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1 **Toward a better identification of naringin and narirutin dispersion state in**  
2 **grapefruit peel press liquor**

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7 **Highlights**

- 8 • Filtration-compression as downstream processing in flavonoid extraction from peel
- 9 • Physico-chemical and biochemical characterization of grapefruit press liquor
- 10 • Identification of naringin and narirutin dispersion state in the press liquor
- 11 • Physical stability and evolution over time of flavonoids dispersion

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30 **Abstract**

31 The objective of this work was to describe the dispersion state of flavonoids (i.e. naringin and  
32 narirutin) in the press liquor obtained after filtration-compression of fresh citrus (grapefruit)  
33 peels. An original methodology for the quantification of the dispersion, based on a size  
34 fractionation, and its evolution over time was proposed. Three fractions were identified: (i) a  
35 particulate fraction, (ii) a large-colloidal fraction and (iii) a small-colloidal and soluble  
36 fraction. Narirutin and naringin were initially found on small-colloidal/soluble form. During  
37 time, an aggregation of the compounds present in the press liquor, revealed by turbidity  
38 measurements, was observed. This aggregation resulted in an increase of the particulate  
39 fraction, at the expense of the colloidal one. This aggregation seemed to trap naringin, that  
40 progressively appeared in the particulate fraction, although that no evolution of narirutin  
41 location was observed. The tendency to sediment of the formed aggregates was evaluated, by  
42 a Turbiscan® optical analyser, in the objective of their future isolation. The stability study of  
43 the press liquor over time showed that this suspension was physically stable; the  
44 aggregates/flocs were not able to settle at room temperature, certainly because of interparticle  
45 repulsion or other reasons as low floc density, porous floc structure...

46

47 **Keywords**

48 Naringin, Narirutin, Dispersion state, Grapefruit peel, Physical stability, Press liquor

49

## 50 **1. Introduction**

51 Processing operations of Citrus (orange, mandarin/tangerine, lemon/lime and grapefruit), one  
52 of the largest cultivated fruit crops in tropical and subtropical regions (USDA, 2018), produce  
53 significant wastes, as peels, pulp and seeds. These transformation coproducts have already  
54 shown numerous potential applications in foods (Galanakis, 2018; Galanakis et al., 2018a,  
55 2018b; Goliomytis et al., 2015; Hu et al., 2020; Teng et al., 2018), cosmetics (Galanakis et al.,  
56 2018c, 2018d; Simitzis, 2018) and health (Chen et al., 2016; Cirillo et al., 2016). Thus,  
57 recovery of high added-value components (i.e. flavonoids, carotenoids, insoluble fibres,  
58 pectin...) from food wastes have attracted the scientific community; further conventional and  
59 new emerging technologies are proposed and described in some literature reviews (Deng et  
60 al., 2015; Galanakis, 2013, 2012; M'hiri et al., 2014). Concerning flavonoids extraction, due  
61 to their hydrophobic nature, the conventional extraction is mainly based on the use of organic  
62 solvents resulting in a negative impact on the environment (Lachos-Perez et al., 2018). In  
63 consequence, green and sustainable technologies such as supercritical fluids, pressurized hot  
64 water, pulsed electric field, enzyme-, ultrasound- and microwave-assisted extraction have  
65 emerged (Barba et al., 2015; Garcia-Castello et al., 2015; Giannuzzo et al., 2003; Li et al.,  
66 2006; Nayak et al., 2015; Plaza and Turner, 2015).

67 If Citrus peels are a source of potentially valuable bioactive compounds, as polyphenolic  
68 compounds (i.e. flavonoids), the recovery of flavonoids from peel is still limited by the  
69 absence of cost-effective and simple processes, more especially useful in developing  
70 countries, the main producers of Citrus (Putnik et al., 2017). Accordingly, it could be relevant  
71 to propose robust and easy to implement processes to facilitate the development of waste  
72 processing chain.

73 In a previous study (Zarate Vilet et al., 2020), it was demonstrated that filtration-compression  
74 is of high interest as a preliminary step for the recovery of flavonoids from grapefruit peels.

75 This solid/liquid separation allowed to isolate a liquid phase (i.e. press liquor) containing the  
76 target flavonoids and other compounds released by the disrupted plant tissue (e.g. sugars,  
77 remaining oils, proteins, pectin, cellulose, hemicellulose,...) (Baker and Cameron, 1999).  
78 These compounds can be present in a solubilised form, e.g. sugars (present in high quantity)  
79 and electrolytes (essentially mineral salts), or in a dispersed one (colloids or particles). Due to  
80 Brownian movements and/or agitation, dispersed compounds can get in contact, and interact  
81 giving rise to new colloidal complexes (e.g. protein-pectin complexes, lycopene-pectin,...)  
82 with different properties than the original compounds (Le Bourvellec and Renard, 2012).  
83 Concerning some flavonoids (e.g. naringin from grapefruit peel), their low water solubility at  
84 room temperature seems to indicate that only a small part should be in a soluble form in the  
85 press liquor; a great part of this type of flavonoids could be present either in a crystalline form  
86 or complexed with other compounds present in the liquid (Galanakis, 2015). Mizrahi and  
87 Berk, 1970 observed that hesperidin crystals were formed immediately after orange juice  
88 extraction. In another study, Ben-Shalom et al., 1985, by identifying pectin-hesperidin  
89 colloidal complexes in orange juice, concluded that pectin molecules served as nucleation site  
90 for hesperidin. In the same way, (Nagarajan 2019) describe the formation of colloidal  
91 complex between lycopene and pectin, favourable to a water extraction of lycopene. Proteins  
92 can also interact with flavonoids and/or pectin in a reversible way contributing to form  
93 complex aggregates (Shomer, 1991). The formation of such aggregates is usually detected by  
94 simple methods such as turbidimetry, nephelometry, or dynamic light scattering (Le  
95 Bourvellec and Renard, 2012; Shomer, 1991).

96 As it is developed above, if interactions between solutes, macromolecules and colloids, in  
97 fruit juices or peel extracts have been identified by various authors, to date, little information  
98 is available on the physical state of flavonoids in regards to the technologies to be  
99 implemented for their extraction and concentration. Indeed, depending on the dispersion state

100 and physical-characterisation (size, density...) of these flavonoids, the type of process  
101 (sedimentation, centrifugation, membrane separation, adsorption...) for the final isolation will  
102 be different.

103 In the case of a downstream processing based on a filtration-compression stage, in order to  
104 separate and concentrate flavonoids from the rest of the press liquor compounds, more  
105 information about their dispersion state within this press liquor is required. The investigation  
106 of the ability of flavonoids to interact with certain compounds is also essential to choose the  
107 relevant following extraction/isolation process.

108 In this context, the aim of this work was to describe the dispersion state of flavonoids  
109 (naringin and narirutin) in the press liquor obtained after filtration-compression of fresh citrus  
110 (grapefruit) peels. An original methodology for the quantification of the dispersion, based on  
111 a size fractionation, and its evolution over time was proposed. The physical stability of this  
112 dispersion was also studied through Turbiscan® analyses.

113

## 114 2. Materials and Methods

### 115 2.1. Grapefruit peels supply and storage

116 Grapefruits (*Citrus paradisi*, Star ruby variety from Spain, provided by Terreazur, Saint-Jean-  
117 de-Védas, France) were first bleached. Juice extraction was conducted using an electric juice  
118 extractor (ZX7000, Krups). Flavedo was manually removed from peel, as it can be valorised  
119 through the extraction of essential oils (Zema et al., 2018). Peels, consisting only of albedo  
120 and pulp rests (core, juice sacs and segment rests), were finally cut into small pieces (around  
121 10 x 10 mm) and stored at -20°C.

### 122 2.2. Grinding and filtration-compression stages

#### 123 2.2.1. Grinding stage

124 Peels were defrosted and grinded into a slurry by means of a hand blender  
125 (Moulinex DD873D10 Infiny Force ultimate, France) for 1.5 minutes at 1 kW (corresponding  
126 to  $9 \cdot 10^5 \text{ J} \cdot \text{kg}^{-1}$  of fresh peel). Prior to filtration-compression, a pectinolytic enzymatic treatment,  
127 causing a cell wall degradation resulting in a better slurry filterability and a larger release of  
128 flavonoids (Zarate Vilet et al., 2020), was proposed. The enzymes and the optimal operating  
129 conditions (mass, temperature, time) were provided by Soufflet Biotechnology®.

#### 130 2.2.2. Filtration-compression stage

131 The mechanical separation of the press liquor from the slurry was performed on a 0.60 L  
132 volume pressurized filtration-compression cell of 70 mm of internal diameter (Figure 1); a  
133 cotton cellulose membrane of 6  $\mu\text{m}$  average pore size (Whatman, Maidstone, UK) was used to  
134 ensure the solid/liquid separation. 40 mL of slurry was poured into the cell and compressed at  
135 an optimal transmembrane pressure of 5 bar by a pneumatically driven-piston dragged by  $\text{N}_2$ .  
136 Filtration-compression was systematically stopped after the recovery of 25 mL of press  
137 liquor; in this volume, obtained in a few minutes, 80% and 90% of naringin and narirutin  
138 found in the filtrate at the end of the filtration were already present (Zarate Vilet et al., 2020)

139 2.2.3. *Characterisation of the press liquor*

140 Flavonoids (naringin and narirutin) content was measured in the press liquor. In addition, the  
141 press liquor was characterised through conventional physico-chemical parameters, as pH, dry  
142 matter content, °Brix. These conventional characterizations were completed by less  
143 conventional characterizations (turbidity, particle size distribution, physical stability),  
144 evaluated with an objective to broadly characterise the solids dispersion state in the press  
145 liquor.

146 Towards identifying with more accuracy this dispersion state, a size fractionation of the press  
147 liquor was also proposed.

148 2.2.3.1. *Flavonoids quantification*

149 Naringin and narirutin content was quantified by HPLC. Firstly, a solvent extraction of the  
150 flavonoids from samples was performed based on a modified method by (Mouly et al., 1993).  
151 This allowed the quantification of all flavonoids in the sample regardless of its physical state  
152 (soluble, crystallized or bounded). An aliquot of 500 µl of press liquor was mixed with 5 ml  
153 of dimethylformamide (DMF) and 5 ml of ammonium oxalate (0.05 M) and heated at 90°C  
154 for 10 minutes. Then, samples were cooled and adjusted with distilled water to be within  
155 calibration curve. Finally, solutions were filtered through 0.45 µm cellulose acetate syringe  
156 filters (Whatman, Maidstone, UK) before injection. Quantification was performed on an  
157 Agilent 1200 series HPLC system equipped with a diode array detector DAD and a C18  
158 column (Uptisphere HDO 5 µm, 250 mm × 4.6 mm, Interchim, Montluçon, France). The  
159 mobile phase was constituted by an isocratic solvent system composed of solvent A  
160 (water/acetic acid, 99/1, v/v), solvent B (acetonitrile) and solvent C (tetrahydrofuran) - A/B/C  
161 80/16/4, v/v/v. Column was heated at 25°C and the flow was set at 1 mL/min. UV detector  
162 was set at 280 nm and a sample aliquot of 20 µL was injected.

163

164

165 2.2.3.2. *Conventional physico-chemical characterisation*

166 pH was measured with an automatic Titroline apparatus (Schott Schweiz AG, St. Gallen,  
167 Switzerland). Dry matter (DM) content was determined according to a modified method of  
168 the AOAC (AOAC, 1990); samples contained in aluminium dishes were dried at 70°C for 48  
169 h in a vacuum oven (Heraeus RVT 360, Hanau, Germany). °Brix were evaluated by digital  
170 refractometry (Pocket PAL-1, 0-53% Brix, ATAGO®, Japan) to obtain an indirect  
171 measurement of dissolved solids (essentially sugars for fruit extracts).

172 2.2.3.3. *Solids and flavonoids dispersion state characterisation*

173 Turbidity was evaluated to provide information on the presence of dispersed solids (e.g.  
174 colloids and other insoluble compounds) and on the aggregation phenomena in the press  
175 liquor. Turbidity measurement, expressed in nephelometric turbidity units (NTU), was done at  
176 90° light scattering and 890 nm (LP 2000 Turbidimeter, Hanna instruments, Hungary). All  
177 measures were performed at 25°C and the apparatus was previously calibrated with three  
178 formazine standard solutions at 0, 10 and 500 NTU. Samples were diluted at 1/10 to fall in the  
179 turbidimeter range.

180 The size distribution of dispersed particles present in the press liquor was studied by laser  
181 diffraction. Measurements were performed by a Mastersizer 3000 (Malvern Instruments  
182 Limited, UK) equipped with a HydroMV® cell. This particle size analyser can provide  
183 theoretical particle size distribution from 10 nm to 3500 µm. Refractive indices of 1.73 and  
184 1.33 were respectively used for cloud particles and dispersion phase; 0.1 was used as the  
185 absorption index of cloud particles (Daoudouh et al., 2018). Samples were pumped into the  
186 optical cell until 40% of obscuration. The absence of modifications in the particle size  
187 distribution due to stirring and pumping was verified.

188 The physical stability of the press liquor was characterized by a Turbiscan® TOWER  
189 (Formulation, France). Based on the multiple light scattering principle, this analysis allowed  
190 an accurate detection of the main aggregation and destabilization phenomena affecting the  
191 homogeneity of the suspension without physico-chemical disturb and dilution (Mengual et al.,  
192 1999). A sample of 10 ml was poured into a glass tube and kept inside the analysis cell for a  
193 given time (48 hours) and temperature (10 and 25°C). Inside this cell, a near infrared light ( $\lambda$   
194 = 880 nm) was emitted and backscattered by particles. Destabilization phenomena were  
195 related with changes in the percentage of the backscattered light along the static tube over  
196 time (1 measure per 0.02 mm and per minute).

#### 197 2.2.3.4. *Press liquor fractionation*

198 Press liquor was successively fractionated with an Amicon® filtration cell (model 8050,  
199 Millipore Corporation, U.S.A.) on polyethersulfone (PES) membranes of 0.8 and 0.1  $\mu\text{m}$   
200 (Supor, Pall Corporation, USA). This methodology allowed to separate the press liquor  
201 compounds in three fractions: (i) *particle fraction* (particles > 0.8  $\mu\text{m}$ ), (ii) *large-colloidal*  
202 *fraction* (particles between 0.1 and 0.8  $\mu\text{m}$ ) and (iii) *small-colloidal and soluble fraction*  
203 (particles < 0.1  $\mu\text{m}$ ).

204 The evolution over 24 h of these different fractions was based on the same fractionation  
205 methodology, as the press liquor was stored at 25 °C under continuous rotational mixing (10  
206 mL, 20 rpm).

207

### 208 **3. Results**

#### 209 *3.1. Press liquor characterisation*

##### 210 *3.1.1. Physico-chemical characteristics and flavonoids content*

211 The physico-chemical characteristics of the press liquor obtained immediately after filtration-  
212 compression are presented in Table 1. The pH of the filtrate ( $3.4 \pm 0.2$ ) is close to that of  
213 grapefruit juice (Rouquié et al., 2019) as similar components (i.e. pulp residues and juice

214 bags) are found in both suspensions. The press liquor Brix degree was equal to 7° Brix; this  
215 value, representing dissolved solids (essentially sugars for fruit extracts), was very closed to  
216 the dry matter, representing the totality of solids, and equal to 7.4 g of dry matter per 100 g of  
217 press liquor. Consequently, the press liquor dry matter was mainly constituted of soluble  
218 compounds, as it is usually observed in fruit juices.

219 Press liquor naringin and narirutin content, expressed in g/litre, are given in Table 1. Around  
220 85% (corresponding to 4.5 g/100 g peel dry matter) of the flavonoids present in the initial  
221 slurry were recovered in the total press liquor (Zarate Vilet et al., 2020).

222 Naringin and narirutin contents in the press liquor were in accordance with literature data for  
223 grapefruit albedo, with naringin content approximately 10 times higher than narirutin content  
224 (Jourdan et al., 1983; Nogata et al., 2006). Other authors (Garcia-Castello et al., 2015; Victor  
225 et al., 2018) found similar naringin concentrations using hot methanol extraction (4.1 g/100 g  
226 peel dry matter) and ultrasound-assisted extraction (3.6 g/100 g peel dry matter), which  
227 confirmed the good efficiency of the filtration-compression stage in terms of flavonoids  
228 extraction.

229 Naringin and narirutin contents were compared with their respective solubility limit.  
230 Considering the low concentration in narirutin (0.4 g/L) and its relatively high solubility in  
231 water (3 g/L at 20°C, “VCCLAB, Virtual Computational Chemistry Laboratory,” 2005), it  
232 could be supposed that this compound was entirely on solubilised form in the press liquor.  
233 Concerning naringin, considering its low water solubility (0.5 g/L at 20°C, Pulley, 1936) and  
234 its high concentration in the press liquor (8.2 times higher), it could be envisaged that a great  
235 part of naringin was in an insoluble form in this liquor. To confirm this initial hypothesis, the  
236 value of the naringin solubility was experimentally verified at 25°C; naringin solubility was  
237 measured not only in water but also in aqueous sugar solution of 7°Brix, corresponding to the  
238 °Brix of the press liquor. The experimental data, presented in Table 2, confirmed the poor

239 solubility of naringin. The presence of sugar tended to decrease this solubility, which  
240 supported the hypothesis that naringin could be found on crystallized forms, complexed to  
241 colloids or tissue fragments or auto-associated (Le Bourvellec and Renard, 2012).

### 242 *3.1.2. Characterisation of solids and flavonoids dispersion state*

#### 243 *3.1.2.1 Press liquor particle size distribution and turbidity*

244 Figure 2 presents the dispersed particle size distribution (in volume density, ranged from 0.01  
245 to 3500  $\mu\text{m}$ ) of the press liquor obtained immediately after filtration-compression ( $t = 0$ ). This  
246 distribution revealed a trimodal volumetric size distribution consisting of an important  
247 colloidal population of particles ( $< 1 \mu\text{m}$ ) and a bimodal particulate population with average  
248 diameters around 5 and 20  $\mu\text{m}$ . The presence of this latter population, higher than the pore  
249 filter size (around 6  $\mu\text{m}$ ), could be explained by the soft character of pulp particles that could  
250 so be deformed and pass through the filter. Rapid floc formation or colloidal particles  
251 aggregation could also explain the presence of such size-class of particles (Genovese et al.,  
252 2007).

253 The presence of colloidal and particles in the press liquor was also identified through turbidity  
254 measurements (Table 1); indeed, their presence could possibly contribute to the cloudiness of  
255 the suspension (Beveridge, 2002; Jethra, 1993). A relatively high turbidity (tap water turbidity  
256 is lower to 1 NTU (WHO, 2017) of 1481 NTU was measured in the press liquor. According to  
257 the particle size distribution, this high turbidity could be attributed to the presence of colloids  
258 or particles, despite their low mass fraction. As literature states, the turbidity of citrus juices  
259 known as “cloud” is the result of fine particles (Mizrahi and Berk, 1970) containing  
260 polysaccharides (mainly pectin), protein, lipids and flavonoids (Beveridge, 2002).

#### 261 *3.1.2.2 Size fractioning and flavonoids distribution*

262 In order to obtain a more in-depth information on the presence of colloids and particles and  
263 the location of the flavonoids, a fractionation of the press liquor was proposed. Although a

264 fractionation of various polyphenolics compounds by membrane filtration is described in  
265 literature (Galanakis et al., 2013), the fractioning of the press liquor is a relatively original  
266 approach to identify of naringin and narirutin dispersion state in this press liquor,

267 Three fractions were identified: (i) a *particulate fraction*, containing particles higher to 0.8  
268  $\mu\text{m}$ , (ii) *large-colloidal fraction*, with colloids between 0.1 and 0.8  $\mu\text{m}$  and (iii) *small-*  
269 *colloidal and soluble fraction*, regrouping the smallest colloids, lower to 0.1  $\mu\text{m}$ , and the  
270 solutes.

271 Table 3 presents the repartition of the total flavonoids (in % of the total flavonoids content)  
272 and turbidity in each of this fraction.

273 The results showed that 94% of narirutin were in the small-colloids/soluble fraction. Only 4%  
274 of the narirutin were in the large colloidal fraction and 2% in the particulate fraction, sorbed  
275 or complexed with other compounds.

276 Concerning naringin, as for narirutin, the main part (86%) was located in the small-  
277 colloids/soluble fraction. Considering the low naringin water solubility, a great part of  
278 naringin, could be on an insoluble form in this fraction. The remaining part is distributed in  
279 the particulate (9%) and the large-colloidal fraction (5%). The part of naringin on small-  
280 colloidal form could be estimated from the solubility experimentally measured: according to  
281 the obtained values, 2% of naringin would be on solubilised form although the remaining  
282 (84%) would be on small-colloidal form.

283 The turbidity of the particulate fraction (around 1346 NTU) appeared to be higher to the one  
284 of the other fractions (Table 3): this turbidity was 7 and 100 times higher than the large-  
285 colloids and small colloids /solutes one respectively. Thus, the particles higher to 0.8  $\mu\text{m}$  and  
286 present in a large amount (in terms of volume density of the total dispersed compounds, see  
287 Figure 2) provided a great part of the turbidity of the press liquor.

288 The large-colloidal fraction (identified on Figure 2) generating a significant turbidity (around  
289 200 NTU), could be attributed to the enzymatic action. The degradation of pectin by  
290 pectinases could reduce the electrostatic repulsion of protein-pectin complex (negatively  
291 charged pectin layer around positively charged proteins) and promote the aggregation  
292 between pectin fragments, proteins and probably some flavonoids (Sorrivas et al., 2006). In  
293 addition, this turbidity could also be the result of other compounds interaction (pectin-  
294 lycopene) (Nagarajan et al., 2019).

295 If these results refined the characterisation of the press liquor, the relation between the solids  
296 dispersion and the flavonoids location was not easy to identify, due to the great presence of  
297 these flavonoids on small-colloidal and solubilised forms; indeed, this class of particle doesn't  
298 generate turbidity (see Table 3).

299 In the second time, it was proposed to study the potential evolution of the solids and  
300 flavonoids dispersion during time. Indeed, given the heterogeneity and the complex  
301 composition of the press liquor, it was supposed that its characteristics could change over  
302 time. In consequence, the evolution of the turbidity and flavonoids location in the press liquor  
303 were studied over a period of 24 h at 25°C. The dispersed particle size distribution after these  
304 24 h was also measured.

### 305 *3.2. Characterisation of the press liquor and fractions over time*

306

#### 307 *3.2.1. Press liquor particle size distribution and turbidity after 24 h*

308 The particle size distribution of the press liquor maintained 24 hours at 25°C under rotational  
309 agitation is presented in Figure 2. A sort of homogenisation of the size distribution could be  
310 observed with a predominant presence of particles around 5 µm. A great part of the sub-  
311 micrometric compounds has globally disappeared, certainly to agglomerate themselves and  
312 form the 5 µm- particles.

313 The initial turbidity, around  $1481 \pm 233$  NTU (Table 1), was multiplied by the factor three  
314 and reached a value of  $4144 \pm 180$  NTU (Figure 3). These results seemed in accordance with  
315 the size distribution change since some authors have noted that particles between 0.5 and  
316  $10 \mu\text{m}$  conferred the great part of the cloud of citrus suspensions (Beveridge, 2002). What is  
317 more, the turbidity of citrus suspensions being the result of heterogeneous particles (e.g.  
318 pectin, proteins, oils and flavonoid crystals) dispersed in a liquid medium, any change in the  
319 composition or structure of these heterogeneous particles could result in a change in turbidity.  
320 Therefore, these results demonstrated that, on mixing and at  $25^\circ\text{C}$ , changes in the structure of  
321 the dispersed (i.e. colloidal or particular) compounds of the press liquor occurred; these  
322 changes could potentially modify the flavonoids dispersion state in the press liquor.

### 323 *3.2.2. Turbidity and flavonoids distribution over time*

324 The evolution over time of the turbidity in the three fractions defined above is presented  
325 Figure 3.

326 It can be noticed that, the particulate fraction of the press liquor still generated the greatest  
327 part of turbidity. The turbidity of this fraction increased all along the 24 h until a final value of  
328 4144 NTU; during the first 5 hours, the turbidity increased substantially from 1346 to  
329 3576 NTU. The contribution of the two other fractions on turbidity remained at a low level.  
330 Progressively, the turbidity of the small-colloids and solutes became insignificant (from  
331 approximately 10 NTU to 0 NTU), although the one of the large colloids decreased over time  
332 (from approximately 200 NTU to 60 NTU).

333 These observations could be the result of simultaneous phenomena, including an aggregation  
334 between pectin, proteins and flavonoids, potentially promoted by the contact between these  
335 compounds under continuous agitation (Bordenave et al., 2014; Le Bourvellec and Renard,  
336 2012; Whitaker, 1984). Thus, this type of aggregation could explain the progressive

337 disappearance of small colloids, reinforcing the presence of particles, as it could be visualised  
338 on the size distribution curves (Figure 2).

339 The evolution over time of naringin and narirutin concentration within the different fractions  
340 was quantified and presented on figure 4. As previously discussed, naringin and narirutin  
341 were initially mainly present in the small-colloidal/soluble fraction. However, their behaviour  
342 during time differed significantly.

343 Narirutin dispersion state remained relatively stable over time with the same concentration in  
344 the different fractions over the studied period. Thus, around 94% of narirutin remained on  
345 soluble form or linked to small-colloids; the relatively low concentration of narirutin in the  
346 press liquor and its relatively high solubility could indicate that a great part of this 94%  
347 corresponded to solubilised narirutin. A small part of narirutin (around 6%) seemed associated  
348 to large-colloids or particles.

349 Naringin dispersion state evolved over time. In the 5 first hours, a significant decrease of  
350 naringin content in the small-colloidal/soluble fraction (from 86% to 46% of the total content)  
351 was observed for the benefit of an increase of the content in the particulate fraction (from  
352 10% to 49% of the total content). This change of naringin location subsisted the following  
353 hours of the experiment, resulting in the presence, after 24 h, of 62% of the naringin in the  
354 particulate fraction and 34 % in the small-colloidal/soluble one. No change of flavonoids  
355 content was observed in the large-colloidal fraction where between 4 and 5 % of the  
356 flavonoids were present. According to this last result, it could be supposed that naringin  
357 moved directly from small-colloidal form to particulate form; naringin seemed to have a great  
358 affinity with compounds present in particulate fraction, favoring its sorption or complexation  
359 with particles.

360 The joint analysis of turbidity and naringin evolution over time demonstrated that the increase  
361 of naringin in the particulate fraction seemed simultaneous to the increase of the turbidity of  
362 this fraction. Figure 5 represents the evolution of naringin concentration as a function of the  
363 turbidity in the particulate fraction over the 24-hour period. A quasi-linear relation was  
364 observed between the turbidity and naringin concentration. Although the standard deviations  
365 amplitude did not allow us to identify a particular evolution model, a correlation seemed  
366 effective between these two parameters. If turbidity changes in the press liquor could be the  
367 consequence of some compound's crystallization and/or aggregation, it seemed evident that  
368 these phenomena involved naringin; the formed aggregates or crystals promoted the transition  
369 of naringin from small-colloidal to particulate fraction. Several authors have already  
370 described the interaction between flavonoids and macromolecules present in citrus fruits,  
371 particularly pectin, one of the most abundant polysaccharides of the albedo. Interactions  
372 between pectin and flavonoids was demonstrated by (Le Bourvellec and Renard, 2012) and  
373 (Ben-Shalom et al., 1985) reported that pectin fragments should serve as a nucleation site for  
374 naringin to form physically stable particles.

375 In brief, over time, aggregation between some compounds, including naringin, occurred in the  
376 press liquor, shifting naringin from small-colloidal form to particulate one. This change in the  
377 naringin location could be of great interest, since this location could orientate the choice of  
378 the following extraction/isolation process. For instance, on particle form, naringin could be  
379 separated from the liquid phase by a simple sedimentation. However, this possibility depends  
380 on the stability character of the dispersed forms, notably without stirring. With the objective  
381 to study this stability, Turbiscan® experiments, allowing fast and sensitive identification of  
382 destabilization mechanisms (such as creaming, sedimentation, flocculation, coalescence...),  
383 were carried out.

384 *3.3. Physical stability of the press liquor*

385 Figure 6 presents the % of backscattering along the tube height, all along the experimental  
386 time (48 h). According to the principle of the turbiscan ® technology, this backscattering is  
387 directly correlated to the turbidity of the sample.

388 At 25°C (figure 6.a), a constant backscattering signal throughout the tube, i.e. a homogeneous  
389 turbidity in the sample, was initially observed. Over time, turbidity increased gradually in the  
390 tube, corroborating the aggregation of particles. However, the turbidity remained relatively  
391 constant all along the tube height, indicating a relatively stable character of the press liquor.  
392 After 48 hours, a slight difference in % backscattering between the bottom and the surface of  
393 the tube was observed, indicating a slight settling of the greater aggregates. It was also noticed  
394 a slight creaming at the tube surface probably due to the flotation of the less dense particle,  
395 e.g. oil globules.

396 Turbiscan® experiments were carried out also at 10 °C with the objective to verify if this  
397 stability could be modified by a lower temperature. At this temperature, turbidity of the press  
398 liquor evolved during time along the tube, with a net increase of the turbidity at the bottom of  
399 the tube. This gradually turbidity increase all along the tube, from the surface to the bottom, is  
400 characteristic of the settling behaviour of flocculated suspension. This observation could be  
401 coherent with an aggregates/flocs size increase, resulting from some attractive electrostatic  
402 interactions (Benitez et al., 2007; Pinelo et al., 2010); above a certain size, aggregates/flocs  
403 could no longer be maintained in suspension by Brownian movements and could settle  
404 (Siebert, 2009). In flocculated suspension, the particles interfere each other and the velocity  
405 gradient of each particle is affected by the close presence of other particles, it is question of  
406 hindered settling (Major, 1978).

407 These results showed that, at 10°C, the stability was lowest than the one observed at 25°C,  
408 which allowed a hindered settling of the aggregates. Various physico-chemical phenomena  
409 are impacted by a decrease of the temperature. Among these phenomena, it can be noted (i)

410 the decrease of thermal motion, limiting the diffusion and the aggregation, (ii) the increase in  
411 viscosity, (iii) the solubility decrease of some compounds. It is obvious that the first two  
412 phenomena could not explain the amplification of the sedimentation à 10°C. The increase of  
413 insoluble compounds and/or the generation of different aggregates could explicate the settling  
414 improvement; particles with different settling properties (e.g. density, size, structure...) could  
415 more interfere, favouring a hindered-type settling.

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417

#### 418 **4. Conclusion**

419 In grapefruit peels, naringin and narirutin, flavonoids with a wide spectrum of beneficial  
420 effects on health, are present in quite great quantity and could be recovered from the peel by a  
421 first filtration-compression as downstream process. The recovered liquid phase obtained after  
422 a filtration-compression of grinded peel is a complex mixture containing also polysaccharides,  
423 proteins, cellular fragments and organelles.... The properties of flavonoids are such that they  
424 could be found in the press liquor on different forms, i.e. linked with some particles and/or  
425 colloids or free in the suspension. The objective of this work was to study the dispersion state  
426 of naringin and narirutin in the press liquor, and notably to identify their presence in the  
427 different press liquor compartments (particulate, colloidal and soluble fractions). The  
428 evolution of this location over time was also studied, in order to orientate the choice of the  
429 subsequent extraction step.

430 Narirutin, less present in the press liquor than naringin, was mainly found on soluble form. No  
431 evolution of this location was observed during time. Naringin was found in the press liquor  
432 with higher concentration than its solubility limit. In the press liquor, naringin was initially  
433 essentially found free or linked to some small colloids. During time, an aggregation of the  
434 compounds present in the press liquor, revealed by turbidity measurements, was observed.

435 This aggregation resulted in an increase of the particulate fraction, at the expense of the  
436 colloidal ones. This aggregation seemed to trap naringin, that progressively appeared in the  
437 particulate fraction.

438 The stability study of the press liquor over time showed that this suspension was physically  
439 stable; the aggregates/flocs were not able to settle at room temperature, certainly because of  
440 interparticle repulsion or other reasons as low floc density, porous floc structure...

441 If flocculation is a process that is widely used in industrial applications in order to facilitate  
442 the separation of particle by physical methods (e.g. filtration, sedimentation...), the natural  
443 flocculation observed in the press liquor seemed to not generate flocs easily settleable. In this  
444 case, with an objective to isolate naringin on particulate form, some other post-treatment  
445 could be proposed (e.g. addition of destabilizing agent, addition of thickening agent,  
446 temperature change...) to destabilise the press liquor, so that repulsion between aggregates  
447 could be reduced, or to modify the floc structure. Further investigations will be proposed in  
448 this sense.

449

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455 colleagues, Adrien Servant, Bénédicte Marion and Céline Roques, who provided expertise in  
456 analytical measurements.

457

#### 458 **Declaration of interest**

459 The authors report no declarations of interest.

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635 **Figure 1.** Filtration-compression cell

636 **Figure 2.** Particle size distribution a  $t=0h$  and  $t=24h$  of the press liquor.

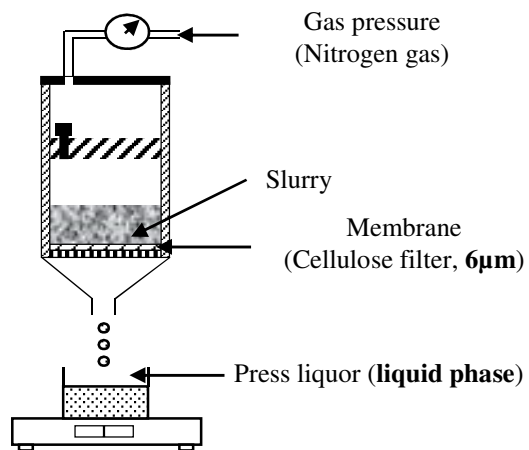
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640 **Figure 6.** Physical stability of the press liquor over time at a)  $25^{\circ}C$  and b)  $10^{\circ}C$

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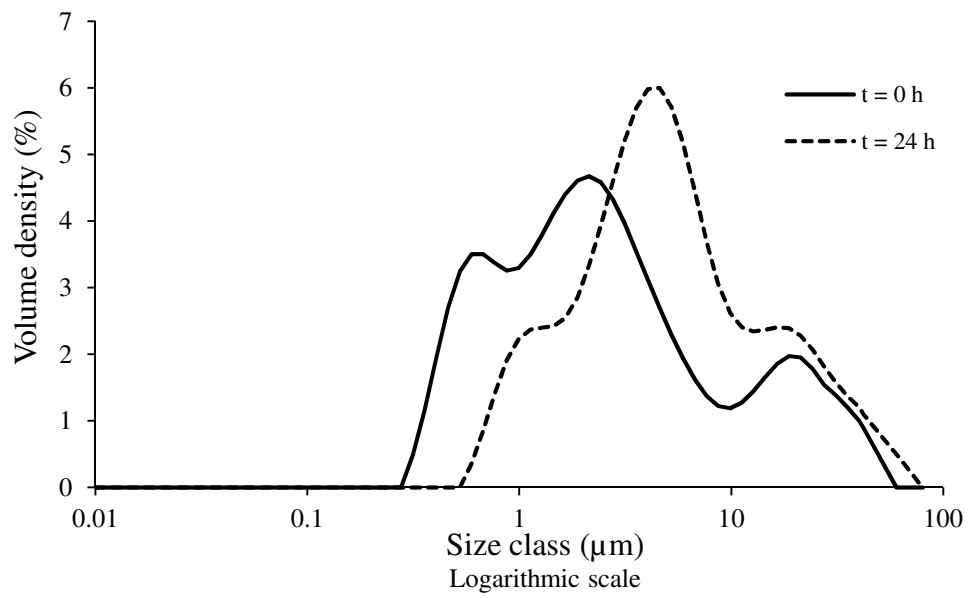
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**Figure 1.** Filtration-compression cell

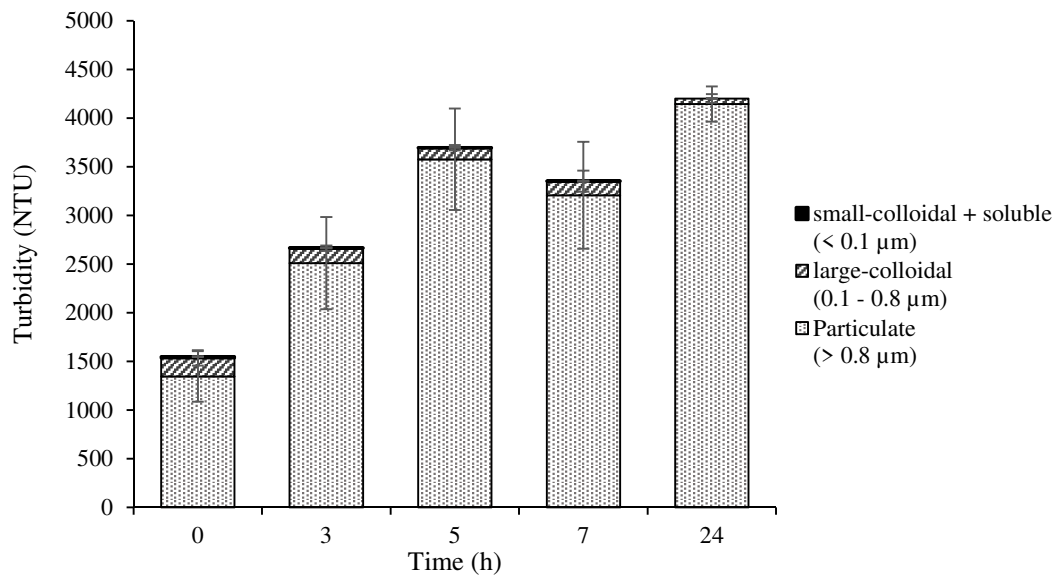


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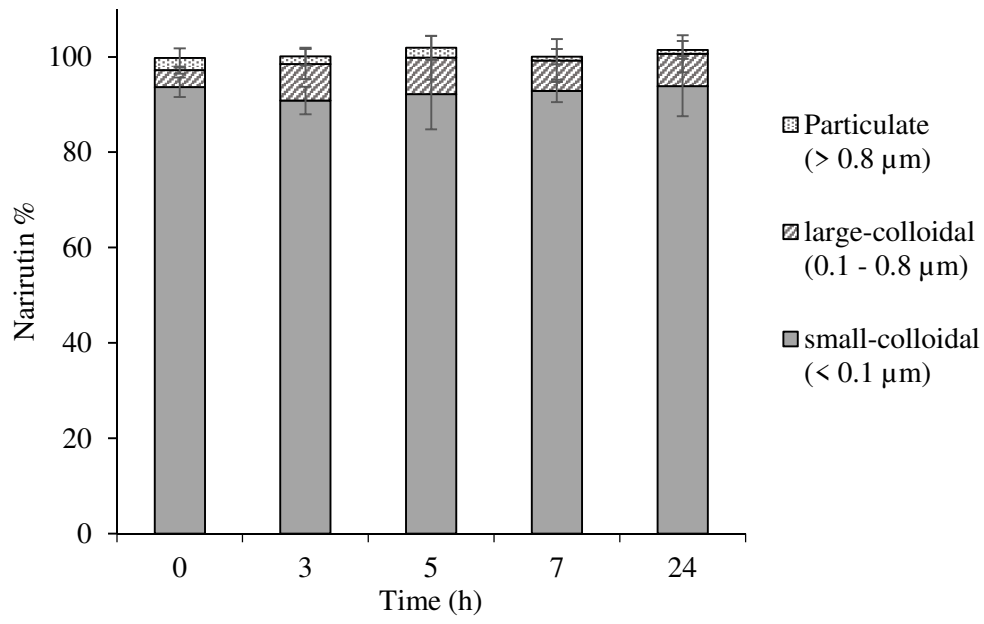
**Figure 2.** Particle size distribution at t = 0 h and t = 24 h of the press liquor

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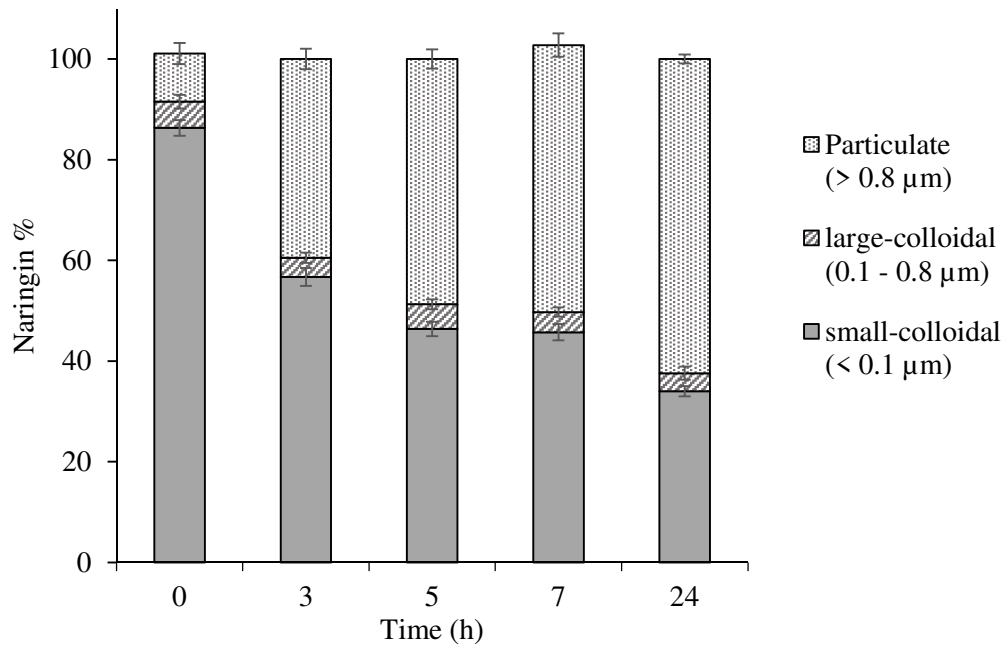


**Figure 3.** Evolution of the turbidity of the press liquor fractions

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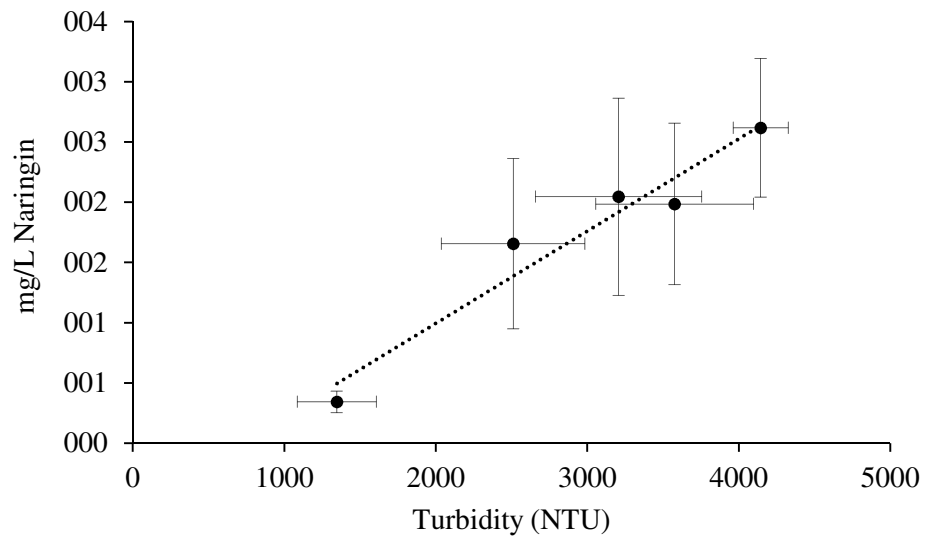
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**Figure 4.** Evolution of naringin and narirutin dispersion state

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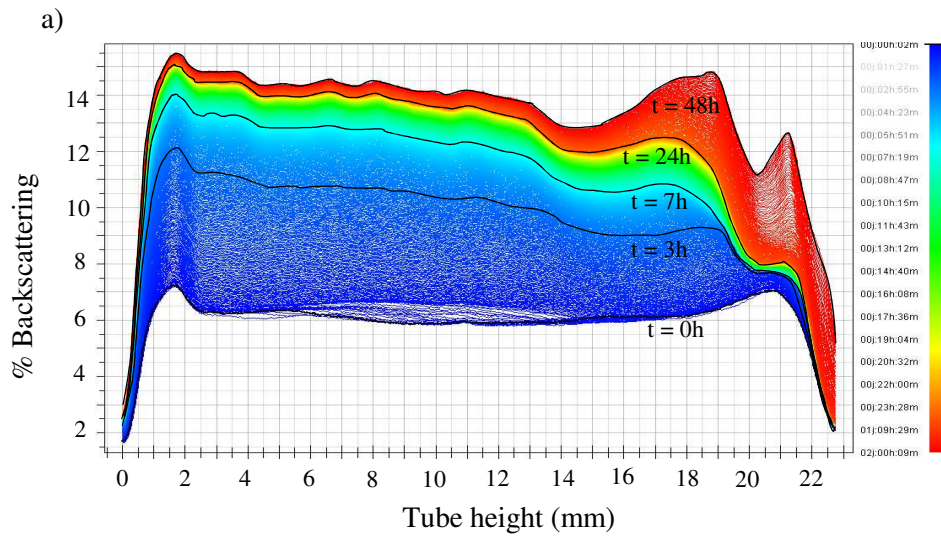


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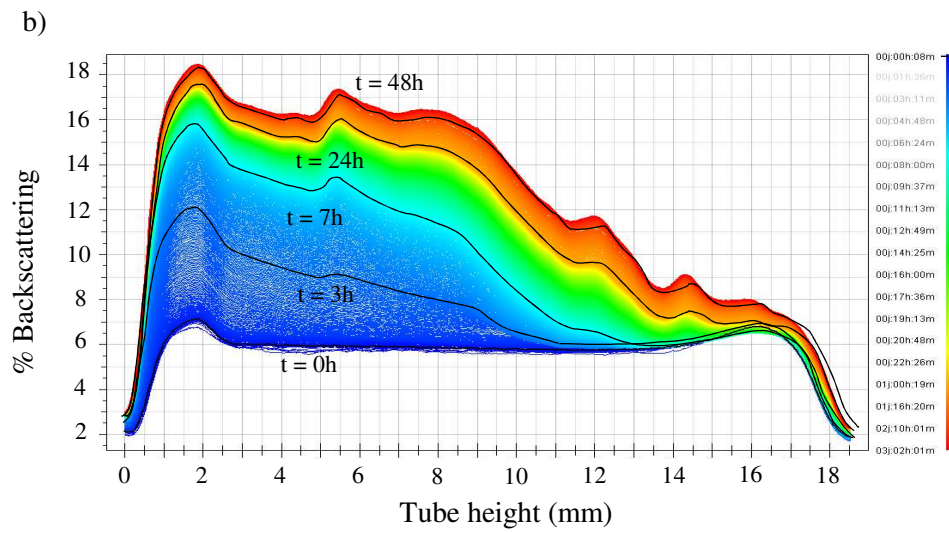
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**Figure 5.** Naringin evolution as a function of turbidity in the particulate fraction

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**Figure 6.** Physical stability of the press liquor over time  
a) 25°C and b) 10°C  
(value 0 corresponding to the bottom of the tube)

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678 Physico- and biochemical characteristics of the press liquor

679

680 **Table 2**

681 Naringin solubility at 25°C (<10 kDa fraction)

682

683 **Table 3**

684 Flavonoids dispersion state in the press liquor

685

686 **Table 1**  
 687 Physico- and biochemical characteristics of the press liquor  
 688

<b>Physicochemical characteristics</b>	<b>pH</b>	3.4 ± 0.2
	<b>Dry matter</b> <i>g/100 g press liquor</i>	7.4 ± 0.1
	<b>° Brix</b> <i>g sucrose/100 g press liquor</i>	7.0 ± 0.5
	<b>Turbidity</b> <i>NTU</i>	1481 ± 223
<b>Biochemical characteristics</b>	<b>Narirutin</b> <i>g/L press liquor</i>	0.40 ± 0.02
	<b>Naringin</b> <i>g/L press liquor</i>	4.1 ± 0.6

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691 **Table 2.** Naringin

solubility at 25°C

692 (<10 kDa fraction)

	<b>Water</b>	<b>7 °Brix water</b>
<b>Naringin (g/L)</b>	0.11 ± 0.01	0.07 ± 0.01

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697 **Table 3.** Flavonoids dispersion state in the press liquor (initial time, t = 0 h)

	<b>Soluble/ small colloidal fraction &lt; 0.1 <math>\mu\text{m}</math></b>	<b>Large colloidal fraction 0.1 – 0.8 <math>\mu\text{m}</math></b>	<b>Particulate Fraction &gt; 0.8 <math>\mu\text{m}</math></b>
<b>Narirutin (%)</b>	93.6 $\pm$ 2.0	3.6 $\pm$ 0.7	2.5 $\pm$ 2.0
<b>Naringin (%)</b>	86.3 $\pm$ 1.6	5.2 $\pm$ 1.4	9.6 $\pm$ 2.1
<b>Turbidity (NTU)</b>	13 $\pm$ 3	191 $\pm$ 80	1346 $\pm$ 261

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