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The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (*Prunus cerasus* var. Marasca) juice

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ABSTRACT

The purpose of this research was to evaluate the effect of cold atmospheric pressure gas phase plasma treatment on anthocyanins and phenolic acids in sour cherry Marasca juice. Plasma treatment was optimized using a response surface methodology regarding the treatment time, sample volume and applied gas flow and compared to thermal pasteurization and untreated juice. Short treatment (3 min) of larger volume of the juice (3 mL) resulted in the highest concentration of both anthocyanins and phenolic acids. Compared to pasteurized and untreated juice, plasma treated sour cherry Marasca juice at optimized conditions had higher amount of phenolic compounds. Observed increase in phenolic compounds content in sour cherry Marasca juices could be a result of undefined small-sized aggregates or particles, which would be dissociated by the plasma treatment.

1. Introduction

Sour cherry Marasca (*Prunus cerasus* var. Marasca Host.) is an autochthonous Croatian cultivar characterized with specific quality reflecting in a high dry matter content, sweet-bitter aroma and intensive dark red color. It is a fruit of high biological value as it is a rich source of polyphenols, especially anthocyanins which are responsible for its specific color. As it is a case with other sour cherry cultivars, all anthocyanins present in sour cherry Marasca are cyanidin derivates differing in sugar moiety bonded to the aglyconic part of molecule, with cyanidin-3-glucosilrutinoside being present in the highest concentration (Elez Garofulić, Dragović-Uzelac, Režek Jambrak, & Jukić, 2013; Pedisić, Dragović-Uzelac, Levaj, & Škevin, 2010). Compared to other sour cherry cultivars, Marasca was reported to have a higher content of total anthocyanins due to their distribution in both fruit flesh and skin (Chaovanalikit & Wrolstad, 2004; Pedisić et al., 2010). Apart of anthocyanins, Marasca cherries contain a significant amount of other colorless polyphenols, especially hydroxycinnamic phenolic acids such as neochlorogenic, chlorogenic, *p*-coumaric and caffeic acid (Bonerz, Wurth, Dietrich, & Will, 2007; Elez Garofulić et al., 2013; Kirakosyan, Seymour, Urcuyo Llanes, Kaufman, & Bolling, 2009). Because of the amount of different polyphenols present in sour cherry Marasca, it could be considered as a fruit with a great processing potential. Nevertheless, it is extremely important to preserve those valuable compounds during processing in the highest possible extent. One of the most widespread sour cherry products is a sour cherry juice. The critical step in juice production is pasteurization, the most widely applied technique for successful inactivation of vegetative microorganisms and enzymes, used for prolongation of the juice shelf life (Dubrović, Herceg, Režek Jambrak, Badanjak, & Dragović-Uzelac, 2011; Kimball, 1999). However, today consumers demand nutritious foods, which are minimally and naturally processed, which has led to the increased interest in non-thermal technologies. One of such techniques is the application of non-thermal plasma as it results in efficient inactivation of microorganisms with moderate heating of the treated sample (Moisan et al., 2001). Non-thermal plasma is generated by subjection of a process gas to a strong electric field, so it presents a partially ionized gas. Apart of ionized gas molecules, this process also leads to formation of other reactive chemical species, such as radicals, heat and UV light, which all together potentially involve in different reactions leading to microorganism inactivation (Keener, 2008).

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Although much work has already been done in understanding the effect of plasma on microorganisms (Ehlbeck et al., 2011; Laroussi, 2005), little is known about its interaction with food components, especially with secondary metabolites such as polyphenols.

In case of the atmospheric pressure plasma treatment, used plasma gas is mixed with ambient air so formation of reactive oxygen and nitrogen species is inevitable. On the other side, polyphenols act as antioxidants protecting the cells against the damaging effects of those reactive oxygen and nitrogen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Haenen, Paquay, Korthouwer, & Bast, 1997; Hu et al., 1995; Van Acker, Tromp, Haenen, Van der Vijgh, & Bast, 1995). Therefore, polyphenols surely interact with plasma-immanent reactive species during the treatment.

Related to the current findings, the aim of this work is to evaluate the effect of gas phase plasma treatment on the content of anthocyanins and phenolic acids in sour cherry Marasca juice regarding the treatment time, gas flow and the volume of treated sample. Plasma treatment conditions were optimized using the response surface methodology in order to determine the treatment parameters resulting in the highest concentration of anthocyanins and phenolic acids and to compare it with traditional thermal pasteurization and untreated juice.

2. Materials and methods

2.1. Material

Sour cherry Marasca juice was prepared by dilution from concentrated juice obtained from company Dona d.o.o. (Gornja Stubica, Croatia) in 2013. Concentrated juice of 65 °Brix was diluted with distilled water to 12 °Brix and used for analysis. Soluble dry matter of concentrated juice and diluted one was measured using a digital handheld refractometer (A. Krüss Optronic GmbH, Hamburg, Germany).

For pasteurized juice, thermal pasteurization was carried out in a small scale laboratory tubular pasteurizator (Euclid d.o.o., Croatia) at 80 °C for 2 min.

2.2. Chemicals and standards

Methanol, acetonitrile and formic acid used for extraction and analysis of polyphenols were HPLC grade, purchased from Grammol (Zagreb, Croatia). Phenolic acids standards (chlorogenic acid, *p*-coumaric acid and caffeic acid) were obtained from Sigma (Steinheim, Germany) while all anthocyanin standards (cyanidin-3-sophoroside, cyanidin-3-glucoside and cyanidin-3-rutinoside) were purchased from Extrasynthese (Lyon, France).

2.3. Plasma treatment of sour cherry juice

The cold atmospheric plasma jet was generated in argon (purity 99.99%; Messer, Sulzbach, Germany) by applying a 25 kHz electric field trough the electrode. The plasma source used was a single-electrode atmospheric jet (End-field Jet type), designed at the Institute of Physics (Zagreb, Croatia) (Fig. 1). It consists of Teflon body to which a glass capillary tube of 7.5 cm length and 0.15/0.1 cm outer/inner diameter is attached. Inside the capillary tube 100 micron diameter Cu wire is placed which is connected to the high voltage source through the vacuum tight connector. High voltage source of nominal 6 W power provides 2.5 kV voltage at 25 kHz. The actual current through the electrode was measured to be typically 3 mA. The actual power of plasma was 4 W as determined from the voltage–current waveforms (Zaplotnik et al., 2014).

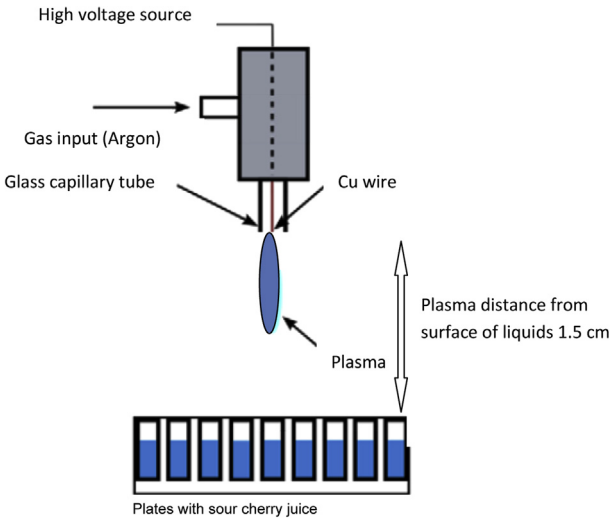

Fig. 1. Schematic description of the used plasma source.

Such plasma source produces a plasma jet extending out of the capillary tube to the length of about 2.2 cm at argon gas flow of 1.5 L/min. Further increase of gas flow causes decrease of the plasma jet length.

Optical emission spectroscopy of Ar plasma jet in the region from 200 to 1000 nm was performed by means of a miniature fiber spectrometer (Avantes 3600, Leatherhead, Surrey, UK) of 0.8 nm spectral resolution. The light was collected from the region near the capillary tube exit by means of a quartz lens and a solar resistant optical fiber (Kregar, Bišćan, Milošević, & Vesel, 2011). It showed the existence of excited NO, OH, O radicals within the plasma jet, as well as excited N_2 and Ar.

For the juice treatment, plasma was running at a constant power of 4 W, varying the gas flow, treatment time and volume of treated juice according the experimental design (Table 1). Distance of the plasma nozzle tip from the samples was fixed at 1.5 cm. Juice samples were placed in a tissue culture test plate consisted of 16 sample positions (TPP Techno Plastic Products AG, Trasadingen, Switzerland) and each treatment was performed in triplicate. Extraction of the polyphenols was performed immediately after the treatment.

2.4. Extraction procedure of sour cherry polyphenols

Sour cherry Marasca polyphenols were extracted from 1 mL of juice with 3 mL of 1:99 (v/v) formic acid in 80:20 (v/v) aqueous solution of methanol. The mixture was stirred carefully, extracted for 15 min in ultrasonic bath preheated to 50 °C, filtered through Whatman No. 40 filter paper (Whatman International Ltd., Kent, UK) and made up to 5 mL in a volumetric flask with extraction solvent. All extracts were prepared in duplicate. Extracts were stored at −18 °C in an inert gas atmosphere before the analysis.

2.5. HPLC analysis

Separations of polyphenols were performed on a Nucleosil 100-5C18, 5 μm (250 × 4.6 mm I.D.) column in an Agilent 1260 system (Agilent Technologies, Santa Clara, California) equipped with a 1260 quaternary pump, 1260 auto sampler, 1260 termostated column and 1260 UV/Vis-Photo Diode Array detector. The mobile phase and the gradient employed were described previously by Mitić, Obradović, Kostić, Micić, and Pecev (2012) with modification regarding the content of formic acid in mobile phases. For gradient

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