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CHANGES IN THE LEVEL AND ANTIOXIDANT ACTIVITY OF POLYPHENOLS DURING STORAGE OF ENZYMATICALLY TREATED RASPBERRY JUICES AND SYRUPS

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ABSTRACT

Background. Berry juices are a rich source of phenolic compounds exhibiting antioxidant activity. Unfortunately, polyphenols and especially anthocyanins are degraded during storage.
Materials and methods. The levels of total phenolic compounds, phenolic acids, flavonoids, and anthocyanins as well as antioxidant activity (radical scavenging ability against DPPH and $ABTS^{+\bullet}$ and chelating power Fe^{2+}) were determined in raspberry juices (obtained after enzymatic treatment with three commercial pectinolytic enzyme preparations) and syrups (obtained by the addition of sucrose at concentrations of 30% and 70%) during storage.
Results. During the five-month storage of juices and syrups at room temperature, there was significant reduction in the level of phenolic compounds, in particular anthocyanins (up to 95% in relation to the initial content). Storage of raspberry juices and syrups also resulted in a reduction in antioxidant activity.
Conclusions. The enzymatic treatment of the raspberry mash generally increased the losses of anthocyanins. The addition of sugar to fruit juices only slightly reduced these losses.

Keywords: raspberry juice, phenolic compounds, antioxidant activity, pectinolytic enzymes, sucrose addition

INTRODUCTION

Raspberries are the most delicate and least durable berries. The harvest must be carried out just before processing, preferably with the fruit being placed in small individual packages. The stability of the fruit at a temperature of 0–4°C is 2 days. They can be processed into juice, concentrated juice, and jams or frozen. The Polana variety is the oldest of the Polish repeated-fruiting varieties (Gasik and Mitek, 2012).

It is very important in the production of juice from berries to extract the maximum amount of flavonoids from them using a suitable heat and enzymatic treatment. One of the factors influencing the extraction of juice is the amount and form of cell wall components, especially pectin compounds, which increase the viscosity, impede extraction of juice, and increase water retention by fruit tissues (Pedrolli et al., 2009). Pectin hydrolysis at a temperature of 55–60°C is used in the production of berry juices, which ensures a better color of juice. Production of non-clarified, turbid juices has a positive impact on the amount of biologically active compounds (Puri et al., 2012).

Enzymes such as pectinases, cellulases and hemicellulases are widely used in the manufacture of fruit juices in order to facilitate juice extraction by enzymatic degradation of cell wall components. In addition, they increase the extraction of bioactive compounds,

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in particular certain phenolic compounds, which are bound with cell wall polysaccharides. Enzymatic extraction also contributes to an increase in the amount of polysaccharides, oils, pigments, or aromatic compounds in juice. From the technological and economic point of view, enzymatic processing of mash and juice helps to shorten the extraction time, increase process efficiency, and reduce energy consumption (Bielecki et al., 2001; Puri et al., 2012).

Fruit syrups are obtained by boiling fruit mash with sugar. Good syrups should have an odor and color similar to the raw material from which they are made and should be transparent. The most popular are cherry and raspberry juices, as well as juices from currants, blueberries, and strawberries (Sivasankar, 2009).

Many scientific studies confirm the health benefits of consuming berries and berry preparations. Health benefits are mostly attributed to phenolic compounds, especially anthocyanins, which give fruits their color. Phenolic compounds exhibit a broad range of biological properties: anti-allergic, anti-atherosclerotic, anti-inflammatory, antibacterial, antioxidant and antithrombotic. The health-promoting effect of phenolic compounds on the human body is mainly related to their antioxidant properties (Heim et al., 2002).

The aim of this study was to determine the effect of enzyme preparations Pectinex Ultra SP-L, Pectinex Yield Mash, and Ultrazym AFP-L used for maceration of the Polana raspberry fruit variety on the level and antioxidant activity of phenolic compounds and the effect of storage time on bioactive compound content in juice. Moreover, the effect that adding sucrose to the obtained juices has on the stability of phenolic compounds during five-month storage was analyzed.

MATERIALS AND METHODS

Enzyme-assisted raspberry juice pressing

Raspberries (1 kg) were blended for 1 minute (blender Braun), heated in a shaking incubator (INCU-Shaker MINI) to a temperature of 45°C. Enzymes were kindly supplied by Novozymes (Warsaw, Poland) and were used at the average dosage recommended by the manufacturers (10 ml/100 kg mash). Each enzyme was diluted with distilled water to a constant volume of 10 ml before adding to the mashed raspberries (Buchert et al., 2005). The enzymatic maceration process was run with shaking for 1 hour. The raspberry mash was then heated to 85°C (2 min) to inactivate the enzymes added. After the treatment, the raspberry mashes were cooled, squeezed (Omega squeezer Eujuicers.com 8004) and centrifuged at 7000 rpm for 15 min at 4°C. The supernatants were stored at −20°C and used for analyses.

We used the following scheme designations:

- C – control (distilled water instead enzymes)
- E1 – Pectinex Ultra SP-L (polygalacturonase activity)
- E2 – Pectinex Yield Mash (pectinmethylesterase activity)
- E3 – Ultrazym AFP-L (polygalacturonase and cellulase activity)

Preparation of raspberry syrups

For the preparation of raspberry syrup, sucrose was added to the raspberry juices (control and treated with pectinolytic enzymes obtained according to the procedure described above) at concentrations of 30% and 70% (w/v). Next, all samples were pasteurized at 95°C for 5 minutes. The resulting juices and syrups were stored at room temperature in closed glass jars and used for further analysis.

Preparation of extracts of raspberry juices and syrups

4 ml of each juice or syrup were extracted with 4 ml of 90% acidified methanol (0.1% HCl) for 1 hour at 4°C. After extraction, they were centrifuged for 15 minutes at 4°C at 7000 rpm, filtered, and used in further assays. The extracts were prepared immediately after pasteurization of the syrups – at "0" time and after 1, 2, 3, 4, and 5-five month storage.

Analytical methods

Total phenolic content. The analysis was carried out with the Folin-Ciocalteu method (Singleton et al., 1974). The total phenolic content was expressed as gallic acid equivalent through the calibration curve of gallic acid.

Phenolic acid content. The total level of phenolic acids was determined with the spectrometric

Arnova method (Szaufer-Hajdrych, 2004) and expressed as caffeic acid equivalents.

Flavonoid content (Lamaison and Carnet, 1990). The flavonoid concentration was expressed in mg/g dry weight [mg·g^{-1} FW] as quercetin equivalent.

Total anthocyanin content. The total anthocyanin content was determined using the pH differential method (Giusti and Worlstad, 2001). The anthocyanin concentration were expressed as cyanidin-3-O-glucoside equivalent.

Antioxidant properties

Free radical scavenging assay. Free radical scavenging activity was measured using DPPH, according to the method used by Brand-Williams et al. (1995) and ABTS$^{\bullet+}$, according to Re et al.'s method (1999) as a source of free radicals. The antioxidant activity was expressed as mmol of Trolox per gram of fresh weight (FW) (TEAC – Trolox equivalent antioxidant activity). The standard curve was prepared in the concentration range of 0–1500 μmol of Trolox (r^2 = 0.978) for the DPPH assay and 0–800 μmol of Trolox (r^2 = 0.985) for the ABTS assay.

Chelating power was determined using the method described by Guo et al. (2003). Chelating power was expressed as EDTA equivalent in mg EDTA per g of fresh weight (FW). The standard curve was prepared in the concentration range 0–150 μg·ml^{-1} of EDTA (r^2 = 0.996).

Statistical analysis

All experimental results were means from measurements performed in triplicate (three extracts and three measurements for each extract). The data in the tables and figures represent mean values ±standard deviation (n = 9). The results were evaluated for statistical significance using univariate analysis of variance (ANOVA) with Statistica 13 software (StatSoft, Inc., Tulsa, OK, USA) and Tukey's post hoc test. Differences were considered significant at p = 0.05. Pearson correlation analysis was performed between antioxidant activity and total phenolic, phenolic acid, flavonoid, and anthocyanin content.

RESULTS

The total phenolic content of the raspberry juices obtained from the raspberry mash subjected to enzymatic treatment and syrups containing sucrose at two different concentrations 30 and 70% (w/v) was determined at monthly intervals during the five months of storage (Table 1). The total phenolic content was similar in the control juice and in juices obtained after raspberry mash maceration with Pectinex Ultra-SPL (E1) and Ultrazyme AFP-L (E3), and was on averaged 1.93 mg·g^{-1} FW. Only treating raspberry mash with Pectinex Yield Mash (E2) significantly lowered the concentration of total phenolic compounds in the juice obtained – 1.76 mg·g^{-1} FW. The same trend was observed during storage. The highest level of phenolic compounds in "0" time was marked for the sample E1 70S, but this result wasn't statistically significant. Phenolic compound content decreased during storage. After five months, the initial level of phenolic compounds in the juices and syrups decreased from 92 to 66.5%. The largest decline was recorded in the case of juice obtained after maceration of the raspberry mash with Ultrazym AFP-L without the addition of sucrose (E3). The lowest total phenolic content (1.32 mg·g^{-1} FW) was determined after 5-month storage in the juice obtained from the mash treated with Pectinex Yield Mash without addition of sucrose (E2), while the highest content, i.e. 1.81 mg/g FW, was found in the control juice containing 70% sucrose. Based on the results obtained, it can be concluded that the degree of degradation of polyphenols depends on the type of enzyme preparation used. The use of polygalacturonase only caused less losses. To minimize polyphenol degradation preparations exhibiting cellulase activity should be avoided. In general, during storage (it is especially apparent after 4 and 5 months) the stability of phenolic compounds was significantly higher in syrups in comparison to fruit juices without the addition of sucrose obtained after the same enzyme preparation treatment.

In juices obtained from raspberry mash after treatment with Pectinex Ultra SP-L (E1) and Ultrazym AFP-L (E3) the enzymatic preparations obtained an approximate phenolic acid content to control juice ratio of (10.82, 11.51 and 11.32 μg·g^{-1} FW, respectively). The lowest content (10.38 μg·g^{-1} FW) of this group of phenolics was detected in the case of Pectinex Yield

Table 1. Changes in the total phenolic content in juices and syrups during five-month storage, mg·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	1.92 ±0.06 b ABCD	1.89 ±0.06 b ABC	1.75 ±0.04 a BD	1.72 ±0.06 a ABC	1.68 ±0.02 a AF	1.48 ±0.06 c ACD
C 30S	1.94 ±0.03 b ABC	1.88 ±0.03 ab ABC	1.87 ±0.04 ab ABC	1.82 ±0.03 a ABD	1.80 ±0.03 a B	1.8 ±0.03 a F
C 70S	1.96 ±0.03 b ABC	1.94 ±0.02 b A	1.87 ±0.02 a ABC	1.85 ±0.02 a D	1.83 ±0.02 a B	1.81± 0.01 a F
E1	1.99 ±0.02 e AB	1.66 ±0.02 d E	1.58 ±0.01 c E	1.56 ±0.04 bc EF	1.51 ±0.02 ab CD	1.49 ±0.02 a ACD
E1 30S	2.02 ±0.04 d A	1.92 ±0.03 c AB	1.84 ±0.04 ac ABCD	1.80 ±0.02 a ABD	1.76 ±0.02 ab BF	1.71 ±0.01 b BEF
E1 70S	2.05 ±0.04 b A	1.93 ±0.04 ab AB	1.93 ±0.09 ab C	1.84 ±0.09 ab BD	1.79 ±0.01 a B	1.74 ±0.04 a EF
E2	1.76 ±0.02 c D	1.69 ±0.02 c DE	1.59 ±0.03 b E	1.52 ±0.05 ab E	1.46 ±0.03 a C	1.32 ±0.01 d C
E2 30S	1.79 ±0.05 a CD	1.76 ±0.04 a CDE	1.73 ±0.02 ad D	1.65 ±0.01 cd CF	1.56 ±0.05 bc DE	1.53 ±0.03 b ABD
E2 70S	1.82 ±0.06 a BCD	1.82 ±0.06 a ABCD	1.76 ±0.08 ab ABD	1.72 ±0.04 ab ABC	1.65 ±0.03 bc A	1.54±0.04 c AB
E3	2.03 ±0.06 b A	1.94 ±0.06 ab A	1.89 ±0.01 a AC	1.77 ±0.01 e ABCD	1.52 ±0.01 d CD	1.35± 0.01 c CD
E3 30S	1.93 ±0.07 d ABCD	1.80 ±0.07 ad BCD	1.78 ±0.03 a ABD	1.71 ±0.03 ac AC	1.64 ±0.04 bc AE	1.56 ±0.02 b ABE
E3 70S	1.98 ±0.14 b AB	1.93 ±0.05 ab AB	1.89 ±0.05 ab AC	1.77 ±0.01 ad ABCD	1.63 ±0.04 cd AE	1.58 ±0.01 c ABE

C – the juice obtained from the control raspberry mash.
C 30S – control juice with 30% (w/v) sucrose.
C 70S – control juice with 70% (w/v) sucrose.
E1 – the juice obtained from the raspberry mash treated with Pectinex Ultra SP-L preparation.
E1 30S – the juice obtained from the raspberry mash treated with Pectinex Ultra SP-L preparation with 30% (w/v) sucrose.
E1 70S – the juice obtained from the raspberry mash treated with Pectinex Ultra SP-L preparation with 70% (w/v) sucrose.
E2 – the juice obtained from the raspberry mash treated with Pectinex Yield Mash preparation.
E2 30S – the juice obtained from the raspberry mash treated with Pectinex Yield Mash preparation with 30% (w/v) sucrose.
E2 70S – the juice obtained from the raspberry mash treated with Pectinex Yield Mash preparation with 70% (w/v) sucrose.
E3 – the juice obtained from the raspberry mash treated with Ultrazym AFP-L preparation
E3 30S – the juice obtained from the raspberry mash treated with Ultrazym AFP-L preparation with 30% (w/v) sucrose.
E3 70S – the juice obtained from the raspberry mash treated with Ultrazym AFP-L preparation with 70% (w/v) sucrose.
Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.

Table 2. Changes in the level of phenolic acids in raspberry juices and syrups during five-month storage, μg·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	11.32 ±0.44 d AB	9.73 ±0.36 c EF	6.22 ±0.29 a CDE	6.10 ±0.41 a ABD	5.63 ±0.07 ab BD	4.93 ±0.08 b DE
C 30S	11.41 ±0.31 c AB	8.65 ±0.63 b AB	7.39 ±0.15 ab AD	7.22 ±0.21 ab ABC	6.62 ±1.17 a AD	6.34 ±0.08 a AB
C 70S	11.34 ±0.19 c AB	9.98 ±0.29 bc F	8.87 ±0.64 ab AB	8.52 ±1.4 ab C	8.21 ±0.17 ab C	7.32 ±0.50 a A
E1	10.82 ±0.46 d AB	7.06 ±0.03 c C	5.73 ±0.08 b C	5.03 ±0.27 ab D	4.62± 0.24 a B	4.53 ±0.44 a CD
E1 30S	10.66 ±0.50 c AB	8.71 ±0.20 b ABE	7.52 ±0.26 ab AD	7.15 ±0.84 a ABC	6.93 ±0.24 a A	6.51 ±0.18 a AB
E1 70S	10.59 ±0.35 d AB	8.54 ±0.28 c ABD	8.14 ±0.17 bc AB	7.51 ±0.16 ab AC	7.44 ±0.54 ab AC	7.09 ±0.04 a A
E2	10.38 ±0.40 e B	7.55 ±0.55 d CD	6.04 ±0.28 c CE	5.10 ±0.25 a D	4.56 ±0.13 a B	3.62 ±0.03 b C
E2 30S	10.71 ±0.40 d AB	7.14 ±0.15 c C	5.41 ±0.05 a C	5.41 ±0.24 a BD	5.32 ±0.26 a B	3.94 ±0.37 b CD
E2 70S	10.57 ±0.08 d AB	8.42 ±0.29 b ABD	7.73 ±0.02 ab AB	7.04 ±0.05 a ABCD	7.06 ±0.01 a AC	5.93 ±0.59 c BE
E3	11.51 ±0.28 d A	9.13 ±0.37 c BEF	7.32 ±1.30 bcADE	6.03 ±0.05 ab ABD	4.91 ±0.22 a B	4.52 ±0.9 a CD
E3 30S	11.46 ±0.53 c AB	8.02 ±0.48 a ACD	7.72 ±0.10 a AB	7.23 ±1.07 a ABC	6.81 ±0.20 a AD	6.71 ±0.24 b AB
E3 70S	11.62 ±0.17 c A	8.81 ±0.07 b ABE	8.41 ±0.23 ab AB	7.52 ±1.31 ab AC	7.20 ±0.16 a AC	7.02 ±0.12 a AB

Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.
Abbreviations as in Table 1.

Mash (E2) application, but even this difference was not statistically significant in comparison to the control (Table 2). Storage of raspberry juices and syrups contributes to the degradation of phenolic acids. After five months, there was a significant decrease in their level, on average by approximately 55% of the initial content. In most cases, the largest reduction in phenolic acids was noted in the first month of storage, while later the reduction in their concentrations was less severe. After the first month, the lowest phenolic acid content was detected for E1, E2 and E2 30S samples in comparison to control juices and syrups. In turn, E3 preparation did not cause significant changes in the phenolic acid level with regard to the control. The lowest contents after five months (3.62 mg·g^{-1} FW) were determined in the juice obtained from the mash treated with Pectinex Yield Mash, without the addition of sucrose (E2), and the highest, 7.32 mg·g^{-1} FW, was determined in the control juice with the addition of 70% sucrose (Table 2). In the case of phenolic acids, the most unfavorable effect was observed for E2 preparation, exhibiting pectinmethylesterase activity.

In the case of flavonoids, no significant differences were found between the control juice and juices

Table 3. Changes in the level of flavonoids in raspberry juices and syrups during five-month storage, mg·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	1.270 ±0.011 d A	1.000 ±0.044 c C	0.960 ±0.029 bc DF	0.900 ±0.001 b C	0.570 ±0.010 a B	0.530 ±0.006 a A
C 30S	1.280 ±0.089 d A	1.210 ±0.013 cd B	1.130 ±0.037 bc ACE	1.090 ±0.016 ab A	0.980 ±0.003 a A	0.670 ±0.007 e BE
C70S	1.300 ±0.020 c A	1.240 ±0.007 bc AB	1.220 ±0.033 b AB	1.110 ±0.014 a A	1.070 ±0.033 a F	0.740 ±0.028 d BCD
E1	1.280 ±0.024 f A	1.130 ±0.007 e D	1.060 ±0.023 d EF	0.840 ±0.012 c BC	0.790 ±0.014 b D	0.590 ±0.010 a A
E1 30S	1.300 ±0.017 a A	1.280 ±0.024 a A	1.220 ±0.019 e AB	1.090 ±0.020 d A	0.970 ±0.005 c A	0.700 ±0.005 b BC
E1 70S	1.330 ±0.004 c A	1.240 ±0.007 bc AB	1.200 ±0.080 ab ABC	1.140 ±0.016 a AD	0.970 ±0.011 e A	0.780 ±0.017 d D
E2	1.270± 0.010 f A	0.960 ±0.007 e C	0.900 ±0.021 d D	0.810 ±0.013 c BC	0.700 ±0.018 b C	0.550 ±0.011 a A
E2 30S	1.280 ±0.004 d A	1.120 ±0.019 c D	1.100± 0.043 c CE	0.740 ±0.002 b B	0.670 ±0.050 ab C	0.600 ±0.011 a AE
E2 70S	1.300 ±0.016 a A	1.280 ±0.006 a A	1.270 ±0.024 a B	1.260 ±0.027 a D	0.970 ±0.052 c A	0.780 ±0.016 b D
E3	1.260 ±0.064 d A	0.950 ±0.039 c C	0.880 ±0.004 bc D	0.850 ±0.023 b BC	0.550 ±0.009 a B	0.540 ±0.011 a A
E3 30S	1.280 ±0.071 a A	1.260 ±0.034 a AB	1.200 ±0.012 ab ABC	1.100 ±0.052 b A	0.860 ±0.022 d DE	0.700 ±0.006 c BC
E3 70S	1.240 ±0.071 a A	1.300 ±0.001 a A	1.210 ±0.021 ab AB	1.140 ±0.002 b AD	0.890 ±0.008 d E	0.760 ±0.037 c CD

Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.
Abbreviations as in Table 1.

obtained after using enzymatic preparations for treating the raspberry mash (Table 3). The average level of flavonoids in the raspberry juice extracts analyzed was 1.27 mg·g^{-1} FW. For flavonoids a similar trend of changes to that noted for phenolic acids was observed (Table 3). Moreover, this class of phenolic compounds underwent significant degradation during the storage of the raspberry juices and syrups. However, although no statistically significant difference between the control juice and the juices obtained from raspberries treated with enzymes was observed at "0" time, the juices obtained after treatment of fruit mash with E1 and E2 for 4 months retained more flavonoids than the control juice. It is only after 5 months that these differences are not statistically significant. In turn, preparation E3 with cellulase activity did not cause a signifficant decrease in flavonoids during storage in comparison to the control juice. The addition of sucrose slowed down the flavonoid degradation process: after five months, all syrup samples containing 30 and 70% sucrose contained more flavonoids than juices with no added sugar. After five-month storage, the flavonoid content in the juice from the Pectinex Ultra SPL-treated raspberry mash containing 70% sucrose

Table 4. Changes in level of anthocyanins in raspberry juices and syrups during five-month storage, mg·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	0.430± 0.006 e A	0.240 ±0.019 d A	0.160 ±0.006 c AB	0.080 ±0.007 b A	0.060 ±0.018 ab ABC	0.030 ±0.001 a D
C 30S	0.410 ±0.021 c A	0.180 ±0.004 b A	0.150 ±0.009 b ABC	0.090 ±0.008 a A	0.060 ±0.022 a ABC	0.055 ±0.003 a AB
C 70S	0.460 ±0.008 d A	0.210 ±0.008 c A	0.140 ±0.016 b AC	0.090 ±0.025 a A	0.070 ±0.003 a AB	0.060 ±0.002 a B
E1	0.425 ±0.012 e A	0.230 ±0.005 d A	0.140 ±0.004 c AC	0.090 ±0.003 a A	0.070 ±0.011 a AB	0.040 ±0.005 b F
E1 30S	0.430 ±0.003 e A	0.190 ±0.010 d A	0.160 ±0.012 c AB	0.090 ±0.004 b B	0.070 ±0.008 ab AB	0.050 ±0.002 a A
E1 70S	0.430 ±0.019 d A	0.200 ±0.007 c A	0.130 ±0.004 b C	0.080 ±0.002 a A	0.070 ±0.003 a AB	0.060 ±0.001 a B
E2	0.430 ±0.016 e A	0.220 ±0.018 d A	0.170 ±0.005 c B	0.100 ±0.009 a A	0.090 ±0.019 a B	0.010 ±0.005 b E
E2 30S	0.440 ±0.005 f A	0.210 ±0.020 e A	0.160 ±0.004 d AB	0.090 ±0.001 c A	0.050 ±0.012 b AC	0.020 ±0.001 a C
E2 70S	0.430 ±0.006 e A	0.190 ±0.010 d A	0.130 ±0.014 c C	0.080 ±0.001 b A	0.060 ±0.001 ab ABC	0.050± 0.002 a A
E3	0.370 ±0.023 e A	0.170 ±0.012 d A	0.130 ±0.010 c C	0.080 ±0.002 b A	0.030 ±0.003 a C	0.020 ±0.003 a C
E3 30S	0.350 ±0.006 f A	0.240 ±0.005 e A	0.160 ±0.011 d AB	0.090 ±0.001 c A	0.060 ±0.007 b ABC	0.030 ±0.003 a D
E3 70S	0.380 ±0.009 e A	0.240 ±0.007 d A	0.160 ±0.010 c AB	0.090 ±0.001 b A	0.080± 0.005 ab AB	0.070 ±0.003 a G

Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.
Abbreviations as in Table 1.

was 0.78 mg·g^{-1} FW (the highest noted content), while the juice with no added sugar exhibited a flavonoid content of 0.59 mg·g^{-1} FW (Table 3).

The anthocyanin content (Table 4) in the individual samples of the juice did not undergo statistically significant changes, even though they are the most unstable compounds in the entire pool of phenolic compounds. Their marked content ranged from 0.37 to 0.43 mg·g^{-1} FW. During the five-month storage of the juices and syrups, there was a significant reduction in the level of anthocyanins in all the samples analyzed (Table 4). Anthocyanins are very unstable compounds, hence there was a significant decrease in their concentration after a month. Their content decreased on average by nearly 50% and ranged from approx. 37% of the output value of the juice obtained from the raspberry mash treated with Pectinex Ultra SPL (E1) to 61% of the output value of the juice obtained from the mash of the control with the addition of 30% sucrose (C 30S). However, it is worth pointing out that practically 4 months of storage the differences in the anthocyanin content between the individual samples are small. After five months, the loss amounted up to 95% of the initial content. The greatest losses were

Table 5. Changes in the ability to scavenge DPPH by raspberry juice and syrup during five-month storage, TEAC μmol·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	0.995 ±0.013 c CD	0.990 ±0.011 bc C	0.810 ±0.009 b E	0.580 ±0.012 a G	0.570 ±0.006 a H	0.460 ±0.004 a B
C 30S	1.060 ±0.021 e F	0.970 ±0.008 d BC	0.770 ±0.006 a AD	0.750 ±0.005 a E	0.670 ±0.004 c D	0.480 ±0.002 b H
C 70S	1.130 ±0.023 c G	0.970 ±0.007 bc BC	0.900± 0.017 bc H	0.760 ±0.004 ab E	0.620 ±0.001 a A	0.590 ±0.003 a H
E1	0.850 ±0.012 e E	0.780 ±0.009 d DE	0.600 ±0.004 a B	0.600 ±0.002 a H	0.510 ±0.006 c F	0.470 ±0.003 b C
E1 30S	0.930 ±0.013 f AB	0.840 ±0.011 e A	0.690 ± 0.007 d C	0.620 ±0.008 c A	0.590 ±0.004 b I	0.530 ±0.002 a A
E1 70S	0.930 ±0.008 e AB	0.790 ±0.006 d E	0.720 ±0.006 a F	0.710 ±0.005 a C	0.690 ±0.004 c J	0.630 ±0.003 b I
E2	0.900 ±0.011 f A	0.760 ±0.009 e D	0.620 ±0.004 d B	0.560 ±0.005 c F	0.540 ±0.003 b G	0.500 ±0.002 a F
E2 30S	0.910 ±0.009 f A	0.850 ±0.007 e A	0.750 ±0.005 d A	0.720 ±0.003 c CD	0.610 ±0.003 b A	0.530 ±0.002 a A
E2 70S	0.920 ±0.009 f A	0.850 ±0.008 e A	0.790 ±0.004 d DE	0.680 ±0.001 c B	0.640 ±0.006 b B	0.530 ±0.001 a A
E3	0.900 ±0.008 e A	0.850 ±0.006 d A	0.680 ±0.007 c C	0.620 ±0.005 b A	0.490 ±0.005 a E	0.490 ±0.001 a E
E3 30S	1.000 ±0.007 f D	0.960 ±0.009 e B	0.760 ±0.003 d A	0.680 ±0.004 c B	0.660 ±0.004 b CD	0.580 ±0.002 a D
E3 70S	0.960 ±0.005 e BC	0.920 ±0.004 d F	0.840 ±0.006 c G	0.730 ±0.007 b D	0.650 ±0.004 a BC	0.640 ±0.002 a J

Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.
Abbreviations as in Table 1.

observed in the juice obtained from the mash treated with the Pectinex Yield Mash preparation without the addition of sucrose (E2) – a decrease in anthocyanin content from 0.43 to 0.01 mg·g^{-1} FW was noted. This is also the lowest anthocyanin content recorded in juice after five months of storage. The highest anthocyanin content (0.07 mg·g^{-1} FW) after five months' storage was determined in the juice obtained from the mash subjected to the maceration of raspberry with the Ultrazym AFP-L enzyme preparation (E3) supplemented with 70% of sugar, accounting for 18.4% of the content determined at time 0. Moreover, in the case of this group of phenolics, it can be observed that sucrose addition is a good way to improve their stability (Table 4).

Applying enzymatic treatment to fruit mash can also affect the antioxidant activity of the juices obtained. Tables 5–7 show the antioxidant properties of raspberry juice extracts obtained from the controls and treated with enzymatic preparations raspberry mash and syrups produced from these juices, determined by three different methods.

Table 6. Changes in antioxidant properties determined against $ABTS^{\bullet+}$ of raspberry juices and syrups during five-month storage, TEAC μmol·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	1.150 ±0.050 a AB	1.140 ±0.021 a D	1.130 ±0.004 a E	1.110 ±0.015 a E	0.810 ±0.090 b ABE	0.730 ±0.007 b ABC
C 30S	1.200 ±0.044 c B	1.160 ±0.023 c D	1.090 ±0.038 bc DE	1.010 ±0.039 ab AD	0.980 ±0.006 ab AC	0.940 ±0.065 a DE
C 70S	1.130 ±0.045 a AB	1.120 ±0.025 a CD	1.110 ±0.028 a DE	1.060 ±0.020 a AE	1.040 ±0.030 a C	0.980 ±0.116 a E
E1	1.090 ±0.063 a AB	1.040 ±0.017 a AB	1.020 ±0.042 a ACD	1.010 ±0.004 a AD	0.800 ±0.105 b D	0.710 ±0.021 b ABC
E1 30S	1.070 ±0.009 b AB	1.050 ±0.011 b ABC	0.990 ±0.076 b ABC	0.810 ±0.002 a B	0.700± 0.107 a BDE	0.740 ±0.078 a ABC
E1 70S	1.130 ±0.065 b AB	1.090 ±0.036 b ACD	1.080 ±0.044 b CDE	1.040 ±0.007 b AE	0.910 ±0.005 a AC	0.820 ±0.076 a BCDE
E2	1.070 ±0.050 a AB	1.020 ±0.019 a AB	0.940 ±0.006 ac AB	0.850 ±0.042 bc BC	0.720 ±0.006 b BDE	0.530 ±0.094 d F
E2 30S	1.020 ±0.007 b A	1.000 ±0.008 b B	0.810 ±0.015 a F	0.830 ±0.005 a B	0.830 ±0.005 a AB	0.620 ±0.098 c AF
E2 70S	1.060 ±0.084 c A	1.020 ±0.023 bc AB	0.960 ±0.012 abc AB	0.950 ±0.017 ab DF	0.930 ±0.022 ab AC	0.900 ±0.024 a CDE
E3	1.110 ±0.024 c AB	1.060 ±0.050 c ABC	0.920 ±0.013 b B	0.860 ±0.038 b BC	0.650 ±0.069 a DE	0.640 ±0.009 a ABF
E3 30S	1.110 ±0.022 c AB	1.090± 0.019 bc ACD	1.030 ±0.017 ab ACD	1.030 ±0.033 ab A	1.020 ±0.018 a C	0.750 ±0.023 d ABCD
E3 70S	1.050 ±0.018 a A	1.020± 0.011 a AB	0.990 ±0.002 ad ABC	0.910 ±0.009 cd CF	0.840 ±0.080 bc AB	0.790 ±0.027 b ABCDE

Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.
Abbreviations as in Table 1.

The changes in the antioxidant activity of the stored juices and syrups (DPPH method) expressed as a Trolox equivalent are shown in Table 5. Enzymatic raspberry mash treatment caused a statistically significant decrease in the juices' ability to neutralize DPPH free radicals in relation to the control, and the same trend was maintained for four months. It was only after 5 months that the result obtained for the control juice was lower than for juices obtained after enzymatic treatment. The highest antioxidant activity after five months was determined for raspberry syrup containing 70% sugar (E3 70S) obtained from juice after Ultrazym AFP-L treatment – 0.64 μmol Trolox·g^{-1} FW, while the lowest value was found for the control juice (C) – 0.46 μmol Trolox·g^{-1} FW. In all the samples analyzed, the antioxidant activity gradually decreased, but still somewhat slower in the case of juices with the addition of sucrose. Statistically significant differences were observed after one month of storage, compared to the initial activity for all juices obtained from raspberry mash treated with all enzyme preparations, both with and without added sugar. A reduction

Table 7. Changes in the ability to chelate Fe (II) of raspberry juice and syrups during five-month storage, mg EDTA·g^{-1} FW

Sample	Storage time, months					
	0	1	2	3	4	5
C	0.130 ±0.005 d DE	0.120 ±0.005 d CD	0.090 ±0.003 c BC	0.080 ±0.004 bc AB	0.074 ±0.006 ab AB	0.064 ±0.003 a EF
C 30S	0.136 ±0.007 b D	0.132 ±0.006 b D	0.110 ±0.005 c E	0.095 ±0.006 a D	0.090± 0.005 a D	0.080 ±0.002 a G
C 70S	0.130 ±0.006 c DE	0.129 ±0.005 c D	0.110 ±0.004 b E	0.100 ±0.002 ab D	0.095 ±0.001 a D	0.080 ±0.001 d G
E1	0.093 ±0.005 c AB	0.089 ±0.004 c A	0.074 ±0.005 b D	0.069 ±0.005 b BC	0.042 ±0.003 a E	0.040 ±0.001 a AB
E1 30S	0.097 ±0.004 c AB	0.084 ±0.004 a A	0.079 ±0.003 a ABD	0.078 ±0.003 a AB	0.074 ±0.002 a AB	0.045 ±0.001 b A
E1 70S	0.110 ±0.004 e AC	0.093 ±0.004 d AB	0.083 ±0.003 c ABCD	0.072 ±0.004 b ABC	0.061 ±0.004 a C	0.060 ±0.002 a DE
E2	0.102 ±0.005 d ABC	0.086 ±0.004 a A	0.084 ±0.003 a ABCD	0.081 ±0.004 a A	0.067 ±0.005 c AC	0.037 ±0.004 b B
E2 30S	0.108 ±0.006 d AC	0.088 ± 0.005 b A	0.087 ±0.004 b ABC	0.072 ±0.002 a ABC	0.067 ±0.003 a AC	0.047 ±0.003 c AC
E2 70S	0.138 ±0.008 d D	0.106 ±0.007 c BC	0.095 ±0.006 bc C	0.081 ±0.003 ab A	0.080 ±0.002 a B	0.068 ±0.002 a F
E3	0.085 ±0.005 b B	0.079 ±0.004 ab A	0.077 ±0.003 ab AD	0.076 ±0.003 ab ABC	0.072 ±0.001 a AB	0.041 ±0.004 c AB
E3 30S	0.116 ±0.007 b CE	0.114 ±0.006 b C	0.084 ±0.005 ab ABCD	0.076 ±0.004 a ABC	0.074 ±0.002 a AB	0.047 ±0.003 c AC
E3 70S	0.093 ±0.006 a AB	0.092 ±0.005 a AB	0.089 ±0.004 a ABC	0.066 ±0.004 a C	0.065 ±0.001 a AC	0.053 ±0.002 a CD

Mean ±standard deviation. Average in the rows designated with the same lower case letters do not differ significantly at $p = 0.05$. Average in the columns designated with the same capital letters do not differ significantly at $p = 0.05$.
Abbreviations as in Table 1.

in the ability to neutralize free radicals, ranging from 4 to 14% in relation to the "0" time, was noted. Only in the control juice with no sugar added (C) and with 70% addition of sucrose (C 70S) were statistically significant changes recorded after two or three months of storage. After five months, only the control juice and syrups retained more than 50% of their initial activity. Other juices and syrups retained from 30% (juice obtained from the raspberry mash treated with Pectinex Ultra SP-L supplemented with 70% sugar (E1 70S)) to 42% (juice obtained from the raspberry mash treated with Ultrazym AFP-L (E3 30S)) of the initial activity. During five-month storage there were strong positive correlations between DPPH radical scavenging and contents of phenolic compounds, phenolic acids, flavonoids and anthocyanins ($r = 0.765$, $r = 0.869$, $r = 0.836$ and $r = 0.831$, respectively).

Table 6 shows the ability of raspberry juices and syrups to neutralize ABTS cations during storage. There were no statistically significant differences between the control juice and juices obtained after enzymatic maceration of raspberry mash at time "0", but after the first month of storage the control juice had higher antioxidant activity, and this tendency was maintained

up to the end of the experiment. Generally, the longer the storage time, the weaker the antiradical activity was, but after the first month, there were no statistically significant changes in the same samples. Statistically significant differences compared to the "0" time were observed after two months in the case of juice obtained after treatment with Pectinex Yield Mash containing 30% sugar addition (E2 30S) and juices obtained from mash treated with Ultrazym AFP-L without the addition of sucrose (E3) and with 30% of the additive (E3 30S). After three months of storage, there was a statistically significant decrease in the ability of samples C 30S, E1 30S, E2, E2 70S and E3 70S to scavenge $ABTS^{\bullet+}$. Strong positive correlations between ABTS radical scavenging and levels of phenolic compounds, phenolic acids, and flavonoids were shown ($r = 0.772$, $r = 0.752$ and $r = 0.776$, respectively).

The ability to chelate Fe(II) is shown in Table 7 as an EDTA equivalent. The highest chelating capacity was obtained for control juice (0.130 mg EDTA·g^{-1} FW), the lowest for raspberry juice obtained after Ultrazym AFP-L mash treatment (0.085 mg EDTA·g^{-1} FW).Using Pectinex Yield Mash (E2) and Pectinex Ultra SP-L (E1) also caused a statistically significant decrease in Fe^{2+} chelating ability in comparison to the control of about 21 and 28% respectively. During the five-month storage of the raspberry juices and syrups, like changes in antiradical activity, the ability to chelate iron was reduced (Table 7). The average chelation ability after five months of storage is approx. 50% of the value determined at time "0". Frequently, a statistically significant change can be observed after a month or two from the preparation of juices. The highest ability to chelate Fe(II) was exhibited by juice containing sucrose (30 to 70%) obtained from the control mash throughout the storage period. After five months, the activity analyzed was 0.08 mg EDTA·g^{-1} FW. During the entire storage period juices obtained from raspberry mash after enzymatic treatment exhibited the lowest chelating ability. In turn, syrups obtained from these juices (especially with 70% sugar addition) showed statistically significant higher chelating power. After five months, the lowest Fe^{2+} binding capacity was determined for the juice obtained from the raspberry mash treated with Pectinex Yield Mash (E2) – EDTA 0.037 mg·g^{-1} FW. Futhermore, the results revealed that there was a strong positive correlation between chelating ability and the content of individual polyphenol groups determined during the five months of storage. Chelating power was strongly correlated with the levels of total phenolic ($r = 0.706$), phenolic acids ($r = 0.762$), flavonoids ($r = 0.755$), and anthocyanins ($r = 0.72$).

DISCUSSION

For the production of fruit juices from berries a hot enzymatic process is recommended. The pulp is heated to 45–50°C followed by its enzymatic process. The production of clear juices and concentrates of strawberries, raspberries or blackberries requires enzymatic depectinisation. Juices from these fruits contain large amounts of pectin, which form a colloidal suspension in the juice, giving the juice a high viscosity. Hence, it is difficult to obtain clear juice concentrates. Residues of pectin and hemicellulose also bind to proteins and phenolic compounds during the processing and storage of juices, forming complexes resistant to digestive enzymes (Helbig, 2001).

In this work, the juices were obtained from raspberry mash treated with three commercial enzymatic preparations and the level and antioxidant activity of phenolic compounds was determined. Upon enzyme treatment, degradation of pectin leads to a reduction in the water-holding capacity of pectin; hence, free water is released into the system and the juice yield increases (Kashyap et al., 2001). However, only a part (60–65%) of the total phenolic content, including the anthocyanins, passes into the juice from the raw material, while the rest of the bioactive compounds remains in the waste. In the study by Koponen et al. (2008) the anthocyanin content in bilberries was 7.64 mg·kg^{-1}, and in the control juice was 3.52 mg·kg^{-1}. In juices obtained after enzymatic maceration of mash the concentration of pigments ranged from 2.97 to 5.14 mg·kg^{-1} depending on the type and amount of the preparation used. The best result was obtained for the Pectinex Smash XXL preparation. In turn, Landbo and Meyer (2004) stated that the juice yield, anthocyanin level, and the level of total phenols in blackcurrant juice were improved by using pectinolytic enzyme preparations for pre-press maceration. The 10 enzyme preparations that were tested turned out to be almost equally good with respect to the main responses measured in the juices. Nevertheless, one enzyme preparation, Pectinex BE, consistently tended to be slightly better than the others.

The level of bioactive compounds, including phenolic compounds, changes even during a short, several-day storage of raw fruit (Connor et al., 2002; Patthamakanokporn et al., 2008). Processing berry fruits, not only into juices, jellies and jams causes losses in phenolic compound content, in particular anthocyanins at each stage of production. Very often, there is even less than 10% of the initial phenolic content in the final product (especially in the case of clarified juices) (Amarowicz et al., 2009).

In our study, we examined the stability of juices obtained from raspberry mash treated with commercial enzyme preparations and with the addition of sucrose − 30 and 70% (w/v).

During the five-month storage period, the level of total phenolic compounds, flavonoids, and phenolic acids was reduced (Tables 1–4). The greatest losses were observed for anthocyanins, which are the most labile compounds among all groups of polyphenols (Table 4). The addition of sugar to the juices contributed to a slight reduction in the losses of their contents. Most likely, light was the most destructive factor, since the samples were not kept in the dark, although temperature might also have had an impact. The loss of the contents of bioactive compounds in stored juices were accompanied by reduced antioxidant properties (Tables 5–7).

The results obtained correspond well with the results reported by other researchers. Wilkes et al. (2014) demonstrated that storage of chokeberry juices at 25°C not only degrades anthocyanins but also flavonols (quercetin derivatives). To strengthen and maintain the attractive color of juices from berries, e.g. raspberry juice, during storage, Rein and Heinonen (2004) added phenolic acids or commercial stabilizers. They found that the color of juices was strengthened immediately upon the addition of the commercial preparations, but the effect was unstable during storage. The best additive to the raspberry juice was ferulic or sinapinic acid. The protective effect of adding phenolic acids against thermal degradation of anthocyanins in redcurrant juice was also shown by Kopjar et al. (2009).

Kalisz and Wolniak (2006) tested the effect of adding low-methoxy pectin on the stability of anthocyanins in raspberry juice. After 3-month storage, the anthocyanin content in juices without and with pectin decreased by approximately 23% and 16%, respectively.

Ścibisz and Mitek (2005) observed that the jam production process from northern highbush blueberries resulted in the loss of total phenolic compounds, chlorogenic acid, total anthocyanins, and individual anthocyanins, as well as antioxidant activity. When storing jams for half a year, the biggest changes in the content of antioxidant compounds occurred during the first two months of storage, where the biggest loss was recorded in the case of anthocyanins. It was also shown that a high concentration of sugar may stabilize the anthocyanin pigments, confirming the results obtained in this work. In blueberry jams, the half-life of anthocyanins was longer than in blackcurrant jams. The storage temperature resulted in significant changes in anthocyanin content. Jams stored for eight months under refrigeration contain 4–7 times more colorants than products stored at 22°C. During the storage of jams, there were no differences in the degree of degradation of individual anthocyanins (Ścibisz et al., 2011).

During the production of raspberry jams, the concentration of ellagic acid increased. This may have been caused by the formation of this compound from hexahydroxydiphenic acid released from ellagitannins by hydrolysis or thermal degradation of cell wall components. During the storage of raspberry jams, the level of glycosidic derivatives of ellagic acid and flavonoid glycosides decreased (Zafrilla et al., 2001). The composition of anthocyanins also changed during the production of jams produced from both fresh and frozen fruits. García-Viguera et al. (1998) found that during jam processing, the total anthocyanin content was reduced by 17–24% and 37–40% when Heritage or Zeva red raspberries were used, respectively. Higher losses were found in jams made of frozen raspberries. Freezing and thawing causes the destruction of cellular structures, which allows intracellular enzymes (e.g. polyphenol oxidase) to come into contact with phenolic compounds (substrates), and enzymatic degradation. The authors cited above determined changes in the content of specific anthocyanins during jam storage at 20, 30, and 37°C. The degree of anthocyanin degradation increased as temperature rose. Already after the first month of storage at 37°C, there was a decrease in anthocyanin content of over 50%, where cyanidin-3-O-glucoside was the most unstable compound.

Wu et al. (2010) found that the level of phenolic compounds and anthocyanin monomers decreased

after the processing of blackberries into jams and canned fruits in syrup and after drying under a high temperature. Only freeze-drying did not cause significant changes in phytochemical content and antioxidant properties. Similar results were demonstrated in the storage of processed fruits at room temperature. The largest losses were recorded in jams, probably due to the long-term thermal treatment. In this study, it was also demonstrated that anthocyanin pigments are highly unstable compounds and are degraded during storage of juices and syrups at room temperature. It is possible that storage in the dark or in dark vessels would improve the stability of anthocyanins, but this requires experimental verification.

An alternative method of food preservation is the use of high hydrostatic pressure. Suthanthangjai et al. (2005) subjected raspberry puree to a pressure of 200, 400, 600 and 800 MPa for 15 minutes and then stored it for 9 days at 4, 20, and 30°C. The greatest stability of anthocyanins was identified for puree stored at 4°C after the application of pressure of 200 and 800 MPa. A pressure of 400 MPa resulted in the greatest loss of cyanidin-3-O-glucoside and cyanidin-3-O-sophoroside. The smallest losses in phenolic compounds, including anthocyanins, and their biological properties were observed during the storage of frozen fruit (Mullen et al., 2002). Six-month freezing storage of highbush blueberries at –18°C and –35°C had no significant effect on the stability of anthocyanins (Ścibisz and Mitek, 2005). Similar results were obtained by Ancos et al. (2004) and Gonzàlez et al. (2003) during storage of frozen raspberries and blackberries. Much higher losses of anthocyanin pigments, even up to 90% were observed during storage of frozen cherries (Chaovanalikit and Wrolstad, 2004).

In the current study, strong positive correlations were found between total phenolic, phenolic acid, flavonoid and anthocyanin contents and antioxidant capacity. It can therefore be concluded that during five-month storage, antioxidant activity falls, along with the content of individual polyphenol groups. Similar results for bilberry and blackcurrant juices were obtained by Buchert et al. (2005). In turn, Kalisz and Wolniak (2006) noted a positive correlation (r = 0.5936 and r = 0.9014, respectively) between the content of the anthocyanin and polyphenol fractions and the antioxidant capacity of the raspberry juice.

CONCLUSIONS

The five-month storage of juices and juice containing sucrose at room temperature caused a reduction in the level of phenolic compounds, in particular anthocyanins, whose content was reduced by up to 95% in relation to the initial content. Storage of raspberry juices and syrups also resulted in a reduction in antioxidant activity. The enzymatic treatment of raspberry mash generally increased these losses.

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