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QUALITY OF CLOUDY PLUM JUICE PRODUCED FROM FRESH FRUIT OF *PRUNUS DOMESTICA* L. – THE EFFECT OF CULTIVAR AND ENZYME TREATMENT

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ABSTRACT

The quality of cloudy juices produced from two plum cultivars varied in chemical characteristics and native polyphenol oxidase (PPO) activity, and was studied in relation to specific pectinolytic activity of enzyme preparations used for fresh fruit maceration before pressing. Process effectiveness expressed as juice yield, turbidity and the rate of transfer of anthocyanins and polyphenols were determined for five different enzyme preparations, whose activity was also analysed. Juice yields obtained after 1 hour mash maceration (50 ºC, 100 $g \cdot t^{-1}$) were between 86.6 and 95.4%. The anthocyanins content of the obtained juices strongly depended on the cultivar and ranged from 26 to 50 $mg \cdot L^{-1}$ for 'Promis', and from 269 to 289 $mg \cdot L^{-1}$ for 'Čačanska Najbolja', which could be related to the differences in the measured PPO activity (175.4 and 79.8 $nkat \cdot g^{-1}$, respectively). The type of enzyme preparation strongly affected the degradation rate of anthocyanins during juice processing. Peonidin-3-rutinoside proved to be the most stable during plum juice production in contrast to cyanidin-3-glucoside. Irrespectively of the cultivar, the juice prepared with the mixture of Rohapect PTE + Rohament PL (2 : 1) showed the highest turbidity among the investigated combinations. The results suggest that for the production of cloudy plum juice use of a preparation with low pectin methyl esterase and polygalacturonase activities and high pectin lyase activity could be recommended.

Key words: enzyme activity, plum, juice processing, degradation of anthocyanins, PPO activity

INTRODUCTION

According to FAOSTAT (2013), the average production of plums and sloes in Europe in the last decade has reached 1.55 million tonnes, which has placed these species at the top of the fruits harvested in this region. Plums are a significant source of nutrients, dietary fibre and antioxidants, such as phenolic compounds (Stacewicz-Sapuntzakis et al. 2001; Gil et al. 2002; Milala et al. 2013). The phenolic compounds occurring in plums include mainly phenolic acids, such as neochlorogenic acid, chlorogenic acid and anthocyanins: cyanidin-3-rutinoside, cyanidin-3-glucoside and peonidin-3-rutinoside (Tomás-Barberán et al. 2001; Kim et al. 2003a). The purple colour of plums is a result of the high level of anthocyanins, which are located mostly in the fruit skin (Ahmed et al. 2004; Will & Dietrich 2006).

Epidemiological data suggest that phenolic compounds contribute to the protective effect against cardiovascular disease, lung cancer, diabetes, cataracts and Alzheimer's disease (Birt et al. 2001; Knekt et al. 2002; Konczak & Zhang 2004). High amounts of dietary fibre and sorbitol help in the regulation of digestion and sugar metabolism. Moreover, plums contain substantial amounts of potassium, which is beneficial for cardiovascular health and a high amount of boron, which may play a role in the prevention of osteoporosis (Stacewicz-Sapuntzakis et al. 2001).

The postharvest life of plum fruit is relatively short, up to 2 weeks in a cold atmosphere. To increase

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the consumption of the fruit, processing of plums to stabilized food products is needed. Compared to the commonly available plum products (i.e. dried prunes, jams, plum-dumplings or compotes), plum juice produced from fresh fruit emerges as an attractive and nutritionally beneficial proposal. However, plum juices from fresh fruit have not yet been produced. The technical problem in plum juice production is the consequence of the high amount of pectic polysaccharides in plum fruit tissue, which are the principal constituents of the middle lamella and make the pressing operation difficult. For this reason, processing methods allowing, that would overcome the problems with pressing have been searched for. Donovan et al. (1998) and Gorsel et al. (1992) have obtained plum juice from dried prunes by extraction with water. Degradation of pectin in plum fruit by pectinolytic enzymatic treatments has also been successfully used to facilitate the processing of plums into juice. For example, Will & Dietrich (2006) studied the enzymatic treatment of plum puree prior to further mash mechanical breakdown and final decanter extraction. In the latter case, the addition of pectinolytic enzyme preparations to the mash before pressing (100 ppm of Pectinex Smash XXL) made it possible to obtain plum juice suitable for the production of cloud-stable plum nectars. According to Fastyn et al. (2008) and Levaj et al. (2012), the pressing of fresh fruit mash can result in obtaining a satisfactory plum juice quality and yield, providing specific pectin degrading enzymes are applied in proper amounts and conditions. On one hand, advanced maceration of fruit mash leads to strong hydrolysis of pectic substances, which favours both the juice yield and transfer of anthocyanins from the fruit skin to the product (Levaj et al. 2012). On the other hand, in the case of a cloudy product (that should contain more health promoting components, especially dietary fibre), the excessive pectin degradation impairs product turbidity and leads to cloudiness instability (Will & Dietrich 2006; Ribeiro et al. 2010). Moreover, depending on the specific activity of pectinolytic enzymes used for fruit mash maceration before processing, products of different qualitative and quantitative anthocyanin contents (Buchert et al. 2005) as well as different antioxidant activity (as a consequence of aforementioned factors) may be obtained.

Another important factor influencing plum juice quality is the presence of the polyphenol oxidase (PPO) enzyme in the raw material, which is responsible for enzymatic browning Martinez & Whitaker (1995) and degradation of anthocyanins (Wesche-Ebeling & Montgomery 1990; Siddiq et al. 1994; González et al. 2000). PPO is a copper-containing enzyme, which catalyzes the formation of quinones from phenols in the presence of molecular oxygen. Polyphenol oxidase catalyzes two different reactions: the o-hydroxylation of monophenols to o-diphenols (cresolase activity) and the subsequent oxidation of o-diphenols to o-quinones (catecholase activity). Anthocyanins are not directly oxidized by the enzyme but by the quinones formed by PPO from catechol, catechin or chlorogenic acid (González et al. 1999; Siddiq et al. 1992). One of the effective methods of inhibiting PPO action and simultaneously protecting the product's colour during juice production is the addition of edible organic acids considered safety and environmentally friendly. In the case of plum juice production, application of ascorbic acid preserves the anthocyanins, which is clearly noticeable immediately after processing (Will & Dietrich 2006; Fastyn et al. 2008). The disadvantage of the addition of ascorbic acid is that it considerably accelerates the degradation of anthocyanins during storage (Fastyn et al. 2010). Therefore, new technologies are needed for a more effective protection of biologically active components during plum juice production. Regarding the fact that PPO is considered one of the main factors responsible for the intense browning of plum juice, one should expect that using cultivars with low PPO activity will work in favour of obtaining a product with a higher stability of colour and more health promoting compounds.

In the present work, the factors influencing the quality of cloudy plum juice obtained from fresh fruit were investigated. First, the cultivars that varied in chemical composition and PPO activity were evaluated as raw materials for juice production. After that, the impact of pectinolytic enzyme preparations on the effectiveness of the juicing process and juice quality attributes were studied. In particular, the interaction between specific pectin-related activity of the enzyme preparations and the

quality and quantity of anthocyanins in cloudy plum juice was evaluated.

MATERIALS AND METHODS

Fruit samples

Two plum cultivars were chosen: 'Čačanska Najbolja' and 'Promis'. The fruit was obtained from the experimental orchard of the Research Institute of Pomology and Floriculture (Presently Research Institute of Horticulture) in Skierniewice, Poland. The plums were picked at harvest maturity during the 2009 season and stored at -25 °C before analyses and juice pressing.

Enzyme preparations

Commercial pectinolytic enzyme preparations were chosen based on the recommendations of the producers concerning their use for red fruit juice production and because of the high activity of the enzymes at low pH and high temperature. The enzymes used were Rohapect PTE, Rohament PL, Rohapect MA PLUS (kindly supplied by AB ENZYMES, Sieradz, Poland) and Pectinex Ultra Colour, Pectinex BE XXL (kindly supplied by NOVOZYMES S.A., Warsaw Poland). Rohapect PTE and Rohament PL were combined in a 2 : 1 proportion according to the producer's recommendations.

Juice pressing

Plum juices were produced on a laboratory scale according to the flow chart in Fig. 1. using equipment and procedure, which had been already proved to have good reproduction in potential industrial applications (Banaszczyk & Płocharski 1993). Directly before processing, the plums were hand-pitted in the frozen state, using a ceramic knife. After removing the stones, about 2.5 kg of the plums were disintegrated using a Fryma perforated disc mill (BASIS 91/55, Fryma-Maschinen AG, Rheinfelden, Switzerland). The resulting mash was mixed and, for each combination, 200 g test samples were weighed out and treated with the enzyme preparations at a dose of 100 $g \cdot t^{-1}$ for 1 hour at 50 °C. After the enzymatic treatment, the samples were pressed using a texture analyser (Model 4303, Instron Ltd., Buckinghamshire, England) equipped with a facility for juice pressing. The raw juices obtained were directly analysed for basic composition. The pressing yield was determined by weighing the pressed juice and expressing the result as a percentage of the initial sample weight. For each combination at least three replications of pressing operations were performed.

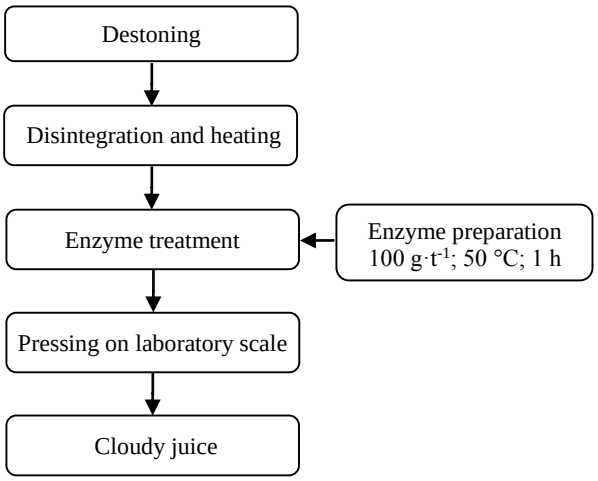

Fig. 1. Flowchart of cloudy plum juice production on a laboratory scale.

Fruit and juice analyses

Soluble solids were measured with an RE50 refractometer (Mettler-Toledo, Tokyo, Japan) at 20 °C. Results were reported as degrees Brix. Titratable acidity was determined using an automatic titration unit (DL 58; Mettler-Toledo, Greifensee, Switzerland). Dry matter of fruit was determined with a gravimetric method – 10 g of fruit tissue was dried at 70 °C and a pressure of 3 kPA to constant weight. The turbidity of plum juices was measured using a HACH Turbidimeter (Hach Company, Loveland, Colorado, USA) and expressed in nephelometric turbidity units (NTU). Prior to turbidity measurements, all the samples were diluted with distilled water at 1 : 5.

Determination of anthocyanins content

Total anthocyanin content was estimated by the pH differential method according to Wrolstad (1976) with some modification using a Cary 3E spectrophotometer (Varian, Melbourne, Australia). Anthocyanin concentrations were calculated using the molar absorptivity of cyanidin-3-rutinoside (28800) and the molecular weight of 595.2 $g \cdot mol^{-1}$. The results were expressed in $mg \cdot 100\ g^{-1}$ or $mg \cdot 100\ ml^{-1}$.

Determination of total phenolic content

Total phenolic content was determined using the Folin- Ciocalteu method (Tsao & Yang 2003), expressing the total phenolic content as gallic acid equivalents (GAE) in mg·100 g^{-1} or mg·100 ml^{-1}. The sample was prepared as follows: **Fruit** – 10 g of disintegrated fruit was mixed with a 100 ml mixture of ethanol and 1.5 N HCl, then homogenized for 2 min. The homogenates were centrifuged at 3 000 × g for 10 min. The supernatant was used for determination of anthocyanin and total phenolic content. **Juice** – centrifuged at 4 200 × g for 15 min. The supernatant was used for the assays.

Antioxidant activity measurements

Free-radical scavenging activity was determined according to the method described by Re et al. (1999) using $ABTS^{\bullet+}$ radical cation, and the detailed measurement procedure was according to Oszmiański & Wojdyło (2007). A 5 ml volume of $ABTS^{\bullet+}$ solution was added to 500 µl of the sample and mixed. The reaction mixture was kept at room temperature in a dark place for 6 min. Absorbance was measured at 734 nm using a Cary 3E UV-Visible spectrophotometer (Varian). For each sample, at least four measurements at different concentrations were made to reduce the initial absorbance of $ABTS^{\bullet+}$ solution from 20% to 80%. Linear regression method was used to calculate the concentration of juice leading to a 50% decrease in the absorbance of $ABTS^{\bullet+}$ solution, and recalculated to mg of Trolox equivalents per ml of juice.

HPLC analysis of anthocyanins

The analysis of anthocyanins by HPLC was performed according to the method by Nielsen et al. (2003) with some modifications. A 5 µl volume of the samples was analysed using an Agilent HPLC Model HP 1100 (Hewlett-Packard, Waldbronn, Germany) equipped with a Diode Array Detector (DAD). Separation was performed on a Synergi Fusion-RP column (250 mm × 4.6 mm; particle size 4 µm), (Phenomenex, Torrence, USA). For the HPLC analysis, the flow rate of the system was 1 ml·min^{-1} using mobile phases – (A) 5% aqueous formic acid and (B) acetonitrile. Elution profile: 0-5 min, isocratic 3% B; 5-32 min, 10% B; 32-54 min, 33% B; 54-58 min, 90% B; 58-62 min, isocratic 90% B. The anthocyanins in the eluate were detected at 520 nm and a temperature of 25 °C. Their amounts were quantified by calibration with the standards of cyanidin-3-glucoside, cyanidin-3-rutinoside and peonidin-3-rutinoside, expressed in mg·100 g^{-1} or mg·100 ml^{-1}.

The sample was prepared as follows: **Fruit** – 5 g of disintegrated fruit was mixed with 50 ml of 1% formic acid in 60% methanol and then homogenized for 2 min. The homogenate was centrifuged at 10 000 × g for 10 min. The supernatant was used for the HPLC assay. **Juice** – centrifuged at 10 000 × g for 10 min. The supernatant was diluted (1 : 2) with 1% formic acid in 60% methanol, then used for the determination of anthocyanin content.

Activity of enzymes

Polyphenol oxidase (PPO) activity in plum fruit

Extraction of PPO enzyme was performed as described by González et al. (1999) with some modifications. The frozen plums were pulverized in liquid nitrogen using an IKA A11 Basic grinder (IKA-Werke). 10 g of the powder was suspended in 25 ml of 0.1 M sodium phosphate buffer (pH 7.0) and homogenized for 2 min. The homogenate was transferred to a 50 ml flask and filled up to the mark with buffer. After 2 h at 4 °C in a dark place, the mixture was centrifuged at 10 000 × g for 15 min using a laboratory centrifuge Sigma 3K30.

The activity of PPO was measured as described by Robb (1988) with some modifications. The assays were carried out by the spectrophotometric method. A 10 mM catechol substrate solution was prepared in the extraction buffer (pH 7.0). A 20 µl of the enzyme solution was added to 1000 µl of the substrate solution. The absorbance at 400 nm was measured immediately after stirring the enzyme solution into the substrate solution every 10 s for 5 minutes. PPO activity (nkat·g^{-1} of fresh fruit) was determined from the linear part of the absorbance curve and calculated using the molar extinction coefficient 1400 (l $mol^{-1}cm^{-1}$).

Pectin Methyl Esterase (PME) activity

PME was assayed by titration of the liberated carboxyl groups of citrus pectin. The PME activity (nkat·ml^{-1} of enzyme preparation) was analysed

using a Toledo T40 (Mettler-Toledo, Greifensee, Switzerland) titrator in a reaction mixture containing 20 ml of 1% citrus pectin (Fluka) in McIlvain buffer, pH 3.5, and enzyme solution (10 ÷ 100 µl) (diluted if needed). A 0.05 M NaOH solution was used for titration.

Pectin lyase (PL) activity

The pectin lyase activity (nkat·ml^{-1} of enzyme preparation) was measured as described by Manachini et al. (1988) with some modifications. Substrate solution – 1000 µl of 0.5% citrus pectin in McIlvain buffer (pH 3.5) was heated up to 40 °C. A 500 µl of enzyme preparation (diluted if needed) was added to the solution. The reaction mixture was mixed and incubated at 40 °C for 10 min. The reaction was stopped by the addition of 3500 µl of 0.5 M HCl. The absorbance was measured at 235 nm against the blank (500 µl of buffer instead of the sample) using a Lambda 20 UV-VIS Spectrometer (Perkin Elmer, Norwalk, CT, USA).

Polygalacturonase (PG) activity

The polygalacturonase activity (nkat·ml^{-1} of enzyme preparation) was measured as described by Bailey & Pessa (1990) with some modifications. 900 µl of 0.4% polygalacturone acid in McIlvain buffer (pH 3.5) was heated up to 30 °C. 100 µl of enzyme preparation (diluted if needed) was added to substrate solution. The reaction mixture was mixed and incubated at 30 °C for 5 min. The reaction was stopped by the addition of 1500 µl of DNS reagent (containing NaOH, K-Na-tartrate and 2-hydroxy-3,5-dinitrobenzoic acid). The mixture was heated in a boiling water bath for 5 min and cooled. 5 ml of distilled water was added and mixed. The sample solution was filtered through a Whatman No. 6 filter paper. The absorbance was measured at 540 nm using a Lambda 20 UV-VIS Spectrometer (Perkin Elmer, Norwalk, CT, USA) against the blank (100 µl of buffer instead of the sample).

Statistical analysis

The data collected for plum fruit was subjected to a one way analysis (factor: cultivar) and for plum juices a two way analysis (factors: cultivar and enzyme) of variance (ANOVA) using Statistica 8.0 software. The differences between sampling dates were estimated with Tukey's HSD test ($p < 0.05$).

RESULTS

Enzyme preparations

Polygalacturonase (PG) – catalyses the hydrolytic cleavage of α-1,4 linkages of pectic acid, was the main activity in all commercial enzyme preparations used (Table 1). Among the enzyme preparations, Rohapect MA Plus was characterized by the highest activity of PG and also of pectin methyl esterase (PME) (138 095 nkat·ml^{-1} and 9 378 nkat·ml^{-1}, respectively), but by the lowest activity of pectin lyase (PL) (14 nkat·ml^{-1}). When PME removes the methyl groups from methylated pectin substances, PL shows specificity for methyl esterified substrates (pectin) (Whitaker 1984; Uhlig 1998). The mixture of Rohapect PTE and Rohament PL (2 : 1) was characterized by the highest activity of PL (993 nkat·ml^{-1}) and the lowest activity of PME (142 nkat·ml^{-1}) in comparison with the other enzyme preparations used. The enzyme preparations Pectinex BE XXL and Pectinex Ultra Colour exhibited similar PG activity to that of the mixture of Rohapect PTE + Rohament PL, but the activity of PME was closer to that of Rohapect MA Plus.

Table 1. Activity of enzyme preparations (nkat·ml^{-1}) at pH 3.5

Enzyme preparation	PG	PL	PME
Rohapect PTE + Rohament PL (2 : 1)	16 565	993	142
Rohapect MA Plus	138 095	14	9 378
Pectinex Ultra Colour	28 087	89	6 789
Pectinex BE XXL	17 276	214	5 915

PG – polygalacturonase, PME – pectin methyl esterase, PL – pectin lyase

Plum fruits

The investigated plum cultivars 'Č. Najbolja' and 'Promis' differed considerably in their physicochemical properties (Table 2). 'Č. Najbolja' had a lower level of soluble solids and dry weight, but about 1.8 times higher acidity than 'Promis'. Gil et al. (2002) reported that the acidity of five studied plum cultivars was in the range of 4.1-5.5 g·kg^{-1}, which is comparable to the acidity found in our study for 'Promis' (5.8 g·kg^{-1}). However, soluble solids and dry matter contents in the fruit of 'Č. Najbolja' were higher than the values reported

by Walkowiak-Tomczak et al. (2007), which might have been the effect of the degree of ripeness or the growing season.

The total phenolic content was 200 mg GAE·100 g^{-1} for 'Promis' *cv*. and 296 mg GAE·100 g^{-1} for 'Č. Najbolja' *cv*. The phenolic content of the plums in our study was within the range reported by Gil et al. (2002) and Chun et al. (2003), and at a much higher level than in apples (Kim et al. 2003b). The fruit of 'Č. Najbolja' was characterized by a higher anthocyanins content with a value 31.4 mg·100 g^{-1} versus 11.0 mg·100 g^{-1} for 'Promis'. This level of anthocyanins was comparable to the results obtained by Usenik et al. (2009). Three anthocyanins: cyanidin-3-rutinoside, cyanidin-3-glucoside and peonidin-3-rutinoside were identified in the fruit of the plum cultivars investigated (Fig. 2). The fourth anthocyanin peonidin-3-glucoside occurred only in trace amount and therefore was not taken into account. The results indicated that the content of cyanidin-3-rutinoside was always higher than that of the other anthocyanins.

The value of PPO activity was much higher in 'Promis' (175.4 nkat·g^{-1}) than in 'Č. Najbolja' (79.8 nkat·g^{-1}) (Table 2). Plums have been reported to be among the fruits with high polyphenol oxidase activity (Łoś et al. 1996).

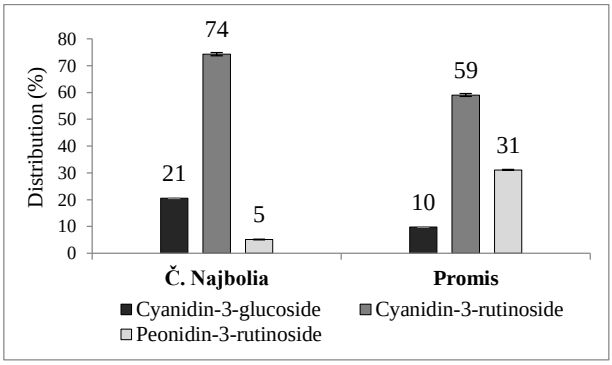

Fig. 2. Distribution of anthocyanins in plum fruit analysed by HPLC method. Bars depict a standard deviation

Table 2. Analytical parameters of plum fruit

Parameters	Unit	'Čačanska Najbolja'	'Promis'
Soluble solids	°Bx	$17.3^{a} \pm 0.0$*	$19.3^{b} \pm 0.0$
Titratable acidity	g·kg^{-1}	$10.1^{b} \pm 0.0$	$5.8^{a} \pm 0.1$
Dry weight	%	$19.5^{a} \pm 0.0$	$21.4^{b} \pm 0.1$
Anthocyanins**	mg·100 g^{-1}	$31.4^{b} \pm 2.9$	$11.0^{a} \pm 0.7$
Total polyphenols	mg GAE·100 g^{-1}	$296.4^{b} \pm 1.3$	$200.1^{a} \pm 0.5$
PPO activity	nkat·100 g^{-1}	$79.8^{a} \pm 19.1$	$175.4^{b} \pm 16.8$

* Means in the row marked by the same letter do not differ significantly at p = 0.05 according to Tukey's test; means ± SD (n=3)
** Total anthocyanin content quantified spectrometrically

Plum juices

Using pectin degrading enzymes in plum processing facilitated juice production, which is in accordance with the literature (Chang et al. 1994; Will & Dietrich 2006). The addition of pectinolytic enzymes to the mash had a positive effect on the production of cloudy plum juices, resulting in a high juice yield (Table 3). The lowest juice yield was obtained when the Rohapect MA Plus preparation was used to macerate of mash of the two plum cultivars: 90.3% for 'Č. Najbolja' and 86.6% for 'Promis'. This was much higher than the value of 60% reported for a plum juice by Siddiq et al. (1994), as well as the 43-73% reported by Chang et al. (1995) and the 78-83% obtained by Levaj et al. (2012). For the other enzyme preparations (Rohapect PTE + Rohament PL, Pectinex Ultra Colour and Pectinex BE XXL), the juice yields were over 93%. The high yields might be partially attributed to the fact that the experiments were conducted on fruit which was frozen after picking and processed off-season. Freezing and defrosting might disrupt cell walls, allowing better penetration by pectic enzymes into individual cells. However, tests conducted on an industrial scale using fresh fruit and an enzyme mixture containing Rohapect PTE + Rohament PL (2 : 1) have given comparable results (data not shown).

The levels of soluble solids and titratable acidity of the plum juices (Table 3) were comparable to those found in the plum fruit (Table 2) and depended mainly on the cultivar. The average soluble solids value for the plum juices was 19.3 ± 0.3 °Bx for 'Promis' and 17.2 ± 0.3 °Bx for 'Č. Najbolja', while the total acidity was 0.60 ± 0.03 g·100 ml^{-1} and 1.01 ± 0.05 g·100 ml^{-1}, respectively.

The combination of Rohapect PTE and Rohament PL (2 : 1) (high PL and low PME) gave the highest turbidity for each of the two plum cultivars, while Rohapect MA PLUS (high PG and PME, low PL) produced the lowest turbidity levels (Table 3). The results are in line with the work of Mieszczakowska-Frąc et al. (2012) in which the turbidity of blackcurrant and plum juices was shown to depend on enzyme activities. Preparations with high activity of pectin lyase (PL) and low activity of pectin methyl esterase (PME) made it possible to produce cloud-stable blackcurrant and plum juices, whereas enzyme preparations exhibiting high activity of both PME and PG and low activity of PL were found not suitable for the production of cloudy blackcurrant and plum juices. The results of the present study confirm the effect of enzyme activity on juice turbidity.

Table 3. Basic analytical parameters of plum juices

Cultivar	Enzyme preparation	Soluble solids °Bx	Titratable acidity g·100 ml^{-1}	Turbidity NTU	Pressing yield %
'Čačanska Najbolja'	Rohapect PTE+Rohament PL	17.0^{a} ± 0.1*	1.01^{bc} ± 0.01	7420^{d}	93.6^{c} ± 0.1
	Rohapect MA Plus	16.8^{a} ± 0.1	1.08^{c} ± 0.02	4485^{a-c}	90.3^{b} ± 1.0
	Pectinex Ultra Color	17.3^{b} ± 0.1	0.99^{b} ± 0.06	5020^{bc}	93.4^{c} ± 0.1
	Pectinex BE XXL	17.4^{b} ± 0.1	0.95^{b} ± 0.05	5910^{cd}	95.4^{d} ± 0.1
'Promis'	Rohapect PTE+Rohament PL	19.4^{d} ± 0.1	0.56^{a} ± 0.01	5390^{b-d}	93.3^{c} ± 0.3
	Rohapect MA Plus	18.9^{c} ± 0.1	0.62^{a} ± 0.01	2190^{a}	86.6^{a} ± 0.5
	Pectinex Ultra Color	19.4^{d} ± 0.1	0.62^{a} ± 0.01	3370^{ab}	93.3^{c} ± 0.5
	Pectinex BE XXL	19.5^{d} ± 0.1	0.60^{a} ± 0.02	3980^{a-c}	93.9^{c} ± 0.5

*Means in the same column marked by the same letter do not differ significantly at p = 0.05 according to Tukey's test; means ± SD (n = 3)

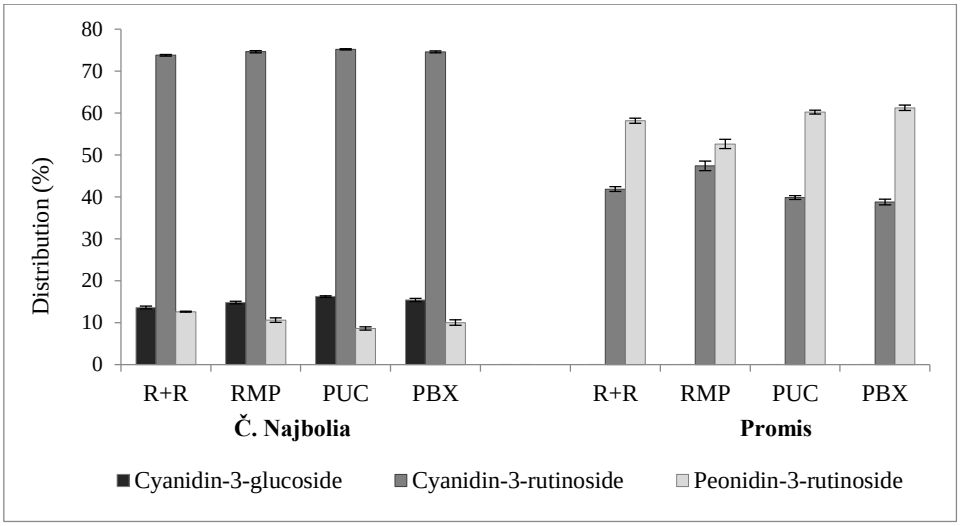

Fig. 3. Distribution of anthocyanins in plum juices analysed by HPLC method. Abbreviation: R+R – Rohapect PTE+ Rohament PL(2:1); RMP – Rohapect MA Plus; PUC – Pectinex Ultra Colour; PBX – Pectinex BE XXL. Bars depict a standard deviation

The enzyme treatments had a varied influence on the contents of anthocyanins and total polyphenols in the plum juices produced (Table 4). The concentration of total polyphenols, determined by the Folin-Ciocalteu method, in the juice from 'Č. Najbolja' plums was the highest after macerating the mash with Pectinex BE XXL (182.4 mg·100 ml^{-1}), and in the juice from 'Promis' plums produced with Pectinex BE XXL (111.3 mg·100 ml^{-1}) and with the mixture of Rohapect PTE and Rohament PL (2 : 1) (111.4 mg·100 ml^{-1}). The anthocyanins content in the plum juices varied between 2.6 and 5.0 mg·100 ml^{-1} for 'Promis' and between 26.9 and 28.9 mg·100 ml^{-1} for 'Č. Najbolja'.

Cyanidin-3-rutinoside was the predominant anthocyanin found in the cloudy plum juices from 'Č. Najbolja" cv. (about 75%), while peonidin-3-rutinoside (53-61%) was predominant in the juices from 'Promis' (Fig. 3).

The effect of enzymatic treatment on the transfer of individual anthocyanins from fruit to plum juices expressed as a percentage (%) of the amount in fresh fruit (HPLC measurements) is presented in Fig. 4. The transfer of anthocyanins from fruit to juice was higher in the case of 'Č. Najbolja' than for 'Promis', which was characterized by much higher PPO activity than 'Č. Najbolja'. According to the literature, PPO is responsible for the degradation of anthocyanins in fruit (Wesche-Ebeling & Montgomery 1990; Oren-Shamir 2009), and higher PPO activity accelerates the degradation process (Łoś et al. 1996). Peonidin-3-rutinoside proved to be the most stable anthocyanin compound during juice processing (Fig. 4). The retention of this anthocyanin in the case of the juice pressed from 'Č. Najbolja' was at a level of 25-42%, depending on the enzyme preparation used. Then again, the lowest stability was observed for cyanidin-3-glucoside, which underwent total degradation in the juice from 'Promis'. A similar effect has been observed by Koponen et al. (2008), who reported that the extraction of anthocyanidin rutinosides was more effective than of glucosides in black currant juice.

The results show that the activity profile of the pectinolytic enzyme preparation used in plum juice processing may impact the yield and distribution of the extracted anthocyanins. The highest yields of anthocyanins in the juice were obtained when the Pectinex Ultra Colour preparation was used for macerating the mash both of 'Č. Najbolja' and of 'Promis', whereas the highest loss of anthocyanins was observed when Rohapect PTE + Rohament PL (2 : 1) was used with 'Č. Najbolja'. Pectinex Ultra Color was characterized by higher activities of PME and PG than the mixture of Rohapect PTE + Rohamnet PL. Pectin methyl esterase demethylates pectins, which is a necessary step before the full action of PG, which cuts pectins (components of the cell wall) into very short fragments. Therefore, the mixture of Rohapect PTE + Rohamnet PL, which exhibited low activity of PME, caused a lower pectin degradation despite having high activity of PG. According to Koponen et al. (2008), liberation of berry anthocyanins into juice is highly dependent on the degradation of the berry cell wall matrix. This is in agreement with a report by Versari et al. (1997), who found that pectinolytic enzymes modified the level of individual pigments and the total anthocyanins content in strawberry and raspberry juices. Moreover, the hydrolysis of anthocyanins in bilberries and black currant has been shown to depend on the anthocyanin structure and the activity profile of the enzyme preparation (Buchert et al. 2005; Koponen et al. 2008). However, the role of anthocyanin degradation in terms of their physiological effects still remains to be elucidated. The results achieved in our experiments revealed no significant effect of the type of enzyme on the total anthocyanin content measured in plum juices by the spectrophotometric method (Table 4). This divergence may be explained by the fact that the enzymatic degradation of anthocyanins leads to the formation of brown pigments, which affect the measurement of absorbance. The half-life of the plum anthocyanins had been investigated in another study, where was observed a fast browning of plum juices.

Plum juices from 'Č. Najbolja' had higher antioxidant activity (AA) (1.60 ÷ 2.35 mg Trolox·100 ml^{-1}) than the juices obtained from 'Promis' (0.68 ÷ 1.45 mg Trolox·100 ml^{-1}) (Table 4). The treatments with Pectinex Ultra Colour and Pectinex BE XXL produced juices with the highest antioxidant activity both from 'Č. Najbolja' and

from 'Promis' cultivars. With 'Promis' (higher PPO activity), the enzyme treatment with Rohapect MA Plus also resulted in a juice with higher AA comparable to Pectinex Ultra Colour and Pectinex BE XXL. In contrast to 'Č. Najbolja' (lower PPO), the juice obtained after the treatment with Rohapect MA Plus exhibited lower AA compared to the other enzyme preparations used.

The information found in the literature indicates that plums have higher antioxidant activity than nectarines, peaches (Gil et al. 2002) and apples (Kim et al. 2003b). Considering their other properties beneficial for human health, for example the effect of dried plums on bowel function (EFSA 2012), it may be concluded that consumption of plums and plum products should be promoted more aggressively and even become part of a daily diet. Our data indicate that the antioxidant activity of cloudy plum juice produced from the selected cultivars could be additionally enhanced by enzymatic macerating under controlled conditions as it might strongly influence both the qualitative and quantitative transfer of bioactive components from the raw material to the final product.

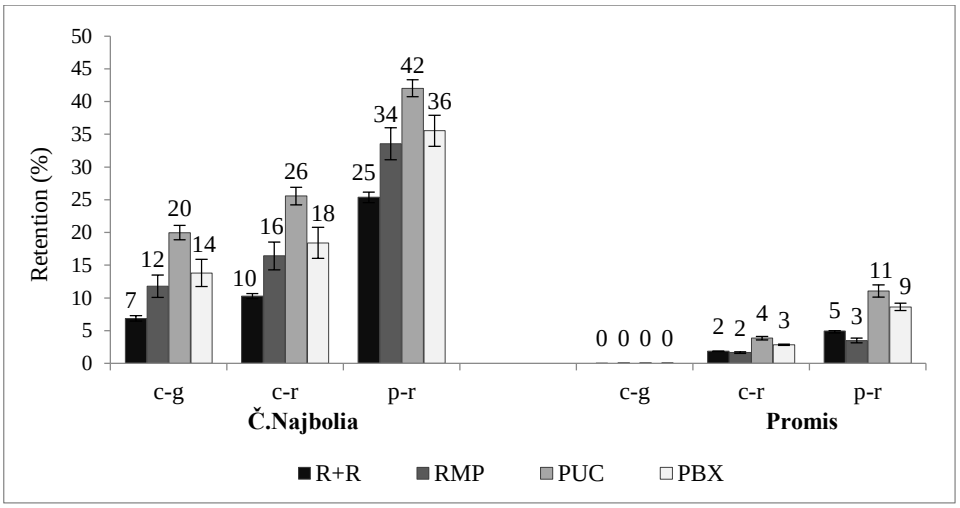

Fig. 4. Effect of enzyme treatment on the anthocyanins identified in plum juices by HPLC analysis. The columns present the retention (%) of individual anthocyanins (c-g: cyanidin-3-glucoside, c-r: cyanidin-3-rutinoside, p-r: peonidin-3-rutinoside) in plum juice in relation to anthocyanins content in fresh fruit. Bars depict a standard deviation. Abbreviation: see Fig. 3.

Table 4. Content of bioactive compounds and radical scavenging activity of plum juices

Cultivar	Enzyme preparation	Anthocyanin* mg·100 ml^{-1}	Total polyphenol mg·100 ml^{-1}	Antioxidant activity mg Trolox·100 ml^{-1}
'Čačanska Najbolja'	Rohapect PTE+Rohament PL	$26.9^{b} \pm 3.1$	$166.9^{d} \pm 4.0$	$1.60^{bc} \pm 0.15$
	Rohapect MA Plus	$27.0^{b} \pm 2.2$	$151.1^{c} \pm 3.7$	$1.71^{c} \pm 0.12$
	Pectinex Ultra Color	$28.9^{b} \pm 1.0$	$175.5^{de} \pm 4.7$	$2.33^{d} \pm 0.09$
	Pectinex BE XXL	$26.9^{b} \pm 2.0$	$182.4^{e} \pm 5.6$	$2.35^{d} \pm 0.17$
'Promis'	Rohapect PTE+Rohament PL	$3.9^{a} \pm 1.0$	$111.4^{b} \pm 2.6$	$0.68^{a} \pm 0.02$
	Rohapect MA Plus	$5.0^{a} \pm 0.2$	$93.3^{a} \pm 2.0$	$1.38^{b} \pm 0.04$
	Pectinex Ultra Color	$2.6^{a} \pm 0.7$	$90.9^{a} \pm 1.0$	$1.37^{b} \pm 0.08$
	Pectinex BE XXL	$4.3^{a} \pm 0.2$	$111.3^{b} \pm 2.5$	$1.45^{bc} \pm 0.06$

* Total anthocyanin content quantified spectrometrically
Means in the same column marked by the same letter do not differ significantly at p = 0.05 according to Tukey's test; means ± SD (n = 3)

CONCLUSIONS

In juice production, particularly important are high soluble solids and anthocyanins content and low polyphenol oxidase activity of plum fruit, which depends on the cultivar.

Using a mixture of enzymes with comparatively low activities of pectin methyl esterase and polygalacturonase, and high activity of pectin lyase high juice yields (over 90%) and a satisfactory turbidity of cloudy juices can be obtained from frozen plum fruit.

Enzymatic degradation of anthocyanins in the juice from 'Promis' cultivar, exhibiting high PPO activity, was much higher than in the juice from 'Čačanska Najbolja' cultivar with low PPO activity.

The activities of the enzyme preparations applied during plum mash maceration affect the extractability of anthocyanins and their final profiles in plum juices.

Among the three anthocyanins analysed, peonidin-3-rutinoside proved to be the most stable and cyanidin-3-glucoside the least stable during plum juice production.

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