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Impact of enzyme on quality of blackcurrant and plum juices

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ABSTRACT

The aim of work was optimization of technology of cloudy blackcurrant and plum juices production. The major concern was the increase of product cloudiness and its stability. Red fruit were processed with commercial pectinolytic preparations (Pectinex BE Colour, Pectinex BE XXL, and mixture of Rohapect PTE with Rohament PL). The effect of enzyme dose, maceration time and addition of ascorbic acid (in case of plum) on juices quality was investigated. Using single enzyme, either polygalacturonase or pectin lyase did not allow obtaining juices with high enough turbidity, however appropriate enzyme mixture allowed to obtain cloudy juice with the turbidity of at least 230 NTU for blackcurrant and above 500 NTU for plum juice. The best pressing-yield for blackcurrant was achieved with polygalacturonase and pectin lyase, 65 g/100 g after 1 h and 74 g/100 g after 4 h of pectinolysis. The macerating mixture gave about 58 ÷ 59 g/100 g yield, irrespectively of enzymation time. Pressing-yield of plum juices was in the range of 94 ÷ 97 g/100 g due to the fact that practically only skins were retained on pressing cloth. Addition of ascorbic acid (AA) during plum juice processing in quantity of 500 mg/kg had protective effect on anthocyanins. In control plum juices range of anthocyanins contents were 12.1 ÷ 16.5 mg/100 ml while in juice with AA addition even 21.4 ÷ 24.5 mg/100 ml.

1. Introduction

Blackcurrants (*Ribes nigrum* L.) and plums (*Prunus domestica* L.) belong to the most popular fruits cultivated in Poland. Several studies have shown that red fruits play an important role in prevention of certain diseases (Beattie, Croziier, & Duthie, 2005; Prior & Gu, 2005) because of high concentration of antioxidants (Ewald, Vaher, & Kaljurand, 2005; McDougall, Gordon, Brennan, & Steward, 2005; Tomás-Barberán et al., 2001; Vangdal & Slimestad, 2006). Unfortunately these fruits are not available for consumer in fresh state during whole year. Moreover, due to high acidity consumption of fresh fruit is often limited (Szajdek & Borowska, 2008). However, in the processed form they may be available year-round. To increase consumption of blackcurrants and plums in off season it is important to produce more attractive than currently available processed products. The promising products are cloudy juices containing more antioxidants and fibre than clear juices (Oszmiański & Wojdyło, 2007). During clear juice production a lot of polyphenols are lost in clarification process (Dietrich, 2004; Hubert, Baron, Quere, & Renard, 2007) compared to whole fruit. In case of cloudy apple juice production, the enzymation and clarification stages are omitted. This process is not justified for berry and stone fruits containing a large amount of pectic substances (Hilz, Bakx, Schols, & Voragen, 2005; Taylor, Rabe, & Dodd, 1995), which have an impact on the structure of raw fruit and juice yield during mash pressing (Grassin, van der Weijden, van der Hoeven, van Dijck, & de Boer, 2005). Pectins and other components of cell wall transferred to pressed juice contribute to cloudiness (Heldt-Hansen et al., 1996; Will & Dietrich, 2006) which should be characterized by the high stability, at least 50 g/100 g according to Dietrich (1995). Unfortunately, cloud destabilization is one of the serious problem for industry (Croak & Corredig, 2006). Pectins depending on their chemical form are categorized as either soluble or insoluble fibre, which cannot be absorbed by the human digestive tract. However, enzymes are able to modify them to short polysaccharide fragments that may be absorbed. Pectin degradation by enzyme action leads to decrease of raw juice viscosity and, in consequence, increasing of juice yield (Płocharski, Szymczak, & Markowski, 1998; Voragen, Schols, & Beldman, 1992) improving production efficiency. Most of commercial enzyme preparations contain pectinases, cellulases and hemicellulases in various ratio, which act in different way. It is important, in cloudy juice production, that the enzyme cut pectin into short fragments to obtain cloud-stable juice (Beldman et al., 1997). The main objective of this

Abbreviations: P.BE.C, Pectinex BE Colour; P.BE.XXL, Pectinex BE XXL; R.PTE, Rohapect PTE; R.PL, Rohament PL; MM, macerating mixture; AA, ascorbic acid.

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work was evaluation of selected commercial enzyme preparations with different main activities and to elaborate suitable enzyme treatment to obtain satisfactory juice yield as well as stable cloudiness and good juice quality.

2. Materials and methods

2.1. Plant material

Juices were produced from blackcurrant, 'Ben Lomond' cultivar, and plum, 'Čačanska Najbolja' cultivar. Fruit were obtained from the experimental orchard of the Research Institute of Pomology and Floriculture[1] in Skierniewice, Poland. Blackcurrants were harvested in 2006 season and plums in 2007. After removing stems and washing fruit were frozen and kept at a temperature below −20 °C until processing. Plums before processing were hand-pitted in the frozen state, after increasing temperature to about −6 °C, using a routine procedure to avoid oxidation in which we use a ceramic knife to cut fruit.

2.2. Chemicals and enzymes

Ascorbic acid (Sigma, Germany) was used. Enzyme preparations: Pectinex BE Colour and Pectinex BE XXL were provided by Novozymes (Bagsvaerd, Denmark), Rohapect PTE and Rohament PL from AB Enzymes (Darmstadt, Germany). The two last enzymes were combined together and called macerating mixture. Information regarding the enzyme preparations' activities is given in Table 1.

2.3. Juice production

The red fruit juice production was made on laboratory scale with Instron 4303 texture press equipped with special attachment (cylindrical container with tightly fitting plunger) for juice pressing using small samples. Prior to incubation with enzyme preparations, about 2.5 kg of blackcurrant and destoned plum fruit was thawed and disintegrated using Fryma perforated disc mill (Fryma-Maschinen AG, Rheinfelden, Switzerland). A 200 g of fruit mash was weighed and heated up to 50 °C than 2.0 ml of water-enzyme solution was added according to experiment design: Blackcurrant juice: *the effect of enzyme dosage*: 100, 200, 400 g/t for 2 h of maceration; *the effect of maceration time*: 1, 2, 3, 4 h for enzyme dose 200 g/t; *the effect of macerating mixture proportion (Rohapect PTE : Rohament PL):* 1:1; 1:2; 2:1 for enzyme dose 200 g/t during 2 h of maceration. Plum juice: *the effect of enzyme dosage*: 50, 100, 200 g/t for 1 h of maceration; *the effect of ascorbic acid addition levels*: 0, 500 mg/kg of fruit mash for enzyme doses 100 and 200 g/t during 1 h of maceration. Then mash underwent pressing for 15 min using texture press Instron 4303 equipped with load cell 5000 N and cylindrical container with plunger (Banaszczyk & Płocharski, 1993). During the pressing cycle the plunger moved at a speed of 8 mm/min; the maximum pressure was 4.5 kN. After extraction the juice sample was immediately heated on a hotplate to a boiling point (in about 5 min) to denature enzymes. Then juice was cooled to ambient temperature in a cold water bath and centrifuged (30 000×*g*; 15 min) in laboratory centrifuge (Sigma 3K30). The juice yield was determined by weighing the pressed juice, which were compared to the initial sample weight (g/100 g). The juice productions were carried out in duplicate.

[1] Presently Research Institute of Horticulture.

Table 1
Activity profiles of enzyme preparations at pH 3.5.

Enzyme preparation	PL	PME	PG
	[nkat/ml]		
Pectinex BE Colour	89	6789	28087
Pectinex BE XXL	214	5915	17276
Rohapect PTE	1586	131	160
Rohament PL	2	10	46957
Rohapect PTE + Rohament PL (2:1)	993	142	16565

PL – Pectin lyase, PME – Pectin methyl esterase, PG – Polygalacturonase.

2.4. Analyses

Fruit juice analyses were done according to the methods described in the manual of the International Fruit Juice Union (IFU), (IFFJP, http://www.ifu-fruitjuice.com/) unless otherwise described.

Soluble solids contents (oBrix) were measured by means of digital refractometer (Mettler-Toledo, type RE50) and density by Mettler-Toledo, type DE51 meter. Total titratable acidity (pH 8.1, expressed as citric acid equivalents in g/100 ml of juice) were determined potentiometrically (Mettler-Toledo, type DL 58).

Turbidity (T_0) of juices was measured nephelometrically using HACH turbidimeter (Hach Company). Turbidity results are given in NTU (nephelometric turbidity units). Stable turbidity (T_S) and the stability of turbidity (T%) were determined according to Stähle-Hamatschek & Gierschner (1989) after centrifugation in a laboratory centrifuge (4200×*g* for 15 min at room temperature) giving a percentage of cloud stability ($T\% = T_S/T_0 \times 100$).

Ascorbic acid (AA) content was determined by reversed-phase HPLC on an HP 1100 system (Hewlett–Packard, Waldbronn, Germany) equipped with a Diode Array Detector (DAD) using two Supelco LC-18 columns (25 cm × 4.6 mm; 5 μm). A 73.5 mmol/L water solution of KH_2PO_4 buffer at pH 2.5 was used as the mobile phase. The column temperature was kept at 30 °C with a flow rate of 0.8 ml/min. Detection of AA was by absorbance at 244 nm. The results were expressed in mg/100 ml. Samples for AA determinations were dissolved in 0.63 mol/L HPO_3.

The total anthocyanins content was quantified spectrometrically (Varian, UV/Vis CARY 300E) by the pH differential method according to Wrolstad (1976). Anthocyanins content was calculated using the molar absorptivity of cyanidin-3-rutinoside (28800 L mol^{-1} cm^{-1}) and the molecular weight of 595.2 g/mol. Losses of anthocyanins during juice production were calculated based on balance of these compounds between mash, juice and pomace as follows. Content of anthocyanins in fruit mash was determined and taken as a reference (100%). Then the contents in the juice and pomace were determined. Sum of these was considered as remained amount. The difference between reference and remained amount was treated as loss of anthocyanins during pressing and recalculated into percentage.

Table 2
Effect of macerating mixture proportion (R.PTE:R.PL) on blackcurrant juice parameters.

Proportion of Rohapect PTE: Rohament PL	1:1	1:2	2:1	*p*-value
Juice yield [g/100 g]	50.1 ± 1.0[b]	43.5 ± 1.8[a]	59.0 ± 0.6[c]	*<0.002*
Total turbidity [NTU]	233.3 ± 9.5	248.3 ± 4.0	247.5 ± 5.3	*0.196*
Stability of turbidity [%]	94.9 ± 1.4	90.7 ± 6.2	82.7 ± 2.2	*0.145*
Soluble solids [°Bx]	18.4 ± 0.1	19.0 ± 0.5	18.2 ± 0.5	*0.394*
Titratable acidity [g/100 ml]	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	*0.190*

Enzyme dose: 200 g/t for 2 h of enzymation at 50 °C. Means marked by the same letter do not differ significantly at $p = 0.05$ ($n = 2$).