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1 **Filtration-compression step as downstream process for flavonoids extraction**
2 **from citrus peels: performances and flavonoids dispersion state in the filtrate**

3

4 **N. Zarate Vilet*¹, E. Gué¹, A. Servent², M. Delalonde¹ and C. Wisniewski¹**

5

6 ¹ QualiSud, Univ Montpellier, CIRAD, Montpellier SupAgro, Univ Avignon, Univ La Réunion,
7 Montpellier, France

8 ² CIRAD, UMR QualiSud, F-34398 Montpellier, France

9

10 **Keywords:** downstream process; filtration-compression; enzymatic treatment; grapefruit peels;
11 flavonoids

12

13 **Highlights:**

- 14 • Filtration-compression for downstream processing of flavonoid extraction from peel
15 • Identification of the maximum extractable liquid of peel by filtration-compression
16 • Cell-wall degrading enzymes favour naringin and narirutin extraction from peel
17 • Identification of the dispersion state of flavonoids for a relevant post-extraction

18

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20 *corresponding author:

21

22 Nuria Zarate Vilet¹

23 CIRAD

24 UMR 95 Qualisud

25 TA-B95/15

26 73 rue Jean-François Breton

27 34398 Montpellier cedex 5

28 nuria.zarate_vilet@cirad.fr

29 +33-4-67-59-38-30

30

31 **Abstract**

32

33 Initiative of waste treatment has to be based on user-friendly technologies, using robust, cost-
34 effective and low-energy consuming processes. The global objective of this study was to reduce
35 technical steps for the extraction of flavonoids (naringin and narirutin) from citrus peel
36 (grapefruit peel). After a first fresh peel grinding, the relevance of a simple filtration-
37 compression, as a first downstream process, of the obtained slurry was studied. An optimization
38 of this solid-liquid separation was proposed and the impact of a pectinolytic enzymatic treatment,
39 resulting potentially in a larger release of flavonoids, was investigated. The results demonstrated
40 that a preliminary step of filtration-compression, directly realized on fresh grinded peels as
41 downstream processing for flavonoids extraction could be pertinent and that the enzymatic
42 treatment improved the slurry filterability. An optimal separation was obtained with a
43 **transmembrane pressure of 5 bar**, leading to highest extractable liquid phase volume and to an
44 extraction around 80% of naringin and narirutin. A modelling of the filtration step, essential for
45 the scaling of a filtration-compression process on site, was proposed. An originality of this work
46 was to identify the dispersion state of the flavonoids within the liquid phase, capital identification
47 for a relevant choice of the subsequent extraction step of these compounds.

48

49 **Abbreviations and nomenclature**

| | | |
|----|-----------------------|---|
| 50 | d_{50} | Mean diameter (μm) |
| 51 | J | Filtration rate ($\text{m}\cdot\text{s}^{-1}$) |
| 52 | J_0 | Initial filtration rate ($\text{m}\cdot\text{s}^{-1}$) |
| 53 | K_b | Blocking constant for complete blocking law (m^{-3}) |
| 54 | K_c | Blocking constant for cake filtration law (m^{-3}) |
| 55 | K_i | Blocking constant for intermediate blocking law (m^{-3}) |
| 56 | K_s | Blocking constant for standard blocking law (m^{-3}) |
| 57 | M | Filtrate mass (g) |
| 58 | $M_{\text{add.}}$ | Mass of water added for the enzymatic treatment (g) |
| 59 | M_{DM} | Mass of dry matter in slurry (g) |
| 60 | $M_{\text{ext-liq.}}$ | Mass of extracted intrinsic liquid |
| 61 | M_F | Maximum filtrate mass (g) |
| 62 | $M_{\text{liq.}}$ | Total liquid mass of the grinded peel (g) |
| 63 | t | Filtration time (s) |
| 64 | TMP | Transmembrane pressure (bar) |
| 65 | V | Filtrate volume (m^3) |
| 66 | w | Enzymatic solution/slurry mass ratio (g/g) |
| 67 | | |
| 68 | Greek letters | |
| 69 | η | Theoretical filtration efficiency (%) |
| 70 | η^* | Experimental filtration efficiency (%) |
| 71 | Ω | Filtration surface (m^2) |
| 72 | | |
| 73 | Index | |
| 74 | DM | Dry matter |
| 75 | ET | Enzyme treated slurry |
| 76 | H | Heating (50°C , 2 hours) |
| 77 | NT | Non-treated slurry |
| 78 | W | Water addition in a ratio 1/1 |
| 79 | W+H | Water addition in a ratio 1/1 + Heating (50°C , 2 hours) |

80

81 **1. Introduction**

82 *Citrus* genus (orange, mandarin/tangerine, grapefruit and lemon/lime) is among the largest
83 cultivated fruit crops in tropical and subtropical regions worldwide, with a global production of
84 92.088 million metric tons in 2017/18, and is expected to increase further in the future [1]. About
85 a quarter of the harvested fruit is transformed into various commercial products such as juice,
86 jam, marmalades and flavouring agents, using only about 50% of the total fruit. Hence, these
87 transformations generate yearly 11 million metric tons of wastes and by-products, which are
88 considered harmful to soil and water bodies due to their low pH (3-4), high water (around 80-
89 90%) and organic matter (95% of total solids) contents [2–4]. Yet today waste management is at
90 the heart of the concerns with the sustainable food systems development aiming at a « 0 waste »
91 production but also with the biorefinery tendency of the food industry [3,5,6]. For Southern citrus
92 industries (ensuring 70-80% of the total citrus production [1,7]), due to their insufficient
93 infrastructure, little or no valorisation are ensured. Thus, peels, pulp and seeds are generally
94 discarded on adjacent land leading to the generation of putrefying waste. Sometimes, these by-
95 products are valorised as cattle feedstock but with a low economic interest as this valorisation
96 requires a previous drying which is a high-energy consuming operation.

97 These practices are particularly unfortunate, as *Citrus* by-products can be considered as a cheap
98 source of high-added value components such as vitamins, minerals, essential oils, fibres and
99 bioactive compounds mainly carotenoids, and polyphenols [2–4,8,9]. Flavonoids are
100 polyphenolic compounds with a wide spectrum of beneficial effects; this includes anticancer,
101 antiatherosclerotic, cardiovascular, anti-inflammatory, neuroprotective, hypolipidemic,
102 antidiabetic, hepatoprotective activities and applications in the treatment of bone disorders [10–
103 13].

104 In the literature, one of the most studied valorisation ways for Citrus by-products is the recovery
105 of flavonoids from peels. This recovery purpose requires not only efficient extraction technology

106 but also various preliminary treatments of the peel, facilitating first its storage before extraction
107 and secondly the solid/liquid separation step. Generally, a safe storage is ensured by drying (hot
108 air, freeze-drying...). Then, with an objective to facilitate the mass transfer during extraction,
109 grinding eventually followed by sifting is carried out to reduce and homogenize the particle size
110 [14]. Extraction of the target compounds is generally combined with treatments or technologies
111 facilitating the flavonoids transfer from the solid to the liquid phase. This step has attracted
112 considerable scientific interest and thus several traditional or emerging extraction technologies
113 are proposed in the literature: solvent extraction [15], hot-water extraction [16], ultrasound-
114 assisted [15,17,18], microwave-assisted [19–21], enzyme-assisted [22], pulsed electric fields
115 [23,24], high voltage electrical discharge [25] subcritical water [26–28], and supercritical CO₂
116 [29–32].

117 However, in Southern countries, the sustainability of waste treatment is threatened by the lack of
118 financial resources and technical support. Moreover, most of the aforementioned processes,
119 mostly studied at laboratory scale, require a high investment or high-energy consumption making
120 these technologies non-suitable for those countries. As the success of valorisation initiative is
121 based on the user's acceptance toward easy and user-friendly technologies, it seems pertinent to
122 think about a high-added-value compounds recovery chain using optimized green, simple, robust,
123 cost-effective and low-energy consuming processes. Our study fell within this context, with the
124 aim to avoid a maximum of preliminary stages, especially the highly energy consuming drying
125 step, before the extraction of flavonoids from Grapefruit (*C. paradisi*), rich source of naringin
126 and narirutin [33].

127 Thus, the objective of this work was to study the relevance of a simple filtration-compression
128 step directly done on the fresh grinded peels as a first downstream process for flavonoids
129 extraction. The optimization of the solid-liquid separation was based on the estimation of the
130 mass of the mechanically extractable liquid phase from the slurry under different **transmembrane**

131 **pressures**, combined with the quantification of the flavonoids (narirutin and naringin) content in
132 the filtrate. Before the filtration in a pressurized filtration-compression cell, fresh peels were
133 grinded to form a slurry with an objective to favour mass transfer by an increase of the exchange
134 surface and a disruption of plant tissues, flavonoids being considered to be largely stocked within
135 the cell vacuoles [24]. The potential impact on the solid-liquid separation of a pectinolytic
136 enzymatic treatment, causing a cell wall degradation resulting potentially in a larger release of
137 flavonoids, was investigated. An originality of this work was also to identify the dispersion state
138 of the flavonoids within the mechanically extractable aqueous phase obtained after this
139 preliminary solid-liquid separation. This last investigation was supposed to be capital for a
140 relevant choice of the following extraction of these compounds.

141

142 **2. Materials and Methods**

143 *2.1. Grapefruit peel supply and storage*

144 Grapefruits (*Citrus paradisi*, Star ruby from Turkey, provided by Terreazur, Saint-Jean-de-
145 Védas, France) were first bleached. Flavedo, which can be valorised through the extraction of
146 essential oils [4], was then manually removed from the peel, the target flavonoids being mostly
147 contained in the albedo [34–38]. Then, juice was extracted using an electric juice extractor
148 (ZX7000, Krups). Albedo and pulp rests (core, juice sacs and segment rests), representing in our
149 experiments about 35% of the weight of the total fruit, were then manually cut into small pieces
150 (around 10 x 10 mm) and stored at -25°C.

151

152 *2.2. Experimental methodology*

153 *2.2.1 Grinding*

154 After defrosting at room temperature (45 min to 1 hour), peels were subjected to grinding. Grinding
155 was ensured using a hand blender (Moulinex DD873D10 Infiny Force ultimate, France) for

156 1.5 minutes at 1 kW (corresponding to a $9.10^5 \text{ J}\cdot\text{kg}^{-1}$ of fresh peel). After grinding, a thick
157 suspension, i.e. a slurry, was obtained.

158

159 2.2.2. *Enzymatic treatment*

160 The enzymatic treatment was based on the use of Pecllyve[®] PR (Soufflet biotechnologies[®]), a
161 commercial pectinolytic enzyme preparation with two main pectinase activities, Pectin-methyl-
162 esterase (EC3.1.1.11; 150 U/g) and Pectin-lyase (EC4.2.2.10; 1100 U/g). An enzymatic solution
163 was prepared with the objective to provide 0.3 g of enzyme per kg of slurry, with a **mass ratio**
164 **solution/slurry (w) of approximately 1/1**. The enzymatic treatment consisted in the mixing of
165 the slurry and the enzymatic solution during 2 h at 50°C. **These operating conditions were**
166 **recommended by Soufflet biotechnologies[®] for an optimal enzyme activity.**

167

168 2.2.3. *Filtration-compression*

169 The liquid phase of the raw (NT) or enzyme treated (ET) slurry was extracted by filtration-
170 compression.

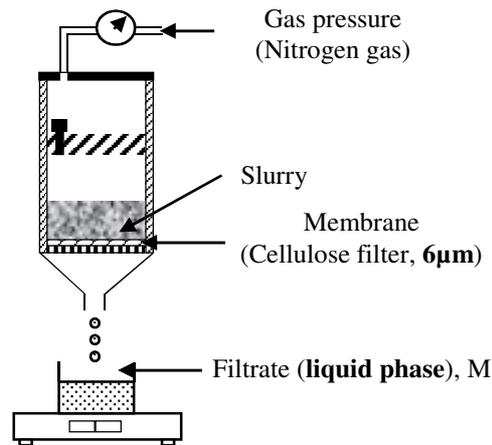
171 Filtration-compression experiments were carried out with a pressurized cylindrical cell of 70 mm
172 internal diameter and 0.60 L volume (Figure 1). A perforated disk was located at the bottom of
173 the cylinder to support a plane cotton cellulose membrane of 6 μm in pore size (Whatman,
174 Maidstone, UK); the pore size of 6 μm was supposed to retain the great majority of the suspended
175 solids, while allowing acceptable filtration rate. **Transmembrane pressure (TMP)** was applied by
176 a pneumatically driven-piston dragged by **nitrogen gas**.

177 **With an objective to compare and discuss flavonoids extraction efficiencies, filtrations were**
178 **operated from same mass of dry matter (around 3.5 g), whatever the type of slurry (ET or NT);**
179 **thus the initial slurry mass (or volume) to be filtered was different for NT and ET slurry (20 g**
180 **and 40 g respectively, according to their different water content).**

181 Filtration-compression experiments were carried out at different transmembrane pressures (1, 3,
182 5, 7 and 10 bar).

183 The filtrate mass (M) was monitored during time until its stabilization; M_F , maximum filtrate
184 mass, was considered as the maximum mass of the liquid phase that could be extracted from
185 slurry by filtration-compression at the defined operating condition (i.e. TMP).

186 At the end of the filtration, filter cake and filtrate were recovered and characterized.



187

188

Figure 1. Filtration-compression cell

189

190 The filtration efficiency, in terms of extracted liquid volume, was estimated for the two slurries,
191 i.e. the raw (NT) one and the enzyme treated (ET) one.

192 The NT slurry filtration efficiency η_{NT} (given by Eq.(1)) was defined by the ratio between the
193 extracted liquid mass, $M_{ext.liq.NT}$, and the total liquid mass of the grinded fresh peel $M_{liq.}$.

$$\eta_{NT} = \frac{M_{ext.liq.NT}}{M_{liq.}} \quad (1)$$

194 Regarding ET slurry filtration, the total liquid mass corresponding to the liquid mass present in
195 the grinded fresh peel $M_{liq.}$ plus the added water ($M_{add.}$ due to addition of the enzymatic
196 solution), η_{ET} was given by Eq.(2):

$$\eta_{ET} = \frac{M_{ext.liq.ET}}{M_{liq.} + M_{add.}} \quad (2)$$

197 With the suppositions that (i) the enzymatic treatment did not modify the part of the mechanically
 198 removable liquid of the slurry and (ii) all the water added ($M_{add.}$) for the enzymatic treatment is
 199 easily removed by filtration, then, the theoretical ET slurry filtration efficiency η_{ET} can be given
 200 by Eq.(3):

$$\eta_{ET} = \frac{M_{ext.liq.NT} + M_{add.}}{M_{liq.} + M_{add.}} \quad (3)$$

201 Considering the enzymatic treatment protocol (with $M_{add.} = w(M_{DM} + M_{liq.})$, where M_{DM} is
 202 the mass of dry matter in slurry), η_{ET} can be deduced from η_{NT} by Eq.(4):

$$\eta_{ET} = \frac{\eta_{NT} + w \left(1 + \frac{M_{DM}}{M_{liq.}}\right)}{1 + w \left(1 + \frac{M_{DM}}{M_{liq.}}\right)} \quad (4)$$

203

204 *2.3. Physicochemical and biochemical characterization*

205 *2.3.1. Physicochemical characterization*

206 *Dry matter content determination:* Slurry, filter cake and filtrate dry matter content was
 207 determined according to the AOAC method with some modifications [39]. Samples were
 208 homogenously distributed on aluminium dishes and kept in a vacuum oven (Heraeus RVT 360,
 209 Hanau, Germany) at 70°C for 48h. Water content was determined from the weight difference
 210 before and after drying. Each measure was done in triplicate.

211 *Particle size distribution:* Particle size distribution (in percentage of volume density) and mean
 212 diameter (d_{50}) of NT and ET slurries were determined by Laser diffraction using a Malvern
 213 Mastersizer (Mastersizer 3000, Malvern Instruments Limited, Worcestershire, UK) equipped

214 with a HydroMV[®] cell. Refractive indices of 1.73 and 1.33 were used for cloud particles and
215 dispersion phase and 0.1 was used as the absorption index of cloud particles [40]. Samples were
216 introduced into the volume presentation unit, which already contained deionized water; in this
217 unit, the diluted sample was gently stirred and pumped through the optical cell.

218 *Flavonoids dispersion state:* Filtrate was considered as an aqueous solution in which colloidal
219 and supra-colloidal particles were suspended. These insoluble particles were supposed to be
220 remaining vegetable tissue and cellular rests and therefore to be susceptible of containing
221 flavonoids. In order to identify the dispersion state of naringin and narirutin within the filtrate,
222 centrifugation of the filtrate for 4 hours at 20 000 g was proposed; this centrifugation operating
223 condition was chosen with the objective of separating the soluble fraction from the rest of the
224 suspension. According to Stokes law, a cut-off size of approximately 100 nm was supposed to
225 be reached with this chosen centrifugation time and acceleration; the separation selectivity has
226 been subsequently confirmed by means of particles-size measurements (Dynamic Light
227 Scattering). Flavonoids (naringin and narirutin) present in the centrifugation pellet, supposed on
228 crystallized form or bound to some larger solids, were considered as *insoluble* flavonoids.
229 Flavonoids present in the supernatant, supposed solubilized or potentially bound with some
230 colloids or macromolecules, were considered as *soluble* ones.

231

232 2.3.2. *Flavonoids biochemical characterization*

233 Naringin and narirutin content of the slurry and filtrates was estimated by High Performance
234 Liquid Chromatography (HPLC) analyses. A solvent extraction was performed using a modified
235 method based on [41] to estimate the total content of flavonoids in the samples.

236 A mass of slurry (corresponding to 250 mg of dry matter) or a volume of 500 μ l of filtrate was
237 withdrawn and diluted with 5 ml of dimethylformamide (DMF) and 5 ml of ammonium oxalate
238 (0.05 M). Afterwards, samples were placed in a hot oil bath (90°C) for 10 minutes, cooled and

239 adjusted to 18 ml for filtrates and 25 ml for slurries. Solutions were then filtered through 0.45 µm
240 Whatman cellulose acetate syringe filters (Sigma-Aldrich, St Louis, MO) before injection.
241 Naringin and narirutin content was quantified using an Agilent 1200 series HPLC system equipped
242 with a degasser, a quaternary pump, an auto-sampler, a thermostated column compartment, and
243 a diode array detector DAD. Separation of flavanones was performed using a C18 column
244 (Uptisphere HDO 5 µm, 250 mm × 4.6 mm, Interchim, Montluçon, France). The mobile phase
245 consisted of an isocratic solvent system combining solvent A (water/acetic acid, 99/1, v/v),
246 solvent B (acetonitrile), and solvent C (tetrahydrofuran) - A/B/C 80/16/4, v/v/v. The flow was
247 set at 1 mL/min and the column was heated at 25°C. UV detector was set at 280 nm, an aliquot
248 of 20 µL was injected.

249

250

251 **3. Results and Discussion**

252 *3.1. Slurry characterization*

253 The physicochemical and biochemical properties of fresh peel and slurries with (ET) and without
254 an enzymatic treatment (NT) are shown in Table 1.

255 The fresh peel and the NT slurry presented similar characteristics in terms of dry matter (DM)
256 and water content, around 17 and 83 g per 100 g of wet matter (i.e. fresh peel or slurries)
257 respectively; these values were coherent with the ones available in the literature [42,43]. The
258 enzymatic treatment, requiring a water addition, increased the water content of the ET slurry until
259 91 g per 100 g of wet matter, coherent value considering the addition of an enzymatic aqueous
260 solution close to a 1/1 ratio.

261

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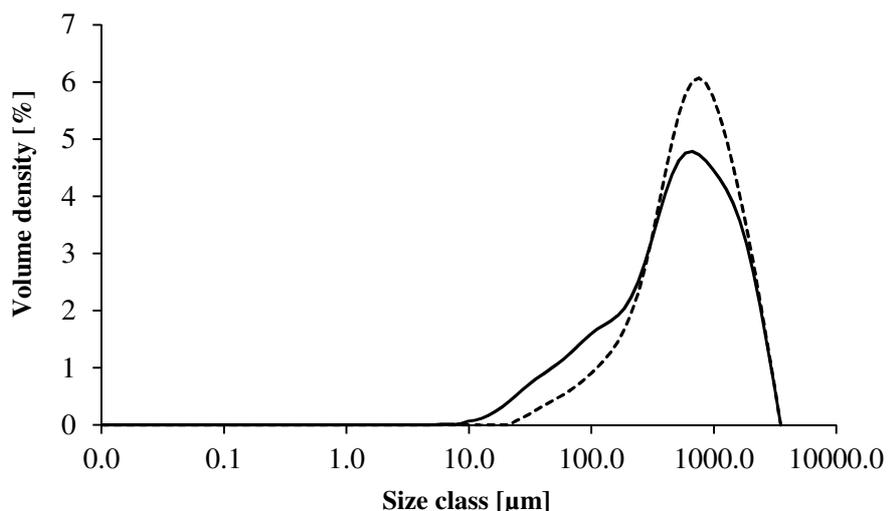
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264 **Table 1**
 265 Physicochemical properties of fresh peel and slurries

| | Fresh Peel | NT slurry | ET slurry |
|-----|---------------------------|------------------|------------------|
| 267 | Dry matter | | |
| | <i>g/100 g wet matter</i> | 17.3 ± 0.9 | 17.3 ± 0.5 |
| 268 | Water content | | |
| | <i>g/100 g wet matter</i> | 82.6 ± 0.9 | 82.7 ± 0.5 |
| 269 | d₅₀ | | |
| | <i>µm</i> | - | 716 ± 19 |
| 270 | Naringin | | |
| | <i>g/100 g dry matter</i> | | 5.1 ± 0.4 |
| 271 | | | 0.9 ± 0.1 |
| 272 | Narirutin | | |
| | <i>g/100 g dry matter</i> | | 0.57 ± 0.02 |
| | <i>g/100 g wet matter</i> | | 0.1 ± 0.01 |

273

274 The particle size distributions of NT and ET slurries are given in Figure 2. The NT slurry was
 275 composed of particles ranging from approximately 25 to 3500 µm, with a relatively important
 276 volume percentage of particles around 1000 µm; the d₅₀ was about 716 µm. If the enzymatic
 277 treatment did not significantly modify the size distribution shape, it was however noted that the
 278 amount of particles with size from 300 to 2000 µm was reduced while the percentage of particles
 279 with size from 10 to 300 µm increased. These results suggested that a part of the larger particles
 280 were broken under the effect of the enzymatic treatment. Pectin-methyl esterase and pectin-lyase
 281 had almost certainly catalysed the demethylation and the depolymerisation of pectin in the albedo
 282 [44], leading to the weakening of cell structures and a consequent particle size reduction [45]. As
 283 a consequence of this size distribution modification, a decrease of the d₅₀ was observed after the
 284 enzymatic treatment.



285
286 **Figure 2.** Particle Size distribution of: – ET slurry and --- NT slurry

287
288 The total concentration of naringin and narirutin in NT slurry, estimated per 100 g of dry matter
289 and wet matter, are given in Table 1. The concentration of naringin was about 10 times higher
290 than the narirutin one. Grapefruit naringin and narirutin concentrations found in the literature for
291 the different parts of the fruit are reported in Table 2. This bibliographic review indicated that
292 the measured concentrations were in the same range of those found in literature for albedo and
293 confirmed the large presence of naringin and narirutin in peel, and specifically in albedo. The
294 significant variability observed in the values reported in Table 2 could be easily explained by
295 various factors (e.g. fruit origin, fruit variety, manufacturing procedures, storage conditions...)
296 but also variation in the degree of maturity of the fruit [35,36,46–48].

297 **Table 2**
298 Naringin and Narirutin content in grapefruit (*C. paradisi*)
299 ^a g/100g juice
300 ^b g/100g wet matter
301
302

| | Juice | Flavedo | Albedo |
|-----------|------------------------------------|----------------------------------|---------------------------------|
| Naringin | 0.006 – 0.087 ^{a,[36-38]} | 0.35 – 0.99 ^{b,[34,35]} | 0.62 – 2.7 ^{b,[34,35]} |
| Narirutin | 0.009 – 0.012 ^{a,[4]} | 0.11 ^{b,[2]} | 0.23 ^{b,[2]} |

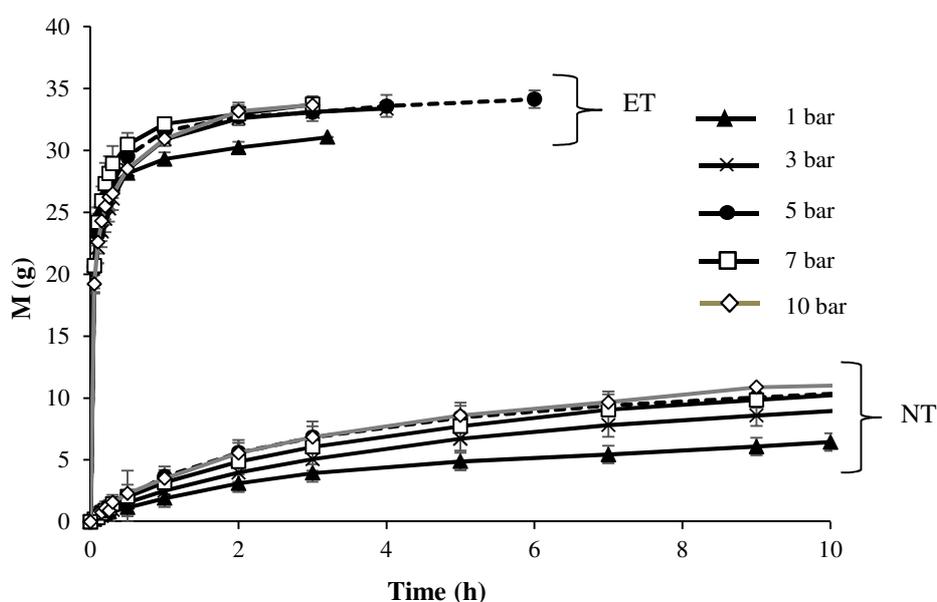
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305 3.2. Filtration-compression of the slurry

306 Figure 3 presents the evolution of the filtrate mass (M) versus time, for NT and ET slurries during
 307 filtration at different **transmembrane pressures**.

308 As it can be observed, the applied **transmembrane pressure** influenced the quantity of collected
 309 liquid at the end of each filtration, i.e. M_F . **Recovered filtrate mass was higher for ET slurry than
 310 for NT one, which was coherent with the filtration protocol (filtration of the same mass of dry
 311 matter, whatever the slurries), leading to the filtration of a higher mass of ET slurry than of the
 312 NT slurry (considering the addition of the enzymatic solution and consequently of water to the
 313 slurry).**



314 **Figure 3.** M_F versus filtration time at different **transmembrane pressures**, for NT and ET slurries

315

316 The influence of pressure and of the enzymatic treatment on the solid/liquid separation efficiency
 317 of filtrations is discussed below. The description of the evolution of the filtrate mass, through the
 318 study of the fouling mechanisms, is also proposed with an objective to provide some indication
 319 about the fouling propensity of NT and ET slurries.

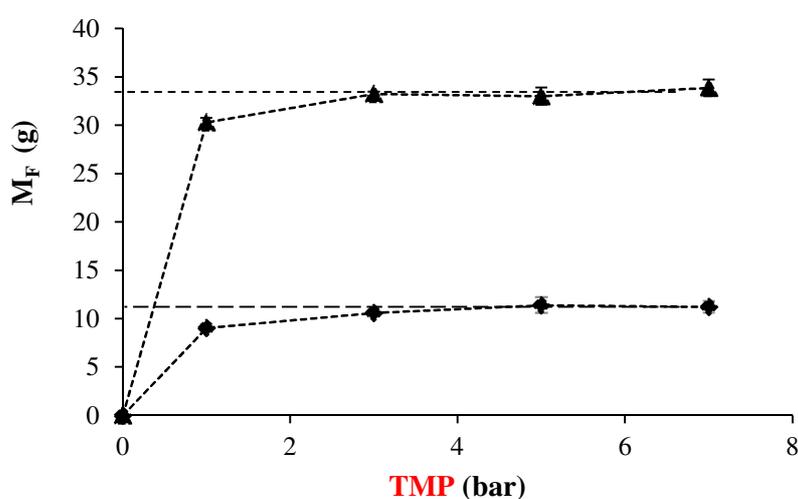
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321 3.2.1. Solid/liquid separation efficiency

322 Figure 4 represents the maximum recovered filtrate mass (M_F), corresponding to the steady
 323 filtrate mass, versus each applied transmembrane pressure.

324 An increase of the transmembrane pressure TMP, from 1 to 5 bar, induced an increase of M_F .

325 For NT and ET slurries, all the curves tend towards an asymptote corresponding to a M_F value
 326 closed to 12 and 34 g respectively.



327

328 **Figure 4.** M_F value versus TMP (NT ◆, ET ▲)

329

330 Figure 4 shows that, in the presence or absence of an enzymatic treatment, a transmembrane

331 pressure of 5 bar appeared to be sufficient to reach the asymptote and thus to extract all the

332 mechanically extractable liquid part of the slurry. The nature of the interactions between the

333 liquid (aqueous solution) and solid (e.g. pieces of vegetable tissues as cell wall fragments,

334 proteins, pectin and fibres ...) phases of the slurry could explain this result. In a complex medium,

335 as slurry, the solid/liquid interactions can be low or high, in relation with the liquid binding forces

336 [49–52]; at least two types of water can be defined: free and bounded water, respectively

337 concerned by low and high interactions with solids. The experimental distinction between these

338 two types of water can be based on water activity [53], freezable and non-freezable ability [54]

339 or extractability by mechanical means [55]. This last definition, according to our experimental
 340 strategy and the obtained results, led us to admit that mechanical action imposed by pressure in
 341 a filtration-compression cell promoted the release of the slurry free liquid. Thus, results revealed
 342 that, for grinded grapefruit peels, with or without enzymatic treatment, a pressure of 5 bar
 343 appeared to be sufficient to extract all the easily removable liquid, i.e. free water. For ET slurry,
 344 the extracted liquid phase corresponded not only to the free liquid of the fresh grinded peel, but
 345 also included the water added for the enzymatic treatment, water considered as free water and
 346 therefore very easily extractable by filtration-compression.

347 However, with an objective to verify if enzyme presence and action were susceptible to modify
 348 the interactions between solids and liquid, i.e. if enzymes were susceptible to modify the quantity
 349 of the mechanically removable liquid part of the slurry, the estimation of the theoretical filtration
 350 efficiency η_{ET} (estimated from η_{NT} , through Eq. 4) was compared to the experimental one, η^*_{ET} ,
 351 estimated through the quantification of dry matter and water content of the filtrate and filter cake.
 352 Table 3 presents the characteristics of the slurry, filter cake and filtrate in terms of dry matter and
 353 water content and extraction efficiencies, from 5 bars-trials (i.e. for pressure conditions allowing
 354 to extract the maximum of the liquid phase). Mass balance was approximately verified,
 355 considering the losses of matter (between approximately 10 and 20%, i.e. stagnant water in
 356 filtrate tube, arduous separation of filter cake from filtration membrane, deposit on the walls of
 357 the filtration-compression cell...).

358 **Table 3**
 359 Slurry, filter cake and filtrate characterization

| | Slurry (g) | | | | Filter cake (g) | | | Filtrate (g) | | | η |
|-----------|------------|------------------|-----------|------------|-----------------|-------------|-------------|--------------|-----------|------------|----------------|
| | Total mass | Added water mass | DM mass | Water mass | Total mass | DM mass | Water mass | Total mass | DM mass | Water mass | |
| NT | 20.1 ± 0.0 | 0.0 | 3.5 ± 0.1 | 16.6 ± 0.1 | 5.21 ± 0.3 | 1.97 ± 0.17 | 3.05 ± 0.20 | 11.6 ± 0.3 | 1.2 ± 0.0 | 10.4 ± 0.3 | 63 ± 2% |
| ET | 39.9 ± 0.3 | 20.0 ± 0.1 | 3.5 ± 0.1 | 36.5 ± 0.2 | 3.14 ± 1.3 | 0.94 ± 0.09 | 2.20 ± 1.06 | 33.5 ± 1.3 | 2.2 ± 0.1 | 31.3 ± 1.2 | 86 ± 2% |

360 The enzymatic action was first observed through the difference between ET and NT filtrate dry
361 matter content, as well as ET and NT filter cake one (Table 3). The evolution of the slurry particle
362 size distribution after enzymatic treatment (Figure 2) had induced a reduction of solids retained
363 by the membrane (pore size around 6 μm) and inversely an increase of solids in the filtrate
364 (greater presence of finer fragments).

365 Concerning the comparison between the experimental and theoretical (i.e. calculated) filtration
366 efficiency, an experimental efficiency of 86% was obtained while the theoretical one, that was
367 based on the fact that the enzymatic treatment did not modify the mechanically removable part
368 of liquid, was equal to 83% (according to η_{NT} and Eq. 4). This comparison demonstrated that, in
369 modifying the cell structures and solids physicochemical characteristics, the enzymatic treatment
370 could slightly increase the mechanically removable part of liquid of the grinding fresh peel
371 (ANOVA, $p < 0.01$).

372

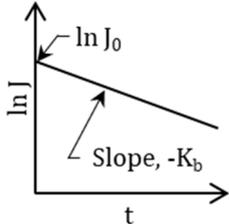
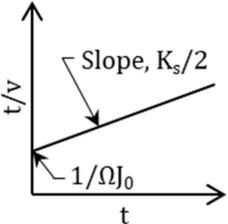
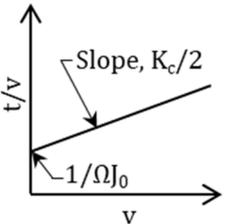
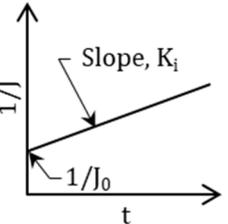
373 *3.2.2. Filtration modelling and fouling propensity of the slurry*

374 We proposed to describe the filtrate mass (M) evolution during filtration time through classical
375 filtration modelling at constant pressure, i.e. Hermia models. The Hermia models consist of four
376 different filtration mechanisms: complete blocking, standard blocking, intermediate blocking,
377 and cake formation. The cake formation model, initially established by [56,57], considers that
378 during filtration, fouling is due to a cake formation on the surface of the filter medium,
379 corresponding to the deposit of particles of larger size than the membrane pore size. Both
380 complete and intermediate blocking laws describe the pore plugging due to foulants reaching the
381 top surfaces of pores. In contrast, the standard blocking law deals with the pore constriction
382 caused by the deposition of foulants onto the pore wall. Table 4 presents mathematical equations
383 giving the relation between the filtrate volume V and the filtration time t , for each filtration law

384 at constant pressure condition [58]. As proposed by [58], the predominant blocking filtration
 385 laws describing fouling pattern were determined from plots based on linear expressions of the
 386 law (Table 4), allowing a rapid identification of the relevant fouling mechanisms. If standard
 387 blocking and cake filtration models could describe experimental pattern for NT slurry, standard
 388 blocking and intermediate blocking were more suitable to describe the fouling evolution of ET
 389 slurry.

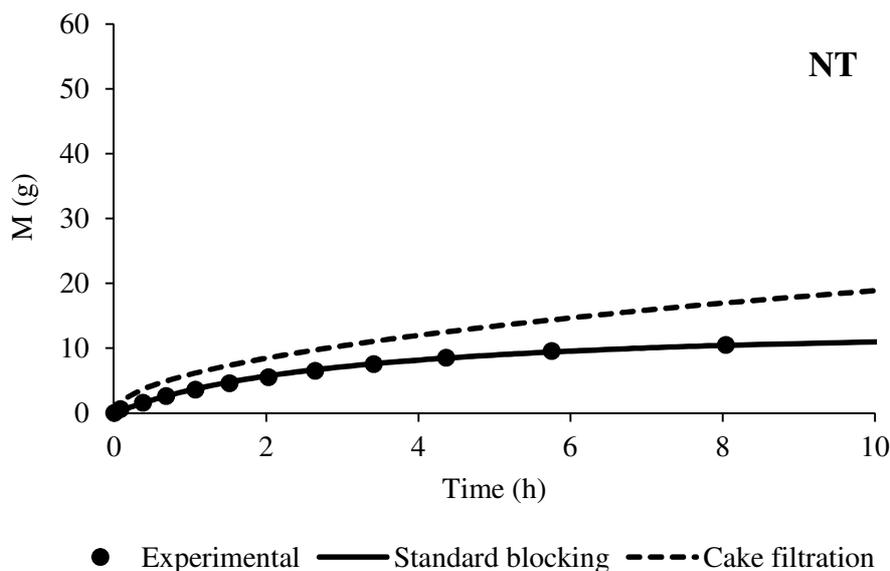
390 **Table 4**

391 Blocking filtration equations for constant pressure filtration [55]

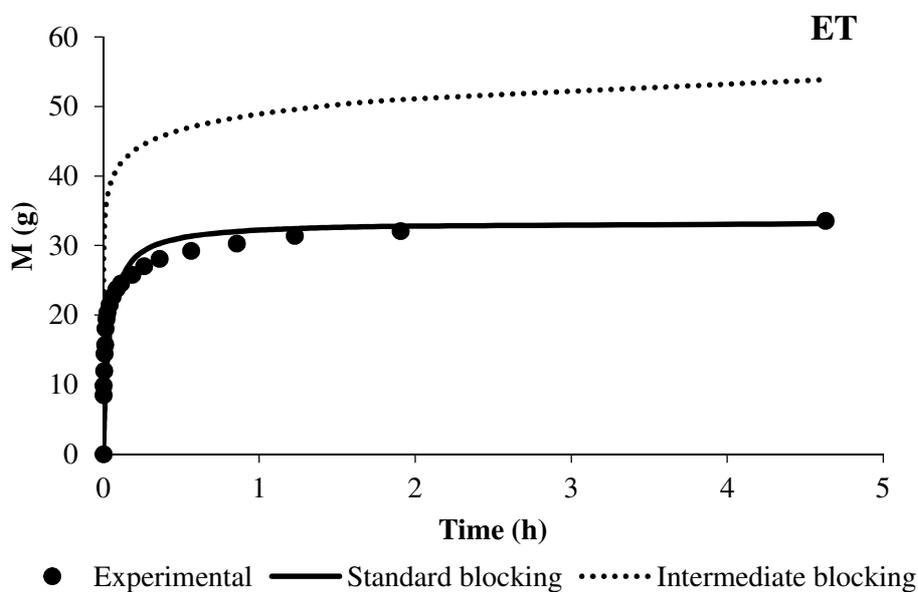
| Function | Complete blocking | Standard blocking | Intermediate blocking | Cake filtration |
|---------------------------------|---|---|--|---|
| $v = f(t)$ | $v = \frac{J_0}{K_b} \{1 - \exp(-K_b t)\}$ | $\frac{t}{v} = \frac{K_s}{2} t + \frac{1}{J_0 \Omega}$ | $K_i v = \ln(1 + K_i J_0 t)$ | $\frac{t}{v} = \frac{K_c}{2} v + \frac{1}{J_0 \Omega}$ |
| $J = f(t)$ | $J = J_0 \exp(-K_b t)$ | $J = \frac{J_0}{\left(\frac{K_s J_0}{2} t + 1\right)^2}$ | $K_i t = \frac{1}{J} - \frac{1}{J_0}$ | $J = \frac{J_0}{\left(1 + 2K_c J_0^2\right)^{1/2}}$ |
| Linear graphical representation |  |  |  |  |

392
393

394 Figure 5 presents the theoretical evolution associated to the models providing the better
 395 description of the experimental one for 5 bar experiments; similar results were obtained for the
 396 other filtration pressures.



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Figure 5. Theoretical and experimental evolution of M versus time
(5 bar, filtrate density of 1040 kg.m^{-3})

402 As it can be observed in Figure 5, the standard blocking law is the only law describing properly
 403 the evolution of M versus time, demonstrating a possible membrane pore constriction caused by
 404 the deposition of foulants into the pore wall, and this, independently of the slurry modification
 405 induced by the enzymatic treatment. Thus, filtration models demonstrated that if the filtration
 406 kinetic of NT and ET slurries was different, the filtration behaviour of these two suspensions
 407 were comparable in terms of fouling mechanisms whatever the applied pressure.

408 Table 5 presents the value of K_s , blocking constant for standard blocking law, in m^{-3} , for the two
 409 types of slurry at 5 bar. Considering that lower is K_s , higher is the filtrate volume V for a filtration
 410 time t , the enzymatic treatment was supposed to be favourable to a weak fouling and
 411 consequently a rapid filtration. This result was consistent with the use of pectin-methyl esterase
 412 and pectin-lyase to enhance juice filterability notably through a reduction of the juice viscosity
 413 [59]. This modelling and the estimation of the K_s constant could be very useful for the
 414 implementation of a filtration-compression process, with an objective of providing and achieving
 415 a sustainable productivity.

416 **Table 5**
 417 Standard blocking constant (5 bar, filtrate density of 1000 kg.m^{-3})
 418

| Standard blocking model | $\frac{t}{v} = \frac{K_s}{2}t + \frac{1}{J_0\Omega}$ | |
|---|--|---|
| | NT | ET |
| $\Omega \text{ (m}^2\text{) } \times 10^4$ | 38 | 38 |
| $J_0 \text{ (m.s}^{-1}\text{) } \times 10^7$ | 3 ± 0.2 | $1\ 210 \pm 800$ |
| $K_s \text{ (m}^{-3}\text{)}$ | $139\ 000 \pm 1400$ | $70\ 000 \pm 10\ 000$ |

419

420 3.3. Flavonoids extraction efficiency

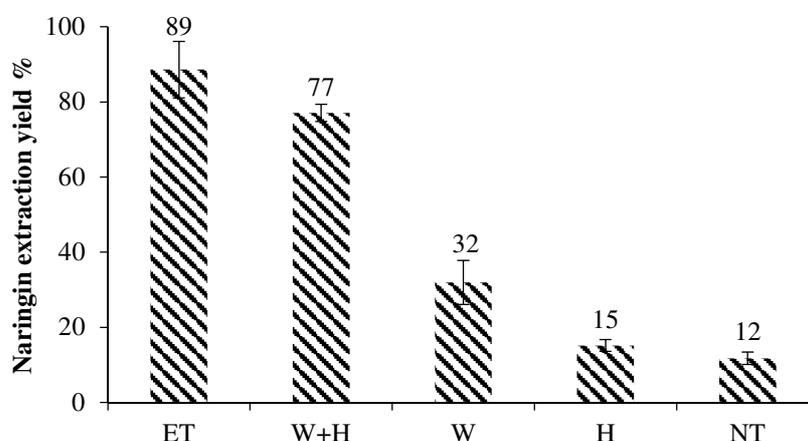
421 3.3.1. Identification of the role of the operating conditions

422 Figure 6 and Figure 7 show the extraction yields of naringin and narirutin for filtration at 5 bar
 423 (i.e. the lower **transmembrane pressure** inducing the maximum liquid extraction).

424 For ET slurry, approximately 89% (**4.5 g per 100 g of peel dry matter**) of the naringin and 92%
 425 (**0.5 g per 100 g of peel dry matter**) of narirutin were recovered in the filtrate; lower yields were
 426 obtained without enzymatic treatment, i.e. 12% and 61% respectively. **Similar data were reported**
 427 **by [60] and [15] with recovery of 4.1 g (hot methanol extraction) and 3.6 g (ultrasound-assisted**
 428 **extraction) of naringin per 100 g of peel dry matter respectively.**

429 The high recovery of naringin and narirutin obtained for the ET slurry could be explained by the
430 hydrolytic effect of the enzyme on pectin, inducing cell wall disruption and consequently
431 enhancing the release of these flavonoids into the extracellular liquid phase recovered by
432 filtration.

433 However, with the objective to discriminate the effect of the added enzymes from that of the
434 operating conditions associated with the enzymatic treatment (heating and water addition),
435 complementary experiments were carried out: (i) heating of the NT slurry in the same conditions
436 as for the enzymatic treatment (50°C for 2 hours) (H), (ii) addition of water to the NT slurry in
437 the same ratio (w) as for the enzymatic treatment (W) and (iii) heating and water addition in the
438 same conditions as for enzymatic treatment (50°C for 2 hours, same w) (W+H). This
439 complementary study could also be pertinent, with an objective to propose other pre-treatments
440 than an enzymatic one to enhance flavonoids extraction by filtration-compression.



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Figure 6. Naringin extraction yield at 5 bar
Enzymatically treated *ET*, raw *NT*, water addition with heating *W+H*, water addition *W*, heating *H*

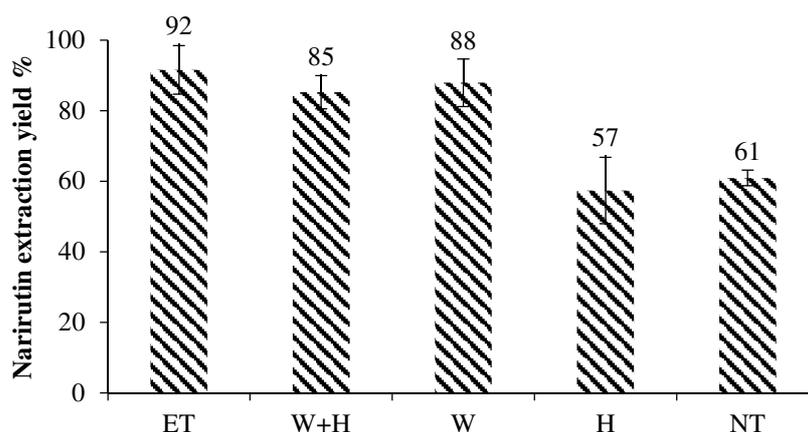
445 As it is observed on Figure 6, heating the slurry (H) did not lead to a significant increase of
446 naringin recovery in comparison with raw slurry. Even if high temperatures are susceptible of
447 increasing the diffusion rates of flavonoids, the low water content limit the transfert phenomena
448 of the flavonoids towards the mechanically extractable liquid. By adding water to the slurry (W),

449 a marked increase on the recovered naringin was noticed; an extraction twice as efficient as the
450 one obtained with heating was observed. The addition of water, which diluted the medium,
451 facilitated the mobility of flavonoids from the solid matrix towards the free extracellular liquid,
452 through an increase of the concentration gradient at the solid/liquid interface and a possible
453 increase of the diffusion coefficient by a decrease of the viscosity of the medium.

454 The twinning effect of heating and water addition (W+H) led to an important increase of the
455 naringin extraction resulting in an efficiency close to 78%. The addition of pectinases, which
456 hydrolysed pectins of the cell walls, increased the naringin extraction efficiency from 78 to
457 89%.

458 These results demonstrated that, if the enzymatic treatment enhanced the naringin extraction,
459 water addition and heating, due to their synergistic effect, could be simple and low-cost slurry
460 pre-treatments to enhance naringin extraction. Thus, the benefit of an enzymatic treatment on the
461 naringin recovery has to be considered, given the enzyme cost.

462 Regarding narirutin recovery (Figure 7), the lower concentration in peel residues combined with
463 its higher solubility allowed a higher release of this flavonoid (between 61% and 92 % depending
464 on the treatment) into the aqueous phase. In this case, the most impacting factor is the water
465 quantity available for rapid narirutin diffusion and dissolution; thus, a single water addition could
466 recover the same quantity of narirutin than an enzymatic treatment.

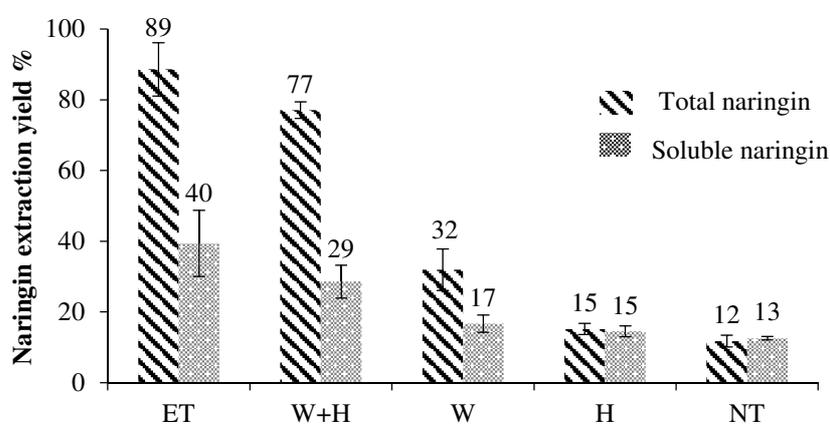


467
468 **Figure 7.** Narirutin extraction yield at 5 bar
469 *Enzymatically treated ET, raw NT, water addition with heating W+H, water addition W, heating H*
470

471 3.3.2. Identification of the dispersion state of extracted naringin and narirutin

472 With an objective to identify the dispersion state of the extracted naringin and narirutin according
473 to the different pre-treatments, centrifugations of the different filtrates were realized to separate
474 the *soluble* and *insoluble* part of naringin and narirutin (see methodologies in 2.3.1).

475 Figure 8 presents the total naringin content compared to the *soluble* one. While naringin was only
476 on a *soluble* state in H and NT filtrates, *insoluble* naringin appeared in the filtrates obtained with
477 other pre-treatments.



478
479 **Figure 8.** Naringin total and soluble extraction yields at 5 bar for different slurries
480 *Enzymatically treated ET, raw NT, water addition with heating W+H, water addition W, heating H*
481

482 The presence of *insoluble* naringin was directly linked to the enhancement of the total naringin
483 extraction. Water addition, alone or combined with heat treatment, had favoured the diffusional
484 transfer and consequently the extraction of a higher part of naringin within the filtrate; the
485 enzymatic treatment had led to a weakening of vegetable cell wall due to pectinases, facilitating
486 naringin diffusion and thus extraction [22]. The results seemed to establish that these released
487 flavonoids, initially mainly on *soluble* state in the filtrate, could rapidly form large aggregates,
488 explaining the increase of an *insoluble* fraction. Thus, about half of the total naringin was on
489 insoluble state after these different pre-treatments. It was supposed that naringin could form
490 colloidal structures with macromolecules (e.g. proteins, phospholipids...) that could be present
491 in large quantity and with a great diversity in the supernatant after such pre-treatment (because
492 of the facility of the diffusional transfer of other compounds or of the enzymatic action on the
493 vegetable tissue). Additional specific tests need to be performed to complete these hypotheses,
494 and notably to quantify the naringin solubility in such complex medium.

495 Concerning narirutin, the supernatant analysis of the filtrates showed that this flavonoid was
496 entirely present under its *soluble* form probably due to its higher solubility and lower
497 concentration within the peel residues (Data not shown).

498

499 **4. Conclusion**

500 In order to promote circular and sustainable food systems, various strategies are proposed to
501 valorise wastes. In the case of Citrus transformation industry, grapefruits flavonoids (naringin
502 and narirutin), presenting interesting pharmaceutical properties, could be extracted from peels,
503 constituting a source of income for Southern countries. Most of the extraction processes require
504 high investment or high-energy consumption making these technologies non-suitable for the
505 Southern countries. Thus, it seems pertinent to think about a high-added-value compounds

506 recovery chain using optimized robust, cost-effective and low-energy consuming processes. The
507 objective of this work was to study the relevance of a simple filtration-compression step directly
508 done on the fresh grinded peels as a first downstream process for flavonoids extraction. The
509 potential impact on the solid-liquid separation of a pectinolytic enzymatic treatment was
510 investigated.

511 The results demonstrated that a preliminary step of filtration-compression, directly realized on
512 fresh grinded peels, as downstream processing for flavonoids extraction could be pertinent. The
513 optimization of solid/liquid separation was obtained with a **transmembrane pressure of 5 bar**,
514 providing the removal of the maximum of the extractable liquid phase from peel. Filtration
515 modelling demonstrated that the standard blocking law properly described the evolution of the
516 filtrate mass versus time, whatever the operating conditions (pressure, enzymatic treatment or
517 none...). If the fouling mechanisms were similar for enzymatic treated slurry and no-treated one,
518 the enzymatic treatment improved significantly the filterability of the slurry. The blocking
519 constant of the standard blocking law, indispensable for the scaling of a filtration-compression
520 process on site, was, under 5 bar, two times higher for non-treated than enzymatic treated peel.
521 The quantification of naringin and narirutin content in this removable liquid phase (i.e. filtrate)
522 demonstrated that the enzymatic treatment or a hot-water addition were suitable to enhance the
523 naringin and narirutin extraction yield, which could reach 80 and 90% respectively. The
524 dispersion state of the flavonoids within the mechanically extractable aqueous phase obtained
525 after this preliminary solid-liquid separation was identified; the part of the *insoluble* (crystallized
526 or bound to some larger solids) naringin could represent half of the total extracted naringin.
527 These last results require further investigations, with an objective to provide pertinent elements
528 to guide the choice of the subsequent extraction step.

529

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535

536 **Declaration of interest**

537 The authors report no declarations of interest.

538

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727

728 **Suggested Referees**

- 729 1. I. Ioannou
730 Université de Lorraine, ENSAIA - Laboratoire d'Ingénierie de biomolécules (LIBio), 2 avenue
731 de la Forêt de Haye, TSA 40602, Vandoeuvre Cedex 54518, France
732 irina.ioannou@univ-lorraine.fr
733
- 734 2. Alfredo Cassano
735 Institute on Membrane Technology, ITM-CNR, c/o University of Calabria, via P. Bucci, 17/C,
736 I-87030 Rende (Cosenza), Italy
737 a.cassano@itm.cnr.it
- 738 3. Noelia Rosales-Conrado
739 Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense
740 de Madrid, Madrid 28040, Spain
741 nrosales@ucm.es
742
- 743 4. Rosângela Bergamasco
744 State University of Maringa, Department of Chemical Engineering, Maringa 87020-900,
745 Parana, Brazil
746 rosangela@deq.uem.br
747
748

Grapefruit peel (without flavedo) and pulp rests



Grinding

Pectinolytic
treatment

NT
Slurry

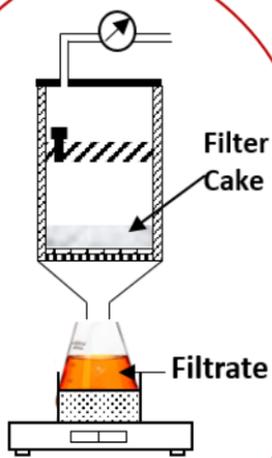


ET
Slurry



Slurries characterization
Particle size measurement
Dry matter content
Naringin and Narirutin content

Estimation of
filtration efficiency



Estimation of the maximum of the extractable
liquid phase (filtrate volume)

Identification of the size-location of flavonoids
in filtrate



Filtrate

Dry matter content
Naringin and Narirutin content

Centrifugation

*Quantification of the
soluble flavonoids
(supernatant)*

Naringin and Narirutin
content