CHEMICAL ENGINEERING TRANSACTIONS
VOL. 87, 2021
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Laura Piazza, Mauro Moresi, Francesco Donsì

ISBN 978-88-95608-85-3; **ISSN** 2283-9216
DOI: 10.3303/CET2187092

Processing of Black Carrot Juice by Nanofiltration and Forward Osmosis

Maria Cinta Roda-Serrat[a,*], Julie Florup Schytt-Nielsen[a], Sylvie Braekevelt[b], Tsaneta Dzhanzefova[c], Bjarne Joernsgaard[c], Birgir Norddahl[a], Massimiliano Errico[a]

[a] Department of Green Technology, University of Southern Denmark. Campusvej 55, 5230 Odense M, Denmark
[b] Aquaporin A/S, Nymøllevej 78, 2800 Kongens Lyngby, Denmark
[c] Chr. Hansen Natural Colors A/S, Agern Alle 24, 2970 Hørsholm, Denmark
mcs@igt.sdu.dk

Juice concentration is traditionally achieved with thermal evaporators. However, many nutrients and sensorial attributes suffer from degradation at high temperature. In order to preserve the sensorial and nutritional attributes of the juice, non-thermal processes based on membranes have arisen as an alternative.
In the present work, 6.7 °Brix black carrot juice has been subjected to a sequence of processes based on nanofiltration (NF) and forward osmosis (FO) operated at room temperature with the aim of concentrating acylated anthocyanins. Four polymeric membranes with molecular weight cut-offs in the range 150 – 2000 Da were compared in terms of flux and anthocyanin rejection. The membrane showing the best performance was a polyamide thin film composite NF membrane with a reported cut-off of 600 – 800 Da. With this membrane, the rejection of anthocyanins was 98% and the rejection of total soluble solids was 65 %. The juice was pre-concentrated to 10 °Brix with minimal anthocyanin loss. The resulting retentate was further concentrated to 43 °Brix using FO biomimetic membranes and a solution of $MgCl_2$ as draw.

1. Introduction

Concentration is a very important step in juice processing that aims at improving preservation, long term storage and transportation of products. Up to now, it is generally achieved by multi-stage vacuum evaporation, where a concentration in the range of 45 – 65 °Brix is targeted (Jiao et al., 2004). This process entails the use of high temperatures which can result in partial loss of heat-sensitive nutrients, bioactives, aromas, flavors and colors. Membrane technology can offer an alternative to evaporators where separation can be achieved without involving high temperatures or phase change. Pressure-driven membrane processes have been widely used for juice dewatering, reverse osmosis (RO) being one of the most popular (Bhattacharjee et al., 2017). The main limitation of RO is that it can only achieve up to 25 – 30 °Brix due to the high osmotic pressure difference across the membrane (Jiao et al., 2004). Nanofiltration (NF) has also been applied for concentration of bioactive components in juice (Arriola et al., 2014). Once the rejection of the target components is shown to be acceptable, NF benefits from lower operating pressures than RO and different component selectivity, thus offering the possibility of selective component enrichment. Unfortunately, the osmotic pressure limitation remains, and a secondary concentration step is required to achieve the high concentration needed in the final product.
Moving away from thermal and pressure-based alternatives, osmotically-driven processes like forward osmosis (FO) appear as plausible candidates for juice concentration (Mohammadifakhr et al., 2020; Wenten et al., 2020). In FO, water is transported from a low osmotic pressure solution (feed) to a high osmotic pressure solution (draw) via a semipermeable membrane (Cath et al., 2006). The FO membranes reject most solutes and ions, while only relatively pure water can pass through. Reverse salt flux from the draw towards the feed is also common, and appropriate selection of draw solutions is of paramount relevance for a successful product (Achilli et al., 2010).

Paper Received: 28 August 2020; Revised: 25 January 2021; Accepted: 19 April 2021
Please cite this article as: Roda-Serrat M.C., Schytt-Nielsen J.F., Braekevelt S., Dzhanzefova T., Jørnsgaard B., Norddahl B., Errico M., 2021, Processing of Black Carrot Juice by Nanofiltration and Forward Osmosis, Chemical Engineering Transactions, 87, 547-552 DOI:10.3303/CET2187092

In this study, a method based on nanofiltration and forward osmosis for concentration of anthocyanins in black carrot juice is presented as an alternative to thermal evaporation. The targeted application of the anthocyanin-rich concentrate is as source of natural red pigments for food coloring (Assous et al., 2014). The aim of the study was thus to increase the anthocyanin content and coloring abilities of the juice. The performance of the processes has been assessed in terms of flux, component rejection, membrane cleanability and color properties of the product.

2. Materials and methods

2.1 Juice preparation

Black carrot juice was reconstituted from commercial black carrot juice concentrate by 10-fold dilution using demineralized water. The characteristics of the juice are shown in Table 1 as initial juice.

Table 1: Characteristics of the process streams

	TSS (°Brix)	MAC (g L^{-1})	BI	VI
Initial juice	6.6 ± 0.3	0.75 ± 0.03	0.37 ± 0.01	0.13 ± 0.00
NF	10.4 ± 0.1	1.54 ± 0.04	0.38 ± 0.02	0.15 ± 0.01
FO	43.4 ± 1.2	5.60 ± 0.30	0.46 ± 0.02	0.40 ± 0.08

2.2 Ultrafiltration (UF) and nanofiltration (NF)

Four flat sheet polymeric membranes in the tight UF and NF range were tested for pre-concentration of the juice and color enrichment. The characteristics of the membranes used in the study are shown in Table 2. The cross-flow laboratory scale filtration unit used was described in a previous study (Roda-Serrat et al., 2015).
The membrane screening tests were performed using 50 mL feed volume and a feed flowrate of 10 mL min^{-1}.
An initial screening at a constant pressure of 6 bar was performed, where the membranes were evaluated in terms of permeate flux and anthocyanin rejection. The system was operated in full recycle mode, where both retentate and permeate were continuously recycled to the feed, except during sampling. The permeate flux was monitored every 5 min.
One membrane was selected for further study and tested at five different trans-membrane pressures in the range 2 – 12 bar. The selected membrane and operating pressure were tested for a feed sample of 150 mL operated in concentration mode, where the permeate was collected separately.
Membrane cleaning was conducted by an initial water rinse, an alkaline cleaning cycle (0.1 % NaOH, 55 °C, 20 min) and a final water rinse until the pH was neutral. The pure water flux was measured before operation, after rinsing and after alkaline cleaning. The resistances of the membrane (R_M), the cake layer (R_C), the reversible fouling layer (R_F) and the irreversible fouling layer (R_{IRREV}) were calculated using Equation (1) and data acquired during filtration of pure water in different scenarios: before use ($R = R_M$), after operation and rinsing with clean water ($R= R_M + R_F$) and after cleaning in place ($R = R_M + R_{IRREV}$). The resistance during operation that could not be accounted for by the rest of parameters was assumed to be the resistance of the cake layer (R_C).

$$J = \frac{\Delta P - \Delta \Pi}{\mu R} \quad (1)$$

where J is the measured permeate flux, ΔP is the applied pressure, $\Delta \Pi$ is the difference in osmotic pressure across the membrane and μ is the permeate viscosity.
The rejection factor (R_i) was calculated using Equation 2 and the separation factor ($S_{i/j}$) was calculated using Equation 3 (Koros et al., 1996).

$$R_i = 1 - \frac{C_{i,permeate}}{C_{i,feed}} \quad (2)$$

$$S_{i/j} = \frac{\left(\frac{C_i}{C_j}\right)_{permeate}}{\left(\frac{C_i}{C_j}\right)_{retentate}} \quad (3)$$

Table 2: Characteristics of membranes utilized in this study

Process	Membrane	Manufacturer	Configuration	Material	MWCO (Da)	Area (cm^2)
Ultrafiltration	GR95PP	Alfa Laval	Flat sheet	Polyethersulfone	2000	30
	XT	Synder Filtration	Flat sheet	Polyethersulfone	1000	30
Nanofiltration	NFG	Synder Filtration	Flat sheet	Polyamide TFC	600-800	30
	DK	GE Osmonics	Flat sheet	Polyamide TFC	150-300	30
Forward osmosis	HFFO2	Aquaporin A/S	Hollow fiber	Polyamide TFC	-	23000

2.3 Forward osmosis (FO)

Concentration by FO was performed using biomimetic membrane modules that contain integrated aquaporin proteins for water transport enhancement. The characteristics of the membrane are also shown in Table 2.
The feed consisted of 17 L reconstituted juice and was continuously recirculated during operation. The draw was a 1.1 M $MgCl_2$·solution that was run in counter-current mode and not re-circulated. The flowrates were 1000 and 420 mL min^{-1} for the feed and the draw, respectively. The cleaning procedure consisted of a rinse with water, an alkaline cleaning (0.02% NaOH, 50 °C, 30 min), and a final flush with water until neutrality. The pure water flux and salt flux were monitored after each cleaning cycle.

2.4 Analytical methods

The anthocyanins in black carrot juice were separated by high pressure liquid chromatography equipped with photodiode array detection (HPLC-PDA) following the method by (Gras et al., 2015).
The total monomeric anthocyanin concentration (MAC) was assessed by UV-Vis spectroscopy (Hach, USA) following the pH differential method as described in (Barba-Espín et al., 2017). The coloring properties of the juice were assessed by monitoring the brown index ($BI = A_{430}/A_{520}$) and violet index ($VI = A_{580}/A_{520}$) (Cisse et al., 2012). The total soluble solids (TSS) in the process streams were measured using a digital refractometer (Krüss, Germany) and expressed as °Brix.
All experiments were performed in triplicates and the results are expressed as the arithmetic mean ± the standard deviation.

3. Results

3.1 Profile and stability of anthocyanins in black carrot juice

The individual anthocyanins were assigned based on the elution profile and spectral properties (Gras et al., 2015). Their individual percentages based on absorbance are shown in Table 2. The black carrot juice thus showed a predominant fraction of acylated anthocyanins (83.2 %) as opposed to non-acylated (16.8 %).
In order to assess stability at room temperature, the reconstituted juice was incubated at 25 °C for 6 hours and no significant anthocyanin degradation was observed.

Table 2: Anthocyanins in black carrot juice

Type	Anthocyanin	MW (g mol^{-1})	% Abs
Non-acylated	Cyanidin-3-xylosyl-glucosyl-galactoside	743	Trace
	Cyanidin-3-xylosyl-galactoside	581	16.8
Acylated	Cyanidin-3-sinapoyl-xylosyl-glucosyl-galactoside	949	8.8
	Cyanidin-3-feruloyl-xylosyl-glucosyl-galactoside	919	66.5
	Cyanidin-3-coumaroyl-xylosyl-glucosyl-galactoside	889	7.9

3.2 Color enrichment by UF and NF

As shown in Table 2, the acylated anthocyanins in the black carrot juice have molecular weights in the range 889 – 949 g mol^{-1}, while the main non-acylated anthocyanin has a molecular weight of 581 g mol^{-1}. The total soluble solids in the juice are assumed to be mainly monosaccharides, sugar alcohols and small organic acids, which have molecular weights mostly < 200 g mol^{-1}. The difference in molecular weight between anthocyanins and TSS poses the basis for size exclusion separation using tight ultrafiltration and nanofiltration. In the screening experiment at 6 bar, both of the fine UF membranes showed low anthocyanin rejection, being 60.9 ± 1.0 % and 81.0 ± 0.3 % for GR95PP and XT, respectively. The NF membranes NFG and DK showed superior performance with anthocyanin rejections higher than 98 % and high selectivity for

permeation of TSS with respect to anthocyanins. Based on these results, the membrane NFG was selected for further study since it provided the highest flux and a comparable anthocyanin rejection and selectivity towards permeation of TSS. The study of the resistances showed that for the NF membranes the resistance due to the cake layer (R_c) was predominant over the resistance of the membrane (R_M) and that of the fouling layer (R_F). In all cases, no irreversible fouling was observed and the term R_{IRREV} is thus not included in Table 3. The difference in the distribution of the resistances could be attributed to either the membrane morphology, the material or even a combination of both. On one hand, larger pores in UF could allow for permeation of foulants and blockage in the interior of the pores, while narrow pore distributions in NF could improve foulant rejection thus promoting their accumulation on top of the membrane surface as a cake layer. On the other hand, chemical interactions or between the foulants and the two different membrane materials could also play a role on the differences in resistance observed. Analyses of fouled membranes would be needed to assess whether the foulants were different for the two membrane materials tested.

Table 3: Performance of Ultra and Nanofiltration membranes for color enrichment operated at 6 bar

Membrane	MWCO (Da)	Flux (L m^{-2} h^{-1})	R_{MAC} (%)	$S_{TSS/MAC}$ (%)	R_M (%)	R_C (%)	R_F (%)
GR95PP	2000	14.5 ± 0.7	60.9 ± 1.0	2.2	35.8	40.8	17.4
XT	1000	11.2 ± 0.3	81.0 ± 0.3	5.2	13.8	65.5	20.6
NFG	600-800	10.3 ± 0.9	98.9 ± 0.0	56.4	9.7	81.7	7.2
DK	150-300	5.8 ± 1.0	99.1 ±0.1	42.1	6.9	89.5	3.0

The permeate flux for the membrane NFG at different pressures s is shown in Figure 1A). A linear increase in the flux is observed between 3 – 8 bar that at higher pressures converges into a limiting flux of ~18 L m^{-2} h^{-1}. As shown in Figure 1B, the rejection of anthocyanins is acceptably high throughout the whole pressure range, while the rejection of TSS experiences an increase with applied pressure. In order to reach a compromise between high permeability and low TSS rejection, a moderate pressure of 5 bar was selected for further testing.

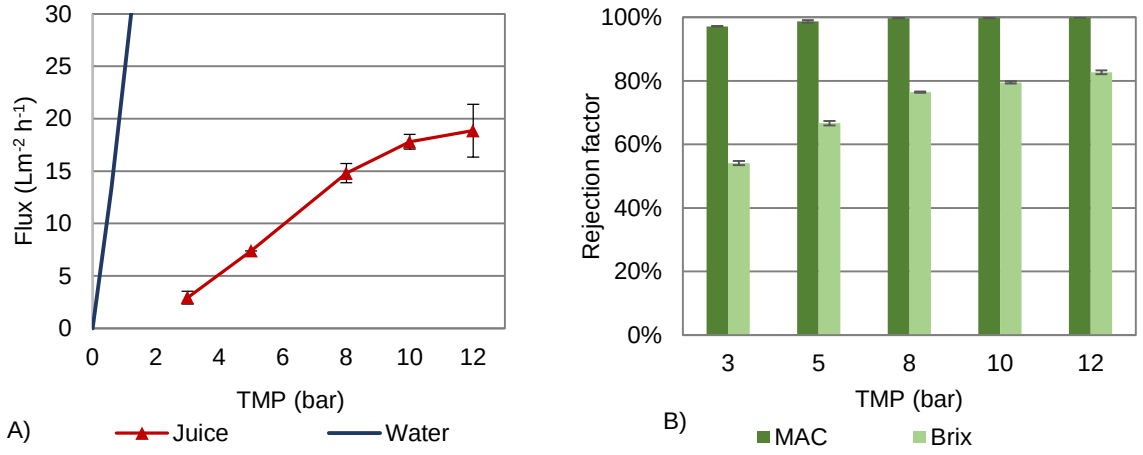

Figure 1: A) Flux and B) Rejection of anthocyanin (MAC) and impurities (Brix) for the membrane NFG at different pressures

Figure 2 A) shows the permeate flux for the experiment run in concentration mode for 300 min, where the volume concentration factor (VCF) reached was ~1.6. The pure water flux is also shown for orientation. The flux decline follows the driving force decay due to the increasing osmotic pressure difference across the membrane as a consequence of the concentration process. Following the heuristic assumption that the juice behaved osmotically as a pure sucrose solution, the maximum achievable VCR for the separation at 5 bar would be 1.93, and higher pressures would be needed to overcome this barrier. This would come at the cost of decreased separation selectivity and higher energy consumption. The concentrations of MAC and TSS on the retentate side are shown in Figure 2B. Anthocyanins were concentrated from 0.75 to 1.54 g L^{-1} while the Brix in the feed increased only from 6.6 to 10.4. The loss of anthocyanins associated with the process was ~3 %. The characteristics of feed and product can be seen in Table 1. During NF, the spectroscopic properties of the juice were affected very marginally as it can be seen from the moderate increase of the brown and violet indexes.

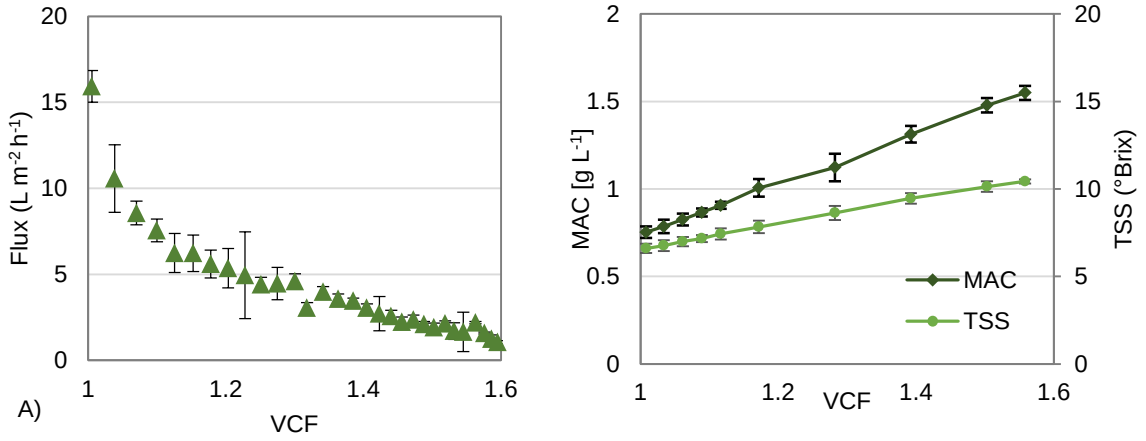

Figure 2: A) Permeate flux and B) anthocyanin concentration (MAC) and total soluble solids (Brix) during the nanofiltration

3.3 Concentration by forward osmosis (FO)

Compared to other common draw agents, $MgCl_2$ has a low specific cost, defined as the cost of solute needed to produce 1 L of draw with a given osmotic pressure; and moderate replenishing cost, accounting for the losses of solute both via FO reverse salt flux and RO salt permeation during draw recovery (Achilli et al., 2010). Furthermore, $MgCl_2$ is approved for use in foods by the European Food Safety Agency (EFSA, 2019) and as a divalent salt it is expected to exhibit a relatively low reverse salt flux. A draw concentration of 1.1 M was selected to exceed the estimated osmotic pressure of the final concentrate by a factor of 1.2.

Figure 3A) shows the FO flux declining from the initial value of 9.9 L m^{-2} h^{-1} down to 1.5 L m^{-2} h^{-1} for a VCF of 5.1. Figure 5B) shows the increase in concentration of both MAC and TSS associated with the volume reduction process. The characteristics of the FO product are also shown in Table 1. The anthocyanin losses associated with the concentration experiment were ~12 %, but are considered acceptable since these may account for losses of product that remained in the system when it was emptied. Permeation of anthocyanins to the draw solution was not observed. In this experiment the pure water flux was fully recovered after membrane cleaning. Table 4 shows a BI factor slightly higher in the FO product as compared to the initial feed and NF product. The VI, on the other hand, experienced a continuous increase during FO which final value was 3-fold that of the initial material. A plausible explanation is the complexation of anthocyanins with Mg^{2+} which could shift the visible absorption maximum towards longer wavelengths even at the pH of the measurement (pH 1.0). This phenomenon has been reported for Al^{3+} and Fe^{3+} (Sigurdson et al., 2017) and to a lesser extent for Mg^{2+} (Sigurdson et al., 2016). Thus, possible interactions of the draw material with the feed should be further studied and taken into consideration.

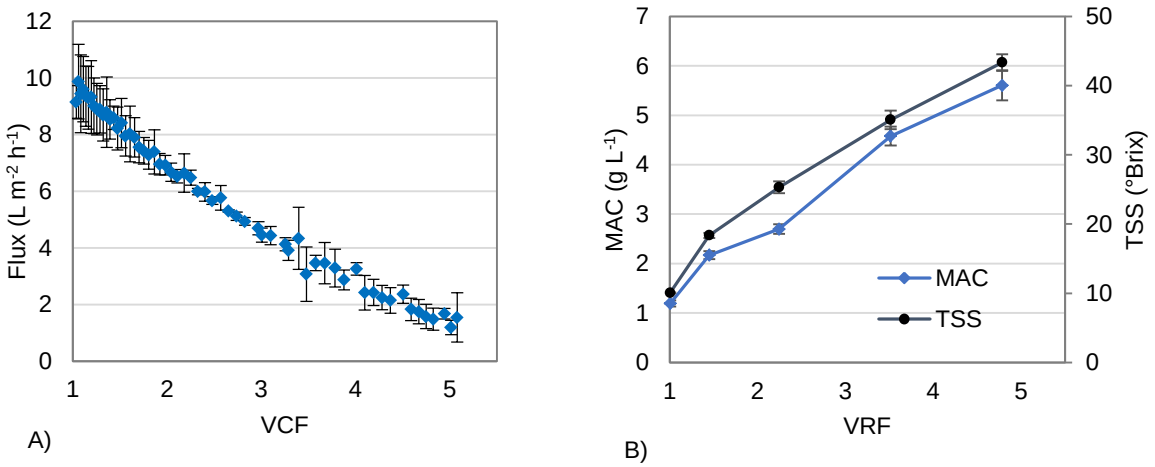

Figure 3: A) Permeate flux and B) anthocyanin concentration (MAC) and total soluble solids (Brix) during forward osmosis

4. Conclusions

Black carrot juice can be processed via a sequence of nanofiltration and forward osmosis. Firstly, taking advantage of the difference in molecular size of acylated anthocyanins (~900 Da) and small impurities (< 200 Da), the juice can be enriched in anthocyanin via nanofiltration at room temperature at a minimal anthocyanin loss (~3 %). The membrane NFG (polyamide, 600-800 Da) had the capability of successfully retaining anthocyanins (R_{MAC} 98%) while partially permeating total soluble solids (R_{Brix} 65 %).
Further concentration by forward osmosis was achieved using 1.1 M $MgCl_2$ as a draw solution. The juice was concentrated from 10.4 to 43.4 Brix. A change in the color properties of the FO product was observed, resulting in an increase of the violet index that could be attributed to metal-anthocyanin interactions with Mg^{2+} due to reverse salt flux. Different flow patterns in industrial scale could have an influence in the separation efficiency and process performance. Therefore, further research in the scalability of the process is recommended.

Acknowledgments

This work was supported by Grønt Udviklings og Demonstrations Program (GUPD) from Miljøstyrelsen in Denmark under the project ProBioFa [J. nr. 34009-18-1358] and the European Union's Horizon 2020 research and innovation program under Marie Skłodowska-Curie [grant agreement No 778168].

References

Achilli A., Cath T.Y., Childress A.E., 2010, Selection of inorganic-based draw solutions for forward osmosis applications. Journal of membrane science, 364(1-2), 233-241.

Arriola N.A., dos Santos G.D., Prudêncio E.S., Vitali L., Petrus J.C.C., Castanho Amboni, R.D., 2014, Potential of nanofiltration for the concentration of bioactive compounds from watermelon juice. International Journal of Food Science & Technology, 49(9), 2052-2060.

Assous M., Abdel-Hady M., Medany G.M., 2014, Evaluation of red pigment extracted from purple carrots and its utilization as antioxidant and natural food colorants. Annals of Agricultural Sciences, 59(1), 1-7.

Barba-Espín G., Glied S., Crocoll C., Dzhanfezova T., Joernsgaard B., Okkels F., Lütken H., Müller R., 2017, Foliar-applied ethephon enhances the content of anthocyanin of black carrot roots (Daucus carota ssp. sativus var. atrorubens Alef.). BMC plant biology, 17(1), 70.

Bhattacharjee C., Saxena V., Dutta S., 2017, Fruit juice processing using membrane technology: A review. Innovative Food Science & Emerging Technologies, 43, 136-153.

Cath T.Y., Childress A.E., Elimelech M., 2006, Forward osmosis: principles, applications, and recent developments. Journal of membrane science, 281(1-2), 70-87.

Cisse M., Vaillant F., Kane A., Ndiaye O., Dornier M., 2012, Impact of the extraction procedure on the kinetics of anthocyanin and colour degradation of roselle extracts during storage. Journal of the Science of Food and Agriculture, 92(6), 1214-1221.

EFSA, 2019, Re-evaluation of hydrochloric acid (E 507), potassium chloride (E 508), calcium chloride (E 509) and magnesium chloride (E 511) as food additives. in: EFSA Journal, Vol. 17, pp. e05751.

Gras C.C., Carle R., Schweiggert R.M., 2015, Determination of anthocyanins from black carrots by UHPLC-PDA after ultrasound-assisted extraction. Journal of Food Composition and Analysis, 44, 170-177.

Jiao B., Cassano A., Drioli E., 2004, Recent advances on membrane processes for the concentration of fruit juices: a review. Journal of food engineering, 63(3), 303-324.

Koros W., Ma Y., Shimidzu T., 1996, Terminology for membranes and membrane processes (IUPAC Recommendations 1996). Pure and Applied Chemistry, 68(7), 1479-1489.

Mohammadifakhr M., de Grooth J., Roesink H.D., Kemperman A.J., 2020, Forward Osmosis: A Critical Review. Processes, 8(4), 404.

Roda-Serrat M.C., Christensen L.P., Christensen K.V., Fretté X., 2015, Optimization of removal of D-trehalose from commercial food colorant by diafiltration with ultrafiltration membranes. Chemical Engineering Transactions, 43, 1027-1032.

Sigurdson G., Robbins R., Collins T., Giusti M., 2016, Evaluating the role of metal ions in the bathochromic and hyperchromic responses of cyanidin derivatives in acidic and alkaline pH. Food Chemistry, 208, 26-34.

Sigurdson G., Robbins R., Collins T., Giusti, M., 2017, Spectral and colorimetric characteristics of metal chelates of acylated cyanidin derivatives. Food chemistry, 221, 1088-1095.

Wenten I., Khoiruddin K., Reynard R., Lugito G., Julian H., 2020, Advancement of forward osmosis (FO) membrane for fruit juice concentration. Journal of Food Engineering, 110216.