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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.

All analyses were performed in triplicate and results were expressed as the mean ± standard deviation (SD).

2.4. Degradation study

A systematic study on the degradation of beetroot juice pigments for both raw and cooked juice was undertaken with a range of conditions such as light (Fluorescent lamp – 12 W) and dark, temperature (25 °C/4 °C) and pH (4.5, 5.5, 6.5) tested over a total storage time of nine days. Samples were collected at regular intervals of time every day and analysed for betalain concentration.

3. Results and discussion

3.1. Bench-top membrane fractionation

Bench-scale membrane separation experiments of raw and cooked beet juice samples were conducted using 50% (w/w) juice.

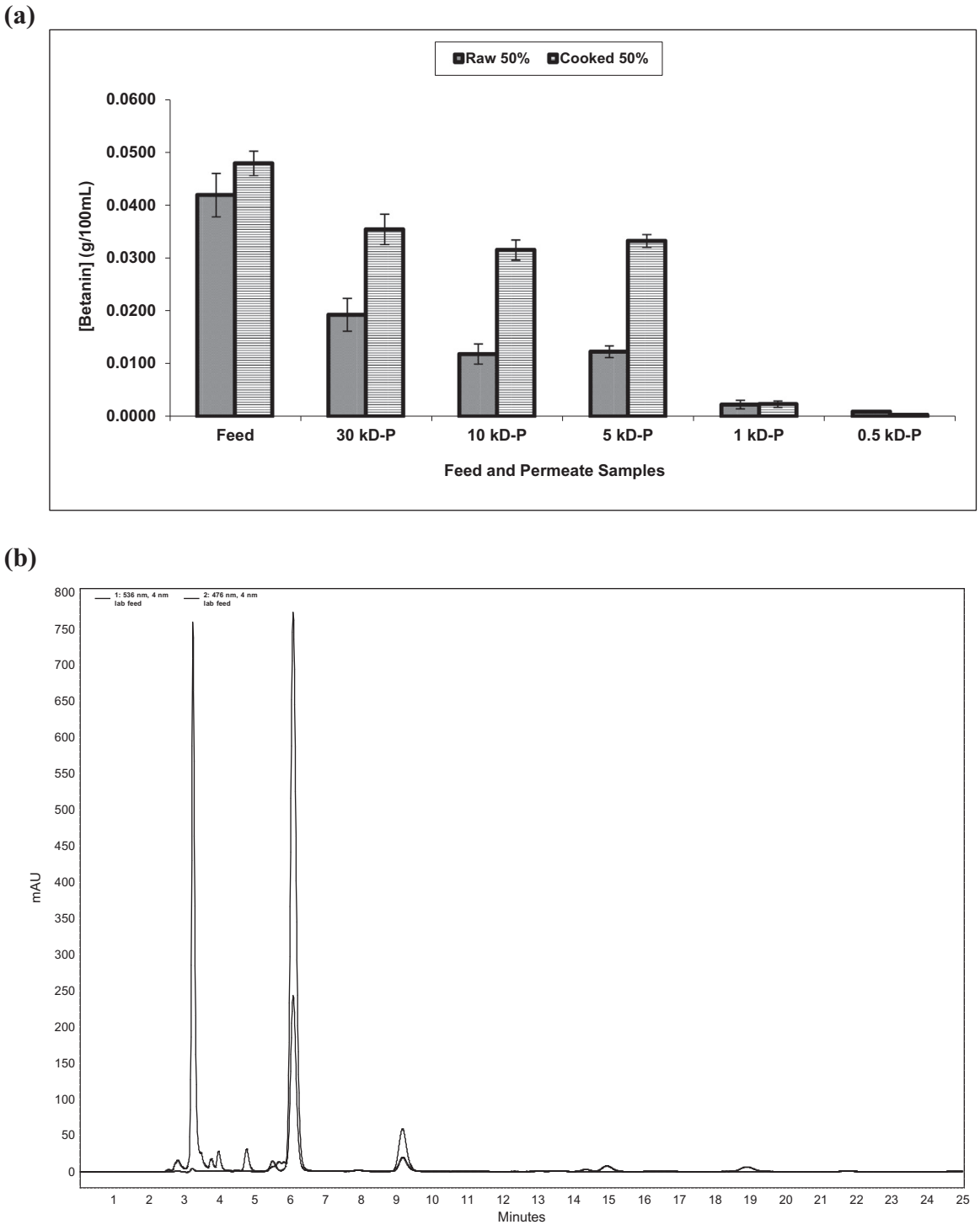

Fig. 1. (a) Betanin concentrations of feed and permeate fractions of raw and cooked juices (50%) obtained using different membranes, Mean ± SD (n = 3); (b) Representative chromatogram recorded at 476 nm and 536 nm of 'feed' beetroot juice showing vulgaxanthin (Rt = 3.2 min) and betanin (Rt = 6.1 min) the two major betalain pigments in the beetroot juice.

Fig. 1a shows concentration values of betanins in permeates from each of the membranes for raw and cooked juices. There was a marked decrease in the colour of permeates obtained from low MWCO membranes especially those from 1 kD and 0.5 kD. This shows that there was a significant separation of colourants occurring in this range which was also clearly observed by looking at the samples. Betanin and vulgaxanthin were the major betalains in the beetroot juice, as illustrated by HPLC (Fig. 1b). Earlier studies conducted on different varieties of beetroot also reported similar results (Knuthsen, 1981; Magdolna, Daood, Hájos, & Biacs, 2001). Even though these initial investigations revealed separation of most betalains at 1 kD and 0.5 kD laboratory system did not give greater flexibility in control of parameters such as pressure and temperature. Further, separation of salts and other dissolved solids such as sugars from betalains was also important for their improved stability and use as a functional ingredient in health, and food applications (Adams, Elbe, & Amundson, 1976; Azeredo, 2009). To address these issues a more detailed investigation of beetroot juice fractionation was conducted on a pilot scale based on the preliminary laboratory results. Furthermore, samples from raw juice were found to have comparatively lower concentrations of pigments than cooked ones as the extraction from raw beet was tougher than much softer cooked beet. A relatively faster degradation was observed for raw juice samples as compared to cooked juice as noticed from physical appearance. This observation prompted to take-up a detailed degradation study of both raw and cooked juices before proceeding to pilot scale trials.

3.2. Degradation studies

Stability of both raw and cooked beet juice samples was measured under different storage conditions and pHs as described in the experimental section. Betalain degradation is affected by temperature, light and oxygen (Herbach, Stintzing, & Carle, 2006). The results obtained from this study are given in Fig. 2. The trends in Fig. 2a clearly show significant degradation of beet juice in dark as well as under light for both raw and cooked cases. Juice samples stored in refrigerator were found to be quite stable. The degradation of betalains could occur by different chemical reactions during different storage conditions. The most possible chemical reactions involved are 1) deglycosylation of betanin at high temperature and under acidic condition. 2) hydrolysis of aldimine bond of betalains above pH 6 and elevated temperature. 3) decarboxylation at C_2, C_{15}, and C_{17} of betacyanins and at C_{11} and C_{13} of betaxanthins. 4) dehydrogenation of betacyanin during thermal treatment (Herbach et al., 2006). At all the storage conditions,

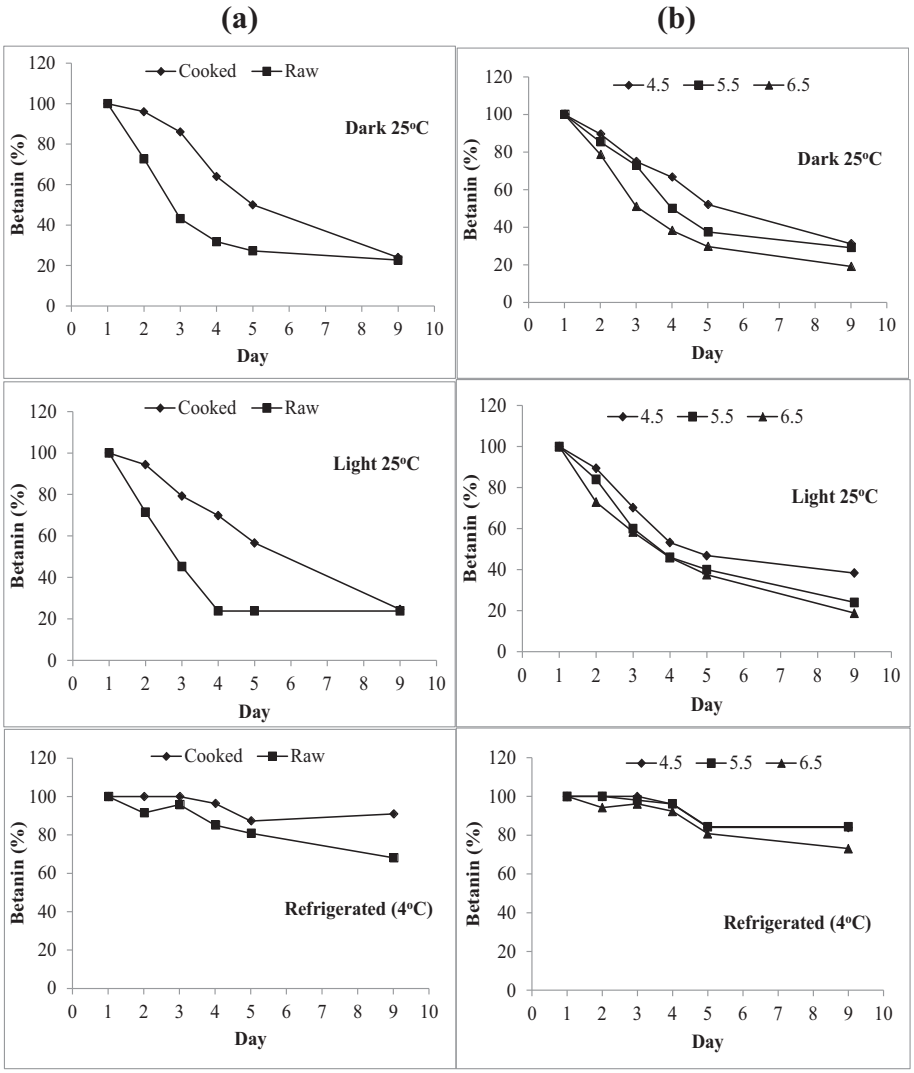

Fig. 2. (a) Betanin degradation profiles of raw and cooked beet juice in dark, light and refrigerated conditions; (b) Betanin degradation profiles of cooked beet juice at three different pHs in dark, light and refrigerated conditions.

raw juice showed faster degradation signs compared to cooked juice. Even though heating of beetroot while preparing cooked beet juice was expected to breakdown betalain compounds to some extent, no considerable difference was observed in the concentration of pigments. In fact cooked juice had a slightly higher betalain concentration than raw juice. It was reported that betalain regeneration after processing increased overall colour retention. A gain of 3% was found for unheated juice, up to 10% for heat-treated juice stored at 4 °C (Stintzing, Trichterborn, & Carle, 2006). The possible mechanism involves partial resynthesis of betanin from its hydrolysis products by condensation of the amine group of *cyclo*-Dopa-5-O-glycoside with aldehyde group of betalamic acid. Betanin is rapidly formed when these two compounds are present in solution at temperatures below 10 °C (Huang & Elbe, 1985). Processing of beetroot at higher temperature (~95 °C) also might help to kill microorganisms thus stabilizing the juice. pH changes also did not influence the stability of juice in the given range (4.5–6.5). Fig. 2b shows the degradation trends of cooked beet juice samples at pHs 4.5, 5.5 and 6.5 kept in light, dark and refrigerator (4 °C). Similar to previous results, only the samples kept in refrigerator showed highest stability irrespective of pH changes. Earlier studies also reported pH stability of beetroot pigments between 3 and 7 (Henriette, 2006; Herbach et al., 2006). Betalains pH stability makes them suitable as natural colourants in a broad palette of low acid and neutral foods such as dairy and beverage products. Betalains are also considered as substitutes for less hydrophilic anthocyanins which under similar conditions fade quickly (Stintzing & Carle, 2004; Stintzing et al., 2006).

3.3. Pilot-scale membrane trials

Microfiltration using 0.1 μm stainless steel membrane removed 99% turbidity from the juice. This produced more stable juice which was clearly observed in the subsequent nano and RO

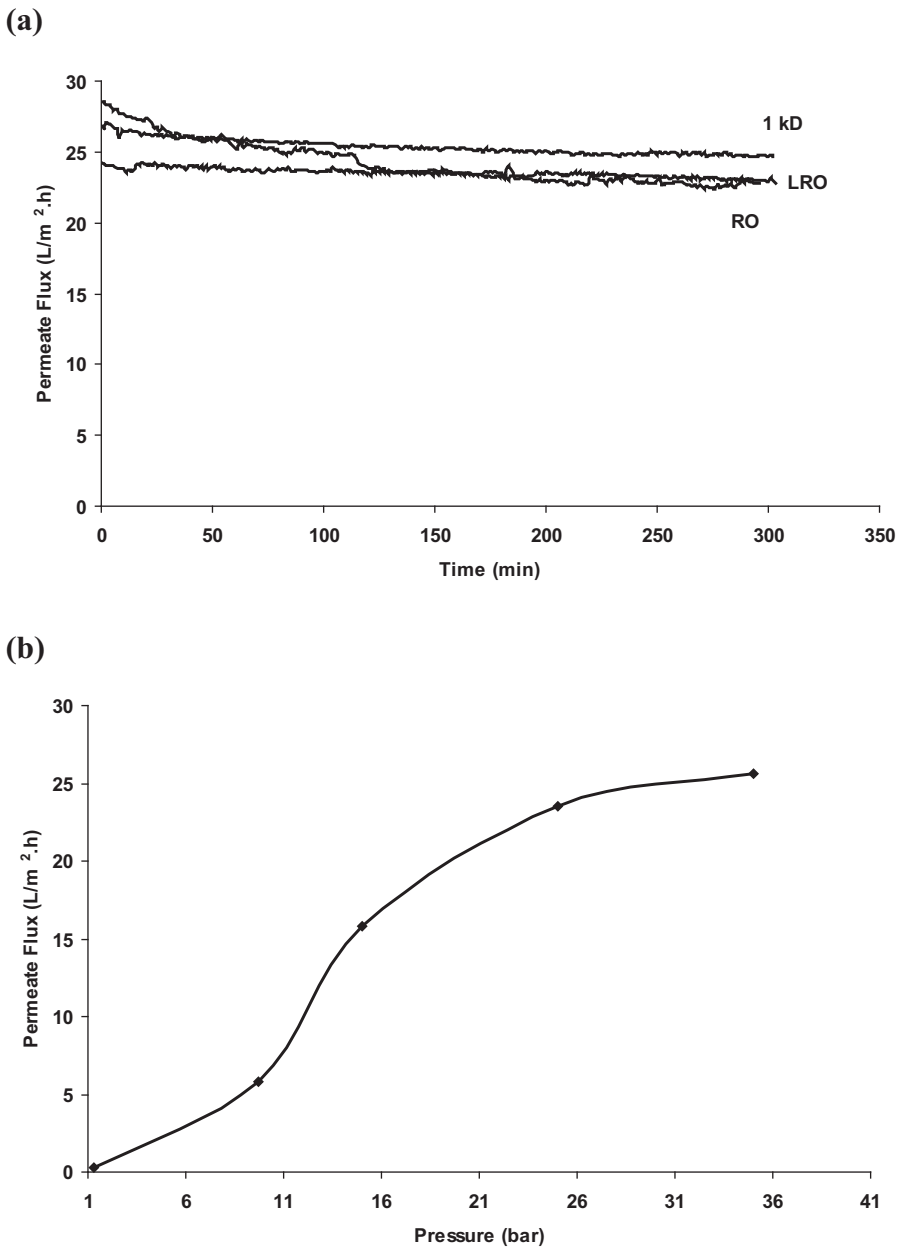

Fig. 3. (a) Permeate flux profiles for three different membranes at 25 bar and 35 °C; (b) Permeate flux profile of LRO membrane for different feed pressures at 35 °C.

Table 1
Membrane rejection coefficients of betalains, salts and dissolved solids and total phenolics.

Membrane type	Membrane rejection coefficient			
	Betalains	Salts	Dissolved solids	Total phenolics
LRO	0.99 ± 0.08	0.07 ± 0.0	0.64 ± 0.04	0.41 ± 0.03
RO	0.99 ± 0.06	0.96 ± 0.1	1.0 ± 0.11	0.99 ± 0.05
1 kD[a]	0.92 ± 0.05	0.06 ± 0.0	0.50 ± 0.02	0.33 ± 0.02

Mean ± SD (n = 3).
[a] Membrane of 1 kD was used in pilot scale trials.

Table 2
Percentage of betalains, salts and dissolved solids retained in the juice at the end of each trial.

Trails	Retained (%)		
	Betalains	Salts	Dissolved solids
Concentration	99.70 ± 2.53	25.10 ± 1.12	72.85 ± 3.11
Diafiltration I	99.30 ± 3.65	10.29 ± 0.98	60.60 ± 2.56
Diafiltration II	98.50 ± 1.57	5.85 ± 0.89	54.60 ± 1.54
Diafiltration III	98.11 ± 1.05	4.02 ± 0.54	53.23 ± 1.07

Mean ± SD (n = 3).

filtrations in terms of consistent permeate flux. Initial clarification of juice with microfiltration expected to remove any microorganisms in addition to other suspended particulate. Fig. 3a shows the permeate flux profiles of beetroot juice for 1 kD, LRO and RO membranes monitored for 5 h. These trials were run at 25 bar *trans*-membrane pressure and 35 °C. Flux remained nearly constant at 25.5 L/m^2 and 23.5 L/m^2 all through the trial for 1 kD and LRO membranes respectively, however in the case of RO membrane flux values dropped marginally during the first 2 h from 27.9 L/m^2 to 23.1 L/m^2 and remained constant thereafter. The overall consistency in the flux values was attributed to the juice quality achieved through microfiltration. The drop in flux observed with RO membrane may be due to some initial fouling. Table 1 gives the rejection coefficients of these three membranes for betalains, salts and dissolved solids. LRO and RO membranes retain 99% of betalains while 1 kD retains 92%. On the other hand 1 kD and LRO membranes permeate up to 94% salts and 50% dissolved solids which was good if salts and sugars need to be separated from the juice while RO membrane retains all the salt and dissolved solids. RO membrane was found to be ideal for concentration of juice without losing any of the dissolved solids. LRO and 1 kD are appropriate for separating and concentrating betalains from other dissolved solids even though 1 kD membrane lets small amounts of colourants (up to 8%) into the permeate compared to LRO. Removal of non-betalaine components from juice will help to make betalains suitable for natural colourants and functional ingredients with increased stability. From the results obtained using these three membranes, it has been identified that LRO was most efficient in terms of retaining betalains while removing of salts and other dissolved solids. Fig. 3b shows the plots of fluxes obtained using the LRO membrane at different pressure values. In all cases fluxes remained highly consistent throughout the period of trials. In general, increase in flux was observed with increased pressure values. A significant difference in flux (~8 L/m^2) was noticed between 15 and 25 bar pressures, however the difference between 25 and 35 bar was quite small (~2 L/m^2). This indicated that 25 bar pressure was more productive than 35 bar when energy consumption and output were taken into account.

During the concentration and diafiltration trials a volume concentration factor (VCF) of 4.5 was achieved. This value was limited by the minimum holding volume of the unit in the current concentration trial. Percentage of total betalaines, salts and dissolved solids retained at the end of each of these trials are presented in Table 2. After three diafiltrations 98% of the betalains were retained while 96% of salts and 47% of the total dissolved solids present in the juice were removed. Betalains constituted 46% of the total dissolved solids in the concentrated juice after three diafiltrations. Unlike enzyme treated clarification, no considerable loss of pigments was observed in the current membrane fractionation and concentration method (Thakur & Gupta, 2006). More dissolved solid impurities mainly sugars could be removed by using a 1 kD membrane filtration, however it caused loss of 15% betalains in the permeate. Results of anion and cation analysis of inorganic salts present in the feed, permeate and concentrate fractions are shown in Table 3. These values clearly indicate the separation of majority of the salts more significantly sodium. Beetroot juice was also found to have very high quantities of nitrate (964 mg/L). These beetroot nitrates were found to be responsible in reducing hypertension in adults. (Bailey et al., 2009). However, high doses of nitrates are not advisable in the diets of children (EFSA, 2010; FSANZ, 2010). Interestingly, more than 95% of the nitrates were removed from the beetroot concentrate after the three diafiltrations. Hence, this process helps to successfully reduce the nitrate content in the final product and separate the betalain pigments in a much purer form than other extraction methods. Overall, for the first time membrane fractionation technology was successfully employed to purify and concentrate betalains from beetroot juice. Membrane processing which is a clean, green and environmentally friendly technology, has been widely used for recovery of bioactive compounds in various large scale industrial operations such as dairy, wine and waste water (Avula, Nelson, & Singh, 2009; D'Antuono et al., 2014; Kumar et al., 2013; Rektor et al., 2004).

3.4. Industrial processing recommendation

Based on the membrane fractionation results obtained from both bench-scale and pilot-scale trials a concept industrial processing model for beetroot processing has been proposed. A schematic representation of the industrial processing model is provided as Supplementary material. As cooked beetroot produced more juice yields and was found to be more stable, extraction from cooked beet was recommended in this process. Beetroot cake obtained as a co-product after extraction could be rich in fibre in addition to small amounts of betalains and other phytochemicals. This could be used for human and animal feed applications. In

Table 3
Inorganic salt (cation and anion) analysis of beetroot juice samples.

Sample	Salts (mg/L)									
	NH_4	Mg	Na	Ca	K	PO_4	SO_4	NO_3	Cl	CO_3
Feed	281 ± 39.13	180 ± 11.84	2723 ± 12.50	16 ± 2.56	1164 ± 67.21	964 ± 52.01	254 ± 62.00	964 ± 60.22	1644 ± 89.02	198 ± 32.76
LRO-P	209 ± 33.12	41 ± 9.01	2142 ± 43.71	3 ± 0.87	963 ± 47.11	421 ± 38.90	23 ± 8.90	957 ± 44.57	1786 ± 43.33	82 ± 17.21
LRO-C	203 ± 21.01	233 ± 9.99	1026 ± 38.78	30 ± 3.31	468 ± 34.44	724 ± 21.21	491 ± 11.91	23 ± 5.05	28 ± 3.32	36 ± 4.09

Mean ± SD (n = 3); LRO-P = LRO permeate; LRO-C = LRO concentrate.

the membrane fractionation section, microfiltration was recommended as a pre-filtration step for the removal of any suspended particulate. In the next step a choice of either LRO or 1 kD membranes could be used depending on purity and concentration levels required in the final betalain solution. If necessary the betalain concentrate may be further fractionated into individual betalains or may be concentrated and dried into powders. Permeates obtained from these membranes contain good amounts of potassium (963 mg/L), other dissolved solids mainly sugars and polyphenolics as seen from Table 1 and could be used as health drink or used in other juice formulations.

4. Conclusions

Membrane technology successfully separated and concentrated betalaines. Microfiltration of beet juice helped to remove 99% turbidity and achieve stable flux with subsequent filtrations. The LRO membrane effectively separated betalains with 98% rejection. Up to 96% salts and 47% of dissolved solids were separated from betalains on concentration and diafiltration. A VCF of 4.5 was achieved in the current trials however; 5–6 times concentration levels could be reached with slight modification to the current set-up. Permeate fraction from LRO/0.5 kD membrane was rich in potassium, sugars and polyphenols making another value added product available from the process. To the best of our knowledge this is the first report on the use of membrane technology for complete removal of salts, nitrate and reduction of other dissolved solids to a significant level in addition to obtaining a betalain rich concentrate from beetroot juice. Moreover, membrane filtration can be the potential green technology for beetroot juice industries.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.07.132.

References

Adams, J. P., Elbe, J. H. V., & Amundson, C. H. (1976). Production of a betacyanine concentrate by fermentation of red beet juice with *Candida utilis*. *Journal of Food Science, 41*, 78.

Avula, R. Y., Nelson, H. M., & Singh, R. K. (2009). Recycling of poultry process wastewater by ultrafiltration. *Innovative Food Science and Emerging Technologies, 10*, 1–8.

Azeredo, H. M. (2009). Betalains: Properties, sources, applications, and stability – A review. *International Journal of Food Science & Technology, 44*(12), 2365–2376.

Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., DiMenna, F. J., Wilkerson, D. P., et al. (2009). Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology, 107*(4), 1144–1155.

D'Antuono, I., Kontogianni, V. G., Kotsiou, K., Linsalata, V., Logrieco, A. F., Tasioula-Margari, M., et al. (2014). Polyphenolic characterization of olive mill wastewaters, coming from Italian and Greek olive cultivars, after membrane technology. *Food Research International, 65*(Part C), 301–310.

EFSA (2010). *Statement on possible public health risks for infants and young children from the presence of nitrates in leafy vegetables*. Europian Food Safety Authority.

Fincan, M., DeVito, F., & Dejmek, P. (2004). Pulsed electric field treatment for solid-liquid extraction of red beetroot pigment. *Journal of Food Engineering, 64*(3), 381–388.

Frank, T., Stintzing, F. C., Carle, R., Bitsch, I., Quaas, D., Straß, G., et al. (2005). Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. *Pharmacological Research, 52*(4), 290–297.

FSANZ (2010). *Survey of nitrates and nitrites in food and beverages in Australia*. Food Standards Australia New Zealand.

Henriette, M. C. A. (2006). Betalains: Properties, sources, applications, and stability – A review. *International Journal of Food Science & Technology, 44*(12), 2365–2376.

Herbach, K. M., Stintzing, F. C., & Carle, R. (2006). Betalain stability and degradation structural and chromatic aspects. *Journal of Food Science, 71*(4), R41–R50.

Huang, A. S., & Elbe, J. H. V. (1985). Kinetics of the degradation and regeneration of betanine. *Journal of Food Science, 50*(4), 1115–1120.

Kim, D.-O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry, 81*(3), 321–326.

Knuthsen, P. (1981). Investigations on beetroot colours for the purpose of regulation. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 172*, 195–200.

Kumar, P., Sharma, N., Ranjan, R., Kumar, S., Bhat, Z. F., & Jeong, D. K. (2013). Perspective of membrane technology in dairy industry: A review. *Asian Australasian Journal of Animal Sciences, 26*, 1347–1358.

Lee, Y. N., Wiley, R. C., Sheu, M. J., & Schlimme, D. V. (1982). Purification and concentration of betalaines by ultrafiltration and reverse osmosis. *Journal of Food Science, 47*(2), 465–471.

Magdolna, N. G., Daood, H., Hájos, M. T., & Biacs, P. (2001). Comparison of red beet (*Beta vulgaris* var *conditiva*) varieties on the basis of their pigment components. *Journal of the Science of Food and Agriculture, 81*(9), 932–933.

Pourrat, H., Lejeune, B., Regerat, F., & Pourrat, A. (1983). Purification of red beetroot dye by fermentation. *Biotechnology Letters, 5*(6), 381–384.

Rektor, A., Pap, N., Kókai, Z., Szabó, R., Vatai, G., & Békássy-Molnár, E. (2004). Application of membrane filtration methods for must processing and preservation. *Desalination, 162*, 271–277.

Schwartz, S. J., Hildenbrand, B. E., & Elbe, J. H. (1981). Comparison of spectrophotometric and HPLC methods to quantify betacyanins. *Journal of Food Science, 46*(1), 296–297.

Stintzing, F. C., & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science & Technology, 15*(1), 19–38.

Stintzing, F. C., Trichterborn, J., & Carle, R. (2006). Characterisation of anthocyanin–betalain mixtures for food colouring by chromatic and HPLC-DAD-MS analyses. *Food Chemistry, 94*(2), 296–309.

Takhistov, P. (2006). Pulsed electric field in food processing and preservation. In Y. H. Hui (Ed.). *Handbook of food science, technology and engineering* (vol. 3). New York: CRC Press, Taylor & Francis Group, pp. 126-121 to 126-120.

Thakur, V., & Gupta, D. D. (2006). Studies on the clarification and concentration of beetroot juice. *Journal of Food Processing & Preservation, 30*(2), 194–207.

von Elbe, J. H., & Schwartz, S. J. (1984). Ultraviolet and visible spectrophotometry. In D. W. Gruenwedel & J. R. Whitaker (Eds.), *Food analysis: Principles and techniques* (pp. 220). New York: Marcel Dekker.