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Research Article

Anthocyanin Retention of Cranberry *(Vaccinium macrocarpon)* Juice Subjected to Different Nanofiltration Conditions

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The aim of this work was to evaluate the retention of anthocyanin during a nanofiltration process of cranberry juice. Nanofiltration membranes, HC-50P DDS with an effective area of 0.36 m^2 in a plate/frame nanofilter system, DDS Lab Module, were used for the experiments. Juice feed flow rate varied from 1.0 to 12.0 L min^{-1} at transmembrane pressures between 20 and 40 bar (2026 and 4052 kPa). Permeate flux reached a maximum value of 41.3 L h^{-1} m^{-2} at a pressure of 40 bar and a feed rate of 12 L min^{-1}, showing a direct dependency on these two parameters. Retention coefficients of anthocyanin of 0.94 to 0.99 corresponding to percentage recovery between 93 and 99% were obtained. Total anthocyanin content increased to values between 237 and 287 mg L^{-1} from original concentration of 82 to 97 mg L^{-1} in feed solution. Total soluble solids were also retained on the nanofilter. Both anthocyanin retentate and permeate obtained by nanofiltration could be potential functional ingredients for the food and nutraceutical industry.

1. Introduction

The use of natural colorants in the food industry is actually not widespread for numerous reasons. The different food pigments have showed a weak stability due to oxidation, hydrolysis, and/or polymerization phenomena, together with the complexity of the diverse purification methods as well as during thermal processing techniques (drying, freezing, etc.). Anthocyanin is a natural colorant that is widely distributed in nature; however, the coloration it imparts to foods is determined by the pH value of the medium, with a consequent restriction of its use to foods that have a pH value below 3.5 if a reddish tone is to be achieved [1, 2]. Efforts to extract anthocyanin from natural sources and to use it with reasonable efficiency as a food colorant have not ceased till now. Rodríguez and Wrolstad [3] pointed out that the polar character of the anthocyanin molecules allows its extraction with solvents such as alcohol, acetone, and water.

Most extraction methods are based on criteria like maximum purity, low degradation, or least alteration of the natural molecular structures, such that extraction methods using methanol or a mixture of acetone and chloroform are quite common. There is nowadays some reluctance to use organic solvents, so membrane technology that has been applied for more than four decades is gaining ground in newer methods to extract natural colorants. Lee et al. [4] reported on concentration techniques using ultrafiltration and reverse osmosis applicable on different liquid foods, like egg, maple juice, fruit, and vegetable juices as well as plant pigments that include anthocyanin. Chung et al. [5] tried an extraction of anthocyanin from the leaves of *Perilla ocymoides* using a solution of 10% citric acid, followed by an ultrafiltration process, and achieved a 60% recovery of the anthocyanin pigments. Woo et al. [6] also studied a similar extraction of anthocyanin from pressed cake of cranberry *(Vaccinium macrocarpon)*, obtained as a by-product from juice production. They used acidified alcohol for the extraction and ultrafiltration for the purification process and finally reverse osmosis was applied to concentrate the anthocyanin extract.

Application of ultrafiltration, nanofiltration, and likewise reverse osmosis to obtain anthocyanin concentrate of high

quality for use as food colorants was also studied by Gil-Martínez [7] on pomegranate *(Punica granatum)* juice at different operating pressures and flow rates. It was found that the application of ultrafiltration technology followed by a reverse osmosis process would leave in the retentate 75% of the original anthocyanin content. The effects of different types of membranes on concentration process of apple and pear juices by nanofiltration were studied by Warczok et al. [8] in batch systems at pressures between 800 and 1200 kPa and temperatures between 25 and 35°C, showing that this technology was useful in juice processing. Effects of operating temperature and pressure on nanofiltration membrane were also studied by Ferrarini et al. [9] while comparing membranes for nanofiltration and reverse osmosis of grape juice. They found that permeability of the nanofiltration membrane would double if pressure increases from 3242 to 4559 kPa, and a linear relationship was established between flow rate of permeate and transmembrane pressure. Flow rate of permeate would increase by 3% for each degree rise in temperature. Rektor et al. [10] applied microfiltration and reverse osmosis to concentrate wine must. As process parameter, retention of anthocyanin was considered. They operated a reverse osmosis process at 5065 kPa, 35°C, and flow rate of 300 L h^{-1} and found a very low content of anthocyanin in the permeate, but a very high retention of anthocyanin of 99.5% on the membrane used. Therefore, following the step of previous works on application of membrane technology for pigment extraction, the main objective of this study was to find out how transmembrane pressure and juice feed flow affect the retention capacity of a nanofiltration membrane and the permeate flux during the concentration of a cranberry juice.

2. Materials and Methods

2.1. Cranberry Juice. A concentrate of cranberry *(Vaccinium macrocarpon)* juice with soluble solids content of 49.8°brix, titrable acids content of 11.18% expressed as citric acid, and pH value of 2.53 was obtained from a cranberry processing plant, Agrícola Cran Chile, Chile. For the nanofiltration experiments the juice was diluted with deionized water to an initial concentration of 12.5°brix and maintained at the operating temperature of 20°C.

2.2. Nanofiltration Process. Nanofiltration equipment (De Danske Sukker Fabrikker, DDS Lab, module 20-0.36 Lab, Copenhagen, Denmark) with 20 flat membranes (HC-50P DDS) and a total filtration area of 0.36 m^2 was used in a batch processing with recirculation of the retentate. As specified by the manufacturer the membranes had a permeability to 40–60% NaCl, rejecting organic molecules with molecular weights greater than 400 dalton or particles in the approximate size range of 1 nm. They withstand pH values between 2 and 10, operating temperature between 0 and 60°C, and pressure between 101.3 and 6078 kPa (1 to 60 bar), respectively, 1 nanometer.

During each filtration cycle, samples of permeate and retentate were taken at time intervals of 20 min, and kept at 0°C in an ice-water bath until analysis. Permeate volume was monitored, while feed rate of juice was maintained constant and adjusted to values between 1 and 12 L min^{-1} by a three-piston, positive displacement pump (Rannie, Ranniepumpe 16.50, Copenhagen, Denmark). Operating temperature was kept at 20°C by a water bath provided by a circulator (High Technology, Heto, Circulator RC 2500 N, Scandinavia). Filtration cycle was stopped when a threefold concentration of feed juice was reached, after which the membrane filters were washed with deionized water until initial permeability was achieved again. Washing procedure with deionized water and solutions A (0.5% NaOH and 0.5% EDTA; pH 12.6), B (0.3% HNO3; pH 2.3), and C (1.0% NaOH; pH 12.9) is described in Table 1. Permeate flux was calculated in L h^{-1} m^{-2} from the volume of permeate with respect to filtration time and filtration surface area.

Table 1: Scheme for the washing procedures of the nanofiltration membranes.

Rinsing step	Washing liquid	Temperature (°C)	Duration (min)	Initial pressure (bar)
1	Deionized water	40	15	4
2	Solution A	75	60	4
3	Deionized water	40	10	4
4	Solution B	55	20	4
5	Deionized water	55	10	4
6	Solution C	75	30	4
7	Deionized water	30	10	4

2.3. Statistical Analysis. The effects of two parameters, operating transmembrane pressure, and feed rate on permeate flux and retention capacity of anthocyanin on the membranes was studied. A factorial design of 3^2 was applied with three replicates each time. The three pressure levels were 20, 30, and 40 bar (2026, 3039, and 4052 kPa), while the three feed rate levels were 1, 6, and 12 L min^{-1}. Contents of total anthocyanin, soluble solids, and titrable acids expressed as citric acid, as well as pH values in permeate and retentate, were determined in triplicate for all samples taken every 20 minutes during each filtration cycle. Statgraphics Plus® 5.1 (Statistical Graphics Corp., Herndon, VA, USA) was used for the analysis of variance. Differences between the media were analysed using the least significant difference test with a significance level of $\alpha = 0.05$ and a confidence interval of 95% ($p < 0.05$). In addition, the multiple range test was used to demonstrate the existence of homogeneous groups.

2.4. Analysis of Juice Samples. The feed juice as well as the filtration products was characterized for their anthocyanin content, soluble solids, titrable acids, and pH value. Soluble solids were determined by an Abbé refractometer and expressed in °brix. Titrable acids expressed as percent citric acid was determined using a glass electrode according to procedures described in the AOAC Official method 942.15 for fruit products [11]. Density was determined using a pycnometer

of known volume and analytical balance (Mettler Toledo, XS205-DU, Zürich, Switzerland). Total anthocyanin content was determined by the pH differential method described by Wrolstad [12]. Reference cyanidine-3-galactoside, with molecular weight 445.2 g/mole and molar absorbance of 46230, was used. Absorbance was determined by a spectrophotometer (Spectronic Instruments, Genesys 5, USA).

2.5. Definitions of Parameters Used in Evaluation of Nanofiltration Process. The model used is that described by Cheryan [13], where it is assumed that in a batch system the probability of a particle going through the membrane remains constant throughout the process. Therefore, a concentration factor F_C can be defined as follows:

$$F_C = \frac{V_o}{V_R}. \quad (1)$$

Under the assumption that the probable magnitude of error in measurement is proportional to magnitude of observations and the probability of a particle going through the membrane remains constant throughout the process, a coefficient of retention, R, can be defined in terms of solute concentrations as given in (2) that resulted from a mass balance to a given solute and to the definition of R and F_C.

$$C_R = C_o F_C{}^R. \quad (2)$$

From (2) it can be seen that concentration of the solute at any time is given by a reduction in volume, which responds to a coefficient of retention R that is equal to (Cretentate − Cpermeate)/Cretentate as determined independently to (2). This implies that if the probability of a solute passing through the membrane is 100%, no solute will be retained on the membrane (Cretentate = Cpermeate), coefficient of retention will be zero, and solute concentration in the retentate will remain equal to that of the initial feed solution. On the other hand, if probability that no solute passing through the membrane is zero (Cpermeate = 0), coefficient of retention will be equal to 1.0 and solute concentration in retentate will increase accordingly.

From (1) and (2) percentage recovery Y of anthocyanin can be defined and expressed as a function of the concentration factor and coefficient of retention as given in (3).

$$Y = \frac{C_R V_R}{C_o V_o} \times 100 = 100 F_C{}^{R-1}. \quad (3)$$

3. Results and Discussion

3.1. Juice Analysis. In Table 2 results of the juice analysis on samples taken during nanofiltration process at a concentration factor F_C of 3, for all the experiments carried out in triplicate, are summarized. As can be observed, in all filtration cycles soluble solids, titrable acids, and anthocyanins were retained on the membrane. The pH values and the densities of the feed solutions, the permeates, and the retentates did not differ significantly from each other at $p < 0.05$, since it was only the case of low changes in concentration of weak organic acids. However, effects of the nanofiltration process can be observed in the concentrations of soluble solids, titrable acids, and total anthocyanin. Soluble solids, as determined by refractometry, increased in the retentates, where they reached more than twice the concentration found in the feed solutions. Soluble solids content in permeate was reduced to about 60%. Similar results were also observed in the case of the titrable acids; concentration increased in retentate and decreased in permeate to the same extent. As for anthocyanin, concentration in retentate almost tripled that of the feed solution, while only small quantities of anthocyanin passed through to permeate. Concentrations of total anthocyanin in permeate and retentate are significantly different at $p < 0.05$, and the filtration effects have been expressed as coefficient of retention and percentage recovery.

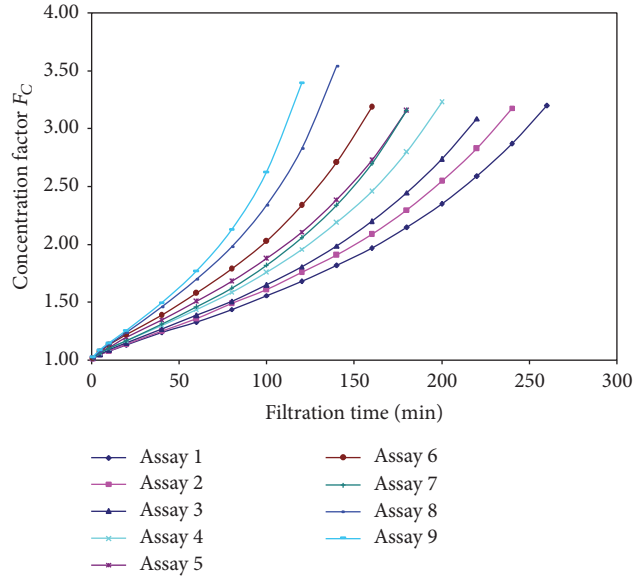

Figure 1: Variation of average concentration factor during nanofiltration process.

3.2. Retention and Recovery of Anthocyanin. Retention capacity of the nanofilter was evaluated throughout the filtration process, by calculating the retention coefficient, R, as given in (2). Concentration factor F_C was also calculated according to (1) at regular time intervals. In Figure 1 the variation of the average concentration factor for all the assays is illustrated. As can be observed at higher transmembrane pressures a concentration factor of 3 is reached in less time. At 40 bar this value is reached in less than 120 minutes, while at 20 bar it took more than 240 minutes. Increase in feed flow rate would also reduce the time taken to reach a threefold concentration of anthocyanin in the retentate (Table 3).

Permeate flux would also decrease during the nanofiltration process as shown in Figure 2; at higher transmembrane pressures the initial permeate flux would be higher. It varied from 20.48 L h^{-1} m^{-2} at 20 bar to 41.31 L h^{-1} m^{-2} at 40 bar. At higher transmembrane pressures, the minimum permeate flux is reached in less time, at 40 bar permeate flux dropped to a value near to 7 L h^{-1} m^{-2} in less than 120 minutes, while it reached a value near to 3 after more than 240 min at 20 bar.

Table 2: Juice analysis of feed, permeate, and retentate at transmembrane pressures of 20, 30, and 40 bar and feed rates of 1, 6, and 12 L min^{-1}.

Analysis	FR	TMP								
		Feed			Permeate			Retentate		
		20 bar	30 bar	40 bar	20 bar	30 bar	40 bar	20 bar	30 bar	40 bar
pH	1	2.7 ± 0.0^{aA}	2.6 ± 0.1^{abA}	2.5 ± 0.0^{bA}	2.9 ± 0.1^{xX}	2.8 ± 0.0^{xX}	2.9 ± 0.1^{xX}	2.6 ± 0.0^{pP}	2.6 ± 0.0^{pP}	2.6 ± 0.0^{pP}
	6	2.6 ± 0.1^{aA}	2.7 ± 0.1^{aA}	2.7 ± 0.1^{aB}	2.8 ± 0.1^{xX}	2.8 ± 0.1^{xX}	2.8 ± 0.0^{xX}	2.6 ± 0.0^{pP}	2.5 ± 0.0^{qP}	2.6 ± 0.0^{pP}
	12	2.6 ± 0.1^{aA}	2.7 ± 0.1^{aA}	2.6 ± 0.1^{aAB}	2.8 ± 0.0^{xX}	2.9 ± 0.0^{xX}	2.8 ± 0.1^{xX}	2.6 ± 0.0^{pP}	2.6 ± 0.1^{pP}	2.5 ± 0.0^{pQ}
ρ	1	1.02 ± 0.03^{aA}	1.01 ± 0.04^{aA}	0.97 ± 0.01^{aA}	1.04 ± 0.01^{xXY}	1.04 ± 0.02^{xX}	1.03 ± 0.01^{xX}	1.06 ± 0.02^{pP}	1.07 ± 0.02^{pP}	1.05 ± 0.02^{pP}
	6	0.99 ± 0.01^{aA}	1.03 ± 0.03^{aA}	1.01 ± 0.03^{aA}	1.03 ± 0.01^{xX}	1.04 ± 0.02^{xX}	1.03 ± 0.01^{xX}	1.04 ± 0.02^{pP}	1.06 ± 0.02^{pP}	1.05 ± 0.01^{pP}
	12	1.04 ± 0.01^{aA}	0.98 ± 0.01^{aA}	1.09 ± 0.01^{aA}	1.05 ± 0.01^{xY}	1.04 ± 0.01^{xX}	1.08 ± 0.02^{yY}	1.07 ± 0.01^{pP}	1.06 ± 0.01^{pP}	1.11 ± 0.03^{qQ}
SS	1	12.5 ± 0.0^{aA}	12.5 ± 0.0^{aA}	12.5 ± 0.0^{aA}	7.0 ± 0.5^{xXY}	7.5 ± 0.0^{xX}	7.0 ± 0.9^{xX}	29.5 ± 1.0^{pP}	27.5 ± 0.0^{qP}	29.0 ± 1.3^{pqP}
	6	12.5 ± 0.0^{aA}	12.5 ± 0.0^{aA}	12.5 ± 0.0^{aA}	7.5 ± 0.0^{xY}	7.0 ± 0.5^{xX}	7.0 ± 0.0^{xX}	27.5 ± 0.9^{pQ}	30.0 ± 0.9^{qQ}	29.0 ± 0.5^{pP}
	12	12.5 ± 0.0^{aA}	12.5 ± 0.0^{aA}	12.5 ± 0.0^{aA}	6.5 ± 0.5^{xX}	7.5 ± 0.0^{xX}	6.5 ± 0.9^{xX}	28.0 ± 1.0^{pPQ}	29.5 ± 0.5^{pQ}	29.0 ± 1.3^{pP}
TA	1	2.8 ± 0.8^{aA}	2.8 ± 0.4^{aA}	3.4 ± 0.5^{aA}	1.8 ± 0.2^{xX}	1.7 ± 0.1^{xX}	1.8 ± 0.1^{xX}	4.8 ± 0.3^{pP}	4.7 ± 0.3^{pP}	5.1 ± 0.3^{pP}
	6	2.9 ± 0.6^{aA}	3.0 ± 0.8^{aA}	2.8 ± 0.6^{aA}	1.6 ± 0.1^{xXY}	1.8 ± 0.1^{xXY}	1.5 ± 0.1^{xY}	5.2 ± 0.1^{pP}	5.0 ± 0.4^{pP}	5.0 ± 0.2^{pP}
	12	2.4 ± 0.5^{aA}	2.8 ± 0.5^{aA}	2.6 ± 0.5^{aA}	1.5 ± 0.0^{xY}	1.9 ± 0.1^{yY}	1.7 ± 0.1^{zX}	4.9 ± 0.2^{pP}	4.8 ± 0.3^{pP}	4.9 ± 0.1^{pP}
ACN	1	82 ± 4^{aA}	97 ± 2^{bA}	93 ± 3^{bA}	5 ± 1^{xX}	1 ± 0^{yX}	3 ± 1^{zX}	237 ± 14^{pP}	288 ± 15^{qP}	274 ± 14^{qP}
	6	85 ± 2^{aAB}	97 ± 1^{bA}	88 ± 2^{aAB}	1 ± 0^{xY}	4 ± 1^{yY}	2 ± 0^{xX}	254 ± 6^{pPQ}	284 ± 23^{pP}	259 ± 16^{pPQ}
	12	90 ± 3^{abB}	92 ± 4^{aA}	83 ± 5^{bB}	2 ± 1^{xY}	4 ± 0^{yY}	8 ± 1^{zY}	267 ± 14^{pqQ}	271 ± 24^{qP}	233 ± 16^{pQ}

TMP: transmembrane pressure; FR: feed rate (L min^{-1}); SS: soluble solids (°brix); TA: titrable acids expressed as citric acid (%); ACN: total anthocyanin (mg L^{-1}); ρ: density (g mL^{-1}). Values in the same column having the same uppercased letter for each parameter are not significantly different at a confidence level of 95%. Values in the same row having the same lowercased letter for each parameter are not significantly different at a confidence level of 95%.

Table 3: Filtration time and permeate flux at different transmembrane pressures and feed flow rates.

Assay	Transmembrane pressure bar	Feed flow rate L min^{-1}	Filtration time at $F_C \approx 1$ min	Maximum permeate flux at $F_C \approx 1$ L h^{-1} m^{-2}	Filtration time at $F_C \approx 3$ min	Minimum permeate flux at $F_C \approx 3$ L h^{-1} m^{-2}
1	20	1	1	20.48	260	3.05
2	20	6	1	24.44	240	3.24
3	20	12	1	26.96	220	3.48
4	30	1	1	24.97	200	3.95
5	30	6	1	31.62	180	4.13
6	30	12	1	36.30	160	4.64
7	40	1	1	25.25	180	4.52
8	40	6	1	37.78	140	5.83
9	40	12	1	41.31	120	7.11

Table 4: Retention coefficient and percentage recovery of anthocyanin on nanofiltration membrane HC-50P DDS at TMP of 20, 30, and 40 bar and feed flow rates of 1, 6, and 12 L min^{-1}.

	Retention of total anthocyanin								
	TMP 20 bar			TMP 30 bar			TMP 40 bar		
Feed rate, FR (L min^{-1})	1	6	12	1	6	12	1	6	12
Retention coefficient, R	0.94	0.99	0.98	0.99	0.96	0.98	0.97	0.98	0.90
Recovery, Y (%)	96	99	99	99	98	98	98	98	93

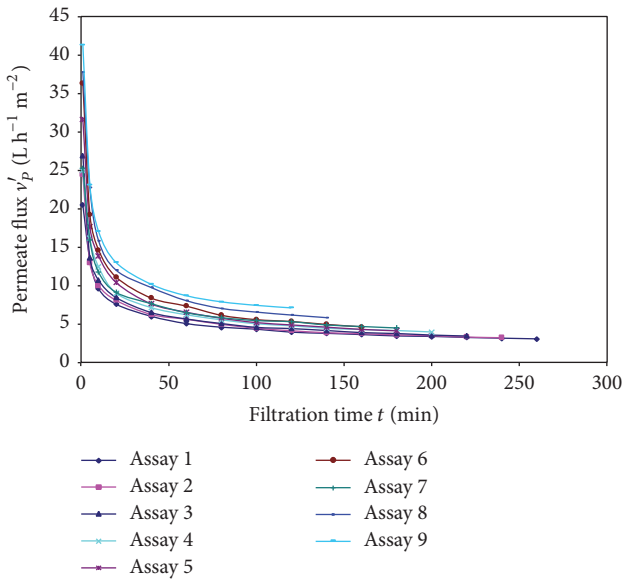

Figure 2: Variation of average permeate flux during nanofiltration process.

The initial value of 41.31 L h^{-1} m^{-2} was obtained at a feed flow rate of 12 L min^{-1} at a concentration factor F_C of 3.40. Under that condition of operation, foam formation was noticeable and caused disturbance in flow regulation and control, setting thus an upper pressure limit to the nanofiltration process.

A lower retention coefficient of 0.94 and a lower percentage recovery of anthocyanin of only 93% were also found at that point, as shown in Table 4, where it can be seen that in the other experiments coefficient of retention is above 0.97, even reaching values of 0.99 with percentage recovery between 96 and 99%. This shows that the nanofilter HC-50P used has a very high capacity to retain anthocyanin and can be useful for any purpose to concentrate that pigment. This value is comparable to that obtained by Rektor et al. [10], where in a process of reverse osmosis a recovery of 99.5% of the anthocyanin from grape juice was reported. Nanofiltration process could have the advantage of being cheaper with respect to energy consumption.

Neither transmembrane pressure nor feed flow rate appeared to interfere in the retention capacity of the nanofilter. An analysis of variance for coefficient of retention showed that neither transmembrane pressure nor feed flow rate gives significantly different retention coefficients at $p < 0.05$. There was also no interaction between these two parameters, which, however, do have a significant effect at $p < 0.05$ on the permeate flux, as to be expected. D'Souza and Wiley [14] also reported the effects of these two parameters on an ultrafiltration process of whey. According to this study, transmembrane pressures over 30 bar but lower than 40 bar at feed flow rate between 6 and 12 L min^{-1} could be used in the nanofiltration of anthocyanin.

As to the behavior of the nanofiltration process itself, it can be seen in Figure 3 that permeate flux (v') would follow a monotonous decrease during the process conducted up to a concentration factor of 3. At all transmembrane pressures for all feed flow rates the same type of variation of the permeate flux can be observed. Logarithmic functions can be used to describe the decrease of permeate flux. Similarity of the gradients (around −0,45) showed that mass transfer would not be a strong function of the feed flowrate and concentration polarization at the upstream membrane/solution interface should be reduced [15]. High coefficients of determination above 0.96 were obtained for the experiments at 20 and 30 bar. At 40 bar a lower coefficient of determination of 0.86 was

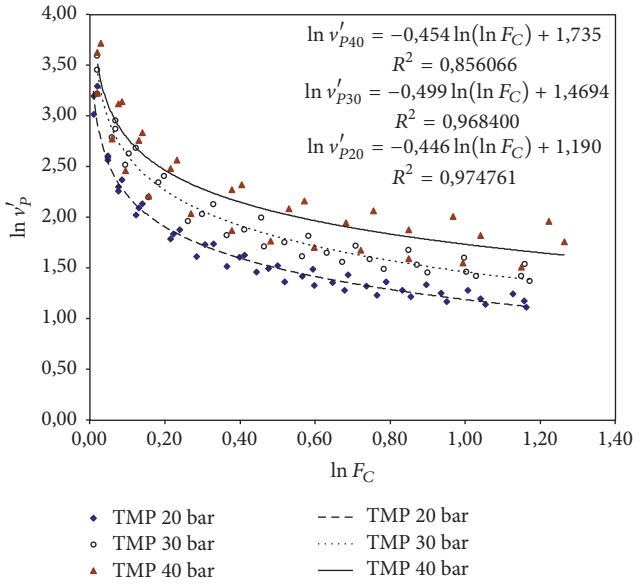

Figure 3: Variation of permeate flux during nanofiltration process at transmembrane pressures of 20, 30, and 40 bar.

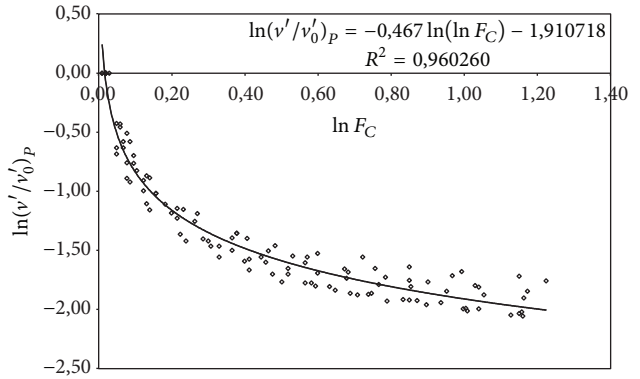

Figure 4: Relative permeate flux as a normalized function of concentration factor at transmembrane pressures between 20 and 40 bar and feed flow rates between 1 and 12 L min^{-1}.

obtained, probably due to the formation of foam at a feed flow of 12 L min^{-1}, which caused deviation from the normal course of the nanofiltration process.

In Figure 4 the permeate flux was normalized with respect to initial permeate flux (v'_0) for all transmembrane pressures and feed flow rates. It can be observed that the normalized function, as shown in Figure 4, had a high coefficient of determination above 0.96, which shows that the nanofiltration process has a typical behavior, where filter resistance would increase as the filter cake thickness increased. Transmembrane pressure and feed flow rate would not interfere in the formation of the filter cake; they would only influence the net filtration time.

4. Conclusions

Nanofiltration process of a cranberry juice has a significant effect on soluble solids, titrable acids, and total anthocyanin contents. The experiments showed that a nanofiltration process can be applied in the concentration of natural pigments, like anthocyanin. High retention coefficients near to 0.99 and high percentage recovery of 99% can be achieved at transmembrane pressures between 30 and 40 bar and feed flow rate between 6 and 12 L min^{-1}. A nanofilter that rejects organic molecules with molecular weights greater than 400 dalton or particles in the approximate size range of 1 nm has a very high capacity to retain anthocyanin and can be useful to concentrate that pigment. Transmembrane pressure and feed flow rate would affect filtration time, but not the retention capacity of the nanofilter. Within the studied range of transmembrane pressures and feed flows, concentration polarization would probably be reduced due to the high degree of turbulence at the upstream membrane/solution interface. Nanofiltration process can be more advantageous in comparison to reverse osmosis.

Nomenclature

C_0: Concentration of a solute in volume V_0 in mg L^{-1}
C_R: Concentration of a solute in volume V_R in mg L^{-1}
F_C: Concentration factor
R: Coefficient of retention
V_0: Volume of feed at time $t = 0$ in L
V_R: Volume of retentate at time t in L
Y: Recovery factor of anthocyanin
v'_P: Permeate flux at time t in L h^{-1} m^{-2}.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper as well as with received funding.

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