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EFFECT OF PROCESSING AND STORAGE CONDITIONS ON PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF HIGHBUSH BLUEBERRY JAMS

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A decrease in contents of anthocyanins, total phenolics, chlorogenic acid and antioxidant capacity in different highbush blueberry (*Vaccinium corymbosum* L.) jams was studied during processing and storage. Jams prepared with sucrose (high-sugar and low-sugar jams), sweeteners (light jam), sweeteners and oligofructose (light jam with oligofructose) were processed with the use of the same parameters. The content of phenolic compounds and antioxidant capacity were determined directly after production and after 2, 4 and 6 months of storage of the jams.

Bearing in mind the content of fresh fruit in jams, the processing of berries evoked a reduction in the antioxidant capacity by 13 to 19%. The difference in total phenolics and anthocyanins contents of all blueberry jams was significant. The highest content of phenolic compounds was reported in the high--sugar jam, whereas the lowest one in the product with oligofructose. The HPLC analysis showed changes in blueberry anthocyanins profile during jam processing. Arabinosides were more labile than galactosides and glucosides. In contrast, stability of individual anthocyanins during storage was very similar. The rate of anthocyanins and antioxidant capacity degradation was influenced, to a substantial extent, by temperature and time of storage.

INTRODUCTION

Highbush blueberries are native to North America and extensively cultivated in the United States and Canada. More recently they have become a popular commercial crop in Central Europe. These fruits are known to contain a high level of antioxidant compounds. Their antioxidant capacity has been attributed to their high concentration of phenolics, particularly anthocyanins and chlorogenic acid (the most predominant cinnamic ester in blueberry) [Skrede *et al*., 2000]. Compared with other fruit, highbush blueberries have a complex mixture of anthocyanins. Kalt *et al.* [1999] reported that blueberries contain cyanidin, delphinidin, petunidin, peonidin and malvidin glucosides, arabinosides and galactosides. Quantitatively, delphinidin and malvidin glycosides were present in the largest quantities, and derivates of peonidin were the least abundant.

Although most blueberries are marketed fresh, substantial quantities are processed into shelf-life products, available to consumers all year round. The most popular product is jam. The colour of blueberry jam is an important factor influencing consumer acceptability, thus minimizing anthocyanin losses during processing is in primary concern. It is common knowledge that anthocyanins are unstable during processing and storage of processed fruits. Temperature, oxygen, pH, light illumination, water activity, presence of saccharides and their degradation products and activities of various enzymes are considered to be important factors influencing anthocyanin stability [Wrolstand, 2000]. Generally, temperature and duration of boiling and pasteurization, jam recipe (sugar and citric acid content), cultivar and degree of fruits ripeness as well as storage conditions of products are the most important factors determining the quality of blueberry jam [Kim & Zakour, 2004; Garcia-Viguera *et al.,* 1998, 1999].

Recently, blueberries have become of special interest to researchers studying anthocyanin content and antioxidant capacity. It is common knowledge that the content of antioxidant compounds is widely affected by cultivar or environmental factors [Connor *et al*., 2002], but scarce information is available on changes in phenolics content of blueberries processed into jam and stored.

The aim of this work was to determine contents of anthocyanins, total phenolics, and chlorogenic acid as well as the level of antioxidant capacity in jams and to compare them with the values found in fresh fruit. The study focused on four types of jam: high-sugar, low-sugar, light, and light with oligofructose. In addition, we monitored changes in antioxidant compounds during long term storage of processed fruits at 6°C and 22°C.

MATERIALS AND METHODS

Plant material

Blueberries (*Vaccinium corymbosum* L.) of the "Bluecrop" cultivar used in this study were grown at a plantation in Piskórka near Warsaw (Poland). Agronomic practices were done in accordance with recommendations for commercial growing. Sample ripeness was judged on the basis of skin

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colour of representative berries. The berries were not selected for size, but reflected the typical weight for the Bluecrop cultivar. After the harvest, the fruits were stored in a cold room for less than 3 days, until jams preparation.

Materials

High methoxyl pectin (type WEC-1) was purchased from ZPOW Pektowin Jasło (Poland) and low methoxyl pectins (type LM 104 AS and LM 102 AS) were obtained from Hercules In. (Denmark). Sweeteners (Aspartam and Acesulfam K) were provided by Hoechst (Germany) and Ajinomoto (Switzerland). Preparations of oligofructose were obtained from Orafti (Belgium).

Preparation of blueberry jams

Blueberry jams were prepared in the laboratory, according to a traditional procedure, by boiling in an open kettle, with manual stirring. The recipe is provided in Table 1. Blueberries, crystallized sucrose (or preparation of oligofructose) and water were gently mixed together. The mixture was allowed to boil for 20 min, after which sweeteners (in light jams) and a pectin solution were added (commercial pectin dissolved in 100 mL hot water with the aid of a mixer). Afterwards, the mixture was boiled for 10 min. At the end of boiling, citric acid and water were poured (in the volume equivalent to water that evaporated during boiling). The jam was hot-packed into glass jars with screw caps and pasteurized at 85°C for 20 min. When the jams were cooled to room temperature they were divided into batches stored at 6°C and 22°C for 6 months. Samples were taken after 0, 2, 4 and 6 months of storage and stored at –35°C until analysed.

Analytical methods

Concentrations of soluble solids were determined in an Abbe refractometer at 20°C and expressed as degrees Brix (°Brix). Total acidity was determined by potentiometric titration with NaOH 0.1N to pH 8.2. Total phenolics content was determined with the Folin-Ciocalteau method at 700 nm, using chlorogenic acid as a standard [Peri & Pompei, 1971]. Total anthocyanin pigment contents of berries and jams were determined by using the pH differential methods [Sondheimer & Kertesz, 1948]. Total antioxidant capacity values in blueberries and jams were assayed with the ABTS radical cation decolourization method [Miller & Rice-Evans, 1996]. The results were expressed as μmol Trolox equivalent/g sample.

Determination of the contents of anthocyanins and chlorogenic acid was conducted with the method of high performance liquid chromatography. Anthocyanins and chlorogenic acid were extracted two times from 4-9 g samples of homogenized fruit or jams using 80 mL of a solution containing methanol/acetone/water/acetic acid (30:30:35:0.1, v/v/v/v). The extracts were filtered and combined. Methanol and acetone were removed by rotary evaporation. The extract was mixed with 0.1% phosphoric acid to a final volume of 25 mL. Ten mL of the extract were injected onto a Sep-Pak C_{18} cartridge (Waters). The cartridges were preconditioned by washing with 5 mL of methanol followed by 10 mL of 0.1% phosphoric acid. Anthocyanins and chlorogenic acid were absorbed into the cartridge, while sugar and organic acid were removed by flushing with water containing 0.01% phosphoric acid. Chlorogenic acid and anthocyanin compounds were recovered with 5 mL of acidified methanol (0.01% HCL). Samples were filtered through PTFE 0.45 μm filters before analyses. The chromatographic system consisted of an LC10-ATpt pump fitted with a SPD-10Avpt UV-visible detector and column heated. A Luna RP–18 (5 μm, 250×4.6 mm) column from Phenomenex was used. Anthocyanins were eluted with a gradient of 10% formic acid (mobile phase A) and 100% acetonitrile (mobile phase B), elution profile by linear gradient steps: start condition 6% phase B in A, then, 9% B in 7 min, 11% B for 11 min, 14% B for 3 min, 22% B for 5 min, 30% B for 4 min, 6% B for 4 min, used at the flow rate of 1 mL/min. Peak areas were monitored at 520 nm. Standards of anthocyanins available from the previous works [Gao & Mazza, 1994; Kalt *et al*., 1999] were used to identify anthocyanins. The cyanidin-3--glucoside standard was used for quantification of monomeric anthocyanins in blueberry jams. Chlorogenic acid was separated by isocratic elution. The eluent was a mixture of water, acetonitrile and formic acid (81:9:10 v/v/v) used at the flow rate of 1 mL/min. Chlorogenic acid was detected at 320 nm and identified according to retention time by comparing with the standard.

Statistical analysis

Statistical analyses were performed with Statgraphics Plus 4.1. Significant differences between the jams were calculated by one-way analysis of variance (ANOVA). The influence of temperature, time of storage and type of jam was determined by three-way analysis of variance. Significant differences ($p \leq 0.05$) between mean values were tested with the Tukey's method. The jam processing was done in triplicate.

RESULTS AND DISCUSSION

Chemical composition of blueberry fruits

The chemical composition of blueberry is shown in Table 2 and the contents of individual anthocyanins and their

TABLE 1. Jam recipe.

Type of jam	Ingredients (g/1000 g jam)						
	blueberries	sucrose	sweeteners	pectin	oligofructose	citric acid	water
High-sugar (e=60%)	460	515	-	7	-	8	10
Low-sugar (e=38%)	460	295	-	7	-	8	230
Light (e=8.6%)	460	-	1.5	7	-	8	523.5
Light with oligofructose (e=20%)	460	-	1.5	7	115	8	408.5

e-soluble solids of jams

TABLE 2. Soluble solids, titratable acidity, contents of total phenolics, total anthocyanins, chlorogenic acid and antioxidant activity in fruits and jams of highbush blueberry.

Parameters	Fruits	High-sugar jam	Low-sugar jam	Light jam with oligofructose	Light jam
Soluble solids (%)	14.2	59.8[d]	38.1[c]	20.4[b]	8.6[a]
Titratable acidity (%)	0.8	1.2[a]	1.2[a]	1.1[a]	1.1[a]
Total phenolics (mg/100 g)	383.3	163.2[c]	158.5[b]	146.8[a]	158.1[b]
Chlorogenic acid (mg/100 g)	84.9	38.6[a]	37.4[a]	37.3[a]	38.6[a]
Total anthocyanins (mg/100 g)	94.6	32.6[c]	28.9[b]	19.8[a]	28.0[b]
Antioxidant capacity (μmol Trolox/g)	28.6	11.5[b]	11.3[b]	10.7[a]	11.0[ab]

Values in rows with different letters are different ($p<0.05$) based on Tukey comparison test.

TABLE 3. Contents of individual anthocyanins and their percentage contribution to the total anthocyanins in fruits and jams of highbush blueberry.

Anthocyanins (peak no.)[a]	Fruits		High-sugar jam		Low-sugar jam		Light jam with oligofructose		Light jam	
	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%	mg/100 g	%
Mv3gal (12)	17.2	22	7.5[c]	25	6.9[b]	27	4.4[a]	28	7.2b[c]	26
Mv3glc (13)	10.1	13	4.3[c]	15	3.8[b]	15	2.5[a]	16	4.1b[c]	15
Mv3ara (14)	14.5	18	3.9[d]	13	2.6[b]	10	1.5[a]	9	3.4[c]	12
Dp3gal (1)	7.2	9	3.3[b]	11	2.9[b]	11	1.8[a]	11	2.9[b]	11
Dp3glc (2)	4.6	6	2.0[b]	7	2.2[b]	8	1.3[a]	8	2.0[b]	7
Dp3ara (4)	7.9	10	2.1[c]	7	1.6[b]	6	1.1[a]	7	1.9b[c]	7
Pt3gal (6)	5.7	7	2.6[b]	9	2.3[b]	9	1.4[a]	9	2.3[b]	8
Pt3glc (8)	4.0	5	1.8[c]	6	1.5[b]	6	1.1[a]	7	1.7[bc]	6
Pt3ara (11)	3.4	4	0.4[b]	1	0.5[b]	2	0.2[a]	1	0.4[b]	1
Cy3gal (3)	1.0	1	0.5[b]	2	0.5[b]	2	0.1[a]	1	0.5[b]	2
Cy3glc (5)	0.7	1	0.3[a]	1	0.2[a]	1	0.1[a]	1	0.2[a]	1
Cy3ara (7)	0.9	1	0.4[b]	1	0.4[b]	2	0.1[a]	1	0.4[b]	1
Pn3gal (9)	0.6	1	0.3[b]	1	0.3[b]	1	0.1[a]	1	0.3[b]	1
Pn3glc (10)	0.7	1	0.2[a]	1	0.3[a]	1	0.1[a]	1	0.2[a]	1
Total	78.5	100	29.6	100	26.0	100	15.8	100	27.5	100

[a]Peak number corresponds to the peaks shown in Figure 1. Peak assignments according to Gao & Mazza [1994]. Values in rows with different letters are different ($p<0.05$) based on Tukey comparison test.

percentage contributions to the total anthocyanins are presented in Table 3. "Bluecrop" cultivar was chosen in our experiment because it is the most widely grown highbush blueberry in Poland. The titratable acidity and contents of soluble solids in blueberry were in accordance with those found by other authors in the berry fruit [Haffner *et al.*, 1998; Skrede *et al.*, 2000].

The content of total phenolics in blueberries was comparable to results reported by Moyer *et al*. [2002] and Connor *et al*. [2002] for Bluecrop blueberries, but differed considerably from results reported by Prior *et al.* [1998]. Chlorogenic acid is the major cinnamic derivative found in large amounts in highbush blueberries [Gao & Mazza, 1994; Skrede *et al*., 2000]. The concentration of chlorogenic acid was determined at *ca*. 85 mg/100 g, which is in good agreement with a previously reported value of 97.7 mg/100 g for "Bluecrop" cultivar [Gao & Mazza, 1994]. Total anthocyanins content in the blueberries used for jam processing was 94.6 mg/100 g. This is much lower than the value of 182 mg/100 g reported by Ehlenfeldt & Prior [2001] for "Bluecrop" berries, but similar to the values reported by Prior *et al.* [1998], Moyer *et al*. [2002] and Connor *et al*. [2002]. Differences in the concentration of phenolic compounds found for the same cultivar by other authors might be due to the use of different extraction solvents and the pre-harvest climatic conditions.

Blueberries are very good sources of natural antioxidants. Antioxidant capacity of fruit had previously been determined by others using different methods. Results achieved by Connor *et al.* [2002], who used the oxygen radical absorbing capacity (ORAC) method, were similar to our observations made for "Bluecrop". In turn, Moyer *et al*. [2002] reported a higher antioxidant capacity for "Bluecrop" cultivar (50 μmol Trolox/g fruits), whereas Ehlenfeldt & Prior [2001] reported a lower antioxidant capacity for "Bluecrop" cultivar (10.4 μmol Trolox/g fruits) in respect of results reported in our study (28.6 μmol Trolox/g fruits).

Anthocyanin composition of highbush blueberry has been well characterised [Gao & Mazza, 1994; Kalt *et al.*, 1999; Skrede *et al.*, 2000]. Peak assignments were made by comparison with results reported by Gao & Mazza [1994],

who used a similar reverse phase HPLC separation system. The chromatogram recorded at 520 nm (Figure 1) exhibited the presence of fourteen major compounds. Several minor peaks of acylated anthocyanins were also detected but not identified herein, due to the fact that they represented less than 7% of total anthocyanins content.

Chemical composition of blueberry jams

Basic composition of the jams was determined in the study, including acidity, soluble solids, total phenolics, total anthocyanis, chlorogenic acid content and antioxidant capacity (Table 2). The obtained blueberry jams were characterised by acidity and soluble solids in accordance with the recipe (Table 1). Acidity of the tested jams was fairly similar, not exceeding a difference of 0.1%.

The content of total phenolics in the jams ranged from 146.8 to 163.2 mg/100 g (Table 2). The high-sugar jam showed consistently the highest total phenolics content compared with the other products. Total anthocyanin contents ranged between 32.6 mg/100 g (high-sugar jams) and 19.8 mg/100 g (light jam with oligofructose) expressed as cyjanidin-3-glucoside equivalents. Anthocyanins content of different jam samples suggests a close relation between the amount of sugar and the degradation of anthocyanins during processing. The effect of sugar on the blueberry anthocyanins has still not been elucidated explicitly. Results in this study indicate that the addition of a high dose of saccharose provided some protection during the heating process. In jam processing this might be due to reduced water activity and acting as partial oxygen barrier. This is in agreement with earlier reports [Rubinskiene *et al.*, 2005; Tsai *et al*., 2004], showing that a high dose of sucrose (40-50%) stabilizes anthocyanins during heating.

Anthocyanins content in the light jam with an addition of oligofructose was significantly lower than that in the other samples. These results suggest that the addition of oligofructose leads to a decrease in stability of anthocyanins during processing. During boiling, oligofructose may be hydrolyzed and degraded to fructose. A number of authors noticed that fructose was more deleterious to the pigment during the heating process than sucrose and glucose [Rubinskiene *et al.*, 2005; Wrolstand, 2000]. Krifi *et al*. [2000] reported that the effect of sugar on anthocyanins was due to a destructive effect of sugar degradation products, namely furfural and 5-hydroxymethylfurfural. These degradation products are formed when sugars are heated in acidic medium. However, because we were not able to follow changes in the contents of oligofructose, fructose and fructose degradation product during the jam production process, we cannot determine factors responsible for the decreased stability of anthocyanins.

In our studies only negligible differences in chlorogenic acid contents were found between the jams. The high-sugar jam had the highest antioxidant capacity and jams with oli-

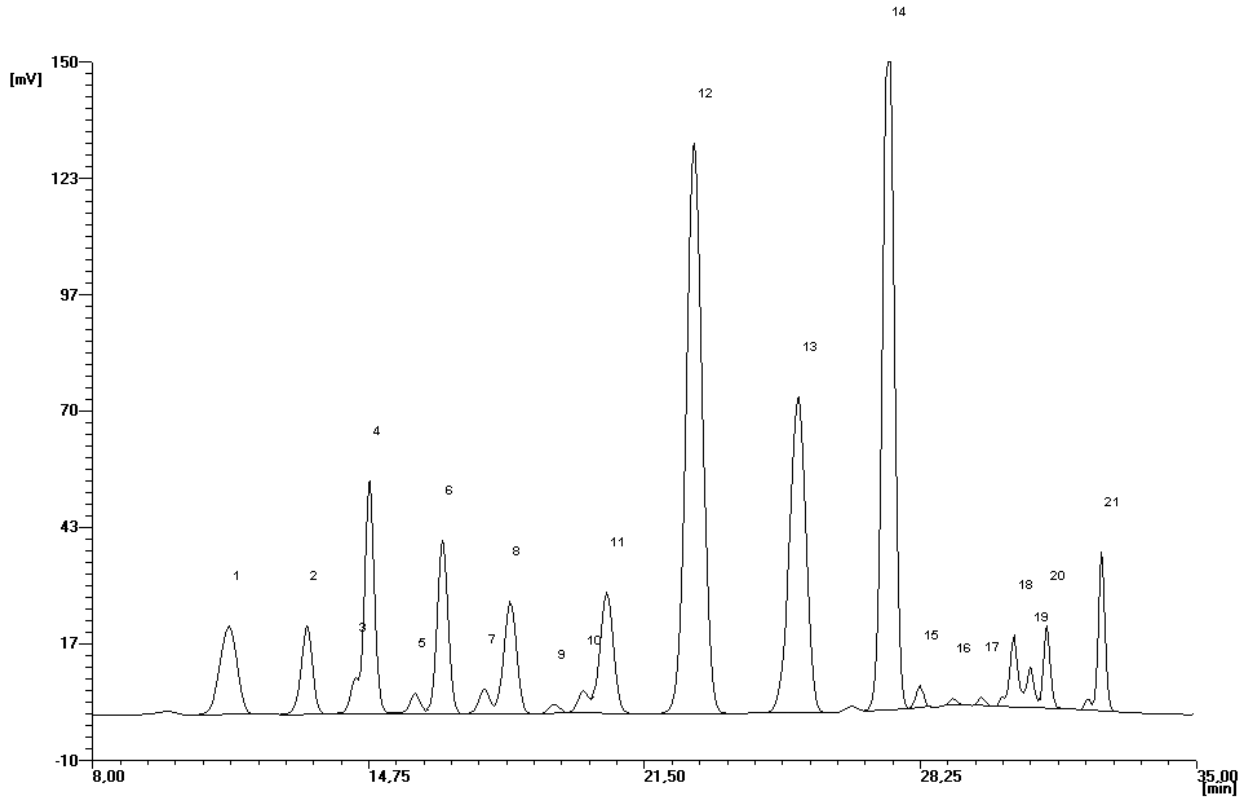

FIGURE 1. HPLC chromatogram of anthocyanins in highbush blueberry fruits.
Peak assignments according to Gao & Mazza [1994]: 1. Dp3gal (delphinidin-3-galactoside), 2. Dp3glc (delphinidin-3-glucoside), 3. Cy3gal (cyjanidin-3-galactoside), 4. Dp3ara (delphinidin-3-arabinoside), 5. Cy3glc (cyjanidin-3-glucoside), 6. Pt3gal (petunidin-3-galactoside), 7. Cy3ara (cyjanidin-3-arabinoside), 8. Pt3glc (petunidin-3-glucoside), 9. Pn3gal (peonidin-3-galactoside), 10. Pn3glc (peonidin-3-glucoside), 11. Pt3ara (petunidin-3--arabinoside), 12. Mv3gal (malvidin-3-galactoside), 13. Mv3glc (malvidin-3-glucoside), 14. Mv3ara (malvidin-3-arabinoside), 15-21 acylated anthocyanins.

TABLE 4. Changes in levels of total phenolics, chlorogenic acid, total anthocyanins and antioxidant activity of jams during storage.

Type of jam	Temperature of storage	Time of storage	Total phenolics (mg/100 g)	Chlorogenic acid (mg/100 g)	Total anthocyanins (mg/100 g)	Antioxidant capacity (μmol Trolox/g)
High-sugar	6 °C	2 months	162.3	37.6	30.3	10.8
		4 months	161.4	37.5	27.3	10.5
		6 months	159.5	36.6	24.3	9.6
	22°C	2 months	140.4	35.3	19.9	8.8
		4 months	136.6	33.8	17.4	8.2
		6 months	130.9	30.5	14.1	7.1
Low-sugar	6 °C	2 months	156.3	36.5	25.9	10.2
		4 months	154.8	36.0	21.4	9.3
		6 months	154.0	35.2	19.2	7.8
	22°C	2 months	132.5	34.4	12.7	8.4
		4 months	129.7	33.2	9.3	7.5
		6 months	121.9	30.8	8.6	5.6
Light with oligofructose	6 °C	2 months	141.8	35.9	12.9	9.1
		4 months	139.4	35.4	10.5	8.3
		6 months	138.5	34.6	9.2	7.2
	22°C	2 months	125.7	34.6	7.6	6.4
		4 months	115.4	32.5	7.2	5.3
		6 months	107.7	28.8	5.9	4.4
Light	6 °C	2 months	156.8	37.9	27.4	10.5
		4 months	156.2	37.5	24.2	10.0
		6 months	155.5	37.3	21.9	9.4
	22°C	2 months	140.3	35.9	22.5	8.6
		4 months	134.4	34.4	21.0	8.0
		6 months	129.3	32.7	18.8	7.4
Level of significance of main effects:						
type of jam			**	*	***	**
temperature of storage			***	**	***	***
time of storage			***	***	***	***

*p≤0.05, **p≤0.01, ***p≤0.001.

gofructose the lowest, following the trends observed for their contents of total phenolics and anthocyanins.

The HPLC analytical results for the quantity of anthocyanins in jams of blueberry are presented in Table 3. Total anthocyanins content of jams obtained in the colorimetric (pH differential) analysis were higher than the results from HPLC assays, but provided the same general trends. The high-sugar jams showed relatively high contents of individual anthocyanins, while the light-jam with oligofructose had a lower anthocyanin content.

Impact of processing on phenolic substances in blueberry jams

A comparison of the content of phenolic substances in fruits and jams (in relation to the dilution of fruit with sugar and water) showed that during jam preparation from 7% to 17% of total phenolics and from 25% to 54% of total anthocyanins contents in berries were lost. Although jam samples were made using the same procedure, the level of losses of phenolic compounds depended on ingredients. In general, losses of anthocyanins during the production of jam were similar to those reported by Kim & Zakour [2004] for raspberry and cherry and by Garcia-Viguera *et al*. [1998] for red raspberry. Thermal degradation, oxidation, enzymatic reaction (especially polyphenoloxidase, which is known to play the main role in degradation of anthocyanins pigments) and other factors can alter anthocyanins content during processing. In fact, during the production of jam the fresh fruits undergo long heating, which causes complete inactivation of native blueberry enzyme. Probably, the enzymatic reaction could not proceed during high temperature processing. Heating treatments seem to be the most destructive processing steps for anthocyanins retention during jam manufacturing. Thermal degradation of anthocyanins is a first order reaction, and the presence of oxygen and fructose increases the negative effect of high temperature [Wrolstand, 2000].

In our study, chlorogenic acid was reduced by 1-4% during jam processing. Minor changes in chlorogenic acid content suggest that this phenolic compound of blueberry fruits

TABLE 5. Changes in contents of total individual anthocyanins (mg/100 g product) of jams during storage.

Type of jam	Temperature of storage	Time of storage	Anthocyanins (peak no.)[a]														Total
			Mv3gal (12)	Mv3glc (13)	Mv3ara (14)	Dp3gal (1)	Dp3glc (2)	Dp3ara (4)	Pt3gal (6)	Pt3glc (8)	Pt3ara (11)	Cy3gal (3)	Cy3glc (5)	Cy3ara (7)	Pn3gal (9)	Pn3glc (10)	
High-sugar	6°C	2 months	4.4	2.3	2.3	1.9	1.4	1.8	1.8	1.1	0.3	0.3	0.2	0.3	0.2	0.1	18.4
		4 months	4.2	2.3	2.3	1.7	1.4	1.6	1.7	1.1	0.3	0.3	0.2	0.3	0.2	0.1	17.7
		6 months	4.1	2.2	1.9	1.6	1.2	1.4	1.6	1.0	0.3	0.3	0.2	0.3	0.2	nd	16.3
	22°C	2 months	2.9	1.9	1.8	1.4	0.7	0.9	1.2	0.7	0.2	0.1	0.1	0.1	0.1	0.1	12.2
		4 months	2.8	1.6	1.7	1.3	0.9	0.9	1.4	0.8	0.1	0.1	0.1	0.1	0.1	nd	11.7
		6 months	1.7	1.0	0.9	0.8	0.6	0.6	0.6	0.6	0.1	nd	nd	nd	nd	nd	6.9
Low-sugar	6°C	2 months	4.1	2.0	1.5	1.7	1.4	1.3	1.6	0.9	0.3	0.3	0.1	0.3	0.2	0.2	15.9
		4 months	3.8	1.7	1.4	1.5	1.3	1.2	1.5	0.9	0.3	0.3	0.1	0.3	0.1	0.1	14.5
		6 months	3.2	1.6	1.2	1.3	1.2	1.1	1.3	0.9	0.2	0.3	0.1	0.3	0.1	0.0	12.8
	22°C	2 months	1.7	0.9	0.6	0.8	0.6	0.6	0.6	0.5	0.1	0.2	0.1	0.1	0.1	nd	6.9
		4 months	1.0	0.5	0.4	0.4	0.4	0.3	0.4	0.3	0.1	0.1	0.1	nd	nd	nd	4.0
		6 months	0.7	0.4	0.3	0.3	0.3	0.2	0.3	0.1	nd	nd	nd	nd	nd	nd	2.6
Light with oligofructose	6°C	2 months	1.6	1.0	0.6	0.7	0.5	0.4	0.3	0.4	0.1	0.0	0.1	0.1	0.1	0.0	5.9
		4 months	1.5	0.9	0.6	0.6	0.5	0.4	0.3	0.4	0.1	nd	0.1	0.1	0.0	nd	5.5
		6 months	1.4	0.8	0.6	0.6	0.4	0.3	0.3	0.4	0.1	nd	0.1	0.0	nd	nd	5.0
	22°C	2 months	0.7	0.4	0.2	0.3	0.4	0.1	0.3	0.2	0.1	0.0	0.1	0.0	0.0	nd	2.8
		4 months	0.5	0.3	0.2	0.3	0.3	nd	0.2	0.1	0.1	nd	0.1	nd	nd	nd	2.1
		6 months	0.4	0.3	0.2	0.3	0.2	nd	0.1	nd	nd	nd	nd	nd	nd	nd	1.5
Light	6 °C	2 months	5.0	2.6	2.2	1.9	1.4	1.7	1.6	1.0	0.3	0.3	0.1	0.3	0.2	0.1	18.7
		4 months	4.6	2.5	2.0	1.6	1.2	1.5	1.5	1.0	0.3	0.3	0.1	0.3	0.2	0.0	17.1
		6 months	4.6	2.2	2.0	1.5	1.1	1.3	1.5	1.0	0.3	0.3	0.1	0.3	0.2	nd	16.4
	22°C	2 months	4.2	2.3	2.1	1.7	1.3	1.6	1.5	1.0	0.3	0.3	0.1	0.3	0.2	0.1	17.0
		4 months	4.0	2.0	1.9	1.4	1.1	1.3	1.3	1.0	0.3	0.3	0.1	0.3	0.2	nd	15.2
		6 months	2.8	1.4	1.2	0.9	0.9	1.0	1.0	0.6	0.2	0.2	0.1	0.2	0.1	nd	10.6
Level of significance of main effects:																	
type of jam			***	**	**	*	*	*	**	*	ns	ns	ns	ns	ns	ns	**
temperature of storage			***	***	***	**	*	*	*	ns	ns	ns	ns	ns	ns	ns	***
time of storage			***	***	***	**	**	**	**	*	*	*	ns	*	ns	ns	***

nd – not detected, *p≤0.05, **p≤0.01, ***p≤0.001, ns-not significant. [a]Peak number corresponds to the peaks shown in Figure 1. Peak assignments according to Gao & Mazza [1994].

is stable during the heating process. This is in agreement with a previous study conducted in the model system [Murakami *et al.*, 2004].

Our study showed that 13-19% of antioxidant capacity in fruits were lost during jam making. These results were consistent with data reported previously by Kim & Zakour [2004] who demonstrated that the antioxidant capacity of plum, cherry and raspberry decreased after jam processing. The decrease of the antioxidant capacity during jam processing may be attributable to the destruction of anthocyanins, which was observed in this investigation.

The chromatographic analysis of individual anthocyanins showed that processing caused 18-56% decrease in anthocyanins content. The proportion of individual anthocyanins was affected by processing as well. It is interesting that anthocyanins with arabinose were more labile than other anthocyanins, *e.g*. malvidin-3-arabinoside constituting 18% of the total anthocyanins in blueberry fruits and only 9-13% in jams. Ichiyanagi *et al*. [2001] reported that hydrolysis rate of anthocyanins did not depend on the aglycone structure, but on the type of conjugated sugar. During heating in 1% trifluoroacetic acid arabinosides were more unstable than glucosides and galactosides, when the glycosides with the same aglycone were compared. In our study, similar hydrolysis might have occurred during jam production, particularly during pasteurization of glass jars with citric acid.

Impact of time and temperature of storage on phenolic substances in blueberry jams

The statistical analysis showed that the type of jams, temperature and time of storage significantly affected the contents of total anthocyanins, total phenolics, and chlorogenic acid as well as the antioxidant capacity of blueberry jams stored at 6°C and 22°C for 2, 4, 6 months (Table 4). In the samples stored at 6°C their levels were higher than in the samples stored at 22°C. In respect of the content of total phenolics in jams directly after production, after 6 months of storage at 6°C and 22°C their losses accounted for 2-6% and 18-27%, respectively. These findings of more extensive degradation of phenolics compounds during storage of preserves at a higher temperature are consistent with previously reported data [Garcia-Viguera *et al*., 1998, 1999].

Time of storage was the main factor responsible for losses of anthocyanins. The higher loss in pigment composition at 22°C was observed during the first two months, while the jams stored at 6°C showed a progressive decrease of anthocyanins. Losses of anthocyanins compared to the jams directly after production were about 20-61% after 2 months and 32-70% after 6 months of storage at 22°C, respectively. Similar changes were observed for the levels of total phenolics, chlorogenic acid and antioxidant capacity.

The type of jam significantly effected the contents of total anthocyanins, total phenolics, and chlorogenic acid as well as antioxidant capacity of jams. In comparison to the jam analysed directly after production, the smallest losses in anthocyanins content during storage were found in the light product, and the highest ones in the light jam with oligofructose and in low-sugar jam.

The decay of individual anthocyanins was similar to that of total anthocyanins (Table 4, 5). None of the anthocyanins showed a higher degradation than the others and all were more degraded at higher temperature. Complete destruction of cyanidin and peonidin derivatives occurred during 6 months of storage. The proportions of major anthocyanins in products (percent distribution) were quite similar regardless of the time of storage. For example, malvidin-3-galactoside constituted *ca.* 26% of the total pigment content in jams directly after production and in jams at the end of the storage period.

Prolonged storage may affect hydrolysis of compounds and lead to gradual reduction in anthocyanins content, as observed in our study. Nevertheless, it may be assumed that the oxidative reaction proceeds in jams during storage, even if the jams in our experiment were hot-packed into glass jars. A strong reduction of anthocyanins during storage was also reported for pigments of red raspberry jams [Garcia-Viguera *et al*., 1998].

CONCLUSIONS

In conclusion, this investigation demonstrates that Polish-grown blueberries are a rich source of phenolic compounds and antioxidant capacity. Results obtained in this study indicate relationships between ingredients used for jam processing and levels of anthocyanin compounds in blueberry preserves. The product with oligofructose had the lowest levels of anthocyanins and total phenolics. Interestingly, we found that during jam processing anthocyanins with glucoside and galactoside showed higher stability than those with arabinoside. Storage of blueberry jams tends to reduce the content of phenolic compounds compared to their content of products analysed directly after production. Nevertheless, the jams still contain their useful amounts when consumed this way. It can also be concluded that the anthocyanins content and antioxidant capacity of blueberry jams is significantly affected by temperature and time of storage. The recommended storage temperature for blueberry jams is 6°C. The intake of anthocyanins and phenolic compounds from fresh highbush blueberry and blueberry jams should provide an excellent means of increasing the level of antioxidants in the diet. Further studies are, however, required to fully understand the role of added sugar on the stability of blueberry anthocyanins during the heating process and during storage of products.

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