Food Chemistry 153 (2014) 114–120

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of ultra-high pressure homogenisation processing on phenolic compounds, antioxidant capacity and anti-glucosidase of mulberry juice

Yuanshan Yu, Yujuan Xu, Jijun Wu, Gengsheng Xiao *, Mangqin Fu, Yousheng Zhang

Sericulture and Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510610, PR China

ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form 30 November 2013
Accepted 9 December 2013
Available online 14 December 2013

Keywords:
Mulberry juice
Ultra-high pressure homogenisation
Phenolic acids
Anthocyanins
α-Glucosidase

ABSTRACT

In this study, the effects of ultra-high pressure homogenisation (UHPH) processing at 200 MPa for 1–3 successive passes (inlet temperatures at 4 °C) were compared with pasteurisation (95 °C, 1 min) processing on phenolic compounds, antioxidant capacity (ORAC value) and anti-glucosidase of mulberry juice. Compared with thermal pasteurisation processing, the more reductions in the anthocyanins, phenolic acids (gallic, protocatechuic, caffeic and p-coumaric acids, and a unknown hydroxycinnamic acid) and quercetin aglycone contents, as well as ORAC value were observed during UHPH processing of mulberry juice, whereas all reductions above during UHPH processing could be inhibited by adding ascorbic acid to mulberry juice. Besides, no significant change ($p > 0.05$) in the α-glucosidase inhibitory activity was observed during UHPH processing of mulberry juice, but showed a 14% reduction in mulberry juice processed by thermal pasteurisation.

1. Introduction

Mulberry, *Morus* genus, is a deciduous tree native to warm and subtropical regions of Asia, Africa, North America and southern Europe (Pérez-Gregorio, Regueiro, Alonso-Gonzalez, Pastrana-Castro, & Simal-Gandara, 2011). In most mulberry-growing countries, particularly China, mulberry is grown for its foliage to feed the silkworm (*Bombyx mori* L.). At present, many mulberry-growing areas in China have focused on enhancing fruit production, and new fruit *Morus* species were also bred. Mulberry fruit was welcome in the market, because it can provide plentiful phenolic compounds and naturally occurring α-glucosidase inhibitors, which may be protective against certain human diseases, such as cancer or chronic diseases (Du, Zheng, & Xu, 2008; Perez-Gregorio et al., 2011).

Consumer demand for fresh mulberry juice is increasing, but these products are susceptible to spoilage and thus have a limited shelf-life. Thermal processing (pasteurisation) is the most commonly used preservation technique to extend the shelf life of juice. However, thermal treatment causes irreversible loss on nutritional qualities, as well as undesirable change in its sensory properties (Patras, Brunton, Da Pieve, & Butler, 2009; Cao et al., 2012; Zhou, Wang, & Liao, 2009). Moreover, consumer demand for minimally processed and fresh-like food products has also led to an increased interest in innovative "non-thermal" processing technologies, which aim to achieve similar microbial and enzymatic inactivation with reduced or no application of heat. The concept of minimal processing is becoming a reality with some non-thermal technologies such as high-pressure processing, pulsed electric fields, ultrasound, and ultra-high pressure homogenisation (UHPH) processing (Marx, Moody, & Bermudez-Aguirre, 2011; Zhang et al., 2011).

UHPH has been popular as a novel non-thermal technology for the inactivation of microorganisms in fruit vegetable juices and milk-based beverages (Bevilacqua, Corbo, & Sinigaglia, 2012; Bevilacqua, Costa, Corbo, & Sinigaglia, 2009; Maresca, Donsi, & Ferrari, 2011). UHPH allows to process in continuous fluid foodstuffs and its great potential to inactivate pathogenic and spoilage microorganisms in fruit juices has been demonstrated (Bevilacqua et al., 2012). Studies had found the reduction of microorganisms significantly increased with increasing the homogenisation pressure, but the temperature in the homogenisation valve also increased with the increase of the homogenisation pressure (increasing about 0.15–0.2 °C/MPa) due to frictional heating (Corbo, Bevilacqua, Campaniello, Ciccarone, & Sinigaglia, 2010; Bevilacqua, Corbo, & Sinigaglia, 2012; Calligaris, Foschia, Bartolomeoli, Maifreni, & Manzocco, 2012; Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2012). For this reason, when the UHPH treatment was used as an alternative to thermal treatment for inactivating the pathogenic and spoilage microflora, the homogenisation pressure was often set at 200–300 MPa to avoid the raise of juice temperature, and further it is necessary to in the valve of homogenizers in a rapid cooling system (Bevilacqua et al., 2012; Velázquez-Estrada, Hernández-Herrero, Guamis-López, & Roig-Sagués, 2012; Velazquez-Estrada, Hernandez-Herrero, Rufer, Guamis-Lopez, &

* Corresponding author. Address: Sericulture and Agri-Food Research Institute, DongGuangZhuang RD., TianHe District, Guangzhou 510610, PR China. Tel.: +86 15975596649; fax: +86 (20) 872 37273.
E-mail address: 499072725@qq.com (G. Xiao).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.12.038

Roig-Sagues, 2013). Moreover, it was found the effectiveness of UHPH treatment on inactivation microorganisms can be improved by performing multiple UHPH passes, so a multi-pass ultra-high pressure homogenisation treatment was often applied for the pasteurisation of fruit juices (Maresca, Donsi, & Ferrari, 2011). Recently, Suárez-Jacobo et al. (2011) evaluated the effect of UHPH treatments on the antioxidant capacity, phenolics composition, vitamin C and provitamin A contents of apple juice, reporting that UHPH treatments significantly reduced the degradation of most of these compounds compared with pasteurised samples. Velázquez-Estrada et al. (2013) evaluate the effect of UHPH treatments on bioactive compounds and antioxidant activity of orange juice, and found that no significant differences ($p > 0.05$) were observed on the total phenolics content and antioxidant capacity values between the fresh and the UHPH-treated orange juice samples while these values where significantly lower in the thermal pasteurised one. However, no references about these aspects are available concerning mulberry juice. Therefore, the aim of this work was to investigate the effect of UHPH processing at 200 MPa for 1–3 successive passes on the phenolic compounds, antioxidant capacity and anti-glucosidase of mulberry juice compared with pasteurisation processing.

2. Materials and methods

2.1. Standards for identification and quantification

Standards of gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, syringic acid, ferulic acid, ellagic acid, quercetin, cyanidin 3-glucoside and cyanidin 3-rutinoside were purchased from Sigma–Aldrich Co., LLC. (St. Louis, MO, USA).

2.2. Mulberry juice preparation

The fruits of *Morus atropurpurea* Roxb. at a full ripe stage were presented by the farm of our research institute. These fruits were processed for 3–4 min in a pulper (Midea brand, Guangdong, China), and then filtrated through filter cloth (100 mesh). The juice obtained was immediately used for further UHPH and thermal pasteurisation processing.

2.3. UHPH processing of mulberry juice

UHPH processing was carried out in a bench-scale high-pressure homogenizer (JC-10C series by Guangzhou Juneng biology & technology Co., Ltd., Guangdong, China). The homogenizer consists of an oil booster pump connected to a high pressure intensifier for continuous pressurization of the process fluids up to 207 MPa. The maximum flow rate of juice that can be achieved in the homogenizer is 10 L/h. Upon pressurization, the fluid is fed into a high-pressure disruption valve. The flow through the micrometric gap causes the physical disruption of the microbial species.

The fresh mulberry juice was subjected to UHPH processing at 200 MPa for one and three passes. A cooling system (4 °C) upstream of the intensifier and in correspondence to the disruption valve completes the equipment. The inlet temperature of juice was 4 °C, and the juice was also readjusted to 4 °C by the cooling system after each pass. The maximum temperature reached by the juice samples did not exceed 35 °C during UHPH treatment. After processed by UHPH, the mulberry juice were collected in sterile plastic bottles and stored at −80 °C until being analysed.

2.4. Pasteurisation processing of mulberry juice

Fresh mulberry juice was thermally processed (95 °C, 1 min) in a tubular heat exchanger (Shanghai pilotech Equipment Co., Ltd., China). After heating, the juice was immediately cooled down to 25 ± 2 °C by a cooled water bath, and then stored at −80 °C until being analysed.

2.5. Determination of ascorbic acid

Ascorbic acid was determined by HPLC method using an Agilent system. The mulberry juice (1 mL) was mixed with 1 mL of metaphosphoric acid (6%, v/v) aqueous solution, centrifuged at 10000*g* (5 min), and then the supernatant was used for further HPLC analysis (Hernandez, Lobo, & Gonzalez, 2006). Separation of ascorbic acid was performed using a Shodex Asahipak NH2P-50 4E (250 × 4.6 mm) column using 80% (v/v) acetonitrile/sodium phosphate (25 mM, pH 2.2) aqueous buffer as the mobile phase at a flow rate 1 mL/min and 30 °C. Its content was detected using DAD detector at 254 nm and reported using external standards (L-ascorbic acid).

2.6. Analysis of anthocyanins

Each mulberry juice sample (10 mL) was mixed 30 mL methanol containing 1% (v/v) HCl. Suspension was kept for ultrasonication (10 min) at 30 °C. Extract was centrifuged at 10,000*g* (10 min), and then the supernatant was diluted by methanol and used for further analysis of anthocyanins.

The anthocyanins were analysed by HPLC in a Agilent system. Samples (5 μL) were injected onto Agilent ZORBAX SB-Aq C18 (4.6 × 250 mm) column, and eluted with solvent A (formic acid–water, 10:90, v/v) and solvent B (acetonitrile) at 1 mL/min and 25 °C. The gradient steps were as follows: 0–7 min, 6–9% B in A; 7–18 min, 9–11% B in A; 18–30 min, 11–30% B in A; 30–34 min, 30–15% B in A and 34–40 min, 15% B in A. Anthocyanins content was detected using DAD detector at 520 nm, while quantification was performed by external calibration based on peak area.

2.7. Analysis of phenolic acids

Phenolic acids were hydrolyzed and extracted according to the methods of Mattila and Kumpulainen (2002) and Fang et al. (2009) with some modifications. Mulberry juice samples (10 mL) were added with 5 mL of 4 M NaOH containing 1% ascorbic acid and 10 mM EDTA, and then kept for 4 h under a nitrogen atmosphere at room temperature. The hydrolyzed solution was adjusted to pH 2 with 6 M HCl, and extracted five times with diethyl ether/ethyl acetate (1:1, v/v). The organic phase was evaporated to dryness under rotary vacuum at 45 °C, and the dry residue (phenolic acid) was dissolved into 5 mL of methanol. The prepared phenolic acid solution (20 μL) was injected onto the same HPLC system as described in analysis of anthocyanins above, and eluted with Solvent A (4% acetic acid aqueous) and solvent B (methanol) with a flow rate of 1 mL/min at 25 °C. The gradient steps were as follows: 0–20 min, 15–50% B in A; 20–25 min, 50–80% B in A; 25–35 min, 80% B in A; 35–40 min, 80–15% B in A and 40–45 min, 15% B in A. The scanning range of DAD was set from 210 to 400 nm. The identification of phenolic acids was accomplished by the retention time and the UV spectra of standards. Quantification of phenolic acids was carried out by an external standard method using calibration curves, and concentration of phenolic acids was expressed as mg/L of juice.

2.8. Analysis of flavonols

Flavonols were extracted and hydrolyzed to aglycons following a modified method from the procedures optimised for berries (Mikkonen et al., 2001). The mulberry juice samples (10 mL) were mixed with 10 mL methanol and 5 mL hydrochloric acid (36%, v/v).