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1 **Coupling of pressure-driven membrane technologies for concentrating, purifying and**
2 **fractionizing betacyanins in cactus pear (*Opuntia dillenii* Haw.) juice**

3

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13

14 **Abstract**

15

16 An integrated process coupling crossflow micro and ultra or nanofiltration was applied to
17 separate the betacyanins in cactus pear juice (30°C). Four microfiltration ceramic membranes
18 (0.1-0.2 µm, 1.8-3.3 bar) and 4 ultra/nanofiltration organic membranes (0.2-4.0 kDa, 5-30
19 bar) were tested. **Microfiltration** was a first step to remove insoluble solids with low
20 retention of soluble solids. By coupling with enzymatic liquefaction, permeate flux J_p was
21 increased by 2 and the retention of betacyanins was limited. **Ultra/nanofiltration** was then
22 used for solute separation. Retentions of solutes could be modulated by varying
23 membrane/pressure combinations that favor rather the concentration of all the solutes or
24 rather the purification of the betacyanins with respect to the total dry matter. Retention of
25 individual betacyanins could be a little different which also made possible fractionation.
26 **Simulations** using simple models allowed to evaluate the interest of the process for
27 concentrating, purifying and fractionating betacyanins with a possible diafiltration step.

28

29 **Keywords:** *Opuntia dillenii*; betacyanins; concentration; purification; fractionation;
30 microfiltration; ultrafiltration; nanofiltration

31

32 **Industrial relevance**

33 Betacyanins are natural colorants that can be obtained from cactus pear juice, a crop of
34 increasing interest for its agricultural potential in sahelian regions. The aim of this study was
35 to evaluate a new integrated process based on membrane separation allowing to concentrate or
36 separate betacyanins from other solutes at low temperature and with a limited environmental
37 impact. This process associates a first step to clarify the cactus pear juice by microfiltration
38 after enzymatic liquefaction and a second step to concentrate or purify betacyanins by ultra or
39 nanofiltration. By choosing different membrane / transmembrane pressure combinations in
40 the 2nd step, solute retentions could be modulated in order to favour rather the concentration
41 of all solutes or rather the separation of betacyanins from total soluble solids or even rather
42 the fractionation of betacyanins themselves.

43

44 **Highlights**

- 45 - Coupling micro and ultra/nanofiltration to separate betacyanins in cactus pear juice
- 46 - Microfiltration with enzymatic liquefaction for clarifying with low solute retention
- 47 - Ultra/nanofiltration for concentrating or purifying betacyanins
- 48 - Separation pattern modulated thanks to different membrane/pressure combinations

49 **Notation**

50

51 C_i concentration of compound i (g kg^{-1})

52 CF_i concentration factor of compound i

53 DV diavolume (or dilution ratio)

54 J_p permeate flux ($\text{kg h}^{-1} \text{m}^{-2}$)

55 MRR mass reduction ratio

56 MWCO molecular weight cut-off

57 R_i retention of compound i

58 $SF_{i/j}$ separation factor of compound i from compound j

59 TDM total dry matter (g kg^{-1})

60 TMP transmembrane pressure (bar)

61

62 β_c total betacyanins

63 β_n betanin

64 $i\beta_n$ isobetanin

65 $n\beta_n$ neobetanin

66

67 **1 Introduction**

68

69 Natural products with beneficial effects on human health, used as colorants and antioxidants,
70 have attracted a lot of attention. Recently, there is a large amount of natural colorants,
71 extracted from various natural raw materials, rich in compounds such as anthocyanins,
72 carotenoids, chlorophylls and betalains. Betalains are water-soluble vacuolar chromoalkaloids
73 found in plants of the order Caryophyllales as well as in some Basidiomycetes (Azeredo,
74 2009; Herbach, Stintzing, & Carle, 2006; Stintzing & Carle, 2004).

75 Structurally, betacyanins are characterized by a cyclo-Dopa structure with additional
76 substitutions through varying glycosylation and acylation patterns at C₅ or C₆ while
77 betaxanthins are condensation products of betalamic acid and various amino compounds.
78 Betacyanins can be classified according to their chemical structures into 4 types: betanin-type,
79 amaranthine-type, gomphrenin-type and bougainvillea-type (Cai, Sun, & Corke, 2005;
80 Stintzing, et al., 2004). The sources of betanin used for food-colouring contain, amongst other
81 substances, a mixture of betanin and its epimer isobetanin (Gonçalves, et al., 2012).

82 Betalains have a number of health properties. Infusions of betalains from the bracts of
83 Bougainvillea mixed with honey, for example, are used to treat coughs in some regions of
84 Mexico (Heinrich, 2003). Certain anticancer, antiviral, antibacterial and antioxidant activities
85 has been attributed to betalains (Cejudo-Bastante, Chaalal, Louaileche, Parrado, & Heredia,
86 2014; Hilou, Nacoulma, & Guiguemde, 2006). They are widely used as natural red food
87 colorant as well as potential antioxidant (Chauhan et al. 2012). Betalains are less commonly
88 used than anthocyanins and carotenoids, although these water-soluble pigments, stable
89 between pH 3 and 7, are well suited for coloring low-acid food (Neelwarne, 2012).

90 The main source of betalains, especially betanins, is the beet root (*Beta vulgaris*), classified as
91 additive E-162 (EU) and 73.40 (FDA, USA), mainly used to color foods, such as dairy
92 products, confectionery, ice cream, desserts, drinks and sausages (Obón, Castellar, Alacid, &
93 Fernández-López, 2009). Nevertheless, the preparations obtained from this root have
94 undesirable earthy flavors (R. Castellar, Obón, Alacid, & Fernández-López, 2003) and the
95 presence of high concentrations of labile betaxanthins limits their use as a food coloring
96 (Calvo & Salvador, 2000; Nemzer, et al., 2011).

97 The purple cactus pear is an interesting alternative as a source of betacyanins for the
98 production of food colouring (R. Castellar, et al., 2003; Delgado-Vargas, Jiménez, & Paredes-

99 López, 2000; Saénz, Tapia, Chávez, & Robert, 2009). Recently, the study of cactus pear has
100 become increasingly important because of its high content of betalains and other phenolic
101 compounds (Cejudo-Bastante, et al., 2014). The cactus pear fruits are characterized by various
102 colors due to the combination of two betalain pigments, purple betanin and yellow-orange
103 indicaxantin (Fernandez-Lopez & Almela, 2001). Cactus pear (*Opuntia sp*) is classified as a
104 spey with high hydric stress tolerance, its cultivation is adequate in arid and semi-arid
105 regions and for poor soils. Research has also indicated that the cactus pear has anti-
106 inflammatory, diuretic, antispasmodic activity (Ballero, Poli, Sacchetti, & Loi, 2001; Loi,
107 Poli, Sacchetti, Senu, & Ballero, 2004; Palmese, Manganelli, & Tomei, 2001). Cactus pear
108 fruit is a source of nutrients and vitamins (Sawaya, Khatchadourian, Safi, & Al-Muhammad,
109 1983; Teles, Stull, Brown, & Whiting, 1984).

110 Numerous studies have revealed the strong potential of membrane processes for the
111 concentration and separation of thermosensitive bioactive compounds in fruit juices
112 (Bhattacharjee, Saxena, & Dutta, 2017). Among the various studies available in the literature,
113 the separation of low molecular weight solutes using a low molecular weight cut-off
114 ultrafiltration or a high MWCO nanofiltration is gaining increasing interest (Acosta, Vaillant,
115 Pérez, & Dornier, 2014; Conidi, Cassano, Caiazzo, & Drioli, 2017; Nath, Dave, & Patel,
116 2018). Indeed, even if this process requires pretreatment to avoid rapid fouling of the
117 membrane, it could be used to achieve separations at low temperature, i.e. without damaging
118 the potential of raw material, and with a fairly low environmental impact.

119 In recent years, some studies have focused on separation of betalains by membrane
120 technologies. For example, (Vergara, Cancino-Madariaga, Ramírez-Salvo, Sáenz, Robert, &
121 Lutz, 2015) clarified a purple cactus pear juice by microfiltration. They obtained very clear
122 permeates, free of turbidity, 70% of betalains retention and also containing polyphenols and
123 high antioxidant activity. Ultrafiltration process has also been used to clarify yellow and red
124 cactus pear pulp (Alfredo Cassano, Conidi, & Drioli, 2010). Mereddy, Chan, Fanning,
125 Nirmal, & Sultanbawa (2017) increased the total betalain content in red beetroot extract up to
126 46% of the total soluble solids in the concentrated juice after three diafiltrations. In this
127 context, this study aimed to evaluate a new integrated membrane-based process to concentrate
128 betacyanins, but also to purify or fractionate them. The method selected consisted of a first
129 clarification step by microfiltration and a second step for solute separation by ultra or
130 nanofiltration. It was evaluated by applying these pressure-driven technologies to the
131 treatment of a cactus pear juice of *Opuntia dillenii* (Ker Gawl.) Haw. from Senegal.

132

133 **2 Materials and methods**

134

135 **2.1 Cactus pear juice preparation**

136 The juice was obtained from purple cactus pears (*Opuntia dillenii* Haw.) harvested from a
137 plantation in Saint-Louis, Senegal. The fruits (45.5 kg) were manually washed with water and
138 peeled. The peeled fruit (22.2 kg) was then refined using a horizontal pulper Auriol PH3
139 (Marmande, France) with 0.5 mm mesh at room temperature in order to separate the seeds
140 from the juice. The 18.2 kg of refined juice obtained were frozen at -18°C until use. All the
141 juice used in the experiments was produced at the same time.

142

143 **2.2 Enzymatic liquefaction and clarification by microfiltration**

144 Part of the juice was enzymatically pretreated using 300 mg.kg⁻¹ of Ultrazym AFP-L
145 (Novozymes, Bagsvaerd, Denmark). The mixture was stirred for 30 min at 30°C without any
146 pH adjustment.

147 Microfiltration experiments were performed using a laboratory pilot already described by
148 (Polidori, Dhuique-Mayer, & Dornier, 2018). The pilot consisted of a 3 L feed tank, 4 tubular
149 ceramic membranes with an effective filtration area of 55 cm², and a tubular heat exchanger to
150 keep the juice temperature at 30±1°C. The details of the 4 microfiltration membranes used
151 throughout the experiment have been listed in Table 1.

152 For the comparison of operating conditions (membrane and transmembrane pressure TMP),
153 the feed composition was kept constant by recycling the entire permeate in the feed tank as
154 usual (MRR mass reduction ratio, defined as the ratio between the total mass of the feed and
155 the mass of the retentate in the circulation loop, kept close to 1). The best operating conditions
156 were selected on the basis of the highest permeate flux (J_p) and the lowest retention of
157 betacyanins.

158 The juice was then clarified according to a classical batch concentration procedure. In that
159 case, the permeate was separately collected keeping the mass of the retentate constant in the
160 circulation loop with the addition of raw juice to the feed tank. Four similar tubular
161 membranes were connected to obtain a total effective surface of 220 cm². The system was
162 implemented to clarify the juice up to a MRR mass reduction ratio of 5. This clarified juice
163 was subjected to a second separation step performed by ultra or nanofiltration.

164

165 and standard deviation evaluated with 3 or 4 measurements.

166

167 **2.3 Separation by nano/ultrafiltration**

168

169 **2.3.1 Equipment and experimental procedure**

170 Nano/ultrafiltration experiments were carried out using the pilot unit already described by
171 (Cissé, Vaillant, Pallet, & Dornier, 2011), which incorporated a Sepa CF II Membrane Cell
172 System (GE Osmonics, Minnetonka, MN, USA) with an effective membrane surface of 155
173 cm². The temperature was maintained at 30 ± 0.5°C using a Julabo F12-ED cryostat
174 (Seelbach, Germany) which fed the double jacket of the feed tank. The permeate mass flux
175 was determined by weighing the amount of permeate extracted Vs. time with a Precisa XL
176 1200C electronic scale (Dietikon, Switzerland). For each trial, 2.5 kg of extract was used.
177 Extracted permeate weight, transmembrane pressure and temperature were recorded every 2
178 min. Four flat-sheet membranes characteristics were used in this experiment and their
179 respective water permeability values are reported in Table 1.

180 Before filtration, membranes were pre-conditioned during 60 min, using deionized water
181 (conductivity < 5 µS.cm⁻¹) at 30°C, at 20 bar of transmembrane pressure and 0.3 m.s⁻¹ of
182 crossflow velocity. Water flux from the last 10 min of preconditioning was used to calculate
183 membrane permeability. Experiments were performed immediately after preconditioning. The
184 permeate and retentate fractions were recycled to the feed tank to maintain a constant feed
185 concentration (MRR ≈ 1).

186 The transmembrane pressures tested for each membrane were 5, 10, 15, 20, 25 and 30 bar,
187 with each pressure level being maintained for 50 min with the except of the Nadir 4 kDa
188 membrane where pressures were limited to 15 bar. All permeate flux values were recorded
189 during the last 10 min for each pressure set point and 30 mL of permeate samples were
190 collected. Samples, feed juice and permeates, were immediately frozen and kept at -18 °C
191 until analysis.

192 Membrane performance was evaluated by its permeate flux (J_p) and its selectivity towards
193 total betacyanins β_c, betanin β_n, isobetanin iβ_n and neobetanin nβ_n. The retention of the
194 compound i R_i was calculated according to Eq. 1. where C_{pi} and C_{fi} the concentrations in g.kg⁻¹
195 of the compound i in the permeate and in the feed, respectively.

$$R_i = 1 - \frac{C_{pi}}{C_{fi}} \quad \text{Eq. 1}$$

196

197 **2.3.2. Modeling betacyanins concentration and separation**

198

199 From mass balance, assuming that the retentions are constant and the system behaves like an
 200 ideal stirred reactor, concentration factor of an element i in the retentate CF_i after
 201 nanofiltration up to a mass reduction ratio MRR and diavolume DV (defined as the ratio
 202 between the volume of water added during the diafiltration phase and the volume of
 203 retentate), could be evaluated using Eq. 2 with C_i the concentration of the compound i in the
 204 retentate and R_i the retention of i (Acosta, et al., 2014; Polidori, et al., 2018; L. Wang, Yang,
 205 Xing, & Xu, 2008; X.-L. Wang, Zhang, & Ouyang, 2002). In order to evaluate the ability of
 206 the process to separate two solutes i and j , a separation factor $SF_{i/j}$ was also defined according
 207 to Eq. 3. In the case where the compound i was the most retained, the higher the $SF_{i/j}$ the more
 208 efficient the separation between i and j . Considering betacyanins for i and total dry matter
 209 TDM for j , this separation factor corresponds to a purification factor. Considering i and j two
 210 different betacyanins, it can be defined as a fractionation factor.

211

$$CF_i = \frac{C_i^{final}}{C_i^{initial}} = MRR^{R_i} e^{DV(R_i-1)} \quad \text{Eq. 2}$$

$$SF_{i/j} = \frac{CF_i}{CF_j} = \exp[(R_i - R_j) (\ln MRR + DV)] \quad \text{Eq. 3}$$

212

213

214 **2.4 Analysis**

215

216 **2.4.1 Physicochemical characterization**

217 Total soluble solids (TSS) were measured using a refractometer Atago PAL-3 (Tokyo, Japan).

218 The total dry matter (TDM) was measured in a vacuum oven at 30 mbar and 70°C according
 219 to (AOAC, 1990) procedure.

220 Conductivity ($\text{mS}\cdot\text{cm}^{-1}$) and pH were measured at room temperature using a conductimeter

221 WTW LF 197 (Weilheim, Germany) and a Schott Titroline apparatus (St. Gallen,

222 Switzerland). Turbidity measurements were performed with a turbidimeter Hanna LP 2000
223 (Rhode Island, USA).

224 The colour was measured using the L*, a*, and b* coordinates of CIE Lab with a
225 chromameter Minolta CR-410 (Tokyo, Japan). The index L* is related to the luminosity,
226 varying between white and black; the color coordinates varying between greenish and reddish
227 colours (a*) and bluish and yellowish colours (b*) (Sant'Anna, Gurak, Marczak, & Tessaro,
228 2013).

229 Glucose, fructose and citric acid contents were determined by HPLC using a UPLC –1290
230 system Infinity II (Agilent, Santa-Clara, USA) equipped with RI and UV detectors. A C18
231 column (SHODEX SH1011, 300x8 mm; Tokyo, Japan) with a mobile phase of H₃PO₄ (0.1%)
232 in water was used, with isocratic elution program at a flow rate of 0.7 mL.min⁻¹ and 40°C.
233 Injection volume was 10 µL and spectrophotometric detection was set at 210 and 245 nm.

234

235 **2.4.2 Betalain analysis**

236

237 **2.4.2.1 Spectrophotometry analysis**

238 The total betacyanins were measured according to (A Cassano, Conidi, Timpone, D'avella, &
239 Drioli, 2007), in which juice samples were analysed using spectrophotometer Specord 600
240 (analytik Jena, Jena, Germany) at 535 nm. Betacyanin contents (β_c , mg kg⁻¹) were calculated
241 according to the Beer-Lambert law using 60 000 L.mol⁻¹.cm⁻¹ as molar extinction coefficient
242 and after diluting until absorbance between 0.2 and 0.8.

243

244 **2.4.2.2 HPLC analysis**

245 HPLC separation and identification of betalains were performed with the same Agilent
246 chromatographic system previously described for sugars and organic acid analysis. The
247 samples were filtered through a 0.45 µm nylon filter. All analyses were conducted in
248 triplicate. Betalains identification was carried out using 1% formic acid in water (v/v, eluent
249 A) and acetonitrile (eluent B). Betalains were separated in a Kinetex XB-C18 column (150 ×
250 4.6 mm, 2.6 µm particle size, Phenomenex, California, USA) maintained at 30°C, at a flow
251 rate of 1 mL min⁻¹. The injection volume for all extracts was 10 µL. Betalain compounds were
252 separated starting with 95% A, followed by a linear gradient from 5 to 10 % B in 5 min, then
253 a linear gradient from 10 to 20% B in 5 min, and from 20 to 5% B in 2 min. To re-establish

254 the initial conditions, this condition was maintained (5% B and 95% A) during 3 min.
255 Betacyanins and betaxanthins were monitored at 535 and 484 nm, respectively. The
256 identification of each chromatographic peak was tentatively assigned by their visible spectral
257 characteristics in comparison with standard (betanin and isobetanin only) and retention times.

258

259 **2.4.2.3 LC-MS and NMR analysis**

260 LC-MS analysis were performed on an Acquity H-Class UPLC system (Waters Corp.,
261 Milford, MA), using a Kinetex C18 100 A 100 × 2.1 mm, 2.6 μm column (Phenomenex)
262 coupled with an Acquity PDA detector and with a mass spectrophotometer Synapt G2-S
263 HDMS system (Waters Corp., Milford, MA) with electrospray ionization source operating in
264 high resolution mode. The elution gradient was set as follow: from 95% formic acid 0.01%
265 and 5% acetonitrile to 100% acetonitrile in 10 min, flow rate was fixed at 0.4 ml/min. The
266 Synapt parameters were optimized as follow: the sample cone was set at 20 V, the source and
267 desolvation temperature were set at 140°C and 450°C, respectively. Each sample were
268 processed with MassLynx (V4.1) software.

269 Fractions for NMR were collected with a semi-preparative HPLC using Ultimate 3000 LC
270 system (Thermo) equipped with an autosampler (WPS3000TFC) and then evaporated to
271 dryness. NMR spectra were recorded at 298 K on a Bruker Avance III 600 MHz NMR
272 spectrometer, using TCI Cryoprobe Prodigy. Spectra were processed and visualized with
273 Topspin 3.5 (Bruker Biospin) on a Linux station. Deuterium Oxide was purchased from
274 Eurisotop, France.

275

276 **3 Results et discussion**

277

278 **3.1 Cactus pear juice composition**

279 The solutes mainly present in the cactus pear juice are glucose, fructose and citric acid (Table
280 2, raw juice). Reducing sugars accounted for 70% of the total dry matter. The high proportion
281 of citric acid in the dry matter, 19%, explains the very acid character of the juice. The pH
282 value was similar to that obtained by (M. Castellar, Obón, Alacid, & Fernández-López, 2008;
283 Medina, Rodríguez, & Romero, 2007) who respectively obtained 3.30 and 3.34 but less than
284 the pH values of 5.3 – 7.1 reported for the cactus pear *Opuntia ficus-indica*, the most studied
285 and cultivated *Opuntia* (A Cassano, et al., 2007; Moßhammer, Stintzing, & Carle, 2006).

286 The high turbidity of the raw juice showed a significant insoluble fraction. Nevertheless, the
287 insoluble solids in suspension represented a small mass fraction of the total dry matter
288 (TDM). Indeed, total soluble solids (TSS) were of the same order of magnitude as TDM.

289 The juice was also characterized by an intense red color due to the presence of betacyanins.
290 The attained concentrations were similar to those presented by many other studies (M.
291 Castellar, Obón, & Fernández-López, 2006; R. Castellar, et al., 2003).

292 Following HPLC analysis, 3 majority peaks were observed at 484 nm (Fig. 1). Compounds 1
293 and 2 had a molecular weight identical to 551.1511 and 551.1512 Da respectively that
294 corresponded with the formula $C_{25}H_{26}N_2O_{13}$. The MSMS confirmed the structure of the
295 betanin. The two compositions presented identical HRMS and MSMS were highly likely to
296 be, the isomers: betanin, isobetanin.

297 Compound 3 gave an exact mass of 549.1399 Da which corresponded to the molecular
298 formula $C_{25}H_{24}N_2O_{13}$. MSMS seemed to indicate that the double bond was in the nitrogen
299 heterocycle of 6 carbon atoms with a characteristic fragment at 148 Da. The difference of -2 in
300 mass with respect to the compounds 1 and 2 would result from oxidation, hence the presence
301 of a double bond which is absent in betanin and neobetainin. In order to confirm this
302 hypothesis, compound 3 was isolated by semi-preparatory HPLC to be analyzed by NMR.
303 The HMBC experiment (1H - ^{13}C long distance) showed a correlation between quaternary C
304 atom. Therefore, the position of the unsaturation was confirmed as being present on the cycle
305 of 6. The presence of the double bond at scale 6 of the cycle also explained the equivalence of
306 the 2H “pyridine” that appeared at the same chemical shift (7.90 ppm) for an integration of 2
307 which allowed identifying as the molecule as neobetainin.

308 Therefore, the three main betacyanins identified in the juice were betanin, isobetanin and
309 neobetainin. Neobetainin (14, 15-dehydro-betanin) is a natural constituent of beet (*Beta*
310 *vulgaris* L.) (Alard, Wray, Grotjahn, Reznik, & Strack, 1985; Kujala, Lojonen, & Pihlaja,
311 2001) or cactus pear (*Opuntia* sp.) (Alard, et al., 1985; Castellanos-Santiago & Yahia, 2008;
312 Wyler, 1986). It is formed by dehydrogenation of betanin (Wybraniec, Starzak, Skopińska,
313 Nemzer, Pietrzkowski, & Michałowski, 2013).

314 These three compounds have already been identified in the cactus pear (Chauhan, Sheth,
315 Rathod, Suhagia, & Maradia, 2013).

316 In order to study the impact of enzymatic hydrolysis on juice and microfiltration, a proportion
317 of the juice was treated with Ultrazym. The results showed that enzymatic treatment has no
318 significant impact on the physiochemical characteristics of the juice (Table 2) even though,
319 enzymatic treatment generally achieves a reduction of turbidity and an increase of TSS mainly
320 due to the hydrolysis of polysaccharides and the release of soluble compounds (F. Vaillant,
321 Millan, Jariel, Dornier, Decloux, & Reynes, 1999; Fabrice Vaillant, Pérez, Acosta, & Dornier,
322 2008).

323

324 **3.2 Membrane and TMP selection for clarification**

325

326 **3.2.1 Interest of the enzymatic liquefaction**

327 By filtering the raw juice without enzymatic liquefaction, the permeate flux was close to 40 L
328 $\text{h}^{-1} \text{m}^{-2}$ whatever the operating conditions (Fig. 2). In this case, the fluxes were constant during
329 filtration that attested membrane fouling reached a steady state very early. On the contrary,
330 flux behaviour was completely modified by the enzymatic pretreatment. First, the flux was
331 significantly improved. Second they were much less stable, with a 35% drop after the first
332 hour of filtration, which indicated that fouling required more time for setting up. These
333 evolutions did not depend on the membrane nor the applied pressure. These results
334 corroborate those of numerous studies that showed that enzymatic treatment very often
335 improves membrane performance during fruit juice clarification (Bahçeci, 2012; Gökmen &
336 Çetinkaya, 2007; Ushikubo, Watanabe, & Viotto, 2007; Watanabe, Ushikubo, & Viotto,
337 2006). Indeed, the enzymatic liquefaction decreases the viscosity of the juice but also
338 modifies the fouling power of the suspension. It helps to solubilize part of the insoluble
339 fraction and modifies the colloidal fraction known to be often involved in fouling (Dahdouh,
340 Delalonde, Ricci, Servent, Dornier, & Wisniewski, 2016). These phenomena contribute to
341 decreasing the overall hydraulic resistance system and lead therefore to an increase in
342 transmembrane flux.

343 By comparing the average permeate fluxes calculated between 60 and 120 min of
344 microfiltration (a steady state is reached in all cases for fouling), the enzymatic hydrolysis
345 made it possible to multiply J_p by 2 to 2.6 (Table 3).

346 Retention and color parameters were also affected by enzymatic liquefaction (Table 3).
347 Retentions of dry matter and betacyanins decreased by a few percent and a few tenths of a
348 percent respectively. The decrease in the retention of the dry matter is probably due to the

349 solubilization of part of the insoluble fraction through enzymatic treatment. Betacyanins are
350 located within the vacuoles of plant cells. Enzymatic hydrolysis which contributes to the
351 deconstructing of pecto-cellulosic cell walls, support their release into the juice. After
352 enzymatic treatment, their apparent retention is lower because the amount of betacyanins
353 associated with the insoluble fraction, which is retained by the membrane, decreases. This
354 result is in line with the color measurements: the red coloring (a^*) of the permeate of the
355 liquefied juice was greater compared to that of the raw juice. The other color parameters (L^* ,
356 b^*) did not vary significantly.

357 The enzymatic treatment of the juice before microfiltration was therefore particularly
358 interesting for our application insofar as it allowed to double the flux of permeate as well as
359 reduce the retention of the desired solutes.

360

361 **3.2.2 Effect of transmembrane pressure**

362 The trend of the curves obtained were quite conventional in MFT (Fig. 3). With the exception
363 of the Pall 0.2 μm membrane, the average permeate flux continuously increased with the
364 transmembrane pressure. However, it was not proportional to TMP. In accordance with the
365 generalized Darcy's law, the permeate flux is a function of the ratio of the transmembrane
366 pressure over the permeate's viscosity and the total hydraulic resistance of the system
367 (membrane / fouling). As the resistance of the membrane is constant (incompressible
368 material), this behavior can be explained by an increase of the resistance generated by the
369 fouling when the pressure increases (compressibility of the external fouling on the surface of
370 the membrane, increase of the internal fouling in the porosity of the membrane material).

371 The best fluxes were obtained with the 0.2 μm Tami membrane and exceeded $100 \text{ kg h}^{-1} \text{ m}^{-2}$
372 for a TMP of 2.8 bar. The high flux of permeate obtained under these conditions is interesting
373 for industrial application.

374 On the other hand, the TMP and the type of membrane do not have a significant impact on the
375 retention of the various compounds present in the juice. Retentions of dry matter and that of
376 betacyanins are very similar. They are on average 9.7%.

377 From the analysis of these results, in terms of membranes, flux and retentions, it is more
378 interesting to clarify the juice with the 0.2 μm Tami membrane at 3 bar TMP using a liquefied
379 feed juice. The selection of these operating conditions makes it possible to obtain a high
380 permeate flux ($> 100 \text{ kg h}^{-1} \text{ m}^{-2}$) and low retentions, in particular for betacyanins. These

381 results are interesting in the perspective of a clarification of the juice at an industrial level. In
382 addition, with the membrane sizes used, the permeate obtained is cold sterilized, which
383 ensures a low microbial load in the clarified juice. This microfiltration is well suited as a
384 pretreatment to nanofiltration in order to reduce the fouling properties of the juice by
385 eliminating the insoluble and colloidal parts without significantly modifying the solute
386 composition profile.

387 The Tami membrane with at 3 bar TMP was therefore logically chosen for clarification with
388 an increasing MRR.

389

390 **3.3 Clarification using optimal operating conditions**

391 Clarification of the liquefied juice was performed up to a MRR of 5.5 with the 0.2 μm Tami
392 membrane at 3 bar TMP (Fig. 4). As it is conventionally observed in crossflow
393 microfiltration, the flux gradually decreased according to the MRR. Up to an MRR of 3, the
394 flow dropped by 38% and then gradually stabilized beyond an MRR of 3. This decrease, in
395 addition to the gradual buildup of the fouling, is attributed to the increase in the viscosity of
396 the juice and its fouling properties. The average permeate flux obtained between 3 and 5.5 of
397 MRR was $83 \text{ kg}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$. The flux remained high even at an MRR of 5, which is not the case
398 during the clarification process of some fruit juices (Fabrice Vaillant, Millan, Dornier,
399 Decloux, & Reynes, 2001). Considering the high average flux, it would be possible to
400 improve the yield of the clarification by microfiltering the product at MRR greater than 6,
401 without drastic reduction in performance. These results are particularly promising for an
402 industrial scale up of the process.

403 The main characteristics of the microfiltered juice under these conditions are shown in Table
404 2. As expected, crossflow microfiltration made it possible to completely clarify the juice, with
405 the turbidity of the permeate reaching less than 1 NTU. The insoluble part contained in the
406 juice was completely eliminated by the process which explained the 9% retention of TDM.
407 The change in the color of the product, especially a^* , was probably mainly related to its
408 clarification. With regard to solutes, the composition profile was only slightly modified by the
409 microfiltration. A retention of betacyanins of the order of 4% was highlighted. This could be
410 explained by the persistence in the liquefied juice of intact cell structures, in which
411 betacyanins would still be present. However, this slight retention does not question the value
412 of the process which makes it possible to clarify and sterilize the juice with good preservation
413 of betacyanins.

414

415 **3.4 Concentration and purification by nanofiltration/ultrafiltration**

416

417 **3.4.1 Permeate flux**

418

419 During ultra and nanofiltration of juice previously clarified by microfiltration, permeate
420 fluxes only slightly varied over time when TMP was constant (Fig. 5). A slight tendency to
421 decrease was noted but it did not exceed 10% after 45-50 min of pressure relief. At higher
422 pressures, greater instability was demonstrated for Nadir membranes. It could be related to a
423 greater sensitivity of these membranes to slight pressure fluctuations generated by the feed
424 piston pump.

425

426 The average permeate fluxes calculated over the last 10 min of each pressure stage were used
427 to compare the membranes with each other depending on the TMP. The permeate fluxes
428 obtained during the ultra or nanofiltration tests, with 4 membranes of MWCO between 0.2
429 and 4 kDa, were very sensitive to the increase of the TMP (Fig. 6). From 5 bar, the evolution
430 of average fluxes according to TMP was linear for all membranes ($r^2 \geq 0.99$) with slopes
431 ranging from 1.05 to 2.66 $\text{kg}\cdot\text{h}^{-1}\cdot\text{m}^{-2}\cdot\text{bar}^{-1}$. The sensitivity of the flux to the TMP was 2 times
432 higher on the Nadir 4 kDa membrane, compared to the average sensitivity of the other
433 membranes. This is probably related to its much higher MWCO than the others. Koch
434 membrane 1 kDa is the least sensitive to pressure.

435 The ordinates at the origin of the flux regression lines for Nadir membranes 1 kDa and 4 kDa
436 were positive, 18.8 and 12.7 $\text{kg}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ respectively; from this we deduced that the average
437 sensitivity of the fluxes according to TMP for these membranes increased considerably at low
438 pressure (TMP < 5 bar). This behavior is characteristic of ultrafiltration membranes in which
439 convective transfers are predominant. In this case the increase of permeate flux with pressure
440 is limited by the phenomena of concentration polarization along the surface of the membrane
441 and by fouling. Nadir 1 kDa and 4 kDa membranes were more efficient in terms of flux; at 15
442 bar for example, their average permeate flux were respectively 41 and 54 $\text{kg}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$.

443 On the other hand, Nadir 0.2 kDa and Koch 1 kDa membranes showed a characteristic
444 behavior of nanofiltration membranes. In nanofiltration, the impact of the pressure on the flux
445 of solvent, i.e. water in our case, could be represented through Eq. 4. Based on the
446 methodology proposed by (Acosta, Vaillant, Pérez, & Dornier, 2017), the water permeability
447 (L_w) was evaluated at 18.8 and 10.6 $\text{kg}\cdot\text{h}^{-1}\cdot\text{m}^{-2}\cdot\text{bar}^{-1}$ respectively for the Nadir 0.2 kDa and

448 Koch 1 kDa membranes. A reflection coefficient σ of 0.54 was obtained for the 2 membranes.
449 In this case, an important part of the transfers through the membrane is probably related to
450 diffusion-solubilization phenomena.

451

$$J_w = L_w(TMP - \sigma\Delta\pi) \quad \text{Eq. 4}$$

452 with

J_w : Water flux ($\text{kg h}^{-1} \text{m}^{-2}$)

σ : Reflexion coefficient

L_w : Water permeability ($\text{kg h}^{-1} \text{m}^{-2} \text{bar}^{-1}$)

$\Delta\pi$: Difference of osmotic pressure
between both sides of the membrane (bar)

1

2 Among the 4 ultra/nanofiltration membranes tested, the Koch 1 kDa lead to the lowest
3 permeate fluxes ($10 \text{ kg.h}^{-1}.\text{m}^{-2}$ at 15 bar). A comparison of the average fluxes obtained with
4 the Nadir 1 kDa and Koch 1 kDa membrane showed that the fluxes were not correlated to the
5 MWCO if the membranes are different. This is probably related to the structural differences
6 in the membranes. If we consider only the Nadir membranes which have *a priori* a similar
7 structure, we found that the fluxes were positively correlated with MWCO and with the
8 permeability to water.

9

10 **3.4.2 Solute retentions**

11 As it is conventionally observed in nanofiltration, the retention of all solutes increased with
12 TMP and tended asymptotically towards a maximum retention value (Fig. 6). For all
13 membranes, the retention of sugars and citric acid, solutes which are very much in the
14 majority of TDM, was lower (from 0.17 to 0.93) than that of betacyanins (from 0.81 to 1.00).
15 These differences in retention are most likely related to the difference in molar mass between
16 these two groups of compounds: 180 and 192 g mol^{-1} for sugars and acid and 549 to 550 g
17 mol^{-1} for betacyanins.

18 In all the cases tested, fructose and glucose were retained in the same way regardless of the
19 operating parameters. On the other hand, citric acid was more or less retained depending on
20 the case. Nadir 1 kDa and 4 kDa membranes retained more citric acid than sugars; the
21 retentions were almost equal with the Koch membrane 1 kDa; the retention of citric acid was
22 lower than that of sugars with Nadir 0.2 kDa membrane. For the same cutoff of 1 kDa, the
23 Koch membrane showed retentions 34% higher on average than the Nadir membrane. This

24 observation is related to the differences in structure and material between the 2 membranes as
25 already mentioned by comparing permeates fluxes.

26 Betacyanins were better retained than major solutes, probably because of their higher
27 molecular weight but also their overall positive charge. Indeed, the ionization of these
28 compounds probably contributes to increase their retention via repulsive electrostatic forces,
29 the membranes being rather positively charged at such pH (Conidi, Cassano, & Drioli, 2012).
30 Betacyanins were completely retained by Nadir 0.2 kDa membrane even at low TMP values.
31 At high TMP (> 15 bar), retentions greater than 96% were obtained with 1 kDa cutoff
32 membranes. In cases where the retention of betacyanins was lower, neobetanin was
33 systematically more retained than isobetanin and betanin. This result is surprising because
34 these 3 compounds have an extremely close molecular structure. This phenomenon could be
35 explained by slight variations in the physicochemical interactions between the pigments and
36 the membranes or with the other solutes present into the product.

37 Although it may happen that practically all the dry matter was retained, the membranes
38 selected in our study were selectively more permeable to major solutes (TDM) than to betanin
39 and isobetanin, which were in turn less retained than neobetanin. As the retention of each of
40 these solutes varied differently depending on the operating conditions, it was then possible to
41 aspire to different separation objectives:

42 1- concentration of the extract: We will seek in this case, a high retention of all the solutes
43 present. The retentions of all the solutes must be similar in order not to generate a distortion
44 of the composition profile;

45 2- Purification of betacyanins: It is about favoring the retention of betacyanins to the
46 detriment of that of the other solutes (sugars, acids) in order to increase the content of
47 pigment compared to TDM;

48 3- Fractionation of betacyanins: Profiling the differences in retention observed between
49 neobetanin and the other 2 betacyanins to separate them.

50 Regarding treating an extract rich in betacyanins as part of this study, the retention of
51 betacyanins $R_{\beta c}$ must be high in all cases. For the concentration of the extract, the difference
52 in retention between the dry matter and the betacyanins ($R_{TDM} - R_{\beta c}$) and the retention
53 difference between the betacyanins themselves ($R_{n\beta n} - R_{(\beta n + i\beta n)}$) must be as small as possible
54 (Eq. 3). On the contrary, for the purification, ($R_{TDM} - R_{\beta c}$) has to be maximized. Finally, to split

55 the betacyanins between them, it is the difference of retentions between neobetanin and
56 betanin/isobetanin ($R_{n\beta n} - R_{(\beta n + i\beta n)}$) that must be sought to be maximized. It is therefore possible
57 to represent the retention results in a 3D space to determine which operating conditions are
58 more favorable for each of the 3 separation objectives described above (Fig. 7).

60
61 The Fig. 8 shows the location of the different couples membrane/pressure tested in this 3D
62 space. The 4 ultra/nanofiltration membranes chosen have quite different specificities. This is
63 interesting insofar as all separation possibilities are conceivable for concentrating, purifying
64 and / or fractioning betacyanins. The concentration of the extract can be implemented without
65 surprise with the Nadir 0.2 kDa membrane but also with the Koch 1 kDa membrane with high
66 transmembrane pressure. The Nadir 1 kDa and 4 kDa membranes are rather indicated for the
67 simultaneous purification and fractionation of betacyanins, and this all the better as the
68 transmembrane pressure is low. Finally, the Koch 1 kDa membrane has a potential for
69 fractionation of betacyanins by limiting TDM losses when used at low pressure. Permeability
70 fluxes obtained under these conditions are however very low.

71 Table 4 provides a summary of the optimal nanofiltration conditions depending on the
72 targeted separation objective. The results show that for fractionation, the ideal is to operate at
73 low pressure, while purification would require a little higher pressure.

74

75 **3.5 Simulation of betacyanin separation by nanofiltration/ultrafiltration**

76 The different situations presented in Table 4, were used to make simulations in order to
77 evaluate the potentialities of using various membrane / pressure pairs. The simulation was
78 conducted for MRR between 2 and 10, and diavolume DV up to 10, by calculating the
79 betacyanin concentration factor ($CF_{\beta c}$, Eq. 2) and the separation factors (Eq.3) either
80 betacyanins compared to TDM ($SF_{\beta c/TDM}$ which corresponds to a purification factor) or
81 among betacyanins ($SF_{n\beta n/(\beta n + i\beta n)}$ which corresponds to a fractionation factor).

82

83 **3.5.1 Concentration**

84 In this case, there is no diafiltration step ($DV = 0$). The concentration factors obtained at a
85 given MRR are very close for the 3 chosen membrane / TMP pairs. For example, with an
86 MRR of 10, the membranes Nadir 0.2 kDa at 5 bar, Koch 1kDa at 15 bar and Nadir 1kDa at

87 25 bar make it possible to obtain CF_{β_c} of 9.9, 9.2 and 9.1 respectively. This is normal because
88 these membrane / TMP pairs lead to high retentions all greater than 0.96.

89

90 **3.5.2 Purification**

91

92 Fig. 9 shows the potential of the 3 selected membrane / TMP pairs to optimize the production
93 of purified betacyanin extracts by diafiltration. These charts, drawn for MRR and DV up to
94 10, allow visualizing the range of CF_{β_c} et $SF_{\beta_c/TDM}$ that can be achieved using a given
95 membrane / TMP pair. These figures also make it possible to evaluate the MRR / DV pairs
96 that must be used to reach various levels of concentration and purification. By way of
97 illustration, with a Nadir 1 kDa membrane at 10 bar, to obtain a $SF_{\beta_c/TDM}$ of 600 and a CF_{β_c} of
98 3, it would be necessary to work with approximately a DV of 9 and to fix the MRR at 7. The
99 order of magnitude of the purification factors varies greatly from membrane to membrane.
100 For the 3 selected case studies, we find that the Membrane / TMP combinations make it
101 possible to reach average concentration factors, less than 6, but with high purification factors.
102 The Nadir 4 kDa / 10 bar combination makes it possible to envisage a very thorough
103 purification of betacyanins (purification factor up to 3800). Finally, the combination Koch 1
104 kDa / 5 bar is very limited in terms of separation whereas Nadir 1 kDa / 10 bar is intermediate
105 and allows a good compromise between concentration and purification.

106

107 **3.5.3 Betacyanins fractionation**

108

109 Fig. 10 presenting the results of the simulation carried out with three membrane / TMP pairs
110 gives us information on the feasibility and efficiency of the fractionation. The Koch 1 kDa / 5
111 bar combination gives slightly higher $SF_{n\beta_n/(\beta_n+i\beta_n)}$ than Nadir 1 kDa / 5 bar, similar
112 concentration factors and much lower purification factors. The Nadir 4 kDa / 5 bar
113 combination is limited in terms of fractionation but has good concentration and purification
114 factors. With these membranes, however, it is impossible to achieve a separation factor
115 greater than 3.3. This result has to be improved a priori to consider an industrial application,
116 by looking for other combinations membrane / TMP more appropriate to realize this type of
117 separation.

118 **4 Conclusion**

119

120 Crossflow microfiltration had great potential for remove insoluble and colloidal fractions
121 contained in cactus pear juice. Under the operating conditions tested, this first step made it
122 possible to clarify the product perfectly without substantially affecting its solute profile. In
123 particular, the retention of betacyanins was very limited, less than 10%, as for the total dry
124 matter. The Association with an enzymatic pretreatment of liquefaction was really interesting
125 because it multiplied permeate flux at least by 2 and decreased the retention of betacyanins.
126 The results were very encouraging for further development of the process on an industrial
127 scale. After microfiltration, different ways could be considered for the separation of
128 betacyanin by ultra or nanofiltration. By selecting different membrane/pressure settings and
129 adding a possible diafiltration stage, we have shown that it was possible to promote either the
130 concentration of all the betacyanins, or their purification from total dry matter, or even some
131 fractionation between them. The overall process that integrates all these unit operations is an
132 attractive alternative to produce, at low temperature, concentrated and purified betacyanin
133 extracts from cactus pear juice. It could be easily used to pre-concentrate or modify the solute
134 composition of the extract without thermal damage before final concentration (evaporation
135 under vacu;, osmotic evaporation) or spray drying for example. For final validation and
136 better evaluation of the process from an economic point of view, further trials should be
137 performed now at a larger scale of production.

138

139

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143

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Table 1. Main characteristics of the different membranes used.

293

	Manufacturer	Designation, material	Average pore size diameter (μm) or nominal molecular weight cut-off (kDa)	Measured initial water permeability* ($\text{kg h}^{-1} \text{bar}^{-1} \text{m}^{-2}$)
Microfiltration	Orelis	Al_2O_3	0.1 μm	144 (10)
	Orelis	Al_2O_3	0.2 μm	158 (7)
	Tami	TiO_2	0.2 μm	170 (16)
	Pall Exekia	Al_2O_3	0.2 μm	112 (4)
Ultrafiltration	Microdyn Nadir	UH004, polyethersulphone	4 kDa	20 (2)
Nanofiltration	Microdyn Nadir	NP030, polyethersulphone	0.2 kDa	4 (0.5)
	Microdyn Nadir	NP010, polyethersulphone	1 kDa	11 (0.7)
	Koch	MPS 36, composite	1 kDa	7 (0.3)

294

*: average and standard deviation evaluated with 3 repetitions.

295

296 Table 2. Main characteristics of raw cactus pear juice, liquefied juice and microfiltered juice
 297 (average and standard deviation evaluated with 3 replicates).

298

Component	Raw juice	Liquefied juice Ultrazym	Microfiltered juice Membrane Tami 0.2 μm TMP = 3 bar $3.0 \leq \text{MRR} \leq 5.5$
TDM (g.kg^{-1})	65.6 (0.5)	64.6 (0.4)	58.8 (0.7)
TSS (g.kg^{-1})	72 (1)	72 (1)	75 (1)
pH	3.35 (0.05)	3.32 (0.05)	3.38 (0.01)
Citric acid (g.kg^{-1})	12.4 (0.4)	12.4 (0.3)	12.9 (0.1)
Glucose (g.kg^{-1})	22.8 (0.1)	22.8 (0.1)	22.9 (0.2)
Fructose (g.kg^{-1})	22.8 (0.2)	22.9 (0.1)	22.9 (0.2)
Betacyanins (g.kg^{-1})	0.76 (0.02)	0.77 (0.01)	0.74 (0.04)
Turbidity (NTU)	1428 (37)	1430 (33)	< 1
Conductivity (mS.cm^{-1})	3.72 (0.22)	3.74 (0.12)	3.53 (0.21)
L*	8.89 (0.17)	8.87 (0.18)	9.68 (0.14)
a*	16.5 (0.4)	16.6 (0.5)	33.4 (3.0)
b*	-1.8 (0.1)	-2.6 (0.1)	-0.9 (0.2)

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302 Table 3. Permeate flux (J_p), retentions and color of permeate obtained during microfiltration
 303 of raw and liquefied cactus pear juice at MRR = 1 using different membrane / transmembrane
 304 pressure (TMP) combinations.

305

Membrane/TMP	Permeate flux ($\text{kg}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$)		Retentions				Permeate colour (a^*)	
			Total dry matter		Betacyanins			
	Raw juice	Liquefied juice	Raw juice	Liquefied juice	Raw juice	Liquefied juice	Raw juice	Liquefied juice
Orelis-0,1 μm /1.78 bar	34.8 (1.5)	75.3 (3.3)	0.12	0.09	0.11	0.09	34.1	37.9
Orelis-0,2 μm /3.32 bar	37.1 (1.8)	97.0 (4.0)	0.10	0.10	0.16	0.08	26.0	41.7
Pall-0,2 μm /2.81 bar	34.0 (1.6)	75.8 (3.6)	0.08	0.05	0.15	0.05	23.3	37.9
Tami-0,2 μm /2.29 bar	43.3 (1.5)	89.5 (2.4)	0.10	0.09	0.13	0.09	35.7	41.6

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308 Table 4. Selection of the optimal operating conditions for concentration, purification and
309 fractionation by nanofiltration of betacyanins present in microfiltered cactus pear juice.

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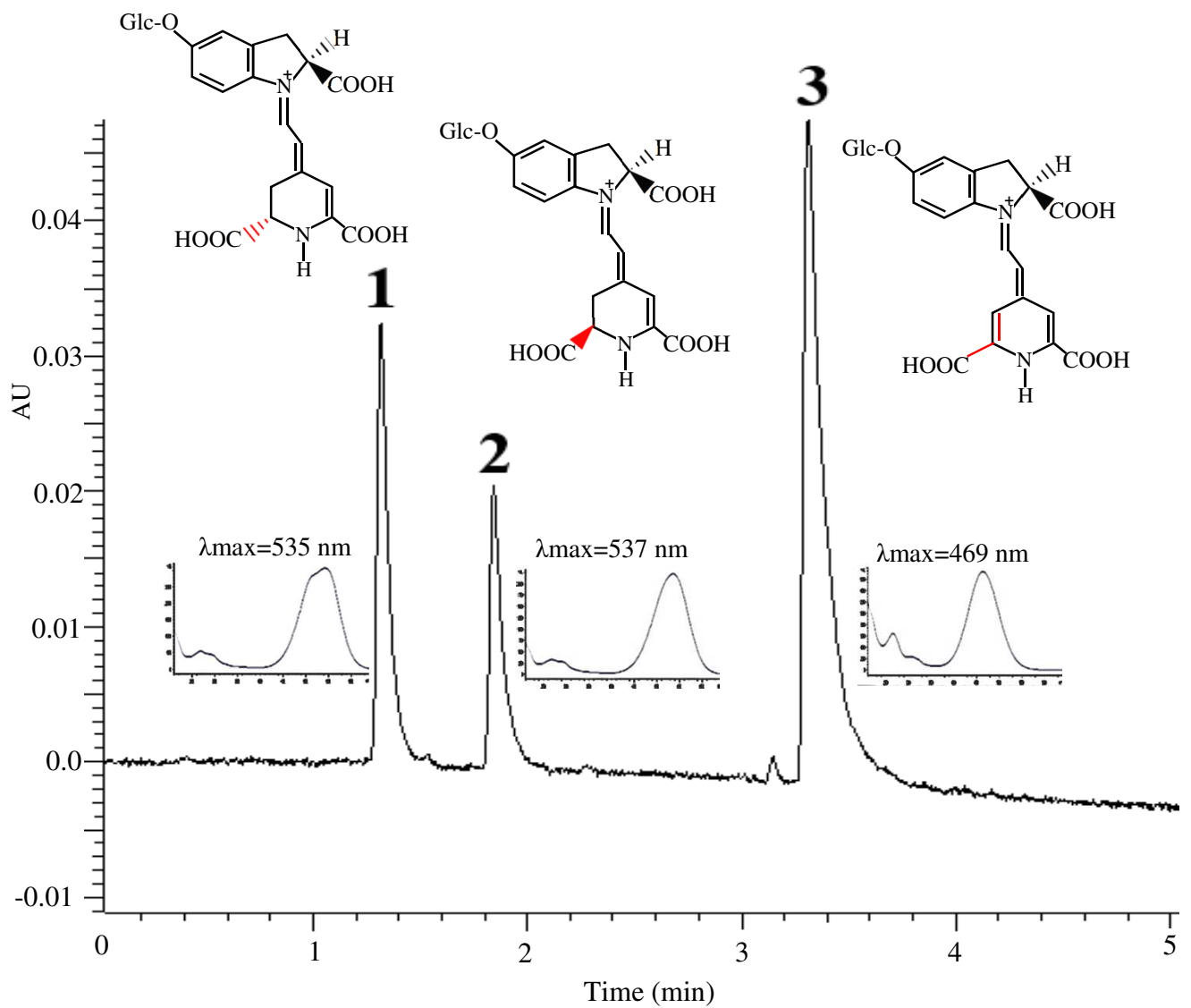
Membrane	Transmembrane pressure TMP (bar)		
	Concentration	Purification	Fractionation
Nadir 0.2 kDa	5	-	-
Koch 1 kDa	15	5	5
Nadir 1 kDa	25	10	5
Nadir 4 kDa	-	10	5

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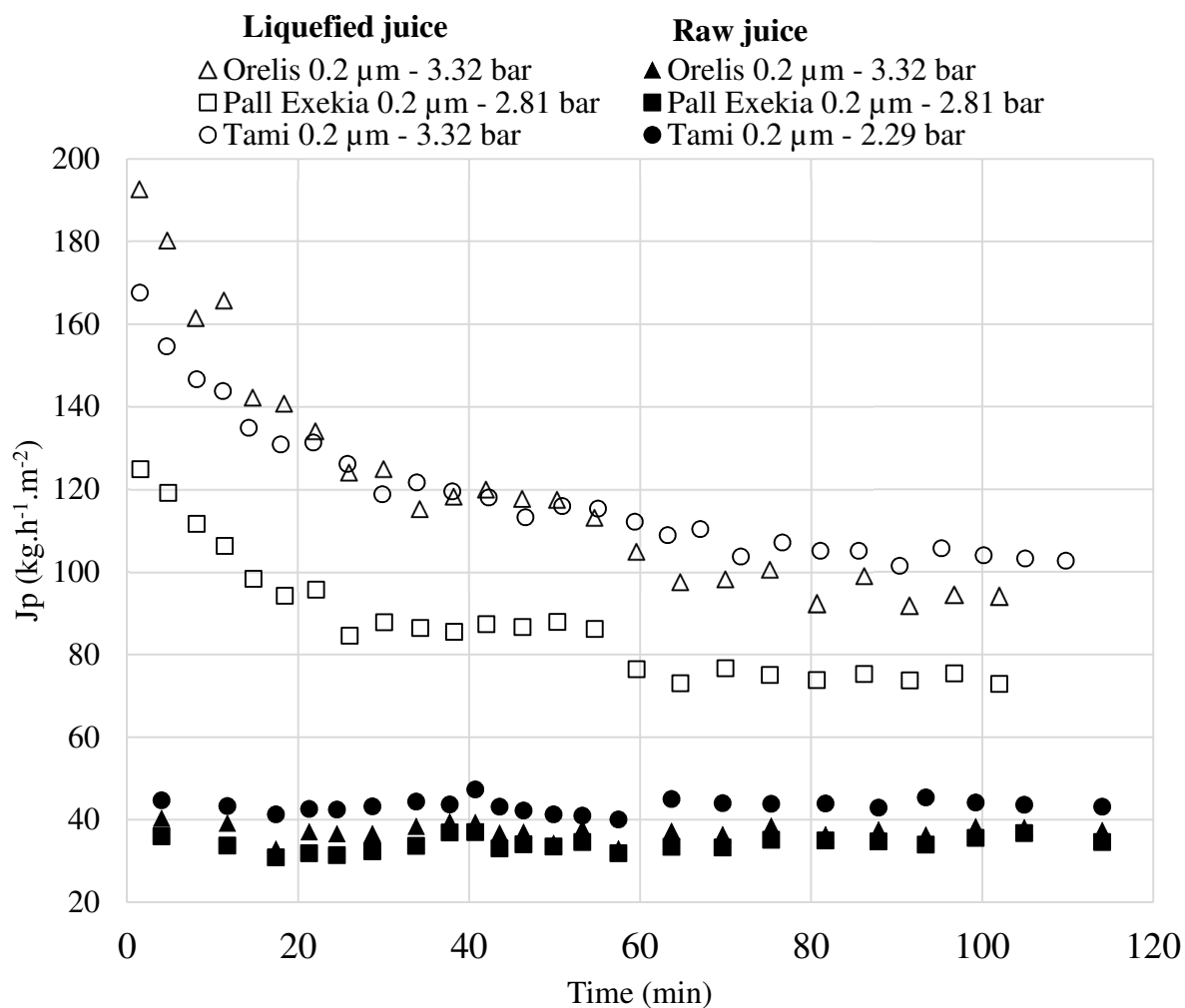
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316 Fig. 1. HPLC chromatograms at 484 nm of the betacyanins extracted from raw cactus pear
317 juice with their visible absorption spectrum: betanin (1), isobetanin (2), neobetanin (3).

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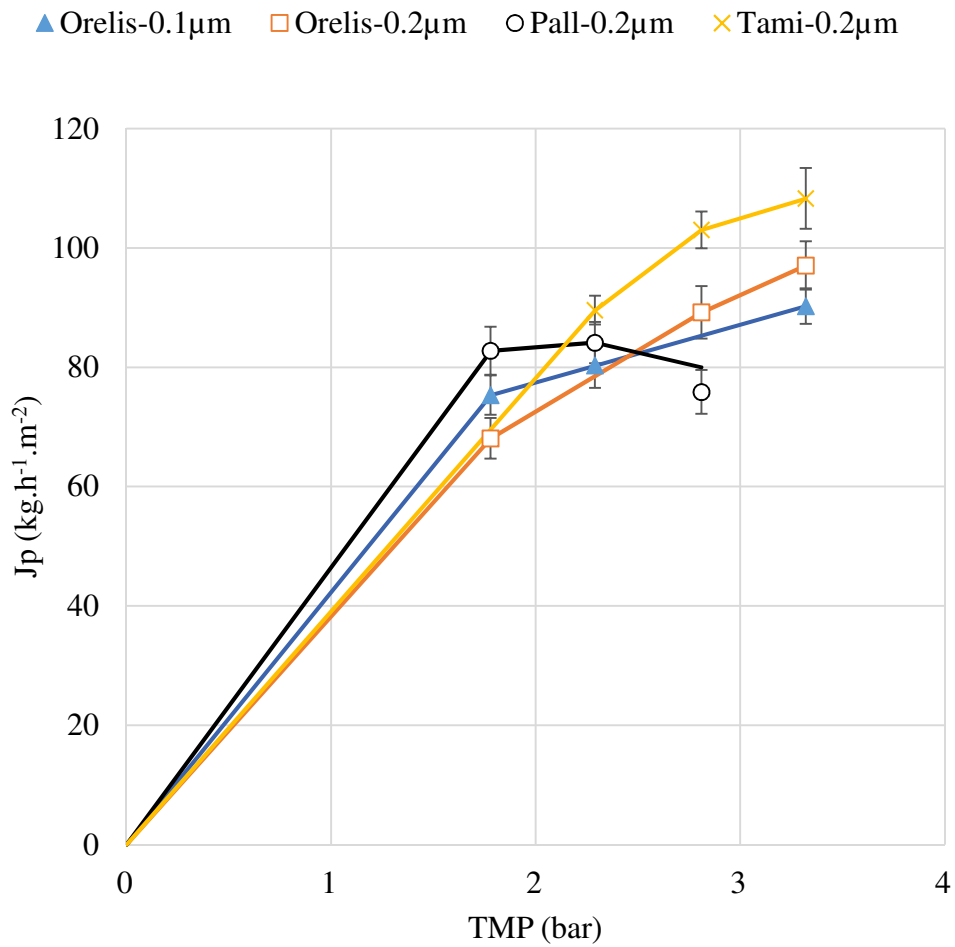


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322 Fig. 2. Examples of permeate flux (J_p) evolution vs. time during the clarification of cactus
 323 pear juice by microfiltration at $\text{MRR} = 1$ with different membranes and operating conditions.

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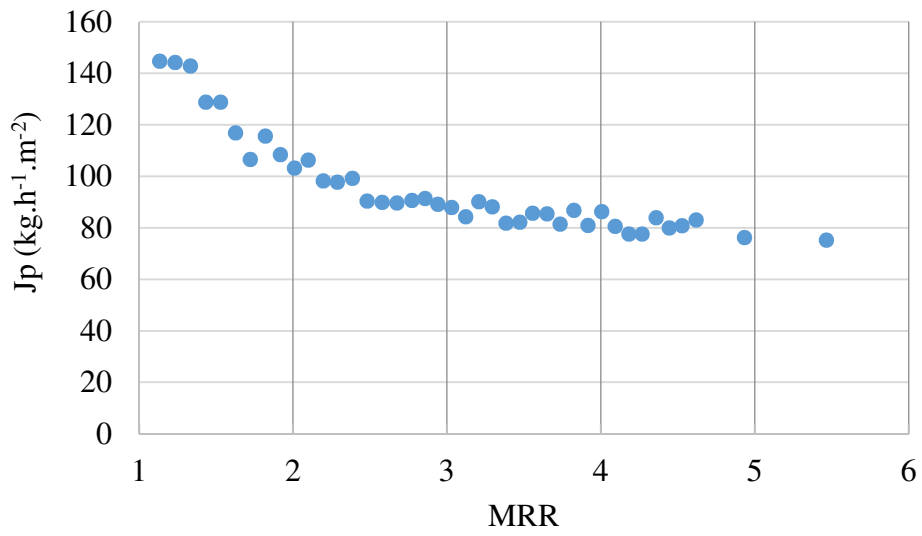
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328 Fig. 3. Effect of transmembrane pressure (TMP) on permeate flux (J_p) during microfiltration
 329 of liquefied cactus pear juice using different membranes at MRR = 1.

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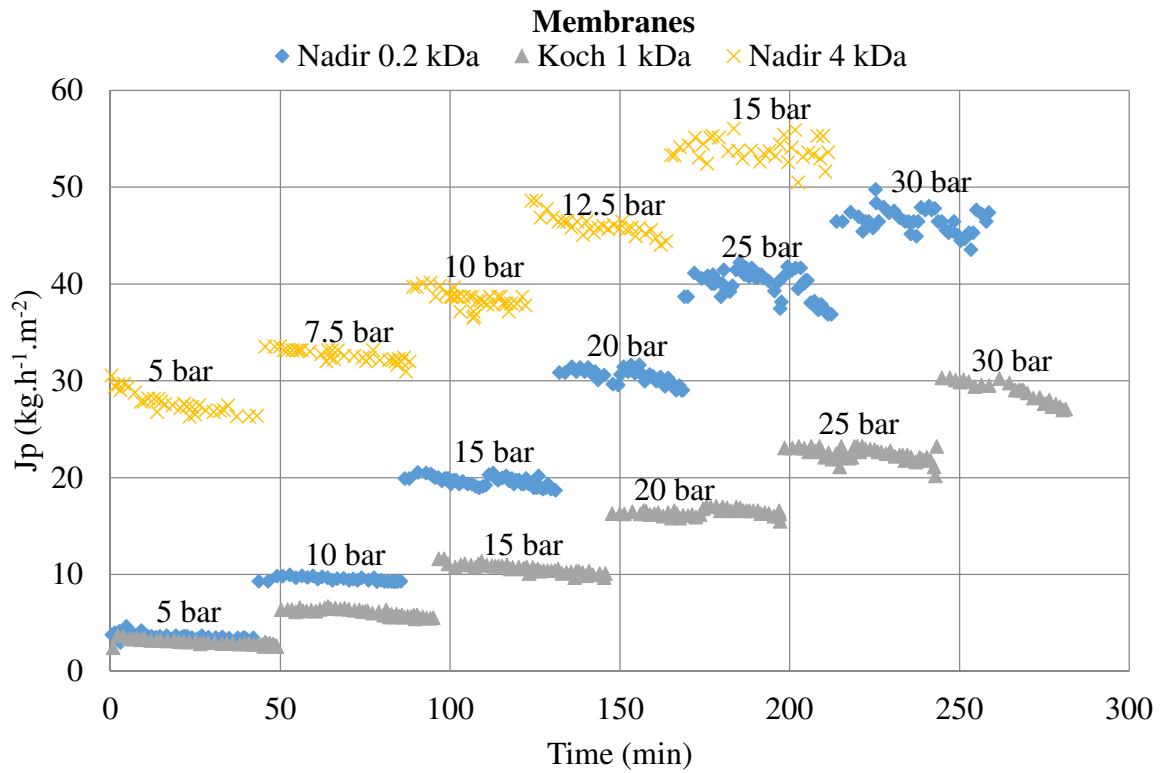


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334 Fig. 4. Permeate flux (J_p) vs. mass reduction ratio (MRR) during the clarification of liquefied
335 cactus pear juice by microfiltration (membrane Tami 0.2 μm , TMP = 3 bar).

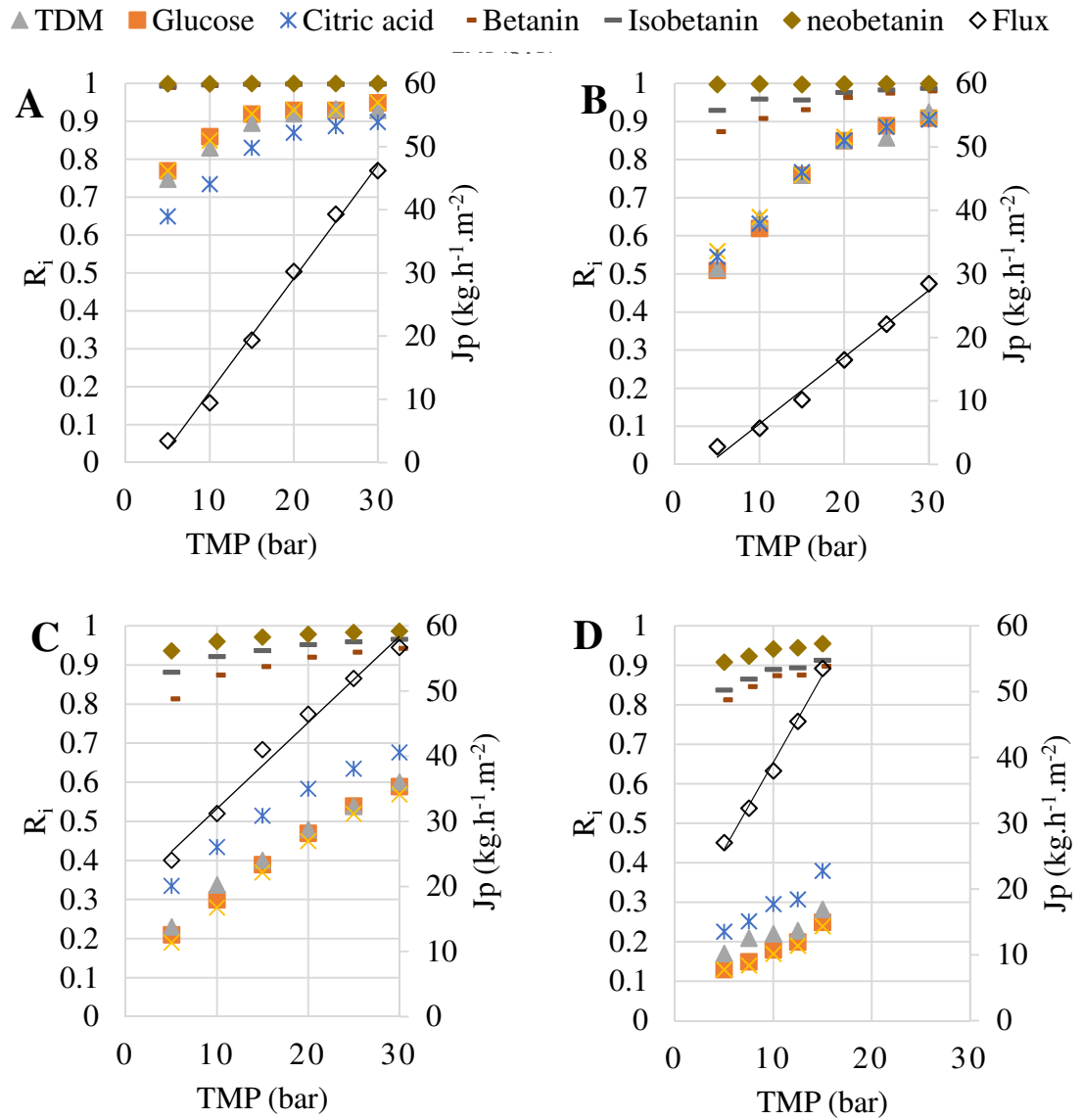
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340 Fig. 5. Examples of the evolution of permeate flux (J_p) vs. time during ultra/nanofiltration of
341 microfiltered cactus pear juice increasing transmembrane pressure.

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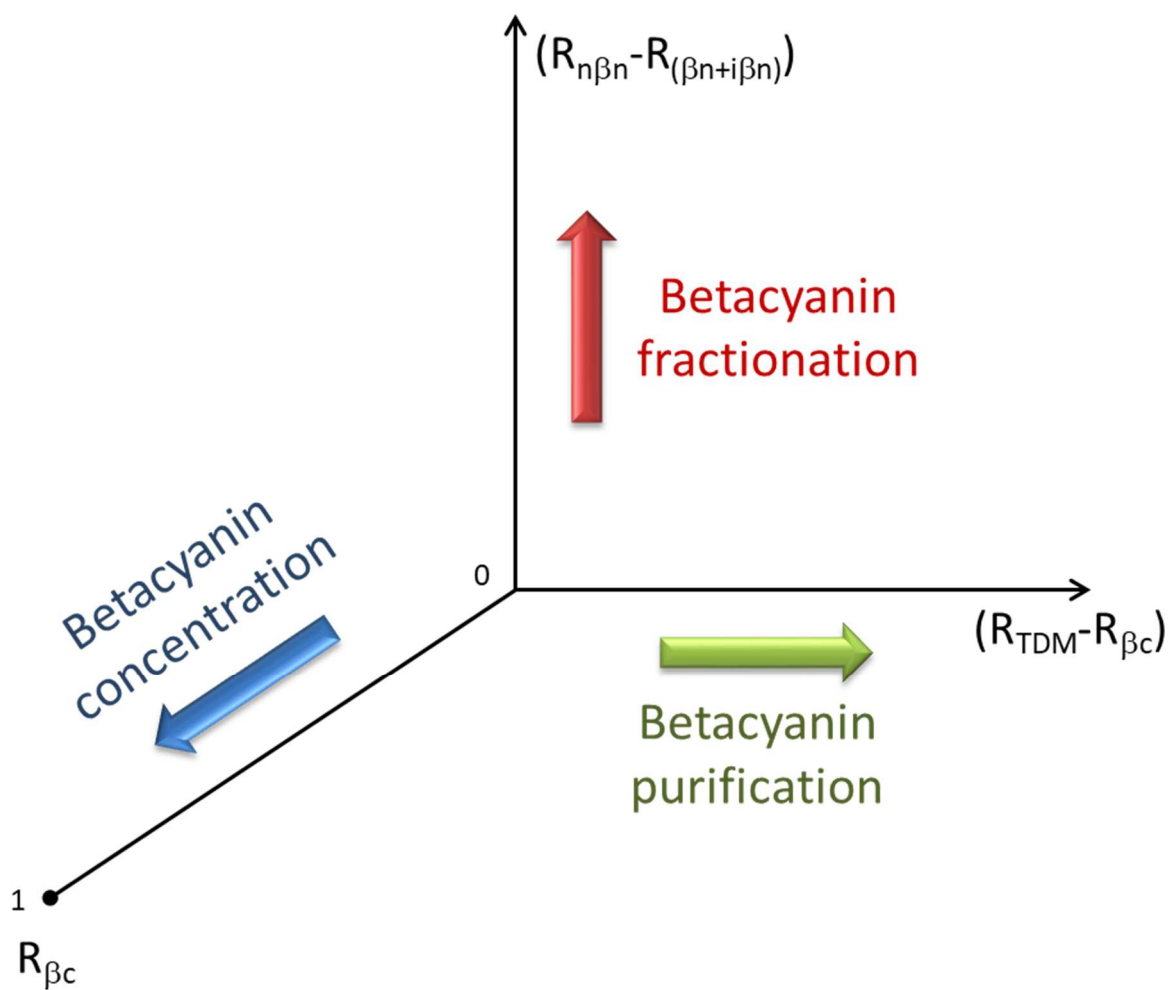
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344 Fig. 6. Permeate flux (J_p) and solute retentions (R_i) obtained at different transmembrane
 345 pressures (TMP) during ultra/nanofiltration of microfiltered cactus pear juice using the
 346 membranes Nadir 0.2 kDa (A), Koch 1 kDa (B), Nadir 1 kDa (C) and Nadir 4 kDa (D).

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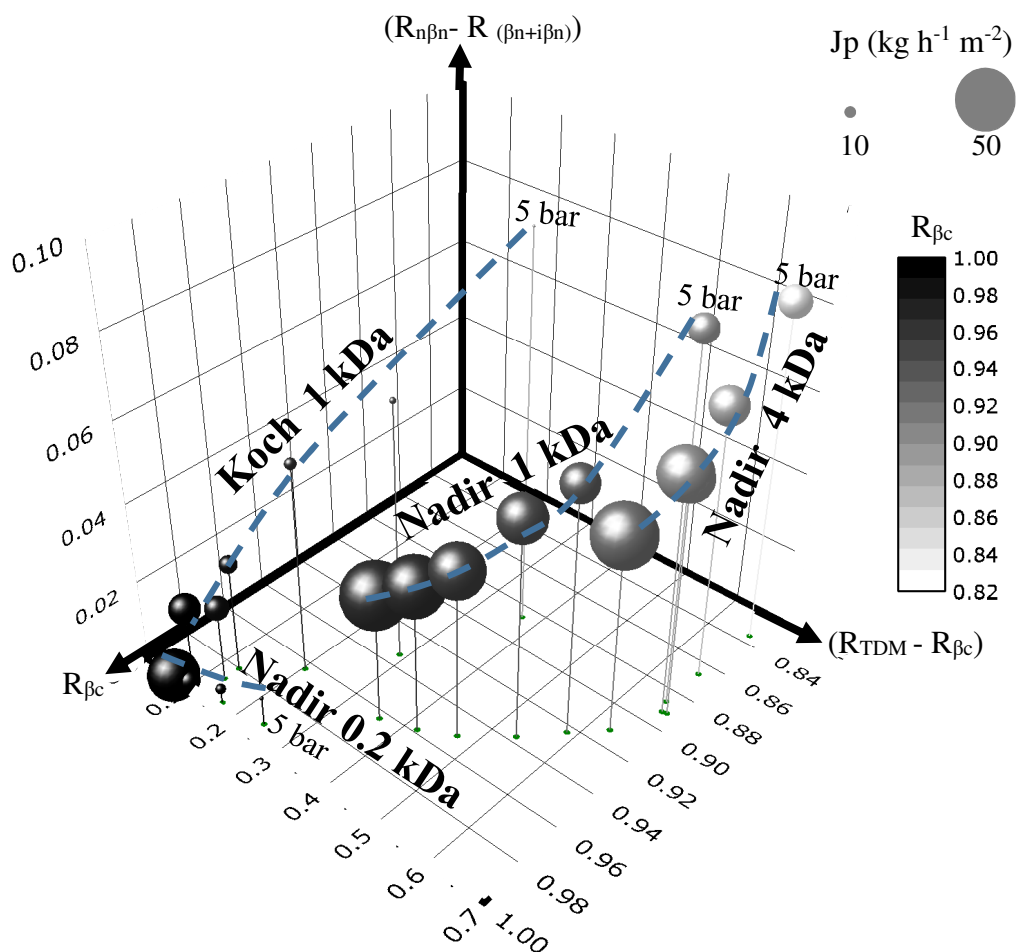
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352 Fig. 7. Potential types of separation by nanofiltration that can be considered according to
353 solute retentions R_i (βc total betacyanins, $n\beta n$ neobetanin, $(\beta n + i\beta n)$ betanin and isobetanin,
354 TDM total dry matter).

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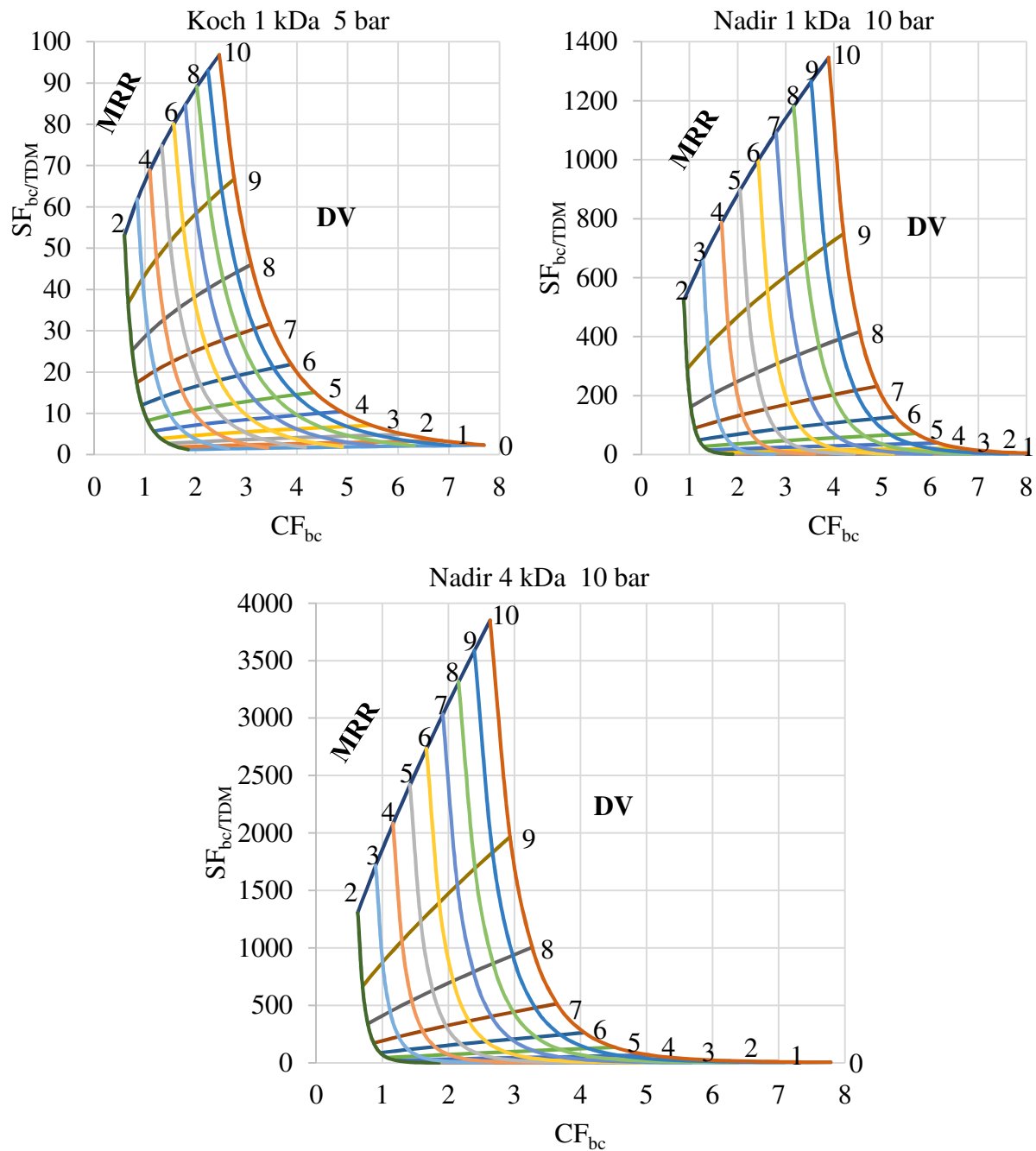
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360 Fig. 8. Representation of the different combinations membrane/pressure tested for
361 ultra/nanofiltration of microfiltered cactus pear juice according to the solute retentions R_i (βc
362 total betacyanins, $n\beta n$ neobetanin, $(\beta n+i\beta n)$ betanin and isobetanin, TDM total dry matter)
363 and permeate flux obtained (J_p).

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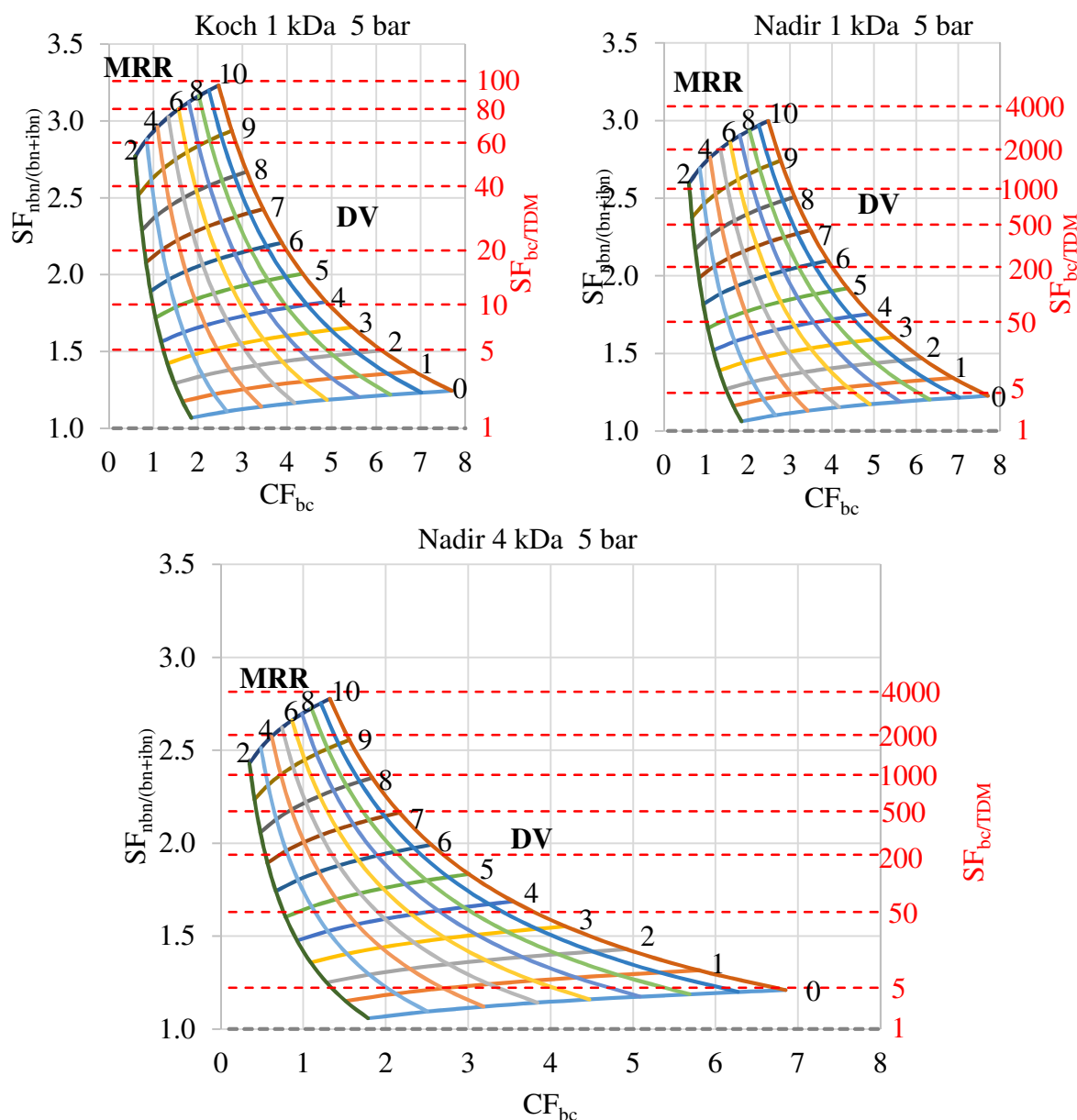
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369 Fig. 9. Concentration factor of betacyanins (CF_{β_c}) and separation factor between betacyanins
370 and total dry matter ($SF_{\beta_c/TDM}$) achievable in the case of 3 membrane/TMP combinations
371 according to the mass reduction ratio (MRR) and the diavolume (DV) for the purification by
372 ultra/nanofiltration of betacyanins of microfiltered cactus pear juice.

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377 Fig. 10. Concentration factor of betacyanins (CF_{β_c}) and separation factors between betacyanins and
378 total dry matter ($SF_{\beta_c/TDM}$) and between neobetainin and the other betacyanins ($SF_{n\beta_n/(\beta_n+i\beta_n)}$)
379 achievable in the case of 3 membrane/TMP combinations according to the mass reduction ratio
380 (MRR) and the diavolume (DV) for the fractionation by ultra/nanofiltration of betacyanins of
381 microfiltered cactus pear juice.