



HAL
open science

Towards a better identification of naringin and narirutin dispersion state in grapefruit peel press liquor

N. Zarate-Vilet, C. Wisniewski, E. Gué, M. Delalonde

► To cite this version:

N. Zarate-Vilet, C. Wisniewski, E. Gué, M. Delalonde. Towards a better identification of naringin and narirutin dispersion state in grapefruit peel press liquor. *Chemical Engineering Research and Design*, 2020, 159, pp.205 - 214. 10.1016/j.cherd.2020.03.023 . hal-03490454

HAL Id: hal-03490454

<https://hal.science/hal-03490454>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

3 N. Zarate Vilet^{1*}, C. Wisniewski¹, E. Gué¹, M. Delalonde¹

4 ¹ *QualiSud, Univ Montpellier, CIRAD, Montpellier SupAgro, Univ Avignon, Univ La Réunion, France*

5 **Corresponding author:** nuria.zarate_vilet@cirad.fr

6

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

12

13

14

15

16

17

18

19

20 *corresponding author:

21 Nuria Zarate Vilet¹

22 CIRAD

23 UMR 95 QualiSud

24 TA-B95/15

25 73 rue Jean-François Breton

26 34398 Montpellier cedex 5

27 nuria.zarate_vilet@cirad.fr

28 +33-4-67-59-38-30

29

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 μm 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 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 (λ
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 μm
200 (Supor, Pall Corporation, USA). This methodology allowed to separate the press liquor
201 compounds in three fractions: (i) *particle fraction* (particles > 0.8 μm), (ii) *large-colloidal*
202 *fraction* (particles between 0.1 and 0.8 μm) and (iii) *small-colloidal and soluble fraction*
203 (particles < 0.1 μm).

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 ± 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 μm) 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 μm . The presence of this latter population, higher than the pore
249 filter size (around 6 μm), 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 μm , (ii) *large-colloidal fraction*, with colloids between 0.1 and 0.8 μm and (iii) *small-*
269 *colloidal and soluble fraction*, regrouping the smallest colloids, lower to 0.1 μm , 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 μm 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 ± 233 NTU (Table 1), was multiplied by the factor three
314 and reached a value of 4144 ± 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°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.

416

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

450 **Acknowledgements**

451 This research was partially supported by CONACYT. We are grateful to Dr. Elena Dibildox
452 Alvarado, who welcomed the PhD student in Mexico for some experiments. We are thankful
453 to Michaël Nigen, who provided expertise in Turbiscan® measurements. We are also
454 immensely grateful to Soufflet Biotechnologies for providing enzymes. We also thank our
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.

460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490

References

- AOAC, 1990. Official methods of analysis of the Association of Official Analytical Chemists, 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Baker, R.A., Cameron, R.G., 1999. Clouds of citrus juices and juice drinks. *Food technology*.
- Barba, F.J., Galanakis, C.M., Esteve, M.J., Frigola, A., Vorobiev, E., 2015. Potential use of pulsed electric technologies and ultrasounds to improve the recovery of high-added value compounds from blackberries. *Journal of Food Engineering* 167, 38–44.
<https://doi.org/10.1016/j.jfoodeng.2015.02.001>
- Benitez, E.I., Genovese, D.B., Lozano, J.E., 2007. Scattering efficiency of a cloudy apple juice: Effect of particles characteristics and serum composition. *Food Research International* 40, 915–922. <https://doi.org/10.1016/j.foodres.2007.03.004>
- Ben-Shalom, N., Pinto, R., Kanner, J., Berman, M., 1985. Model System of Natural Orange Juice Cloud: Effect of Calcium on Hesperidin-Pectin Particles. *Journal of Food Science* 50, 1130–1132. <https://doi.org/10.1111/j.1365-2621.1985.tb13027.x>
- Beveridge, T., 2002. Opalescent and Cloudy Fruit Juices: Formation and Particle Stability. *Critical Reviews in Food Science and Nutrition* 42, 317–337.
<https://doi.org/10.1080/10408690290825556>
- Bordenave, N., Hamaker, B.R., Ferruzzi, M.G., 2014. Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods. *Food Funct.* 5, 18–34. <https://doi.org/10.1039/C3FO60263J>
- Chen, R., Qi, Q.-L., Wang, M.-T., Li, Q.-Y., 2016. Therapeutic potential of naringin: an overview. *Pharmaceutical Biology* 54, 3203–3210.
<https://doi.org/10.1080/13880209.2016.1216131>
- Cirillo, G., Curcio, M., Vittorio, O., Iemma, F., Restuccia, D., Spizzirri, U.G., Puoci, F., Picci, N., 2016. Polyphenol Conjugates and Human Health: A Perspective Review. *Critical Reviews in Food Science and Nutrition* 56, 326–337.
<https://doi.org/10.1080/10408398.2012.752342>

491 Dahdouh, L., Delalonde, M., Ricci, J., Ruiz, E., Wisniewski, C., 2018. Influence of high shear
492 rate on particles size, rheological behavior and fouling propensity of fruit juices during
493 crossflow microfiltration: Case of orange juice. *Innovative Food Science & Emerging
494 Technologies* 48, 304–312. <https://doi.org/10.1016/j.ifset.2018.07.006>

495 Deng, Q., Zinoviadou, K.G., Galanakis, C.M., Orlie, V., Grimi, N., Vorobiev, E., Lebovka,
496 N., Barba, F.J., 2015. The Effects of Conventional and Non-conventional Processing
497 on Glucosinolates and Its Derived Forms, Isothiocyanates: Extraction, Degradation,
498 and Applications. *Food Eng Rev* 7, 357–381. [https://doi.org/10.1007/s12393-014-
9104-9](https://doi.org/10.1007/s12393-014-
499 9104-9)

500 Galanakis, C.M., 2018. Phenols recovered from olive mill wastewater as additives in meat
501 products. *Trends in Food Science & Technology* 79, 98–105.
502 <https://doi.org/10.1016/j.tifs.2018.07.010>

503 Galanakis, C.M., 2015. Separation of functional macromolecules and micromolecules: From
504 ultrafiltration to the border of nanofiltration. *Trends in Food Science & Technology*
505 42, 44–63. <https://doi.org/10.1016/j.tifs.2014.11.005>

506 Galanakis, C.M., 2013. Emerging technologies for the production of nutraceuticals from
507 agricultural by-products: A viewpoint of opportunities and challenges. *Food and
508 Bioproducts Processing* 91, 575–579. <https://doi.org/10.1016/j.fbp.2013.01.004>

509 Galanakis, C.M., 2012. Recovery of high added-value components from food wastes:
510 Conventional, emerging technologies and commercialized applications. *Trends in
511 Food Science & Technology* 26, 68–87. <https://doi.org/10.1016/j.tifs.2012.03.003>

512 Galanakis, C.M., Markouli, E., Gekas, V., 2013. Recovery and fractionation of different
513 phenolic classes from winery sludge using ultrafiltration. *Separation and Purification
514 Technology* 107, 245–251. <https://doi.org/10.1016/j.seppur.2013.01.034>

515 Galanakis, C.M., Tsatalas, P., Charalambous, Z., Galanakis, I.M., 2018a. Control of microbial
516 growth in bakery products fortified with polyphenols recovered from olive mill
517 wastewater. *Environmental Technology & Innovation* 10, 1–15.
518 <https://doi.org/10.1016/j.eti.2018.01.006>

519 Galanakis, C.M., Tsatalas, P., Charalambous, Z., Galanakis, I.M., 2018b. Polyphenols
520 recovered from olive mill wastewater as natural preservatives in extra virgin olive oils
521 and refined olive kernel oils. *Environmental Technology & Innovation* 10, 62–70.
522 <https://doi.org/10.1016/j.eti.2018.01.012>

523 Galanakis, C.M., Tsatalas, P., Galanakis, I.M., 2018c. Implementation of phenols recovered
524 from olive mill wastewater as UV booster in cosmetics. *Industrial Crops and Products*
525 111, 30–37. <https://doi.org/10.1016/j.indcrop.2017.09.058>

526 Galanakis, C.M., Tsatalas, P., Galanakis, I.M., 2018d. Phenols from olive mill wastewater and
527 other natural antioxidants as UV filters in sunscreens. *Environmental Technology &*
528 *Innovation* 9, 160–168. <https://doi.org/10.1016/j.eti.2017.12.002>

529 Garcia-Castello, E.M., Rodriguez-Lopez, A.D., Mayor, L., Ballesteros, R., Conidi, C.,
530 Cassano, A., 2015. Optimization of conventional and ultrasound assisted extraction of
531 flavonoids from grapefruit (*Citrus paradisi* L.) solid wastes. *LWT - Food Science and*
532 *Technology* 64, 1114–1122. <https://doi.org/10.1016/j.lwt.2015.07.024>

533 Genovese, D.B., Lozano, J.E., Rao, M.A., 2007. The Rheology of Colloidal and Noncolloidal
534 Food Dispersions. *J Food Science* 72, R11–R20. [https://doi.org/10.1111/j.1750-](https://doi.org/10.1111/j.1750-3841.2006.00253.x)
535 [3841.2006.00253.x](https://doi.org/10.1111/j.1750-3841.2006.00253.x)

536 Giannuzzo, A.N., Boggetti, H.J., Nazareno, M.A., Mishima, H.T., 2003. Supercritical fluid
537 extraction of naringin from the peel of *Citrus paradisi*. *Phytochemical Analysis* 14,
538 221–223. <https://doi.org/10.1002/pca.706>

539 Goliomytis, M., Kartsonas, N., Charismiadou, M.A., Symeon, G.K., Simitzis, P.E.,
540 Deligeorgis, S.G., 2015. The Influence of Naringin or Hesperidin Dietary
541 Supplementation on Broiler Meat Quality and Oxidative Stability. *PLoS ONE* 10, 1–
542 11. <https://doi.org/10.1371/journal.pone.0141652>

543 Hu, J.-N., Zheng, H., Chen, X.-X., Li, X., Xu, Y., Xu, M.-F., 2020. Synergetic effects of
544 whey protein isolate and naringin on physical and oxidative stability of oil-in-water
545 emulsions. *Food Hydrocolloids* 101, 105517.
546 <https://doi.org/10.1016/j.foodhyd.2019.105517>

547 Jourdan, P.S., Weiler, E.W., Mansell, R.L., 1983. Radioimmunoassay for naringin and related
548 flavanone 7-neohesperidosides using a tritiated tracer. *Journal of Agricultural and*
549 *Food Chemistry* 31, 1249–1255. <https://doi.org/10.1021/jf00120a026>

550 Lachos-Perez, D., Baseggio, A.M., Mayanga-Torres, P.C., Maróstica, M.R., Rostagno, M.A.,
551 Martínez, J., Forster-Carneiro, T., 2018. Subcritical water extraction of flavanones
552 from defatted orange peel. *The Journal of Supercritical Fluids* 138, 7–16.
553 <https://doi.org/10.1016/j.supflu.2018.03.015>

554 Le Bourvellec, C., Renard, C.M.G.C., 2012. Interactions between Polyphenols and
555 Macromolecules: Quantification Methods and Mechanisms. *Critical Reviews in Food*
556 *Science and Nutrition* 52, 213–248. <https://doi.org/10.1080/10408398.2010.499808>

557 Li, B.B., Smith, B., Hossain, Md.M., 2006. Extraction of phenolics from citrus peels: II.
558 Enzyme-assisted extraction method. *Sep. Purif. Technol* 48, 189–196.
559 <https://doi.org/10.1016/j.seppur.2005.07.019>

560 Mengual, O., Meunier, G., Cayre, I., Puech, K., Snabre, P., 1999. Characterisation of
561 instability of concentrated dispersions by a new optical analyser: the TURBISCAN
562 MA 1000. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 152,
563 111–123. [https://doi.org/10.1016/S0927-7757\(98\)00680-3](https://doi.org/10.1016/S0927-7757(98)00680-3)

564 M'hiri, N., Ioannou, I., Ghoul, M., Boudhrioua, N.M., 2014. Extraction Methods of Citrus
565 Peel Phenolic Compounds. *Food Reviews International* 30, 265–290.
566 <https://doi.org/10.1080/87559129.2014.924139>

567 Mizrahi, S., Berk, Z., 1970. Physico-chemical characteristics of orange juice cloud. *J. Sci.*
568 *Food Agric.* 21, 250–253. <https://doi.org/10.1002/jsfa.2740210508>

569 Mouly, P., Gaydou, E.M., Estienne, J., 1993. Column liquid chromatographic determination
570 of flavanone glycosides in Citrus: Application to grapefruit and sour orange juice
571 adulterations. *Journal of Chromatography A* 634, 129–134.

572 Nagarajan, J., Krishnamurthy, N.P., Nagasundara Ramanan, R., Raghunandan, M.E.,
573 Galanakis, C.M., Ooi, C.W., 2019. A facile water-induced complexation of lycopene
574 and pectin from pink guava byproduct: Extraction, characterization and kinetic
575 studies. *Food Chemistry* 296, 47–55. <https://doi.org/10.1016/j.foodchem.2019.05.135>

576 Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Dairi, S., Aoun, O., Khodir, M., 2015.
577 Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for
578 recovery of polyphenols from Citrus sinensis peels. *Food Chemistry* 187, 507–516.
579 <https://doi.org/10.1016/j.foodchem.2015.04.081>

580 Nogata, Y., Sakamoto, K., Shiratsuchi, H., Ishii, T., Yano, M., Ohta, H., 2006. Flavonoid
581 Composition of Fruit Tissues of Citrus Species. *Bioscience, Biotechnology, and*
582 *Biochemistry* 70, 178–192. <https://doi.org/10.1271/bbb.70.178>

583 Pinelo, M., Zeuner, B., Meyer, A.S., 2010. Juice clarification by protease and pectinase
584 treatments indicates new roles of pectin and protein in cherry juice turbidity. *Food and*
585 *Bioproducts Processing* 88, 259–265. <https://doi.org/10.1016/j.fbp.2009.03.005>

586 Plaza, M., Turner, C., 2015. Pressurized hot water extraction of bioactives. *TrAC Trends in*
587 *Analytical Chemistry* 71, 39–54. <https://doi.org/10.1016/j.trac.2015.02.022>

588 Pulley, G.N., 1936. Solubility of Naringin in Water. *Industrial & Engineering Chemistry*
589 *Analytical Edition* 8, 360–360. <https://doi.org/10.1021/ac50103a020>

590 Putnik, P., Bursać Kovačević, D., Režek Jambrak, A., Barba, F.J., Cravotto, G., Binello, A.,
591 Lorenzo, J.M., Shpigelman, A., 2017. Innovative “Green” and Novel Strategies for the
592 Extraction of Bioactive Added Value Compounds from Citrus Wastes—A Review.
593 *Molecules* 22, 680. <https://doi.org/10.3390/molecules22050680>

594 Rouquié, C., Dahdouh, L., Delalonde, M., Wisniewski, C., 2019. New prospects for immersed
595 hollow-fiber membranes in fruit juices microfiltration: Case of grapefruit juice.
596 *Journal of Food Engineering* 246, 75–85.
597 <https://doi.org/10.1016/j.jfoodeng.2018.11.001>

598 Shomer, I., 1991. Protein coagulation cloud in citrus fruit extract. 1. Formation of coagulates
599 and their bound pectin and neutral sugars. *J. Agric. Food Chem.* 39, 2263–2266.
600 <https://doi.org/10.1021/jf00012a034>

601 Siebert, K.J., 2009. Chapter 2 Haze in Beverages, in: *Advances in Food and Nutrition*
602 *Research*. Elsevier, pp. 53–86. [https://doi.org/10.1016/S1043-4526\(09\)57002-7](https://doi.org/10.1016/S1043-4526(09)57002-7)

603 Simitzis, P.E., 2018. Agro-Industrial By-Products and Their Bioactive Compounds—An Ally
604 against Oxidative Stress and Skin Aging. *Cosmetics* 5, 58.
605 <https://doi.org/10.3390/cosmetics5040058>

606 Sorrivias, V., Genovese, D.B., Lozano, J.E., 2006. EFFECT OF PECTINOLYTIC AND
607 AMYLOLYTIC ENZYMES ON APPLE JUICE TURBIDITY: PECTINOLYTIC
608 AND AMYLOLYTIC ENZYMES ON APPLE JUICE TURBIDITY. *Journal of Food*
609 *Processing and Preservation* 30, 118–133. [https://doi.org/10.1111/j.1745-](https://doi.org/10.1111/j.1745-4549.2006.00054.x)
610 [4549.2006.00054.x](https://doi.org/10.1111/j.1745-4549.2006.00054.x)

611 Teng, J., Liu, X., Hu, X., Zhao, Y., Tao, N.-P., Wang, M., 2018. Dihydromyricetin as a
612 Functional Additive to Enhance Antioxidant Capacity and Inhibit the Formation of
613 Thermally Induced Food Toxicants in a Cookie Model. *Molecules* 23, 2184.
614 <https://doi.org/10.3390/molecules23092184>

615 USDA, 2018. Citrus World Markets and Trade.

616 VCCLAB, Virtual Computational Chemistry Laboratory [WWW Document], 2005. .
617 <http://www.vcclab.org>.

618 Victor, M.M., David, J.M., Sakukuma, M.C.K., França, E.L., Nunes, A.V.J., 2018. A simple
619 and efficient process for the extraction of naringin from grapefruit peel waste. *Green*
620 *Processing and Synthesis* 7, 524–529. <https://doi.org/10.1515/gps-2017-0112>

621 Whitaker, J.R., 1984. Pectic substances, pectic enzymes and haze formation in fruit juices.
622 *Enzyme and Microbial Technology* 6, 341–349. [https://doi.org/10.1016/0141-](https://doi.org/10.1016/0141-0229(84)90046-2)
623 [0229\(84\)90046-2](https://doi.org/10.1016/0141-0229(84)90046-2)

624 World Health Organization (WHO), 2017. Water Quality and Health– Review of turbidity:
625 Information for regulators and water suppliers. Technical Brief. (Technical brief).
626 World Health Organization (WHO), Geneva.

627 Zarate Vilet, N., Gué, E., Servent, A., Delalonde, M., Wisniewski, C., 2020. Filtration-
628 compression step as downstream process for flavonoids extraction from citrus peels:
629 Performances and flavonoids dispersion state in the filtrate. Food and Bioproducts
630 Processing 120, 104–113. <https://doi.org/10.1016/j.fbp.2020.01.001>

631

632

633 **List of figures**

634

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

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

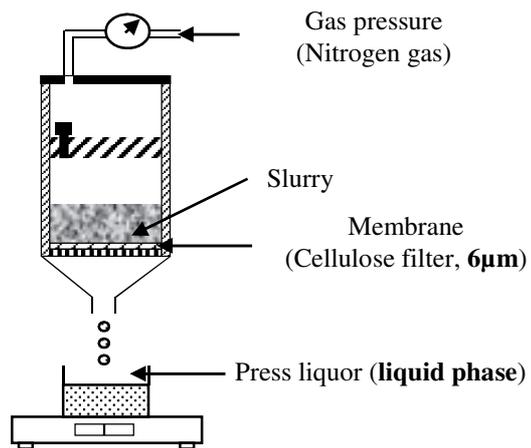
637 **Figure 3.** Evolution of the turbidity of the press liquor fractions.

638 **Figure 4.** Evolution of naringin and narirutin dispersion state within fractions

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

640 **Figure 6.** Physical stability of the press liquor over time at a) 25°C and b) 10°C

641



642

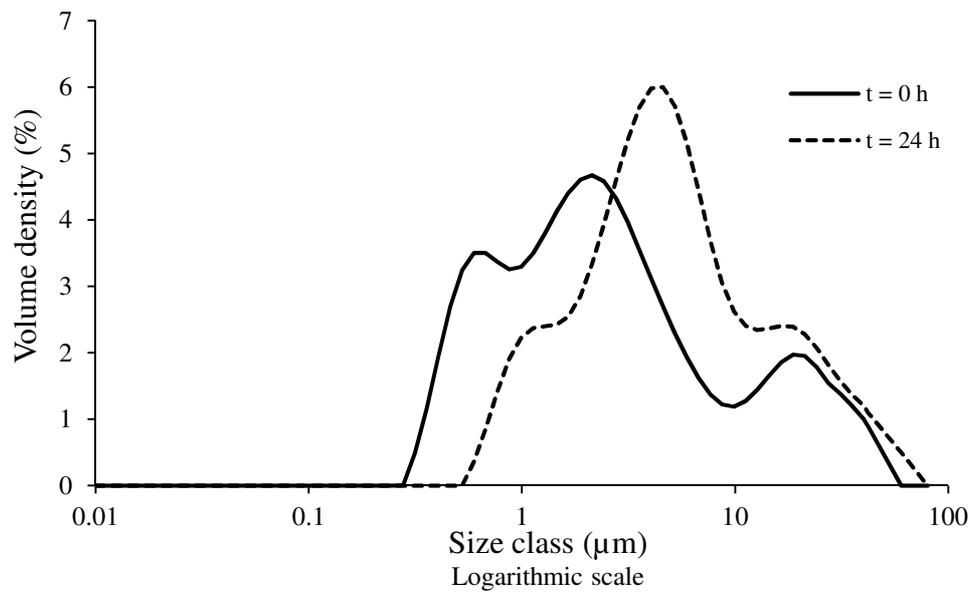
643

644

645

646

Figure 1. Filtration-compression cell



647

648

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

649

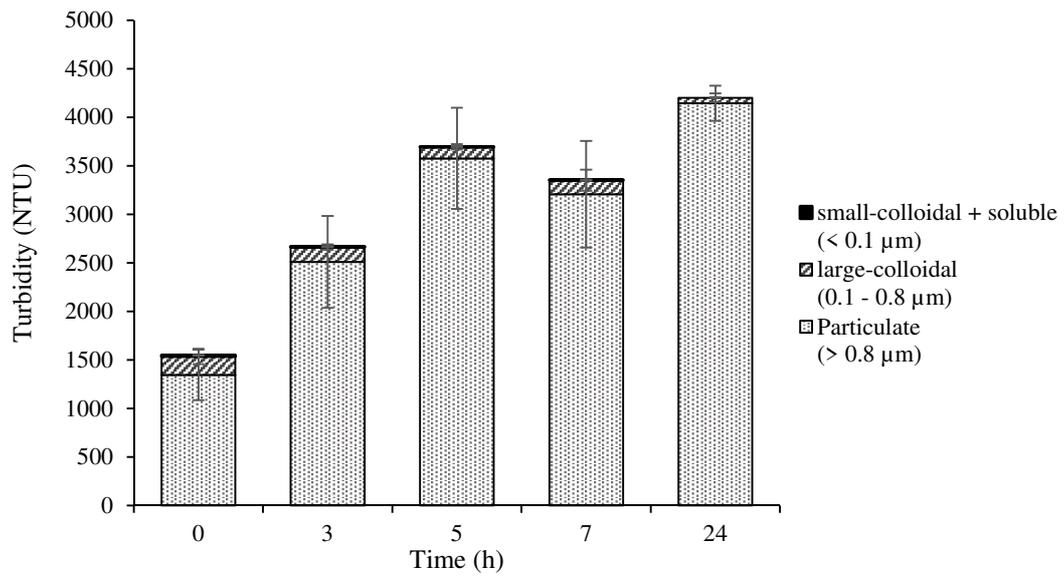


Figure 3. Evolution of the turbidity of the press liquor fractions

650
 651
 652
 653
 654
 655
 656
 657
 658
 659
 660
 661
 662
 663

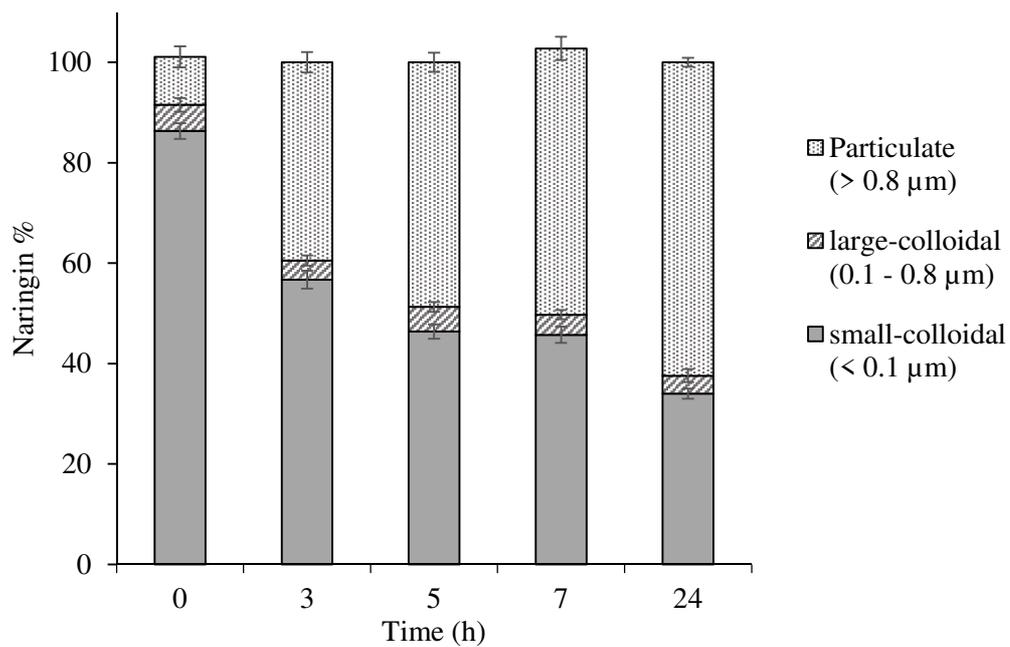
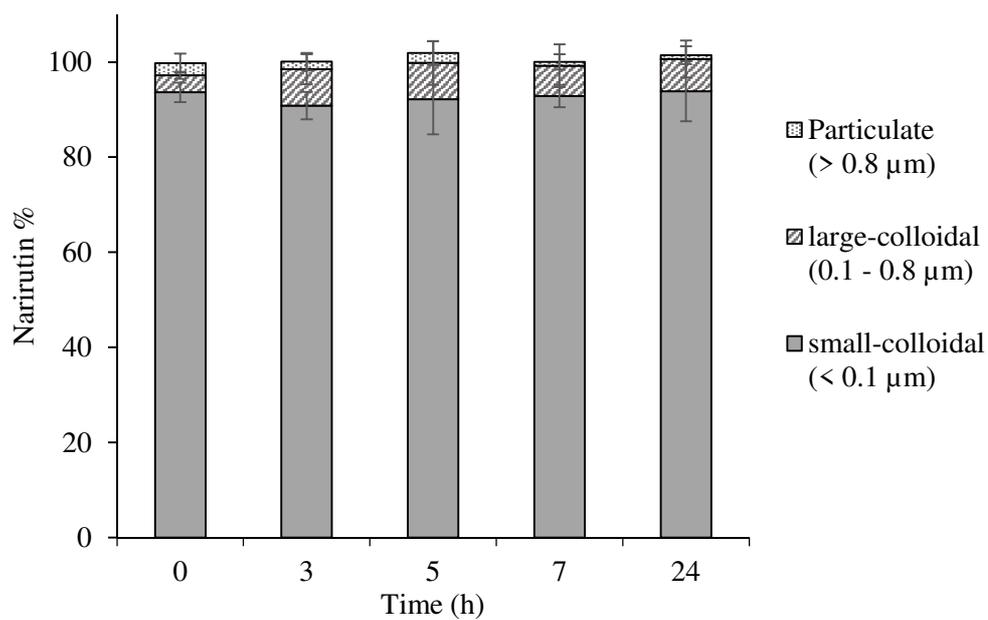
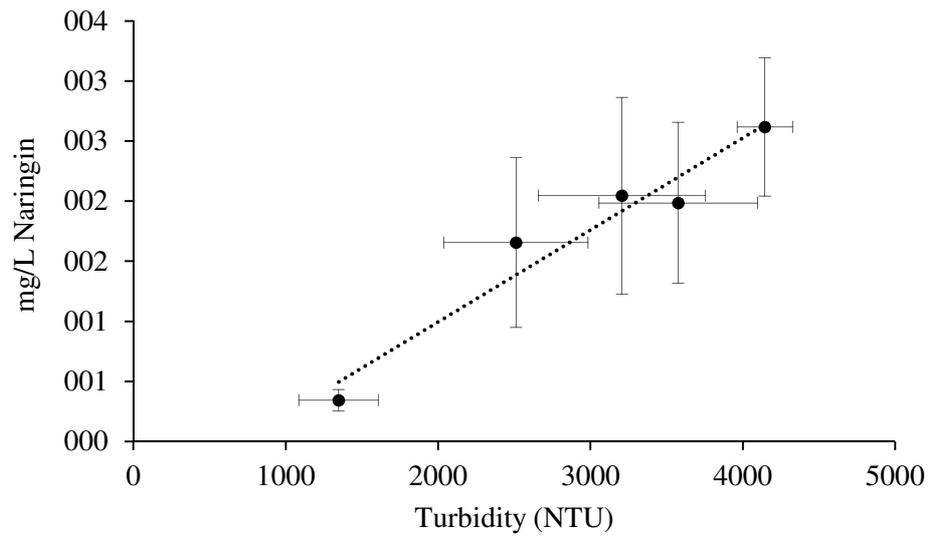


Figure 4. Evolution of naringin and narirutin dispersion state

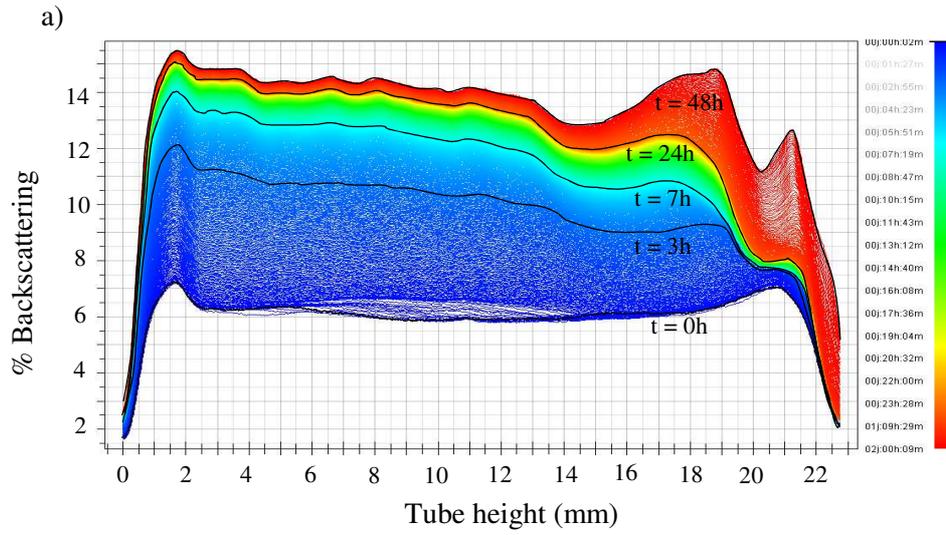


667

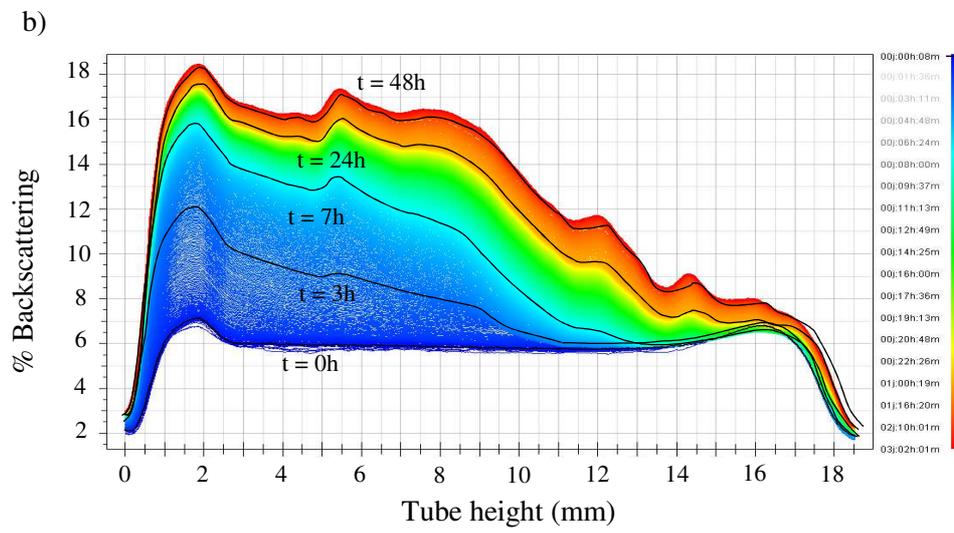
668

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

669



670



671

672

673

674

675

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)

676 **List of Tables**

677 **Table 1**

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

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

689

690

691 **Table 2.** Naringin

solubility at 25°C

692 (<10 kDa fraction)

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

693

694

695

696

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

	Soluble/ small colloidal fraction < 0.1 μm	Large colloidal fraction 0.1 – 0.8 μm	Particulate Fraction > 0.8 μm
Narirutin (%)	93.6 \pm 2.0	3.6 \pm 0.7	2.5 \pm 2.0
Naringin (%)	86.3 \pm 1.6	5.2 \pm 1.4	9.6 \pm 2.1
Turbidity (NTU)	13 \pm 3	191 \pm 80	1346 \pm 261

698

699

700