of aglycones in water.

The significant differences in the degree of hydrolysis of quercetin and myricetin glycosides were found in the pomace from the two successive years. The pomace in which the degree of hydrolysis of the aforementioned glycosides are lower are characterized by a higher content of saccharides, acids and polyphenols, which makes pomace a better source for the isolation of phytocompounds or the obtaining of anthocyanins–sugar extracts.

The results of the antioxidant activity of the seedless fraction of the pomace (Table 4), determined by DPPH free radical method were expressed as the concentration of TEAC (μM)/g of the product. The results presented indicate that black currant pomace have a high antioxidant activity; this value ranged for both seasons from 93.3 μM TEAC/g to 126.5 μM TEAC/g of the dry product. A similar activity was found in the studies by Nawirska et al. [26], where the antioxidant activity of the pomace was 138.8 μM TEAC/g; in addition, it should be pointed out that their pomace was obtained under milder drying conditions. Antioxidant activity, similarly to the content of total polyphenols determined by the Folin–Ciocalteu method, did not depend on the fraction type.

Conclusions

Statistically significant differences were found in the content of fat, ash and total dietary fiber in the granulometric fractions. The fraction of under 0.8 mm is characterized by the smallest content of fat, saccharides and polyphenols, which results from their better transfer to juice comparing to larger size fractions. The seedless fraction of the particle size of 2–5 mm is characterized by the greatest content of phytocompounds, including anthocyanins and quercetin and myricetin glycosides and thus can be taken into consideration as a valuable raw material for obtaining food colorants and antioxidants.

Industrial seedless black currant pomace is a good source of dietary fiber and protein. Seedless black currant pomace has high antioxidative activity due to large amount of polyphenols present. High variability of the components has to be taken into account when industrial pomace is to be used. The composition of the pomace depends on a growing season and technology of juice extraction.

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