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Betalain rich functional extract with reduced salts and nitrate content from red beetroot (*Beta vulgaris* L.) using membrane separation technology

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ABSTRACT

An initial laboratory-scale evaluation of separation characteristics of membranes with nominal molecular weight cut-offs (NMWCO) ranging from 30 kD down to 0.5 kD indicated effective separation of betalains in the 0.5 kD region. Subsequent pilot-level trials using 1 kD, loose reverse osmosis (LRO) and reverse osmosis (RO) spiral-wound membranes showed LRO membrane to be very efficient with up to 96% salt and 47% other dissolved solids removed while retaining majority of the pigment (~98%) in the betalain rich extract (BRE). The total betalain content in the BRE increased up to 46%, the highest recovery reported so far at pilot scale level. Interestingly, more than 95% of the nitrates were removed from the BRE after the three diafiltrations. These studies indicate that membrane technology is the most efficient technique to produce BRE with highly reduced amounts of salts and nitrate content.

1. Introduction

Beetroot (*Beta vulgaris* L.), commonly found in Australians sandwiches is a red rooted vegetable coming from the family of *Chenopodiaceae*. Beetroot is known for its deep and powerful purplish-red pigments, termed as betalains. Betalains are water-soluble nitrogen-containing pigments, which comprise the red–violet betacyanins and the yellow betaxanthins (Henriette, 2006). Betalains are a permitted choice for natural red-purple colour. Betalains extracted from beetroot have extensive applications as natural colourants in the food, pharmaceutical and cosmetic industries. Betalains are more water soluble than anthocyanins. They have three times higher colouring strength than anthocyanins. In spite of this wide range of applications and benefits, this red rooted vegetable is under produced and under consumed world-wide. In Australia, beetroot is mainly sold either as a fresh vegetable or as processed (sliced) product. Roughly 85% of processing beetroot is sliced and up to 30% of the total beetroot produced is wasted on the farm due to over size and uneven shape for slicing. Only a small portion of beetroot is utilised for juicing. Other niche markets for beetroot products such as health drinks, functional foods and natural colourants have not been explored. Good processing technologies with value added product diversification can minimize wastage and further boost the area under cultivation and further contribute to the state and national economies.

With immense interest in the development of functional foods, the extraction of betalains from beetroot is gaining industrial popularity. Extraction techniques such as continuous diffusion (counter current extraction) with subsequent enzyme treatment are currently used. Betalains extracted using these methods contain large amounts of salts, sugars and other impurities such as proteins. Even though fermentation is carried out to remove sugars, it is a time consuming process (3–7 days) and a considerable loss of pigments occurred (Pourrat, Lejeune, Regerat, & Pourrat, 1983; Thakur & Gupta, 2006). Pulsed electric field technique is also used in the extraction which is mainly helpful in increasing juice extraction efficiency and shelf life of the juice. However, a drawback with this method is when it combines with dielectric breakdown non-conductive molecules within the structure become conductive (Fincan, DeVito, & Dejmek, 2004; Takhistov, 2006).

Due to the many factors that influence the stability of betalains, there is a need to select a suitable separation technique which does not interfere with the stability of betalains but enhance their separation. Aqueous membrane technology offers potential for selective separation of betalain compounds in a more efficient and acceptable form. There are only two reported studies on the clarification and filtration of beetroot juice using enzyme

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treatment followed by ultrafiltration/reverse osmosis (Lee, Wiley, Sheu, & Schlimme, 1982; Thakur & Gupta, 2006). Even though sugar concentration was brought down, betalain levels reduced significantly (up to 43%) on enzyme treatment, especially in the later case. The main objective of this study was to determine the feasibility of selective fractionation of betalaines from a complex matrix of minerals and sugars of beetroot juice using membrane technology. An initial screening of membranes to evaluate separation characteristics was done using a laboratory-scale membrane unit. Based on the results pilot scale membrane fractionation and concentration trials were undertaken using selective membranes.

2. Materials and methods

2.1. Juice extraction

Red beetroots (5–10 cm diameter, 10 kg) were purchased from a local market, washed, peeled and manually cut into halves. The cut beetroots were diced into 5 × 5 mm cubes with a CL 50 Gourmet dicer (Robot Coupe, Australia). For the initial laboratory experiments both raw and cooked beet juice samples were prepared. Raw juice was prepared directly from the diced beet (5 kg) with the aid of 10 L of RO water using a Brown's screw press model – 2503 (Brown International, USA). In the case of cooked juice diced beet (5 kg) was cooked with 10 L of RO water in a steam jacketed vessel for 45 min at 95–100 °C and extracted using the screw press. Raw beet yielded roughly 10 L of juice while the cooked beet produced roughly 11.5 L of juice. Both raw and cooked juice were centrifuged to remove suspended particles and stored in 200 mL bottles at −10 °C. For pilot trials juice 50 kg beetroot was cooked with 100 L of RO water and extracted as described above. This resulted in 121 L of cooked beetroot juice.

2.2. Membrane fractionation

2.2.1. Bench-top experiments

Initial membrane fractionation studies of both raw and cooked beet juice (50% w/w) were carried out using a 200 mL Amicon bench-top module. Bench-scale membrane separation experiments of raw and cooked beet juice samples were conducted using 50% (w/w) juice diluted with RO water as the threshold pressure (~4.5 bar) of the lab module was not sufficient to obtain any filtration with 100% juice (w/w) especially with lower range MWCO membranes. Millipore membrane discs (63 mm diameter) with nominal molecular weight cut-off (NMWCO) ranging from 30 to 0.5 kD were used in these screening experiments. All the filtrations were conducted at ~4.5 bar pressure and 25 °C. Permeate fractions were collected from each of the fractionations and analysed for separation of betalains.

2.2.2. Pilot-scale trials

Cooked beetroot juice (120 L) was pre-filtered using a 0.1 μm stainless steel (SS) microfiltration (MF) membrane (Graver Technologies, USA) which resulted in 100 L of permeate and 20 L of retentate fractions. Permeate fraction was pasteurized at 80 °C using Carpigiani Pasto32 (Carpigiani, Italy) and stored at −10 °C before using in the fractionation trials. A GEA – Model L high pressure membrane fractionation unit (GEA Process Engineering Pty. Ltd., Australia) with 500 L/h throughput and on-line data logging facility was employed in the pilot-scale fractionation trials. Fractionation trials were conducted with 1 kD (Koch-spiral wound), loose reverse osmosis (LRO – 300 Da MWCO Dow spiral wound) and reverse osmosis (RO – Dow spiral wound) polymeric membranes using previously microfiltered cooked beetroot juice. The membrane filtration was operated at 25 bar pressure and 35 °C temperature. The permeate flux for LRO was measured at variable pressures at 35 °C. Membrane permeate flux values were measured with continuous recirculation of permeate and retentate streams back into the feed tank. All the recirculation trials using 1 kD and LRO membranes were conducted using 20 L of juice. Juice concentration trials were conducted by continuously removing the permeate fraction while the retentate was recirculated. Concentration and diafiltration trials were conducted using a LRO membrane with a starting juice volume of 30 L microfiltered juice. Diafiltration was conducted in three stages aiming at maximum removal of salts, dissolved solids and other impurities. Diafiltration was carried out by replacing the quantity of permeate (15 L) with RO water (15 L) each time.

2.3. Sample analysis

Brix and conductivity of juice samples were measured using Mettler-Toledo portable instruments and pH was measured using a portable pH meter (TPS WP-80). Mineral analysis was performed by using Inductively Coupled Optical Emission Spectrometry (ICP-OES). For elemental testing the first part of the test was conducted by acid digestion and then mixed with ultrapure water. The results of the tests are traceable to Australian standard and expressed in mg per L of sample. The concentration of the betalain pigments betanins (betacyanins) and betaxanthins was measured spectrophotometrically at wave lengths 538 nm and 476 nm respectively using a UV–vis spectrophotometer (Beckman-Coulter DU-530) following Elbe's method (Schwartz, Hildenbrand, & Elbe, 1981; von Elbe & Schwartz, 1984). Membrane rejection coefficients of different components were calculated using the following equation:

$$\text{Membrane rejection coefficient} = \left(1 - \frac{[Component]_p}{[Component]_f}\right)$$

$[\text{Component}]_p$ = Component concentration in permeate

$[\text{Component}]_f$ = Component concentration in feed

2.3.1. HPLC analysis

Separation and identification of betalains was carried out by HPLC following the method of Frank et al. (2005) with slight modifications. The HPLC system consisted of a SIL-10AD VP auto injector (Shimadzu), SCL-10A VP system controller (Shimadzu), LC-10AT VP liquid chromatograph (Shimadzu) and a SPD-M10 A VP diode array detector (Shimadzu). Samples were diluted in water and filtered prior to injection. 50 μL of each extract was injected onto a Luna C_{18} column, 3 μm, 4.6 × 250 mm (Phenomenex, Australia), kept at 25 °C, with a mobile phase of 50 nM KH_2PO_4 (pH 2.75)/methanol (85:15; v/v). Samples were eluted under isocratic conditions at a flow rate of 1.0 mL/min and were monitored at 538 nm and 476 nm with a run time of 25 min for each sample. Betanin and vulgaxanthin was identified by comparison of retention time and spectra with standard compounds. Standards were obtained commercially from Chromadex, California, USA.

Total phenolics were measured following the method of Kim, Jeong, and Lee (2003). Standard solutions were prepared using 1 mg/mL catechin solution. Next, 650 μL of ultrapure water, 50 μL of diluted beetroot juice sample or standard and 50 μL of the Folin-Ciocalteu reagent (Sigma, Sydney, Australia) were combined in each assay tube. These mixtures were vortexed and allowed to stand for 5 min. Then 500 μL of 7% sodium carbonate was added, the sample vortexed again, and allowed to stand at room temperature for 90 min. The absorbance was recorded at a wavelength of 750 nm.