Modulation of carotenoid/flavonoid profiles and sugar content of a potential functional citrus-based food through crossflow microfiltration

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Abstract

Crossflow microfiltration was implemented to enrich a clementine / pink grapefruit juice in carotenoids, flavonoids and pectins modulating its sugar content thanks to an added diafiltration step. Using tubular ceramic membrane with pore diameter of 0.2 µm at 30-40°C, a crossflow velocity of 5 $m \cdot s^{-1}$ and a transmembrane pressure of 2.6 bar, the impact of the process was assessed focusing on bioactive compounds and physical characteristics of the concentrates. With permeate flux above 30 $kg \cdot h^{-1} \cdot m^{-2}$, the process resulted in an increase of 8 to 10 times in the content of provitamin A carotenoids (33 $mg \cdot kg^{-1}$ in the final products), lycopene (43 $mg \cdot kg^{-1}$), hesperidin (3.2 $g \cdot kg^{-1}$) and pectin (5.5 $g \cdot kg^{-1}$) giving a unique and interesting composition profile. Moreover, diafiltration divided the sugar content by 3. This can be relevant for the product to be recommended as part of a reduced sugar diet for diabetic people. By modelling the retention of bioactive compounds, we showed that it was possible to modulate sugar, flavonoids, carotenoids and pectin contents in the concentrate that could be a key issue for the biological activity of this potential functional citrus-based food.

Keywords

Membrane process; lycopene; provitamin A carotenoids; citrus flavonoids; functional food

1. Introduction

Citrus fruits and their juices represent important sources of phytochemical bioactive compounds such as carotenoids (β-cryptoxanthin: βcx, β-carotene: βc, lycopene: Lyc) and flavonoids (hesperidin: Hes, naringin: Nar) which can contribute with certain key micronutrients such as vitamins (C, folate), minerals and fibers (pectins) to their beneficial effects on health including anti-oxidant activity, cardiovascular desease prevention and obesity control (Lv et al., 2015).

With regards to phytochemicals, the health properties of citrus flavonoids such as Hes and Nar are largely described in the literature. They include antioxidant anti-inflammatory, anti-carcinogenic, anti-diabetic and anti-hypertensive effects (Mahmoud, Hernandez Bautista, Sandhu, & Hussein, 2019). Hes was mainly found in orange and mandarin, and Nar in grapefruit (Dhuique-Mayer, Caris-Veyrat, Ollitrault, Curk, & Amiot, 2005; Fanciullino et al., 2006). Other citrus phytochemicals of interest are carotenoids. The health effects of carotenoids are associated with their antioxidant properties, which reduce the risk of low-density lipoprotein oxidation, heart disease, eye disease, as well as cancer (Ciccone et al., 2013; Karn 2020). Among the major carotenoids identified in citrus, the pro-vitamin A carotenoids mainly βcx and βc have a primordial role for human health, especially for eyesight, growth, development and immune response (Burri 2015). Moreover, *in vivo*, animal and human studies have showed that βcx plays an important role in bone homeostasis (Yamaguchi, 2012). Lyc, another carotenoid found in pink grapefruits has attracted much interest with regard to health benefits mainly for its role in antioxidative, anti-atherogenic and anticarcinogenic activities (Camara et al., 2013). Citrus juices are part of healthy diet and contribute to a healthy lifestyle. European guidelines (NHS National Health Service, PNNS Plan National Nutrition et Santé) recommends a maximum of one glass of 150-200 mL of fruit juice daily as part of the 5 fruits and vegetable a day campaign (Braesco, Gauthier &

Bellisle, 2013). This recommendation is in line with limiting the intake of free sugars recently reduced to less than 5% of total daily energy intake (25g) (WHO, 2015). Knowing that a glass of 250 mL of 100% orange juice contains 20 g of sugars, it would be preferential to consume high fibers foods that promote reduced sugar absorption (Silva et al., 2013). Thus, citrus-based products enriched in nutrients of interest with less or no sugars could meet consumer demands for food offering health benefits and provide an alternative to regular citrus juice consumption.

Crossflow microfiltration (CMF) can be used to produce new citrus based products especially enriched in phytochemicals such as carotenoids and certain flavonoids as well as in pectin without heating (Polidori, Dhuique-Mayer, & Dornier, 2018; Gence, Servent, Poucheret, Hiol, & Dhuique-Mayer, 2018). This membrane process mainly used to clarify or to stabilize fruit juices was used to concentrate insoluble phytochemicals. Carotenoids and flavonoids are often retained by the porous membrane and thereby concentrated in the retentate. Moreover, the process can be used to modulate and control the concentration in bioactive compounds as well as the proportion between insoluble and soluble solids such as sugars in the concentrate depending on the objective. Indeed, a diafiltration step can be added to a microfiltration step in order to remove soluble compounds especially sugars, for health benefits purposes. This step is used in industrial processing for example to reduce bitterness (grapefruit juice) associated to such as Nar and limonoids in order to make a product more desirable by consumers (Ilame & Singh, 2015). Therefore it is of great interest to focus on the advantage of concentrating bioactive compounds (carotenoids and flavonoids) as well as on removing a high part of sugars.

The aim of this work was to modulate the nutritional quality of citrus-based products obtained by crossflow microfiltration (CMF). These citrus-based products can be considered as potential functional foods because they can be enriched in carotenoids, flavonoids, pectins and possibly made with or without sugar. In order to better know their nutritional quality before carrying out a detailed nutrition-health study, two products were obtained with or without a diafiltration step allowing the sugar content to vary. They were characterized through their bioactive compounds (βcx, βc, Lyc, Hes, Nar, and Nat) and pectin content. The impact of the membrane process was evaluated on bioactive compounds by pointing out the correlations with physico-chemical properties of the citrus-based food. Finally, a simple predictive model was proposed in order to forecast composition profiles as a function of operating conditions.

2. Materials and methods

2.1.Citrus juices

Commercial flash-pasteurized 100% pure clementine (*Citrus clementina* Hort. Ex. Tan) and pink grapefruit (*Citrus paradisi* Macf.*)* juices were purchased from a local food supplier (Carrefour, Montpellier, France). Juices were kept at 4°C between 4 and 5 days until processing. Then, the initial citrus juices were formulated by mixing 60/40% clementine juice/pink grapefruit juices (v/v) using juices from 4 different 15 L batches.

2.2.Crossflow microfiltration process

The process of crossflow microfiltration (CMF) was performed at laboratory scale using a TIA device (Bollène, France) already described in details by Polidori et al. (2018). The system was equipped with a 3 L feed tank, a positive-displacement Moineau pump that

allowed to feed the 1 L circulation loop under pressure at high velocity and 4 Pall-Exekia ceramic tubular membranes (Bazet, France) of 0.2 µm average pore diameter and 55 cm^2 filtering area each. Two concentrates were obtained by recovering the retentate up to a mass reduction ratio MRR (Eq. 1) between 8 and 9 with or without a final diafiltration step.

$$\boldsymbol{MRR = 1 + \frac{m_p}{m_r}} \quad \textbf{(1)}$$

With m_p, mass of permeate (kg), and m_r mass of retentate (kg).

During the concentration process, the mass of retentate was maintained constant in the circuit by adding the same mass of fresh juice into the feeding tank to compensate the extracted permeate. The concentration factor of the compound i (CF_i) in the retentate was calculated using Eq. 2.

$$\boldsymbol{CF_i = \frac{C_i}{C_{i0}}} \quad \textbf{(2)}$$

with C_i, final concentration of compound i in the retentate and C_{i0}, initial concentration of compound i in the feed.

The diafitration step was carried out at the end of the concentration phase without modifying the filtration conditions. It consisted in adding distilled water to the system in order to remove the water soluble compounds. The addition of distilled water meant that the mass of retentate remained constant by compensating the mass of extracted permeate up to a diamass ratio DMR of 1 (Eq. 3). From the initial and final purities of the retained compound i calculated on the basis of total dry matter content (TDM), p_i and p_{i0} (Eq. 4), the purification factor of the compound i, PF_i was calculated according to Eq. 5.

$$\boldsymbol{DMR = \frac{m_w}{m_r}} \quad \textbf{(3)}$$

with m_w, mass of added water (kg) and m_r, mass of retentate (kg).

$$p_i = \frac{C_i}{TDM} \quad \textbf{(4)}$$

$$PF_i = \frac{p_i}{P_{i0}} \quad \textbf{(5)}$$

The citrus concentrate obtained by microfiltration without a diafiltration step was labelled (DF0) and the concentrate from microfiltration coupled with diafiltration up to DMR 1 was labelled (DF1). These concentrates were stored in amber sealed bottles under nitrogen and kept frozen (-20°C) until physico-chemical and biochemical analyses. Assuming retentions R_i (Eq. 6) are constant, mass balances are correct (no losses), the retentate compartment is perfectly stirred and changes in product density during the process can be neglected (density differences below 3.10^{-2} kg L^{-1}), CF_i in the retentate can be evaluated thanks to the Eq. 7 from MRR and DMR (Polidori et al., 2018).

$$R_i = 1 - \frac{C_{ip}}{C_{ir}} \quad \textbf{(6)}$$

with C_{ip} and C_{ir}, the concentrations of the compound i in the permeate and the retentate respectively.

$$CF_i = MRR^{R_i}\, e^{DMR\,(R_i - 1)} \quad \textbf{(7)}$$

The purification factor of the compound i compared to TDM can then be expressed as Eq. 8.

$$PF_i = \frac{CF_i}{CF_{TDM}} \quad \textbf{(8)}$$

2.3. Macroanalyses and physical characterization

Total soluble solids (TSS) were measured with a digital refractometer Pal3 Pocket Atago (Tokyo, Japan) at 25°C and total dry matter (TDM) was measured by drying 2.0 g of sample

in an oven at 70°C under vacuum (100 mbar) for 24 h. pH was assessed using TitroLine pH-meter (Schott instruments, Mainz, Germany) at 20°C. Titratable acidity (TA) was measured with the same equipment, by titration with 0.1 mol·L^{-1} NaOH solution. Suspended insoluble solids (SIS) were determined by centrifugation at 3000 x g for 20 min (Gence et al 2018). The rheological analyses were measured using a constrained strain Physica MCR301 rheometer (Anton Paar GmbH, Graz, Austria). The experimental results were analysed by the apparent viscosity representation η (mPa·s) as a function of shear rate. Rheological measurements allowed the calculation of a flow index in order to determine whether the samples rheofluidifyed, rheo-thickened, or were Newtonian by the classical power law.

Particle size was determined according to Gence et al., (2018) by LASER diffraction using a Mastersizer 3000 (Malvern Instruments Limited, Worcestershire, UK). The statistical diameter Dx 50 which corresponds to the diameter for which 50% of particle volume of the suspension has a diameter less than the value indicated, as well as the surface area mean diameter (D [3,2]) were derived from the measured distribution. Turbidity was measured using a Hanna LP 2000 turbidimeter (Hanna Instruments, Woonsocket, USA). The color was determined using a chromameter CR-410 (Minolta, Tokyo, Japan). From the CIE (L*, a*, b*) coordinates obtained, the hue angle (h*) and color difference from the initial juice (ΔE*) were also calculated

2.4. Macronutrients analysis

Simple soluble sugars were analyzed by HPLC according to Gies, Descalzo, Servent, & Dhuique-Mayer (2019). Briefly, 2 g of sample was twice extracted with 8 mL ethanol (80%), then the mixture was heated at 80°C for 10 min. After homogenization and centrifugation (5000 x g, 10 min, 10°C, Beckman Coulter, USA), the supernatant was filtered through a 0.45

192 µm membrane before injection in UPLC. Samples were analyzed using an UPLC – 1290
193 System Infinity II (Agilent, USA) equipped with a refractometer detector. A SHODEX
194 SH1011 column 300x8 mm (Tokyo, Japan) was used with an isocratic system of water with
195 H_2SO_4 (0.01 %) and a flow rate of 0.7 mL·min^{-1}. Temperature was set at 30°C, injection
196 volume at 10 µL and spectrophotometric detection at 210 and 245 nm. External calibration
197 was established for each standard sugar for concentrations from 0 to 10 g·L^{-1}. The pectin
198 content is determined from alcohol–insoluble residue (AIR) and then extracted in acidified
199 water, that was adjusted to pH 1.5 with nitric acid prior to acetone precipitation and drying
200 according to the method reported by De Roeck, Mols, Duvetter, Van Loey, & Hendrickx
201 (2010).
202

203 **2.5. Carotenoid analysis**

204 Carotenoids extraction was carried out in triplicate according to Gies et al (2019). A quantity
205 of 2 g of juice, or 0.5 g of concentrate with 0.5 mL distilled water added, was weighed in a
206 glass tube, mixed with 2 mL of pyrogallol 1% in ethanol and was homogenized 30 s on a
207 vortex. Then the tube was placed for 3 min in a water-bath at 70°C. After cooling, 2 mL of 12
208 mol·L^{-1} KOH was added and the tube was set in a water-bath 30 min at 70°C for
209 saponification. After cooling in an ice-bath, 2 mL of distilled water was added, in order to
210 help for phase shifting. Then, samples were extracted twice with 5 mL of n-hexane. The
211 organic phases were pooled and evaporated under nitrogen. The residue was dissolved in 500
212 µL of CH_2Cl_2 and 500 µL of MTBE/MeOH (4:1, v/v), transferred to an amber vial before
213 injection into the HPLC system.
214
215 Carotenoids were analyzed by HPLC using the Agilent 1100 system (Agilent, Massy, France)
216 with a diode array detector. Carotenoids were separated along a C30 column 250 x 4.6 mm

i.d.,5 µm (YMC, Tokyo, Japan). The mobile phases were H_2O as eluent A, methanol as eluent B, and MTBE (methyl-tert-butyl-ether) as eluent C with the gradient used by Poulaert, Borel, Caporiccio, Gunata, & Dhuique-Mayer (2012). The flow rate was fixed at 1 $mL \cdot min^{-1}$. The column temperature was set at 25°C and the injection volume was 20 µL. The absorbance was measured at 470 and 450 nm. Chromatographic data and UV-Visible spectra were treated using the Agilent Chemstation Plus software. Quantification of carotenoids was achieved using calibration curves with 5 concentration levels from 2 to 15 $mg \cdot L^{-1}$ for the βc standard, from 10 to 40 $mg \cdot L^{-1}$ for βcx and from 7 to 50 for Lyc.

2.6. Flavanone glycoside analysis

The flavanone glycosides hesperidin (Hes), naringin (Nar) and narirutin (Nat) were determined according to the method described in Dhuique-Mayer et al. (2005). Briefly, 5 g of juice or 1 g of concentrate were extracted with 10 mL dimethylformamide and 10 mL ammonium oxalate 0.05 $mol \cdot L^{-1}$ (1:1 v/v) in tightly Pyrex tube. Then, the tubes were heated in an oil bath at 90°C for 10 min. After cooling, the volume were adjusted to 50 mL with distilled water. After centrifugation, the supernatant was filtered through a 0.45 µm membrane and analyzed by HPLC-Agilent 1100 system (Agilent, Massy, France). The isocratic solvent system was made of water/acetonitrile/THF/acetic acid (80:16:3:1, v/v/v/v). Quantification was carried out at 280 nm. The flow rate was fixed at 1 $mL \cdot min^{-1}$ and the injection volume was 20 µL. Flavanone glycoside concentrations were determined using an external calibration method. Standard solutions of Hes, Nat and Nar were diluted in DMF/water (2:1, v/v) to reach maximum concentration of 151 and 158 and 160 $mg \cdot L^{-1}$ respectively.

2.7. Statistical analysis

All statistical analyses were performed using XLSTAT software version 2019.4.1 (Addinsoft, Paris, France). All data were reported as mean and standard deviation from 3 replicates of each experiment. Data were analyzed statistically using one-way analysis of variance (ANOVA) in order to determine significant differences ($p < 0.05$). Tukey's multiple comparison method was used to further examine any significant difference between results.

3. Results and discussion

3.1. Nutritional quality of the initial citrus juices

The initial citrus juice was formulated with 60/40% *C. clementina* / *C. paradisi* juices in order to optimize carotenoid and flavonoid profile. Three main carotenoids were found in this initial citrus juice: βcx and βc, the pro-vitamin A carotenoids coming from clementine juice and lycopene, the well-known antioxidant carotenoid representing the major carotenoid in pink grapefruit juice. Lyc was the major carotenoid of this formulated juice for lots A and B (4.47 $mg \cdot kg^{-1}$) followed by βcx (2.68 $mg \cdot kg^{-1}$) and βc (1.08 $mg \cdot kg^{-1}$) (Table 1). The main flavanone glycosides identified in this initial citrus juice was Hes related to clementine juice and displaying the highest concentration (376.2 $mg \cdot kg^{-1}$). Nat was the minor flavanone glycoside from clementine juice with a content 5 times lower than Hes (71.5 $mg \cdot kg^{-1}$). Finally, Nar was the main flavanone from pink grapefruit (259.6 $mg \cdot kg^{-1}$). Together, these bioactive compounds of nutritional interest with pectin and sugars contained in initial juices (Table 2) can be concentrated or modulated in a same functional citrus-based product by CMF. Note that citrus juices from lot C and D had lower levels of βcx and Hes presenting a less interesting nutritional profiles (Table 1).

3.2. Crossflow microfiltration process

The permeate flux (Jp) decreased (30%) with the MRR (between 1 and 9) for both concentrates (DF0 and DF1) to reach a value between 30 and 40 $kg \cdot h^{-1} \cdot m^{-2}$ at MRR of 8-9, with a sudden drop (MRR of 1 to 3) and then with a pseudo-stabilization from 4 to 9 MRR (Figure. 1). This typical behavior is mainly associated with the increase of the viscosity and of the fouling power of the retentate during concentration, which contributes to decreasing the permeability of the filtration system (Jesus et al., 2007). The permeate flux was quite similar to that obtained by Polidori et al., (2018) during concentration of different orange juices (from 20 to 80 $L \cdot h^{-1} \cdot m^{-2}$). For DF1 concentrate, Jp increased a little during the diafiltration step from 30 to 40 $L \cdot h^{-1} \cdot m^{-2}$. This could probably be explained by the decrease in viscosity of the permeate due to the solute dilution by adding distilled water. These results showed good repeatability for the flux at high MRR (variation coefficient between 6-15%) except for lot D. Conversely, flux was less repeatable at low MRR and this was probably due to the different batches or to slight temperature variation between filtration trials.

3.3. Effect of CMF on physical properties and main constituents of citrus concentrates

The physical characteristics as well as pectin and sugar contents of initial citrus juices and both concentrates are reported in Table 2. The viscosity of concentrates increased comparatively with juices because the membrane process concentrated insoluble solids and pectin almost 10 times (Cho, Lee, & Kim, 2003). The progressive decrease of soluble compounds (e.g. decrease of 67% of sugars), due to the addition of water during the diafiltration step. explained the final lower viscosity ($p < 0.05$) of DF1 compared with DF0.

289 Particle size distribution indicated that both concentrates were very close to each other (Figure
290 2). CMF reduced the size of the particles (40%) and this agreed with previous studies carried
291 out on fruit juice and orange juice (Dahdouh et al 2016; Sentandreu, Gurrea, Betoret, &
292 Navarro, 2011). Gence et al (2018) also reported that the membrane process led to the
293 mechanical disruption of particles by high shear-rate in the circulation loop. The pulp content
294 or suspended insoluble solids (SIS), which represented a small mass fraction of the TDM, was
295 higher for DF0 than DF1 and was correlated to turbidity (Tamba, Servent, Mertz, Cisse, &
296 Dornier, 2019). The higher pulp content in DF0 was due to retention of insoluble solids of
297 initial juice because DF0 had a higher MRR (MRR = 9) than DF1 (MRR= 8). Dahdouh et al.,
298 (2016) showed that there was a relationship between turbidity and SIS content for fruit juices.
299 Total dry matter (TDM), total soluble solids (TSS), titrable acidity, as well as sugars were
300 lower for DF1 compared to DF0. The cause of this significant difference ($p < 0.05$) was
301 mainly due to the elimination of the soluble fraction in permeate during the diafiltration step.
302 Sugars constituted the majority of TDM in the initial juice and the diafiltration led 73% of
303 TSS in DF1 being removed, of which 67% were soluble sugars. CMF membranes only retain
304 the insoluble fraction. Solutes are logically not retained by the membrane because of their low
305 molar mass between 180 and 192 $g \cdot mol^{-1}$ (Tamba et al , 2019). All concentrates presented a
306 bright orange color, which was due to the concentration of carotenoids by the membrane
307 process. Only a slight difference was observed for the color parameter (L* and b*) indicating
308 that DF1 was slightly darker than DF0 probably caused by the diafiltration step which
309 increased the purity of all carotenoids, making the product darker.

310

311 **3.4. Effect of CMF on carotenoids and flavonoids**

312 Similar effects of CMF on carotenoids and flavonoids were observed whatever the lot of
313 initial juice even if the carotenoids/flavonoids profiles of juices C and D were slightly less
314 nutrionnally pertinent in term of composition in bioactive compounds. The two types of
315 carotenoids xanthophylls and carotenes of which βcx, βc and Lyc, were similarly
316 concentrated by approximately 9-fold, the corresponding concentration being 25.9 $mg \cdot kg^{-1}$;
317 10.5 $mg \cdot kg^{-1}$ and 47.2 $mg \cdot kg^{-1}$ respectively in average for lot A and B. This unique profile
318 made the citrus-based product nutritionally interesting because no vegetables nor fruits were
319 equivalent to these carotenoids levels and profile. Among fruit rich in βcx, butternut squash
320 represents the higher content with 34.7 $mg \cdot kg^{-1}$ but all the others ranged from 1.16 to 13.4
321 $mg \cdot kg^{-1}$ (Burri, La Frano, & Zhu, 2016). Lycopene from fresh tomato displayed a content of
322 17.4 $mg \cdot kg^{-1}$ (Karakaya & Yilmaz 2007). Pink grapefruit juices, considered rich in Lyc and
323 βc, displayed a content of 13.4 $mg \cdot kg^{-1}$ and 3.8 $mg \cdot kg^{-1}$ respectively (Achir et al 2016).
324 Moreover, Hes the more hydrophobic flavanone glycoside was also concentrated 8.5-fold
325 showing that this compound, like carotenoids, was completely retained by the membrane.
326 Conversely, Nat and Nar were either incompletely or not retained at all by the membrane and
327 were recovered in permeate fraction. Their concentration factor ranged from 1.3 to 2.8
328 respectively for Nar and Nat. These results were consistent with previous work on CMF of
329 citrus juices where Nat was less retained by membranes (Polidori et al., 2018). The different
330 solubility of the major flavanone glycosides (0.02 $g \cdot L^{-1}$ for Hes and 0.6 $g \cdot L^{-1}$ for Nar) can
331 explain their different behaviors. Probably the number, localization and nature of the
332 substituents on the flavonoid skeleton, as well as the conformation of bound glucosides
333 greatly influenced their solubility. Carotenoids and Hes were associated with the insoluble
334 fraction, and their concentration factors are approximately the same (and very close to MRR)
335 than those reported by Gence et al (2018) or Polidori et al (2018). Adding a diafiltration step
336 to crossflow microfiltration helped to better purify the bioactive compounds. Carotenoids and

Hes were purified from 12 to 16-fold whereas Nat was purified only 3 times and Nar almost not in accordance with the much lower retention of these last two flavonoids.

3.5. Correlations between physico-chemical characteristics and bioactive compounds

Principal component analysis (PCA) was used to differentiate the two citrus concentrates based on nutritional and physico-chemical criteria (Figure 3). The two concentrates were quite distinct from each other and from their initial juice. The main information was explained by axis F1 (69%). Strong correlations (Pearson) were observed between carotenoids (βcx/ Lyc) and colour (0.996), flavonoids (Hes) (0.996), turbidity, viscosity, pulp and pectin (0.967, 0.987, 0.978, and 0.994). These correlations were explained by the fact that carotenoids and flavonoids were hydrophobic molecules (except Nar, the only flavonoid not retained by the membrane) bound to the pulp which lead to a higher viscosity and turbidity as well as a darker color of the concentrates compared to the initial juices. Opposite and negative correlations were observed for the hydrosoluble fraction mainly sugars and Nar and for granulometry. These characteristics corresponded to the initial juices group.

3.6. Retention evaluation and modelling

The retentions of all the compounds R_i were evaluated in both concentrates from the measured concentration factors rearranging Eq. 7 to obtain Eq. 12.

$$R_i = \frac{Ln\ CF_i + DMR}{Ln\ MRR + DMR} \qquad \textbf{(12)}$$

As expected, on one hand the retentions of carotenoids, hesperidin and SIS were complete with R_i close to 1. The retention of pectins was very high but a small amount of these compounds passed through the membrane, probably their soluble fraction (R_i = 0.96). On the other hand, sugars and organic acids were not retained by the membrane ($R_i \approx 0$). The retention of Nat was intermediate (R_i = 0.47) and only 0.09 for Nar. These results are in accordance with Polidori et al. (2018) who observed 97% retention for hesperidin and 63% for Nat during the microfiltration of orange juice in similar conditions. Refractometric measurements showed 15% retention of TSS indicating one part of the soluble fraction was stopped when crossing the porous media, probably by adsorption. Nevertheless, this assumption has to be confirmed through more in depth investigations of the pool of solutes. TDM was logically retained at 17%, this indicator combining TSS and SIS.

As shown in Figure 4 that compares experimental and calculated concentration and purification factors, model accuracy was good in many cases (coefficient of variation below 15%), except for some carotenoids, especially lycopene, in the concentrates obtained with diafiltration. For this carotenoid, the model underestimated both factors. This gap was possibly explained by isomerizations which influenced the response factor in the quantitative analysis of this carotenoid.

In spite of this shortcoming, the model can reasonably be used to predict the compositional profile of final concentrates that could be obtained using other mass reduction and diamass ratios. As an example, Figure 5 can be generated to compare carotenoid, flavonoid and macrosolute profiles of an initial juice with those of concentrates obtained using different MRR (from 8 to 12) and DMR (from 0 to 3). As expected, varying MRR allows to control carotenoids, hesperidine and pectin contents whereas DMR mainly allows to modulate total

385 soluble solids (sugars), titratable acidity and also naringine/narirutine concentrations. So this
386 model can be considered as an interesting simulation tool for selecting operating conditions to
387 be used in order to reach a composition profile target.
388

389 ## 4. Conclusion

390 In summary, this study investigated the possibility of modulating the nutritional properties of
391 a potential functional citrus-based food obtained by membrane technology. The two citrus-
392 based foods were enriched in bioactive compounds such as carotenoids, as well as some
393 flavonoids and pectins but were differentiated by sugar/acid contents. Provitamin A
394 carotenoids, Lyc, Hes and pectins were approximatively cold-concentrated from 8 to 10-fold
395 giving a unique interesting profile to the citrus products. The originality of the product lays in
396 the fact that the high content of bioactive compounds, which is comparable to natural food
397 products, is naturally incorporated in the food matrix. This specific product could be
398 consumed directly as a healthy citrus based-food. Moreover, diafiltered concentrate can be of
399 interest if the product is recommended as part of a reduced sugar diet for diabetic people for
400 instance. By modelling the retention of bioactive compounds, we showed that it was possible
401 and easy to modulate sugar and flavonoid contents in the concentrate that could be a crucial
402 issue for its biological activity. Likewise, carotenoids and pectins contents could be regulated
403 in the same way. Further studies are necessary to validate *in vivo* the functionality of the
404 product in order to confirm a potential beneficial protective effect against several lifestyle-
405 related-diseases and especially diabetes.
406

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Figure captions

Figure 1: Permeate flux (Jp) vs. mass reduction ratio (MRR) during the microfiltration of citrus juices from 4 different lots (lots A, B, C, D).

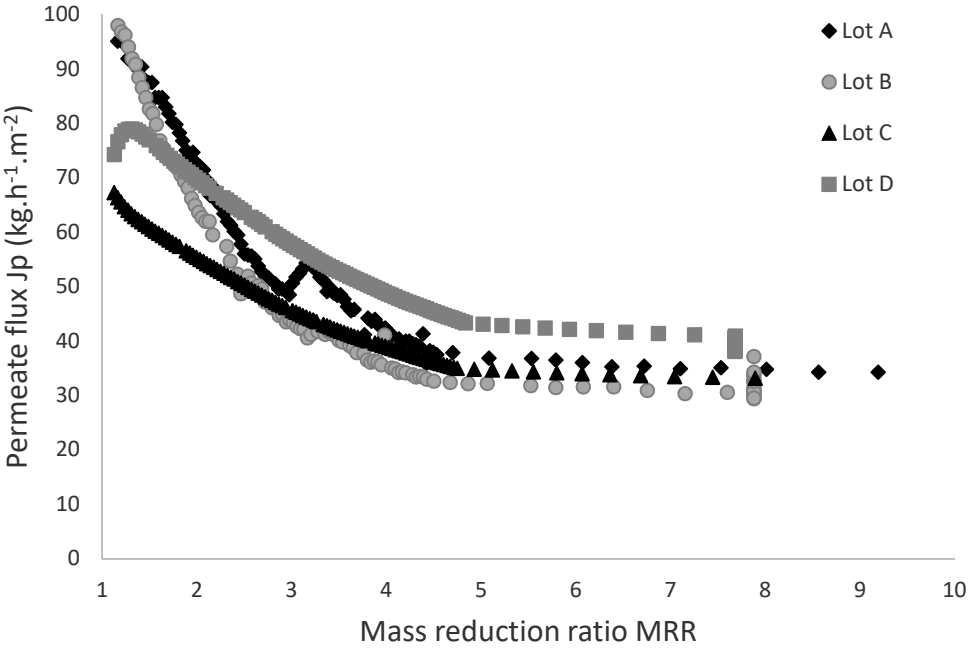
Figure 2: Particle size distribution in the initial juices (lot A and lot B) and their respective concentrates (DF0 and DF1).

Figure 3: (**A**) Results of applying PCA to the data related to the nutritional composition and physico-chemical properties and (**B**) differentiation using PCA of citrus products.

Figure 4: Comparison between experimental and calculated concentration and purification factors for the different compounds analyzed in concentrates without diafiltration (DF0) and with diafiltration (DF1).

Figure 5: Forecasted impact of the mass reduction and diamass ratios (MRR and DMR) used during the microfiltration of a citrus juice on the profiles of carotenoids, flavonoids and macrosolutes in the final concentrate.

Figure 1.


Permeate flux Jp (kg.h-1.m-2)
Mass reduction ratio MRR
Lot A
Lot B
Lot C
Lot D
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8
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10

Figure 2

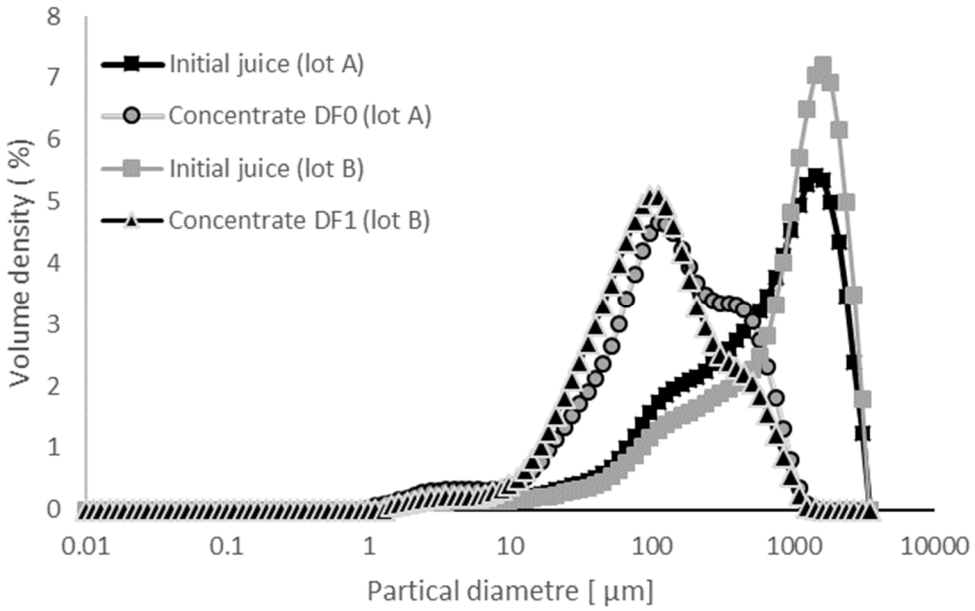

Initial juice (lot A)
Concentrate DF0 (lot A)
Initial juice (lot B)
Concentrate DF1 (lot B)
Volume density (%)
Partical diametre [µm]
0
1
2
3
4
5
6
7
8
0.01
0.1
1
10
100
1000
10000

Figure 3

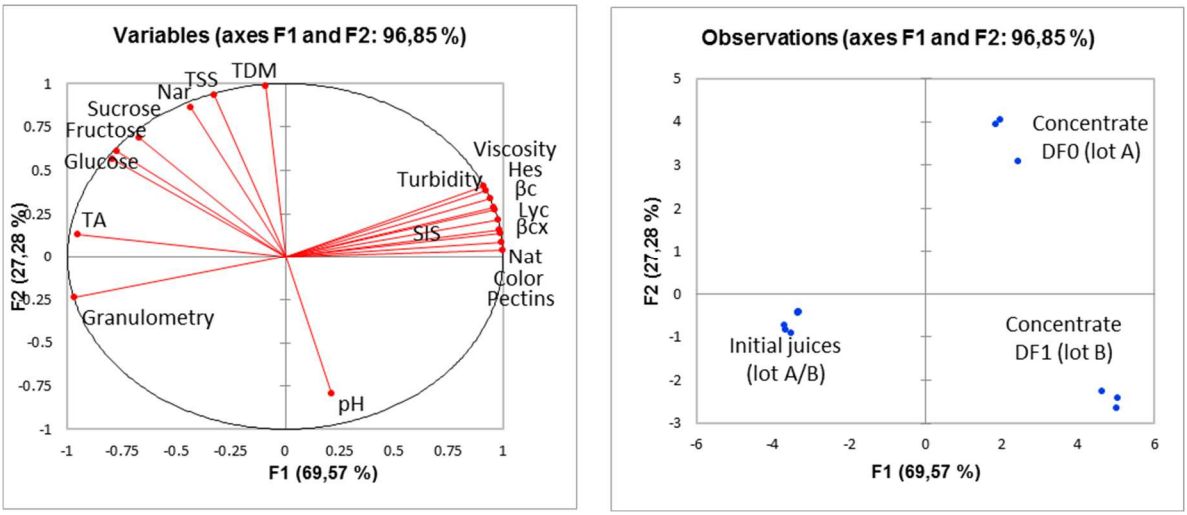
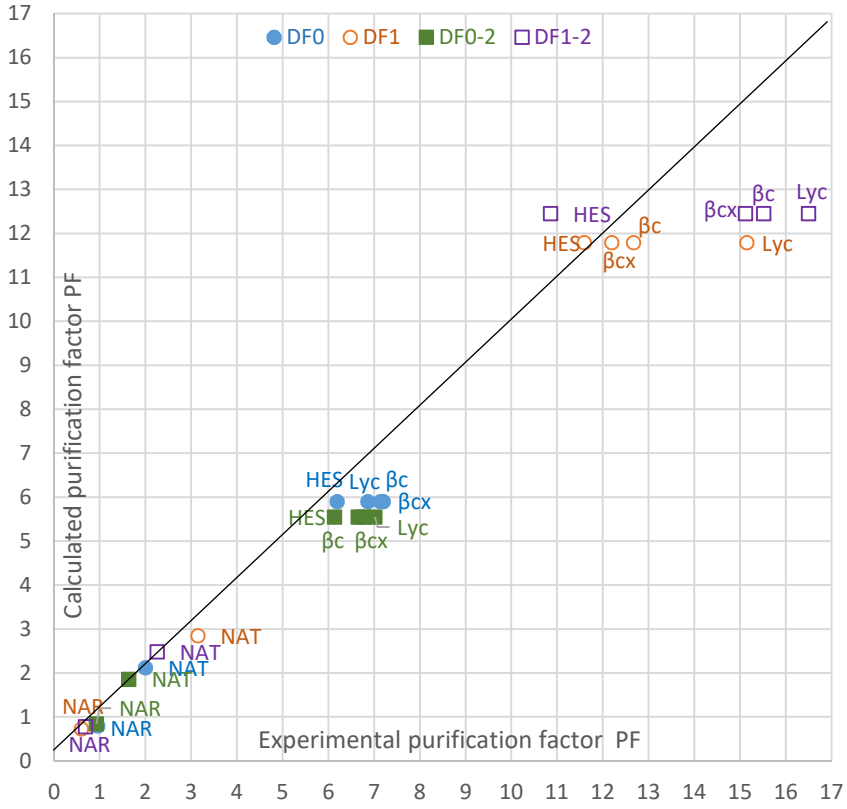
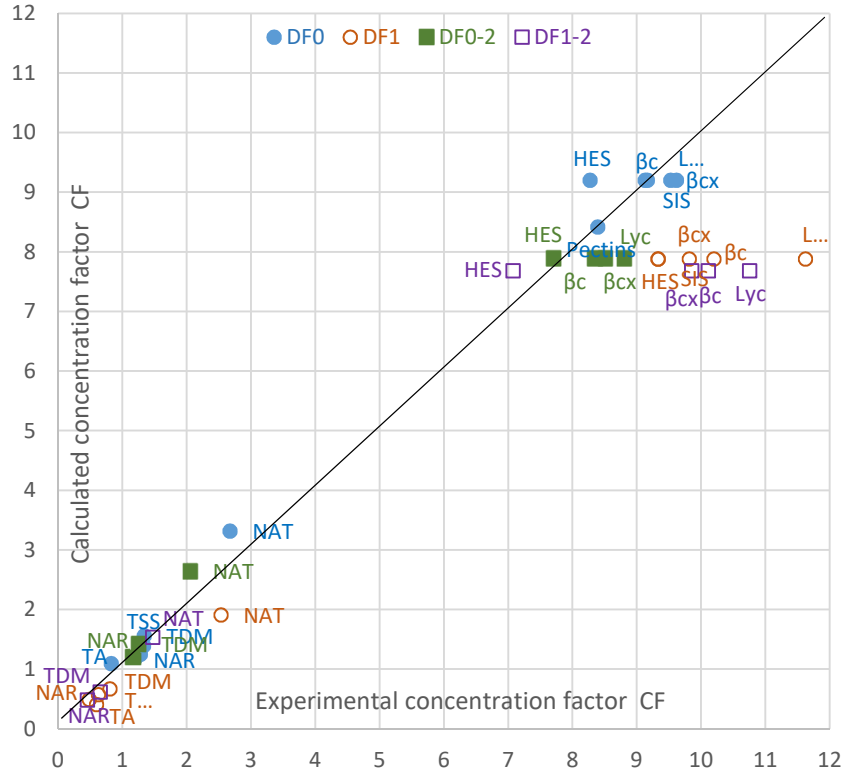


Figure 4


DF0
DF1
DF0-2
DF1-2
Calculated purification factor PF
Experimental purification factor PF
HES
Lyc
βc
βcx
NAT
NAR

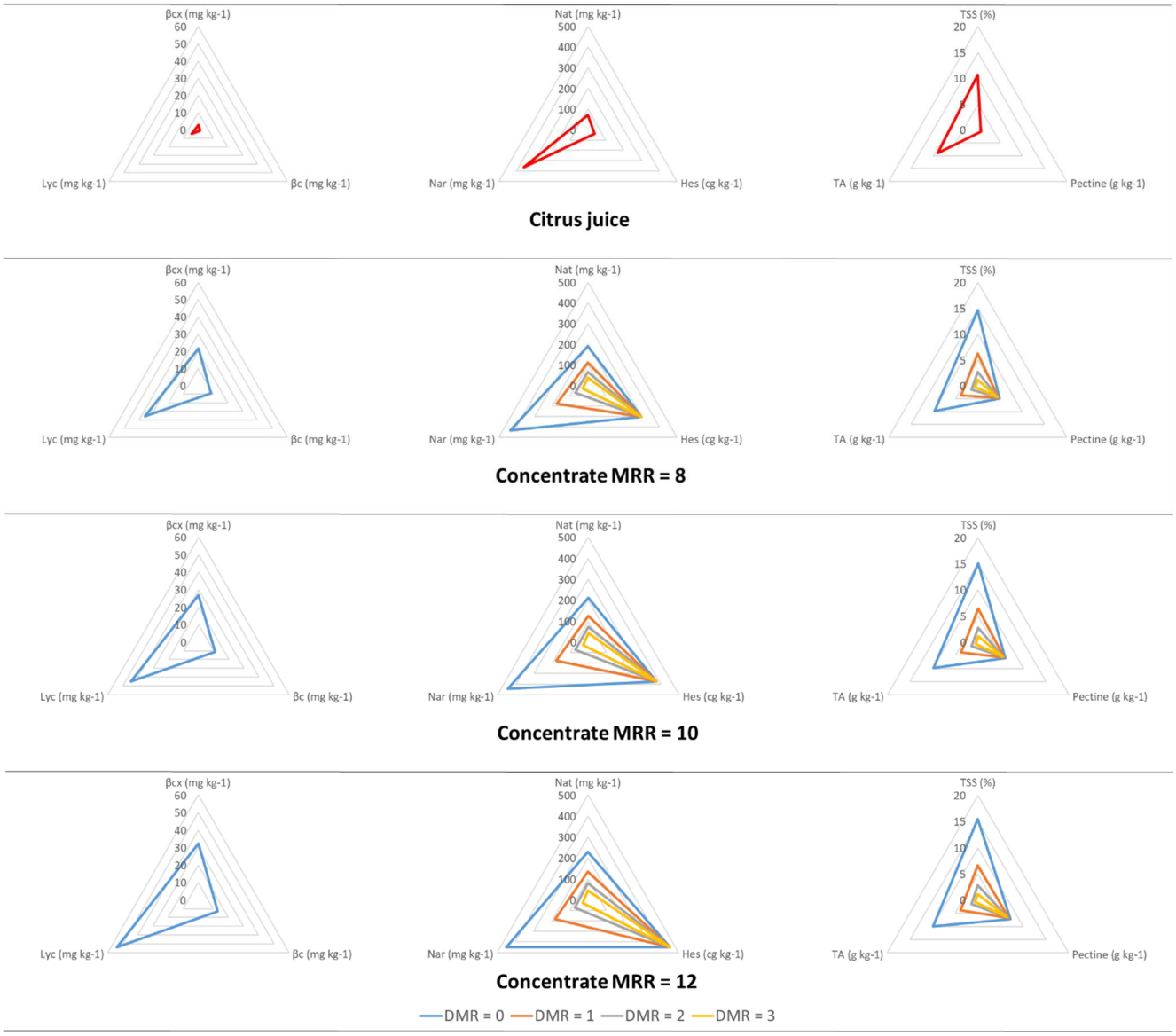
564


DF0
DF1
DF0-2
DF1-2
Calculated concentration factor CF
Experimental concentration factor CF
HES
βc
SIS
βcx
Lyc
Pectin
NAT
TSS
TDM
NAR
TA
T...

565

566

Figure 5


βcx (mg kg-1)
Nat (mg kg-1)
TSS (%)
Lyc (mg kg-1)
βc (mg kg-1)
Nar (mg kg-1)
Hes (cg kg-1)
TA (g kg-1)
Pectine (g kg-1)
Citrus juice
Concentrate MRR = 8
Concentrate MRR = 10
Concentrate MRR = 12
DMR = 0
DMR = 1
DMR = 2
DMR = 3

570 **Table 1:** Carotenoid and flavonoid contents, purity of citrus juice and concentrates (without
571 diafiltration DF0 and with diafiltration DF1) and concentration/purification factors (CF, PF)

Operating Conditions	Concentration (MRR = 9.2 ; DMR = 0 ; 30°C)						Concentration and purification (MRR = 7.9 ; DMR = 1 ; 34°C)					
	Initial juice Lot A Content	Purity	DF0 Content	Purity	CF	PF	Initial juice Lot B Content	Purity	DF1 Content	Purity	CF	PF
Carotenoids (mg·kg^{-1})												
βcx	2.48 (0.14)^{c}	22.4 (1.6)	23.63 (0.52)^{b}	159.8 (5.3)	9.54	7.13	2.88 (0.08)^{c}	27.7 (0.8)	28.29 (0.46)^{a}	338.1 (7.4)	9.82	12.21
βc	1.03 (0.06)^{c}	9. 3 (0.6)	9.42 (0.25)^{b}	63.8 (2.4)	9.17	6.86	1.13 (0.03)^{c}	10.9 (0.4)	11.52 (0.12)^{a}	137.7 (1.2)	10.2	12.69
Lyc	4.45 (0.12)^{b}	40.6 (0.8)	42.80 (0.90)^{a}	289.8 (7.6)	9.70	7.19	4.50 (0.20)^{b}	43.3 (2.1)	51.74 (2.21)^{a}	625.4 (23.3)	11.6	14.29
Flavonoids (mg·kg^{-1})												
Nat	74.3 (0.6)^{c}	669 (8)	198 (2)^{a}	1337 (30)	2.67	1.99	68.9 (1.3)^{d}	662 (14)	174 (1)^{b}	2084 (15)	2.53	3.15
Hes	384.6 (1.6)^{c}	3476 (62)	3180 (18)^{b}	21518 (370)	8.27	6.19	367.9 (3.9)^{c}	3537 (33)	3431 (16)^{a}	41005 (364)	9.33	11.61
Nar	265.4 (2.6)^{b}	2399 (46)	339 (2)^{a}	2296 (43)	1.27	0.96	253.8 (5.7)^{b}	2440 (48)	122.1 (0.1)^{c}	1455 (624)	0.48	0.60
Operating Conditions	Concentration (MRR = 7.9 ; DMR = 0 ; 31°C)						Concentration and purification (MRR = 7.7 ; DMR = 1 ; 39°C)					
	Initial juice Lot C Content	Purity	DF0 Content	Purity	CF	PF	Initial juice Lot D Content	Purity	DF1 Content	Purity	CF	PF
Carotenoids (mg·kg^{-1})												
βcx	2.08 (0.11)^{c}	18.6 (0.9)	17.69 (0.30)^{a}	126.3 (2.0)	8.51	6.77	1.52 (0.04)^{d}	13.6 (0.4)	14.99 (0.15)^{b}	206.2 (3.1)	9.85	15.12
βc	1.85 (0.05)^{c}	16.6 (0.4)	15.44 (0.29)^{a}	110.3 (2.1)	8.35	6.65	1.34 (0.03)^{d}	12.0 (0.3)	13.59 (0.14)^{b}	186.8 (2.2)	10.10	15.52
Lyc	5.57 (0.07)^{c}	49.9 (0.2)	49.03 (1.16)^{a}	350.1 (8.3)	8.80	7.01	4.16 (0.15)^{c}	37.3 (1.4)	44.74 (1.46)^{b}	615.4 (26.6)	10.80	16.50
Flavonoids (mg·kg^{-1})												
Nat	64.8 (2.4)^{c}	581 (19)	133 (3)^{a}	951 (24)	2.06	1.64	60.5 (0.4)^{c}	542 (5)	89 (2)^{b}	1222 (18)	1.47	2.25
Hes	244.6 (3.2)^{b}	2193 (26)	1884 (16)^{a}	13454 (127)	7.70	6.14	263.5 (2.5)^{b}	2360 (28)	1864 (11)^{a}	25635 (464)	7.07	10.85
Nar	246.5 (9.7)^{b}	2210 (97)	287 (5)^{a}	2049 (43)	1.17	0.93	251.3 (2.4)^{b}	2250 (18)	113 (18)^{c}	1554 (262)	0.45	0.68

572 Different letters on the same line indicate significant differences ($p < 0.05$).

573

574 **Table 2:** Physico-chemical characteristics and macronutrient content of citrus juice and
575 concentrates

576

Treatment and operating condition	Concentration MRR = 9.19 / DMR = 0		Concentration / diafiltration MRR = 7.88 / DMR = 1	
Raw material	Initial juice (Lot A)	Concentrate DF0	Initial juice (Lot B)	Concentrate DF1
Rheological measures				
Flow index	0.29 (0.02)^{c}	0.36 (0.01)^{b}	0.31 (0.01)^{c}	0.41 (0.01)^{a}
Consistency index	326.23 (59.22)^{c}	5060.8 (104.6)^{a}	303.59(24.02)^{c}	3484.9 (24.22)^{b}
Limit viscosity at 1000 s^{-1} (mPa.s)	3.46 (0.15)^{c}	71.57 (0.68)^{a}	2.55 (0.23)^{c}	67.53 (0.5)^{b}
Granulometry				
D [3,2] (µm)	98.09 (0.45)^{b}	42.05 (1.07)^{d}	123.94 (2.54)^{a}	48.5 (0.43)^{c}
$Dx_{(50)}$ (µm)	750.9 (11.5)^{b}	139.9 (0.5)^{c}	1105.7 (20.8)^{a}	109.2 (0.6)^{c}
Colour				
L*	49.04 (0.51)^{c}	56.3 (0.09)^{b}	-	57.67 (0.18)^{a}
a*	4.28 (0.01)^{b}	17.59 (1.81)^{a}	-	17.43(0.03)^{a}
b*	40.35 (0.34)^{c}	53.43 (0.27)^{b}	-	55.48 (0.17)^{a}
h*	83.93 (0.07)^{c}	71.79 (1.7)^{b}	-	72.55 (0.03)^{a}
ΔE		20.06 (1.16)^{b}		21.82 (0.27)^{a}
TDM ($g.kg^{-1}$)	110.66 (2.08)^{b}	147.83 (1.76)^{a}	104 (0.30)^{c}	83.69 (0.47)^{d}
TSS ($g.kg^{-1}$)	106.0 (0. 3)^{b}	140.7 (0.6)^{a}	107.3 (0.6)^{b}	67.0 (1)^{c}
TA ($g.kg^{-1}$)	8.69 (0.10)^{a}	7.15 (0.34)^{b}	9.24 (0.15)^{a}	5.52 (1.02)^{c}
pH	3.31 (0.02)^{b}	3.24 (0.18)^{b}	3.59 (0.02)^{a}	3.67 (0.06)^{a}
SIS ($g.kg^{-1}$)	3.78 (0.09)^{c}	34.5 (0.83)^{a}	3.39 (0.17)^{c}	31.7 (0.33)^{b}
Turbidity (NTU)	2326 (46)^{c}	17697 (356)^{a}	2106 (11)^{c}	15386 (215)^{b}
Pectins ($g.kg^{-1}$)	0.66 (0.02)^{c}	5.50 (0.40)^{b}	-	7.43 (0.35)^{a}
Sugars ($g.kg^{-1}$)				
Glucose	23.0 (0.6)^{a}	21.8 (1.1)^{a}	-	7.3 (0.1)^{b}
Fructose	23.5 (0.7)^{a}	22.8 (0.5)^{a}	-	7.9 (0.1)^{b}
Sucrose	52.6 (1.5)^{a}	56.4 (2.6)^{a}	-	19.0 (0.2)^{b}

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