Food Research International 65 (2014) 35–41

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of enzyme-assisted extraction on the chilled storage stability of bilberry (*Vaccinium myrtillus* L.) anthocyanins in skin extracts and freshly pressed juices

Rada Dinkova [a,b], Peter Heffels [b], Vasil Shikov [a], Fabian Weber [b], Andreas Schieber [b], Kiril Mihalev [a,*]

[a] Department of Food Preservation and Refrigeration Technology, University of Food Technologies, 26 Maritza Boulevard, Plovdiv 4000, Bulgaria
[b] Department of Nutritional and Food Sciences, Chair of Food Technology and Food Biotechnology, University of Bonn, Römerstraße 164, Bonn 53117, Germany

ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form 20 May 2014
Accepted 30 May 2014
Available online 7 June 2014

Keywords:
Bilberry juice
Enzymes
Maceration
Shelf-life
Anthocyanins
UHPLC

ABSTRACT

Three different commercial enzyme preparations were evaluated with respect to the extractability and chilled storage (10 °C, 9 days) stability of bilberry skin anthocyanins in model systems. Using ultra high-performance liquid chromatography–diode array detection, 15 individual anthocyanins were characterized, with delphinidin and cyanidin glycosides being the predominant compounds. Except for the galactosides of cyanidin and peonidin, 1.2–4.7 times higher concentrations of individual anthocyanins were observed for the extracts from enzymatically treated bilberry skin suspensions. Anthocyanin degradation followed well (R^2 = 0.89–0.98) a first-order reaction kinetics and the half-life values increased (12–64%) due to enzyme-assisted extraction. The results obtained demonstrate an improved storage stability of anthocyanins, enzymatically released from the bilberry skin matrix. On the basis of the enzyme screening assay, a novel process, composed of partial dejuicing (pressing, ~40% juice yield) of the bilberry mash followed by enzymatic maceration (EMPD), was developed. Interestingly, enzymatic treatment resulted in a decrease among counts of mesophilic and psychrotrophic microorganisms and moulds and yeasts in the bilberry juice upon chilled storage (10 °C, 9 days), implying an inhibition of the microbial growth. Therefore, applying the EMPD process, this two-step juice extraction technology may be very helpful, allowing both enhanced contents of bioactive compounds and extended microbiological shelf-life of "fresh-like" berry juices.

1. Introduction

Anthocyanin pigments are not only important in defining the aesthetic value of foods and beverages but also play a significant role from a nutritional point of view (Stintzing & Carle, 2004). Bilberry (*Vaccinium myrtillus* L.), also known as the native European blueberry, is one of the richest natural sources of anthocyanins. Besides being responsible for the bluish red color of the bilberry fruit, these pigments are believed to be associated with many health benefits for humans, including eye protection, antioxidant, cardioprotection, anti-inflammatory, hypoglycemic and antimicrobial effects (Chu, Cheung, Lau, & Benzie, 2011).

Bilberries, like other berries, generally have a short shelf-life and they are widely processed into juices, jams and fruit preparations for ice-cream, yoghurt and confectionery. Therefore, the effects of processing and subsequent storage on the bioactive compounds, particularly anthocyanins and other polyphenols, need to be considered when assessing potential health benefits of berry-derived foodstuffs and beverages. Recently, while almost equal distributions between the juice and press cake were observed for the lingonberry, the bilberry juice possessed 1.5 and 3.2 times lower recovery rates for the total polyphenolic and anthocyanin content, respectively (Dinkova et al., 2012).

Pectinolytic enzyme preparations are commonly used in industrial berry processing to facilitate juice extraction. These enzymes cause degradation of the cell wall matrix, thus enhancing the juice yield. Concomitantly, an increased extractability of phenolic compounds, particularly anthocyanins (Buchert et al., 2005) and flavonols (Kaponen et al., 2008), has been observed in bilberry and black currant juices after enzyme-aided processing. Moreover, improved enzymatic maceration of the black currant mash has been shown to affect the antioxidant potency of the resulting juices (Landbo & Meyer, 2004). The efficiency of enzyme-assisted extraction has also been demonstrated for recovery of polyphenols, including anthocyanins, from grape (Kammerer, Claus, Schieber, & Carle, 2005), blueberry (Lee & Wrolstad, 2004) and black currant (Landbo & Meyer, 2001) processing waste. However, careful selection of enzyme preparations is required as some glycosidase activities present can hydrolyze anthocyanins to the corresponding aglycones, thus negatively affecting color stability (Wrolstad, Wightman, & Durst, 1994). Skin suspensions have been validated as suitable model systems for screening assay of commercial enzymes applied in grape

* Corresponding author. Tel.: +359 32 603748; fax: +359 32 644102.
E-mail address: kmihalev@yahoo.com (K. Mihalev).

http://dx.doi.org/10.1016/j.foodres.2014.05.066
0963-9969/

pomace (Kammerer et al., 2005) and blueberry (Lee & Wrolstad, 2004) processing.

Therefore, in the current study three different pectinolytic preparations, specially designed for berry mash maceration, were evaluated using bilberry skin suspensions. Following a minimal processing strategy, to reduce the number of unit operations and to keep the sensory profile as natural as possible, freshly pressed bilberry juices were obtained using enzymatic mash treatments for enhanced recovery of polyphenols. Anthocyanin stability both in the skin extracts and freshly pressed juices was monitored during chilled storage at 10 °C, which has been proposed as a challenge testing temperature for microbial quality of unpasteurized fruit juice (Patrignani, Tabanelli, Siroli, Gardini, & Lanciotti, 2013).

2. Materials and methods

2.1. Materials

For quantification purposes by ultra high-performance liquid chromatography–diode array detection (UHPLC-DAD), malvidin 3-*O*-glucoside (Phytoplan, Heidelberg, Germany) was used. For analytical purposes the following reagents were used: DPPH [2,2-diphenyl-1-picrylhydrazyl] and Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); TPTZ [2,4,6-tripyridyl-s-triazine] and gallic acid monohydrate (Fluka, Buchs, Switzerland); and Folin–Ciocalteau's reagent (Merck, Darmstadt, Germany). All other reagents and solvents were of analytical or HPLC grade. Deionized water was used throughout.

Frozen bilberries (*Vaccinium myrtillus* L., harvested in Bulgaria in 2012) were obtained from Cima 99 Ltd. (Striama, Bulgaria) and stored at −20 °C until analysis. Bilberry skins were manually separated from thawed berries (approximately 1 kg) using a laboratory sieve (1.60 mm) and lyophilized (Lyovac GT 2, Leybold-Heraeus GmbH, Köln, Germany).

The following commercial enzyme preparations were used: Pectinex® Ultra Color (Novozymes A/S, Bagsvaerd, Denmark); Panzym® Pro Color and Panzym® BE XXL (E. Begerow GmbH & Co., Langenlonsheim, Germany).

2.2. Enzyme-assisted extractions

2.2.1. Preparation of bilberry skin extracts

Finely ground lyophilized skins (1.0 g) were mixed with 50 ml of water, acidified (pH 3.0) with 1 M HCl. The suspension was placed in a 50 °C water bath for 15 min before 5 mL of an acidified water solution (1%, v/v) of enzyme preparation (Pectinex Ultra Color, Panzym Pro Color or Panzym BE XXL) was added. After incubation for 2 h at 50 °C, the sample was placed in a boiling water bath for 5 min to inactivate enzymes, then immediately cooled in an ice bath and centrifuged (4730 ×*g* for 15 min at 25 °C). The supernatant (bilberry skin extract) obtained was stored in white colored plastic bottles with screw caps at 10 °C for 9 days. Each extraction was performed in triplicate.

2.2.2. Preparation of bilberry juices

Frozen bilberries were thawed and milled (mesh size 4.5 mm) (Fig. 1). Aliquots of the bilberry mash (600 g) were macerated adding diluted enzyme (Pectinex Ultra Color) solution directly or after partial dejuicing (pressing, ~40% juice yield). The juices were extracted using a laboratory rack and cloth press and stored in transparent glass bottles with screw caps at 10 °C for 9 days. Each bilberry processing variant was performed twice.

2.3. UHPLC analyses

The separation of bilberry anthocyanins was performed using a Shimadzu UHPLC (Kyoto, Japan) system, equipped with two high pressure pumps model LC-30AD, a model DGU-20A5R degasser, a model SIL-30AD auto sampling unit (cooled to 10 °C), a model CTO-20AC column oven (at 40 °C), and a model SPD-M20A diode array detector. The column used was a Waters Acquity (Milford, MA, USA) HSS T3 (150 mm × 2.1 mm, particle size 1.8 μm), equipped with a security guard column. The mobile phase consisted of 5% (v/v) formic acid in water (eluent A) and of 5% (v/v) formic acid in acetonitrile (eluent B). The gradient program was as follows: 4% B isocratic (2 min), 4% B to 8% B (5 min), 8% B to 10% B (6 min), 10% B to 17% B (6 min), 17% B to 30% B (4 min), 30% B to 100% B (0.3 min), 100% B isocratic (2 min), and 100% B to 4% B (0.5 min). Monitoring was performed at 520 nm at a flow rate of 0.4 mL/min. The injection volume was 5 μL and the

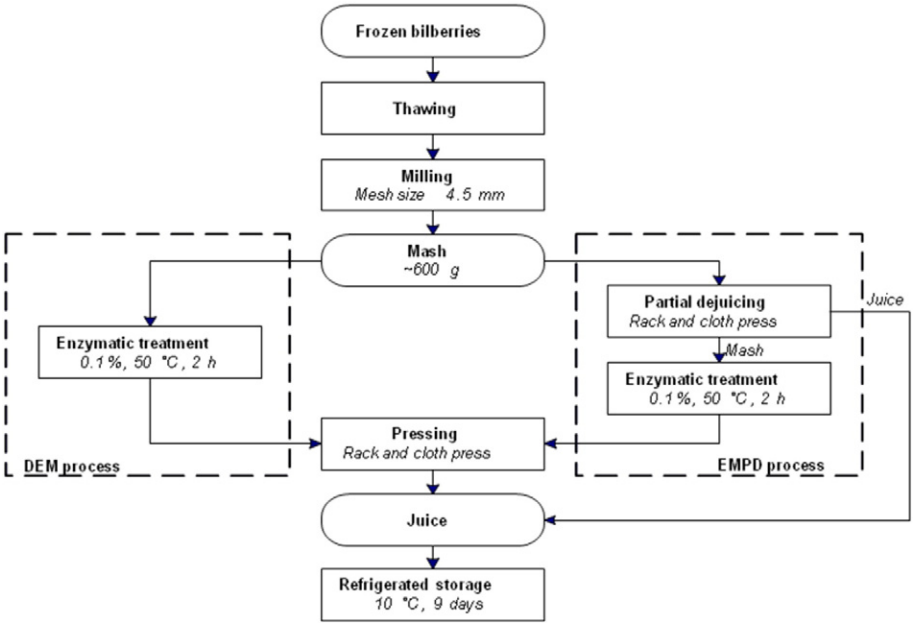

Fig. 1. Flow diagram for the production of bilberry juices applying different mash maceration processes: DEM, direct enzymatic maceration; EMPD, enzymatic maceration after partial dejuicing.