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Reverse osmosis as a potential technique to improve antioxidant properties of fruit juices used for functional beverages

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ABSTRACT

Reverse osmosis (RO) as a potential technique to improve the antioxidant properties of cranberry, blueberry and apple juices was evaluated for the formulation of a functional beverage. The effects of temperature (20–40 °C) and trans-membrane pressure (25–35 bars) on physico-chemical and antioxidant properties of fruit juices were evaluated to optimize the operating parameters for each fruit juice. There was no significant effect on any quality parameters of fruit juices under studied operating parameters of RO. However, total soluble solid, total acidity and colour (a^*) of the concentrated juices increased in proportion to their volumetric concentrations. Antioxidant capacity measured by FRAP assay of concentrated apple, blueberry and cranberry juice was increased by 40%, 34%, and 30%, respectively. LDL oxidation inhibition by concentrated blueberry and cranberry juice was increased up to 41% and 45%, respectively. The results suggest that RO can be used for enhancing the health promoting properties of fruit juices.

1. Introduction

Consumption of plant-based products, such as fruits and vegetables, are associated with a healthier life style with lower risk of chronic diseases like cardiovascular disease (CVD) (Thilakarathna & Rupasinghe, 2012). Polyphenols are one of the most common and ubiquitously distributed plant secondary metabolites, of which flavonoids are a sub-class which has prompted growing interest because of these therapeutic properties (Bravo, 1998). Apple (*Malus domestica* L.), cranberry (*Vaccinium macrocarpon* L.) and blueberry (*Vaccinium angustifolium* Aiton.) are some of the polyphenolic bioactive-rich fruits grown in Canada (Morton, Caccetta, Puddey, & Croft, 2008). Many investigations have shown health benefits associated with the consumption of these fruits (Duthie et al., 2006; Vanduyn & Pivonka, 2000). It is widely accepted that dietary flavonoids improve the cardiovascular health by inhibiting pathophysiological processes such as LDL oxidation, under oxidative stress (Nijveldt et al., 2001). In addition to characterizing and improving the nutritional and pharmacological interest in phenolic bioactives, research to improve process technologies for enhancing the bioactive profile in food has also been growing (Gorelik, Ligumsky, Kohen, & Kanner, 2008).

RO is a membrane separation process in which a hydraulic pressure that is higher than the osmotic pressure of the solution is applied in such a way that permeation of water from high to low solute concentration occurs (Bhattacharyya & Williams, 1992). This process can be applied to concentrate bioactives of fruit juices, reducing the damage caused by thermal evaporation of water and resulting in the maintenance of their nutritional and sensory characteristics (Girard & Fukumoto, 2000). The use of RO in the concentration of many fruits is very promising and this technique partially promotes dehydration, resulting in an increase of total soluble solids (TSS), including phenolic bioactives (Gurak, Cabral, Rocha-Leão, Matta, & Freitas, 2010). This process has been used for various fruit juices such as orange (Jesus et al., 2007) and grape (Gurak et al., 2010). This process can be used to enhance the bioactives and their functionality in fruit juices when preparing functional beverage targeting health benefits. The aim of this work was to evaluate RO as a process for the partial concentration of bioactives present in cranberry, blueberry and apple juices and to determine the effects of the processing parameters on physico-chemical and antioxidant properties of the concentrated fruit juices.

2. Materials and methods

2.1. Raw materials and chemicals

Apple, blueberry, and cranberry juice were purchased from three commercial juice companies: apple juice were obtained from J. W. Mason & Sons Ltd., Windsor, NS, Canada; blueberry juice from Van Dyks Ltd., Caledonia, NS, Canada; cranberry juice from

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Cranberry Acres Ltd., Berwick, NS, Canada, respectively. All the chemicals used were of analytical grade and purchased from Sigma–Aldrich, Oakville, ON, Canada.

2.2. Membrane processing of fruit juices

The membrane technology (GEA filtration model L pilot plant, Hudson, WI, USA) was used to concentrate different bioactive constituents in order to preserve the natural antioxidants and to maintain a high total antioxidant activity of the juice. The system was equipped with a Dow filmtec BW30-2540 RO membrane of surface area of 2.6 m^2 (Dow Chemical Company, MN, USA). This membrane was selected based on previously reported findings (Prince, Cran, Le-Clech, Uwe-Hoehn, & Duke, 2011; Qiu & Davies, 2012). The effects of temperature and trans-membrane pressure on flux of the fruit juices were evaluated to optimize the operating parameters for each fruit juice. Permeate flux and volumetric concentration factor (VCF) were calculated using the following equations, where V is the volume permeated during determined time (t), A is the membrane surface area, X is the total suspended solids maintained on the feed side of the membrane and Y is the concentration of suspended solids in the influent water to the membrane system

$$\text{Permeate flux} = \frac{V}{A * t}$$

$$\text{VCF} = \frac{X}{Y}$$

2.3. Experimental design

For the evaluation of the effects of temperature and trans-membrane pressure on the physico-chemical, nutritional and antioxidant properties of the partially concentrated fruit juices, complete factorial design (2 × 3) was carried out with fruit juice temperatures (20 and 40 °C) and trans-membrane pressures (25, 30 and 35 bars) as independent variables, and permeate flux and parameters related to juice quality (physico-chemical and antioxidant), as dependent variables. All assays were conducted in triplicate and the average values of these assays were used to analyse the data. The range of each processing parameter was chosen based on the scientific literature on concentration of fruit juice using membrane filtration (Gurak et al., 2010; Jesus et al., 2007).

2.4. Analysis of physico-chemical and antioxidant properties of fruits juices

2.4.1. Colour index

Colour of the fresh and membrane filtered fruit juices were determined by using a reflectance colorimeter (Model CR-300, Minolta Camera Co Ltd, Osaka, Japan), based on a^*, b^* and L^* values. L^* ranges, from 0 (completely opaque) to 100 (completely transparent). Positive and negative a^* values indicate reddish and greenish respectively, whereas positive and negative b^* values indicate yellowish and bluish, respectively (Lopez-Nicolas, Perez-Lopez, Carbonell-Barrachina, & Garcia-Carmona, 2007).

2.4.2. pH, total soluble solids and titratable acidity

Samples of single-strength juice and concentrated juice were analyzed in triplicate for pH, using a standardized pH meter (Model Accumet® 10, Denver Instruments Co., Arvada, CO, USA) and total soluble solids (TSS) were tested using a hand-held digital refractometer (Model 300016, Super Scientific Ltd, Scottsdale, AZ, USA). The titratable acidity was measured using a semi-automatic titrator (DMP 785, Metrohm Ltd., Herisau, Switzerland) at pH 8.2, with 0.1 N NaOH as titrant and was expressed as malic acid equivalents for apple juice, citric acid equivalent for blueberry juice, and quinic acid equivalent for cranberry juice. The predominant organic acids in apple (Gokmen, Artik, Acar, Kahraman, & Poyrazoglu, 2001), blueberry (Kalt & McDonald, 1996) and cranberry (Coppola, Conrad, & Cotter, 1978) are malic acid, citric acid and quinic acid, respectively.

2.4.3. Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1999), with some modifications, as described by Rupasinghe, Wang, Huber, and Pitts (2008).

2.4.4. Ferric reducing antioxidant power assay (FRAP)

Antioxidant capacity of fruit juices was measured using FRAP assay, according to Benzie and Strain (1999) with some modifications, as described by Rupasinghe et al. (2008).

2.4.5. LDL oxidation inhibition

Percentage LDL oxidation inhibition was determined spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) assay (Xu, Yuan, & Chang, 2007) with slight modifications as described by Gunathilake. K.D.P.P. (2012).

2.5. Determination of major phenolic compounds

2.5.1. Sample preparation

Both fresh and membrane filtered fruit juice samples were rendered free of sugars and organic acids by using solid phase extraction and passing them through a C18 Bond Elute column® (Agilent Technologies, Mississauga, ON, Canada). The column was conditioned with 3 mL of 100% methanol and was washed with 3 mL deionized water. Ten (10) mL of sample was loaded, followed by a wash of 6 mL deionized water. Phenolic compounds were eluted using 3 mL of 100% methanol. The elutes were filtered through 0.45 μm nylon filters before the HPLC analysis.

2.5.2. Analysis of major polyphenols profile

Polyphenols of fruit juice samples after solid phase extraction were analysed using an ultra-high performance liquid chromatography (UHPLC) (Model H-class system, Waters, Milford, MA, USA) equipped with an acuity UHPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) (Waters, Milford, MA, USA). For the analysis of non-anthocyanin polyphenols, gradient elution was carried out with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with the flow rate of 0.2 mL/min. A linear gradient profile was used with the following proportions of solvent A applied at time t (min) as described by Rathnasooriya, Rupasinghe, and Jamieson (2010) with slight modification; (t, A%): (0, 94%), (2, 83.5%), (2.61, 83%), (2.17, 82.5%), (3.63, 82.5%), (4.08, 81.5%), (4.76, 80%), (6.75, 20%), (8.75, 94%), (12, 94%). The analysis of anthocyanins was performed as described below; the mobile phases were 5% (v/v) formic acid in water (solvent A) and 5% (v/v) formic acid in methanol (solvent B). The linear gradients used were as follows; (t, A%): (0, 10%), (8, 30%), (17, 40%), (19, 40%), (20, 10%), (22, 10%). The flow rate was 0.2 mL/min, with an injection volume of 2.0 μL.

2.5.3. MS/MS analysis

MS–MS analysis was performed with a Micromass Quattro micro API MS/MS system, which is controlled by Masslynx V4.1 data analysis system (Micromass, Cary, NC, USA) as described by