



Effects of various polysaccharide clarification agents and reaction time on content of polyphenolic compound, antioxidant activity, turbidity and colour of chokeberry juice



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ABSTRACT

Chokeberry juice is a good source of health-promoting nutrients, and can be a suitable supplement for a healthy diet. Therefore, the aim of this study was to investigate the changes that occur during clarification using different polysaccharides-based clarification agents (chitosan, xanthan gum (XG), carboxymethylcellulose (CMC), agar-agar (AA), carob gum (locust bean gum, LBG), β -cyclodextrin (BCD), guar gum (GG)), their doses and reaction time on the phenolic compounds (UPLC-PDA-FL), turbidity, change of colour (CIEL*a*b*) and antioxidant activity (ABTS and FRAP) of chokeberry juice. Low turbidity and high antioxidant activity and contents of polyphenol compounds were obtained in chokeberry juice with addition of clarification agents as AA, CMC and XG. The colour of these juices were attractive, intensive red and without browning after reaction time and storage. The dose of clarification agents and after reaction time (1, 5, 16 h) significantly influenced increase in the quality of the finally products. These clarification agents after 5 months storage were also well influenced the stability of clarification process and of physical and bioactive parameterising the centrifuged chokeberry juice. This study suggests that AA, CMC and XG can be used as a clarifying aid of chokeberry juices.

1. Introduction

Chokeberry is characterized by a specific bitter and tart taste due to a high concentration of polyphenolic compounds, mainly procyanidin-condensing tannins, bitter eriodictyol-glucuronide flavanone and phenolic acids. Therefore, fruits of chokeberry are rarely used for direct consumption. The most important products for industry are production of chokeberry juice (Howard, Brownmiller, Prior, & Mauromoustakos, 2013; Kovačević et al., 2016; Oszmiański & Lachowicz, 2016; Tolić et al., 2017). Unfortunately, during pressing of chokeberry the polysaccharides, pectins and tannins pass into the juice, causing turbidity and sedimentation in juice (Erkan-Koç, Türkyılmaz, Yemiş, & Özkan, 2015; Oszmiański & Wojdyło, 2005). Therefore, sedimentation and turbidity occurring in juices and concentrates represent a difficult problem to solve for entrepreneurs. Furthermore, cloudy juices are not accepted by the consumer, because they mainly pay attention to the transparency and clarity of juice.

One solution to obtain clear chokeberry juice may be the use of

fining agents to remove of hydrolysable polyphenols. For the protection of anthocyanins and removal of tannins, in chokeberry juice of cold-clarification with β -cyclodextrin (BCD) is suggested (Howard et al., 2013). The effects of the use of different clarification agents such as chitosan (Erkan-Koç et al., 2015; Oszmiański & Wojdyło, 2007), xanthan gum (Erkan-Koç et al., 2015), carboxymethylcellulose (Genovese & Lozano, 2001), agar-agar (Rehman, Aman, Zohra, & Qader, 2014), carob gum (Cairns, Morris, Miles, & Brownsey, 1986), β -cyclodextrin (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002) and guar gum (Wang et al., 2010) for removal of turbidity in a variety of fruit juices such as pomegranate, bayberry, apple juice, and white wine have been extensively investigated. However, has not still been found an efficient clarification agent removing tannins and the effects of some polysaccharides on the turbidity of chokeberry juice have not been investigated. Therefore, the effects of different polysaccharides clearing agents on the turbidity and content of polyphenolic compounds, should be tested to solve the problems with clarified in chokeberry juice.

Already, Erkan-Koç et al. (2015) and Oszmiański and Wojdyło

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(2007) found that chitosan used in different doses for the clarification of juices from pomegranate and apple had no effect on health-promoting compounds. According to Erkan-Koç et al. (2015), xanthan gum, like chitosan, also did not cause significant differences in the content of the profile of polyphenolic compounds. Genovese and Lozano (2001) found that the addition of carboxymethylcellulose in the amount of 0.4–0.5% for apple juice turbidity results in stabilization during storage. β -cyclodextrin was used mainly to stabilize the anthocyanins in fruit juices (Howard et al., 2013). Compared to carboxymethyl, xanthan gum and chitosan cellulose, substances such as β -cyclodextrin, agar-agar, carob gum and guar gum are rarely used for the clarification of fruit juices.

Therefore, the aim of this study was to investigate the changes that occur during clarification using different polysaccharides-based clarification agents (chitosan, xanthan gum (XG), carboxymethylcellulose (CMC), agar-agar (AA), carob gum (locust bean gum, LBG), β -cyclodextrin (BCD), guar gum (GG)), of dose (0.1, 0.2, 0.5 and 1.0 g/L) and reaction time (1, 5, 16 h) on the phenolic compounds (UPLC-PDA-FL), turbidity, precipitate, change of colour (CIEL*a*b*) and antioxidant activity (ABTS and FRAP) of chokeberry juice. An additional goal of this study was to investigate the stability of physical and bioactive parameters in centrifuged juice after 5 months storage.

2. Material and methods

2.1. Chemicals

2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), methanol acetic acid and phloroglucinol were purchased from Sigma-Aldrich (Steinheim, Germany). (–)-Epicatechin, (+)-catechin, procyanidin B2, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, caffeic acid, dicaffeoylquinic acid, *p*-coumaric acid, myricetin, isoquercitrin, cyanidin-3-O-galactoside and cyanidin-3-O-glucoside were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-phase liquid chromatography (UPLC; gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany). Chitosan (SIHA Profloc), xanthan gum, carboxymethylcellulose (CMC), agar-agar, carob gum (LBG), β -cyclodextrin (BCD) and guar gum (GG) were from Brenntag (Poland).

2.2. Samples

2.2.1. Chokeberry juices

The experimental material consisted of juices of chokeberry (~10 L) of the cultivar Galicyjanka were obtained from the company Tymbark – MWS Sp. z o. o., in Tymbark, near Kraków, Poland (49°43'45"N 20°19'27"E) from 2015 production in September.

2.2.2. Clarification

The chokeberry juice was separately clarified with natural sedimentation (control sample), chitosan (solution 1%, weight/volume (w/v)), xanthan gum (solution 1%, w/v), carboxymethylcellulose (CMC; solution 1%, w/v), agar-agar (solution 1%, w/v), carob gum (LBG; solution 1%, w/v), β -cyclodextrin (BCD; solution 1%, w/v) and guar gum (GG; solution 1%, w/v). The chitosan solution was prepared by adding 50 ml of 2% citric acid solution and 50 ml of 2% chitosan solution and mixed at 9500 g for 3 min. Next the solution was heated to 60 °C and was filtered. To obtain the lowest turbidity values in chokeberry juices, the doses of 0.1, 0.2, 0.5, 1.0 g/L and reaction time: 1, 5, 16 h of clarification agents at 25 °C were determined. After incubation, the precipitate were removed from all the clarified juice samples by centrifugation (19,000 × g for 15 min at 20 °C). Then, the samples were analysed. Additionally, the samples of chokeberry juice after 16 h of reaction and after the separation a layer of liquid from the precipitate, were storage for 5 months at 5 °C with addition of 0.2 g/L dimethyl

dicarbonate (Velcorin) (Lanxess Energizing Chemistry Germany). The aim of storage was to investigate the stability of clarification process on content of physical and bioactive parameters of the chokeberry juices.

2.3. Precipitate values, viscosities and turbidity measurement

Samples of chokeberry juice were weighed to 50 ml centrifuge vessels. After centrifugation at 19,000g for 15 min at 20 °C, the separation a layer of liquid. The centrifuge vessels were weighed again with the precipitate on the using an analytical laboratory scale to the nearest 0.0001 g and then after all reaction times the amount of precipitate was weigh the precipitate without juice and calculated.

The viscosities of the chokeberry juices without precipitate were measured with a rotation viscometer MC1 (DV-II+ PRO VISCOMETER, Brookfield, England), with spindle '61'. The spindle was rotated at 100 × g for 30 s at 20 °C (Kolniak-Ostek, Oszmiański, & Wojdyło, 2013). All measurements were repeated three times. The results were expressed as mPas.

The turbidity of juices without precipitate were measured with a turbidimeter Turbiquant 3000T (Merck, Germany) using 2.5-cm round cuvettes. All measurements were repeated three times. Turbidity was expressed in nephelometric turbidity units (NTU) at 20 °C, respectively (Kolniak-Ostek et al., 2013).

2.4. Qualitative and quantitative assessment of polyphenols

The sample juice was centrifuged at 19,000g for 10 min, and the supernatant was filtered through a Hydrophilic PTFE 0.20 μ m membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatography-photodiode array detector-mass spectrometry method.

Qualitative (LC/MS QTOF) and quantitative (UPLC-PDA-FL) analysis of polyphenols (anthocyanins, flavan-3-ols, flavonols, and phenolic acids) was performed as described previously by Lachowicz, Oszmiański, and Pluta (2017a). Separations of individual polyphenols were carried out using a UPLC BEH C18 column (1.7 μ m, 2.1 × 100 mm, Waters Corporation, Milford, MA) at 30 °C. The samples (10 μ l) were injected, and the elution was completed in 15 min with a sequence of linear gradients and isocratic flow rates of 0.45 ml min⁻¹. The mobile phase consisted of solvent A (2.0% formic acid, v/v) and solvent B (100% acetonitrile). The program began with isocratic elution with 99% solvent A (0–1 min), and then a linear gradient was used until 12 min, lowering solvent A to 0%; from 12.5 to 13.5 min, the gradient returned to the initial composition (99% A), and then it was held constant to re-equilibrate the column. All measurements were repeated three times. The results were expressed as g per 1 L of juice.

2.5. Analysis of proanthocyanidins by phloroglucinolysis

Direct phloroglucinolysis of freeze-dried samples was performed as described by Lachowicz, Wojdyło, Chmielewska and Oszmiański (2017b). Juice lyophilisates were addition in an amount of 0.2 ml into 2-ml Eppendorf vials. Subsequently, 0.8 ml of the methanol solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) were added to samples. After addition of 0.4 ml of methanol HCl (0.3 M), the vials were incubated for 30 min at 50 °C with continuous vortexing in a thermo shaker (TS-100, BioSan, Riga, Latvia). The reaction was terminated by placing the vials in an ice bath, drawing 0.6 ml of the reaction medium and diluting with 1.0 ml of sodium acetate buffer (0.2 M). The samples were centrifuged immediately at 20,000g for 10 min at 4 °C, and stored at 4 °C before reverse-phase HPLC (RP-HPLC) analysis. All incubations were done in triplicate. Phloroglucinolysis products were separated on a Cadenza CD C18 (75 mm × 4.6 mm, 3 μ m) column (Imtakt, Japan). The liquid chromatograph was a Waters (Milford, MA) system equipped with diode array and scanning fluorescence detectors

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