1 **Influence of high-pressure homogenization on functional properties of orange**

2 **pulp**

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12   **_Abstract_**

13   The current work evaluated whether high-pressure homogenization (HPH) could functionalize orange

14   pulp in terms of water holding and swelling capacity and rheological properties. The orange pulp particle

15   size was gradually decreased by applying HPH at increasing pressure (200 and 800 bar) whereby the

16   mechanical impact at 800 bar resulted in the appearance of a more homogeneous, smoother suspension

17   with a twofold increase in yield stress. HPH also affected the pectin properties within the orange pulp

18   cell walls. More specifically, HPH at 800 bar increased the relative presence of water-extractable pectin.

19   By investigating subsamples containing particles with different sizes isolated from orange pulp before

20   and after HPH, it became clear that particle size is inversely related to the water holding capacity and

21   the ability of the particle network to deform prior to flow. Especially highly disintegrated orange pulp

22   material (< 40μm) contributes to the water holding capacity and the soft particle network behavior.

23   **_Industrial relevance_**

24   Orange pulp is of particular interest in the context of producing fiber-rich functional ingredients because

25   of its large quantity available within the juice industry. High-pressure homogenization (HPH) at

26   pressures higher than the ones usually applied in food industry seems required to functionalize orange

27   pulp as HPH at 200 bar (a common pressure applied in the food industry) could not increase the relative

28   presence of small particles, contributing to water holding capacity and soft particle network behavior, as

29   substantially compared to HPH at 800 bar.

30

31   **_Keywords:_** orange pulp, high-pressure homogenization, swelling volume, water holding capacity, pectin

32   **_Abbreviations used_**

33   AIR = alcohol-insolube residue

34   CEC = cation exchange capacity

35   CEP = chelator-extractable pectin

36   DME = degree of methyl-esterification

37   FITC = fluorescein isothiocyanate

38   HF = hemicellulose fraction

39   HPH = high-pressure homogenization

40   LVE = linear viscoelastic

41   MM = molar mass

42   NEP = sodium carbonate-extractable pectin

43   OHC = oil holding capacity

44   PBS = phosphate buffered saline

45   UA = uronic acid

46   WHC = water holding capacity

47   WEP = water-extractable pectin

48

49   *1   Introduction*

50   Consumption of dietary fiber is linked to health benefits associated with bowel function, reduced risk of

51   coronary heart diseases and type 2 diabetes and weight management (Hauner et al., 2012).

52   Unfortunately, the average total dietary fiber consumption (14 to 29 g/day) is far below the

53   recommended intake between 21 and 40 g/day (WHO/FAO, 2003). This recommendation could be

54   achieved by increasing the consumption of fruits and vegetables: it is suggested to eat five to nine

55   pieces a day. On the other hand, fiber-enriched food products could also play a key role in our diet.

56   Fruit and vegetable by-products, mainly consisting of cell wall material rich in dietary fiber, can in this

57   context be considered as potentially interesting functional ingredients. In order to create healthy, fiber-

58   rich food products that consumers like to eat, functional food ingredients should not only possess

59   desired nutritional properties but also particular technological functional properties in terms of

60   hydration properties, surface activity and texturization. Compared to commonly-used hydrocolloid

61   ingredients, the structure-forming properties of plant cell wall-derived fibers, currently available on the

62   market, are however limited. Because of its large quantity available within the juice manufacturing

63   industry, orange pulp is of particular interest in the context of producing fiber-rich functional

64   ingredients. The limited functionality of orange pulp, when dispersed in water, however confines

65   valorization pathways for this by-product.

66   Mechanical processing is known to alter the physicochemical properties of plant-based fiber

67   suspensions. During mechanical processing, particle sizes are reduced to a certain micro-scale level

68   enhancing physicochemical properties such as the water holding capacity (WHC), the swelling capacity,

69 the oil holding capacity (OHC) and the cation exchange capacity (CEC). Mechanical processing

70 techniques based on high-pressure processes, e.g. high-pressure homogenization (HPH) and

71 microfluidization, have been shown more effective in particle size reduction compared to conventional

72 mechanical processing techniques such as ball milling and jet milling (Chau, Wang, & Wen, 2007). In

73 these high-pressure valve homogenizers, the fluid is forced through a small gap between seat and valve

74 creating elongational and turbulence stresses modifying the fluid. Alternative technologies such as

75 steam explosion and supercritical CO_2 explosion have also demonstrated the ability to open up the

76 structures of plant based biomasses (Tanahashi, 1990; Narayanaswamy, Faik, Goetz, & Gu, 2011).

77 However, in the case of steam explosion a degradation of the hemicellulose was observed, which goes

78 paired with a reduction in water binding capacity of the plant cell wall and an increase in brittleness of

79 the structure (Ramos, 2003). These phenomena are more desired for the use of the treated biomasses in

80 power plants rather than for improving their texturizing properties in an aqueous system. Supercritical

81 CO_2 on the other hand shows promising results but still remains challenging in terms of cost

82 effectiveness.

83 The particle size reduction obtained during HPH is shown to go hand in hand with changes in

84 physicochemical properties but changes clearly depend on the microstructure of the plant material.

85 Significant increases in the WHC, the swelling capacity, the OHC and the CEC due to mechanical

86 processing were for example found for the insoluble fiber fraction of carrot pomace, for tomato paste

87 suspensions , and to a lesser extent for carrot suspensions, whereas no clear changes in the WHC of

88 apple sauce and potato pulp suspensions upon HPH were noticed (Chau et al., 2007; Bengtsson, &

89 Tornberg, 2011). The relation between changes in microstructural properties and changes in functional

90 properties due to HPH are however not yet completely understood.

91 The objective of the current work was to evaluate whether HPH could be used to functionalize orange

92 pulp. Hereto, orange pulp was homogenized using different pressure levels (200 and 800 bar) and the

93 resulting microstructural (particle size analysis and microscopy analysis) and functional properties (WHC,

94 swelling volume and rheological properties) were determined and compared to the initial orange pulp

95 properties. In order to evaluate the role of changes in cell wall polysaccharides in changes of functional

96 properties induced by HPH, the alcohol-insoluble residue (AIR) of the samples was analyzed in terms of

97 pectin extractability, degree of methyl-esterification (DME), neutral sugar composition and molar mass

98 distribution. In the second part of this work, subsamples with different particle size ranges were

99 prepared from the non-homogenized and the high-pressure homogenized orange pulp. These

100   subsamples were studied in terms of microstructural and functional properties in order to better

101   understand the functional properties of high-pressure homogenized orange pulp.

102

103   ***2   Materials and methods***

104   <u>2.1   Orange pulp</u>

105   The orange pulp fibers were from Brazilian oranges, cultivar Valencia. They were from the 2010 harvest

106   and provided by Fischer S/A, a Brazilian orange juice company . The pulp, obtained after juice extraction,

107   was pasteurized for food safety reasons and for inactivation of intrinsically present pectin

108   methylesterase. The pulp was subsequently poured in 180 kg vessels and frozen. The vessels were

109   shipped by boat to Europe for the present study under frozen conditions. Moisture content of the

110   starting pulp was about 3.4%. The samples were thawed at ambient conditions to avoid structural

111   damage to the pulp, diluted to 2% dry matter content with standardized tap water (1.00 g NaCl and 0.15

112   g $CaCl_2$ in 1 L reverse osmosis water with a conductivity of 2.2±0.1 mS/cm at 25°C), blended and high-

113   pressure homogenized. The high-pressure homogenization was performed with a NS1001L-Panda 2k

114   equipped with a single R-valve (Niro Soavi). The R-valve, which is preferably used in the context of cell

115   rupture, consists of a cylindrical/flat type of impact head, a small impact ring and a passage head with

116   sharp angles. A homogenization condition commonly used in the food industry was used (200 bar) as

117   well as an elevated pressure level of 800 bar, which is a compromise between industrial process capacity

118   and the intended increased product functionality. Control samples, also further called 'blended

119   samples', underwent the same preparation procedure but no HPH step was applied in this case. Samples

120   were frozen with liquid N_2 and stored at -40°C until further analysis (after thawing at room

121   temperature).

122

123   <u>2.2   Isolation of the subsamples</u>

124   Orange pulp samples were loaded onto a wet-sieving column (Vibratory Sieve shaker AS200, Retsch) in

125   order to isolate relevant particle fractions, further called 'subsamples'. Sieves with pore sizes of 250,

126   125, 80 and 40 μm were used. The material that was not retrieved on the smallest sieve (40 μm) was

127   centrifuged (30 min at 12500 g, Beckman Coulter centrifuge) in order to obtain subsamples that

128   contained the smallest material. The ranges that are used to refer to the different fractions are the pore

129   sizes of the sieves used during the isolation process.

130

131   2.3   Particle size analysis

132   The particle size distribution of the samples was analyzed by laser diffraction (Mastersizer, Malvern). A

133   refractive index of 1.56 was used for the cell wall particles. From the particle size analysis, different

134   diameters can be obtained: (i) D(v, 0.1), D(v, 0.5) and D(v, 0.9) respectively represent the maximum

135   particle diameter below which 10%, 50% or 90% of the sample volume exists, (ii) D[4,3] shows the

136   volume based mean diameter and (iii) D[3,2] indicates the surface area based mean diameter.

137

138   2.4   Microscopy analysis

139   Microscopy pictures of diluted samples (~1:10) were taken using a light microscope (Olympus BX-41). In

140   addition, immunofluorescence using the pectin-specific antibody JIM7 was performed. Hereto, the

141   samples were incubated with the primary antibody JIM7 (fivefold diluted in phosphate buffered saline

142   containing 3% milk powder (MPBS); PlantProbes, Leeds, UK) for 1 h and 30 min at room temperature.

143   After primary labeling, samples were washed with PBS by centrifugation (3 times 5 min at 22 °C and 400

144   g; Microfuge 22R Centrifuge, Beckman Coulter, Germany). For the visualization of JIM7, secondary

145   labeling with an anti-rat Ig antibody coupled to fluorescein isothiocyanate (FITC) (Nordic Immunology,

146   Tilburg, The Netherlands) was used. The secondary antibody was diluted 1/20 in 3% MPBS. After a final

147   washing step with PBS, samples were mounted in an anti-fade agent (Citifluor, Agar Scientific, Stansted,

148   United Kingdom) on glass slides and examined with the Olympus BX-41 microscope equipped with

149   epifluorescence illumination. Immunolabeling experiments were carried out at least in duplicate.

150

151   2.5   Water holding capacity

152   The orange pulp fiber dispersions were diluted to a 1% concentration (on a dry weight basis) in

153   standardised tap water by using a 4-bladed propeller fitted on a RWD 20 digital IKA stirrer (set at 900

154   rpm for 10 min) in a 400 mL glass beaker with diameter of 7.8 cm. Subsequently, the dispersions were

155   transferred into 50 mL centrifuge tubes and centrifuged at 3500 g for 5 min. The WHC was calculated

156   from the gravimetric measurement of supernatant and packed hydrated fiber. All analyses were

157   performed in duplicate.

158

159   2.6   Swelling volume

160   The orange pulp fiber suspensions were diluted to a 1% concentration (on a dry weight basis) in

161   standardised tap water by using a 4-bladed propeller fitted on a RWD 20 digital IKA stirrer (set at 900

162   rpm for 10 min) in a 400 mL glass beaker with diameter of 7.8 cm. The dispersions were transferred into

163　100 mL graduated cylinders and the bed volume was recorded after 24 h (to the nearest 0.5 mL). All

164　measurements were performed in duplicate.

165

166　2.7　　　Rheological properties

167　Flow curves of the orange pulp samples were determined at 20.0 °C on a Physica MCR 301 rheometer

168　(Anton Paar GmbH, Graz, Austria) with a coaxial cylinder configuration (TEZ 150P), a symmetrical blades

169　spindle (ST24-2D/2V/2V-30, diameter 24 mm, total vane length 30 mm, top and medium blades are

170　vertical and crossed, bottom blades are aligned with top blades and tilted vertically by an angle of 45°)

171　and a profiled measuring cup (CC27/T200/SS/P, diameter 28.9 mm, 20 indents of 0.5 mm depth). Since

172　the rheological method was based on Debon et al. (2012), the pulp concentration of the samples was

173　adjusted before the measurement to the concentration validated in that study. Therefore, orange pulp

174　samples were centrifuged at 3500 g for 10 min and 4% fiber pellet was reconstituted with supernatant.

175　24 h after reconstitution, 40.0 (± 0.2) g fiber dispersion was presheared for 300 s at 100 s^{-1} (to avoid the

176　effect of loading). Then, the shear rate was decreased in 20 steps from 100 to 1 s^{-1} and the measuring

177　time increased logarithmically from 6 to 30 seconds. The flow curves were fitted with the Herschel-

178　Bulkley model. All measurements were performed in duplicate. As the amount of subsample was

179　limited, the flow curves of these samples could not been determined.

180　For both the orange pulp samples and subsamples, an oscillatory strain sweep test was carried out at

181　20.0 °C on a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) with a parallel plate (PP25,

182　24.96 mm diameter, concentricity ± 5 µm, parallelity ± 1 µm) at 1 mm gap. Due to the limited amount of

183　subsample, both the orange pulp samples and subsamples were measured with a parallel plate

184　(although the rather large particle size of the samples) to allow comparison of the oscillatory data of

185　both sample types. After sample loading and trimming, the sample was equilibrated 20 min at 20 °C

186　under a Peltier hood. The strain was then increased in 60 steps from 0.1 to 100% and the measuring

187　time decayed logarithmically from 30 to 10 s. The angular frequency was set at 10 rad/s. All

188　measurements were performed in duplicate, 24 h after orange pulp fiber dispersion in standardised tap

189　water and centrifugation at 3500 g for 10 min.

190

191　2.8　　　Characterization of cell wall polysaccharides

192

193　*2.8.1 The alcohol-insoluble residue*

194   The cell wall material of orange pulp samples was isolated as alcohol-insoluble residue (AIR) according to

195   the method of McFeeters and Armstrong (1984). Approximately 10 g of the material was homogenized

196   in 64 mL 95% (v/v) ethanol using a mixer (Buchi mixer B-400, Flawil, Switzerland). The suspension was

197   filtered (Machery-Nagel MN 615 Ø 90 mm) and the residue was rehomogenized in 32 mL 95% (v/v)

198   ethanol. After another filtration step, the residue was homogenized in 32 ml acetone. A final filtration

199   resulted in the AIR, which was dried overnight at 40 °C. The AIR was ground using a mortar and pestle

200   and stored in a desiccator until further use.

201

202   *2.8.2 Fractionation*

203   According to the extractability with different media, the alcohol-insoluble cell wall material was

204   fractionated into different pectin-rich fractions and one hemicellulose-rich fraction. The water-

205   extractable pectin (WEP) fraction was obtained by incubating 0.5 g AIR in 90 mL boiling water while

206   stirring (Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006). After 5 min, the suspension was cooled under

207   running tap water and filtered. The filtrate was collected and adjusted to 100 mL (WEP). The residue was

208   re-suspended in 90 mL 0.05 M cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 M

209   potassium acetate (pH 6.5) for 6 h at 28 °C after which a new filtration step followed (Chin, Ali, & Hazan,

210   1999). After adjusting the filtrate volume to 100 mL, the chelator-extractable pectin (CEP) fraction was

211   obtained. The residue was re-incubated in 80 mL 0.05 M Na_2CO_3 containing 0.02 M $NaBH_4$ for 16 h at 4

212   °C, and subsequently for 6 h at 28 °C (Chin et al., 1999). The mixture was filtered and the volume of the

213   filtrate was adjusted to 100 mL, which resulted in the sodium carbonate-extractable pectin (NEP)

214   fraction. The residue was re-incubated under N_2 atmosphere in 90 mL of 4 M KOH, 0.02 M $NaBH_4$, and

215   3.5% borate for 22 h at room temperature. The suspension was filtered and the filtrate was adjusted to

216   200 mL after pH adjustment (pH 5.0) to obtain the hemicellulose fraction (HF). All fractions, including

217   the remaining residue, were frozen with liquid N_2 and stored at -40 °C until further analysis.

218

219   *2.8.3 Uronic acid content*

220   To determine the uronic acid (UA) content in the AIR and the WEP and the CEP fraction of the

221   (sub)samples, hydrolysis was performed with concentrated sulfuric acid according to the procedure by

222   Ahmed and Labavitch (1977). Briefly, 8 mL of concentrated H_2SO_4 (98 %) was added to 20 mg of AIR or to

223   2 mL of the liquid fraction, after which 2 mL of distilled water was added dropwise. The solution was

224   stirred for 5 min and another 2 mL of distilled water was added dropwise. The sample was stirred until it

225   was completely dissolved (4 h for AIR, 1 h for the fractions) and diluted to 50 mL or 25 mL for

226 respectively the AIR and the fractions. Then, the UA concentration was quantified using the
227 spectrophotometric method described by Blumenkrantz and Asboe-Hansen (1973), according to which
228 0.6 mL of the hydrolysate was heated (5 min at 100°C) in sodium borate (1:6; 0.0125 M sodium
229 tetraborate in 98% H_2SO_4). Subsequently, the solution was cooled down to room temperature and
230 mixed with metahydroxydiphenyl (60 µL of 0.15% metahydroxydiphenyl in 0.5% NaOH) after which the
231 absorbance was measured at 520 nm and 25 °C. The hydrolysis step was carried out in duplicate while
232 three colorimetric analyses were performed for each hydrolysate.

233

234   *2.8.4 Pectin degree of methyl-esterification*
235 The degree of methyl-esterification (DME) of pectin in AIR and in the WEP and the CEP fraction was
236 determined as the ratio of the molar amount of methyl-esters to the molar amount of UA residues. The
237 DME of NEP could not be assayed due to the saponification of methyl-esters during the alkali extraction
238 of this fraction.

239 To quantify the amount of methyl-esters, the ester bonds were saponified with NaOH according to the
240 procedure of Ng and Waldron (1997). Briefly, the samples (20 mg of AIR in 8 mL of distilled water or 8
241 mL of WEP and CEP) were saponified with 2 M NaOH (3.2 mL) for 1 h at 20 °C. The saponified samples
242 were subsequently neutralized by 2 M HCl (3.2 mL) and diluted to 25 mL (for WEP and CEP) or 50 mL (for
243 AIR) with a 0.0975 M phosphate buffer pH 7.5. The amount of methanol released was measured using
244 the spectrophotometric method of Klavons and Bennett (1986). In this procedure, methanol in 1 mL of
245 the hydrolysate was oxidized to formaldehyde with alcohol oxidase (Sigma-Aldrich, Bornem, Belgium) (1
246 ml of 1 unit/mL solution), followed by a condensation with 0.02 M 2,4-pentanedione in 0.2 M
247 ammonium acetate and 0.05 M acetic acid (2 mL; 15 min incubation at 58 °C) to 3,5-diacetyl-1,4-
248 dihydro-2,6-dimethylpyridine. This colored product was determined at 412 nm and 25°C. The hydrolysis
249 step was again carried out in duplicate while three colorimetric analyses were performed for each
250 hydrolysate.

251

252   *2.8.5 Molar mass distribution*
253 All pectin fractions (WEP, CEP and NEP) were lyophilized using a freeze-dryer (Christ alpha 2-4,
254 Osterode, Germany). Lyophilized WEP and NEP samples were dissolved in demineralized water (WEP:
255 0.25% w/v, NEP: 1.5% w/v) and extensively dialyzed against demineralized water during 48 h. CEP
256 samples were also dissolved in demineralized water (7.5% w/v), but dialyzed against 0.1 M NaCl during
257 24 h, followed by dialysis against demineralized water for another 24 h. The dialyzed pectin fractions

258   were adjusted to a concentration of 0.05 M $NaNO_3$ before analysis. The HF fraction was dialyzed against

259   water, lyophilized and dissolved (0.5% w/v) in 0.05 M $NaNO_3$.

260   The molar-mass (MM) distribution of the different fractions was analyzed by means of high-

261   performance size exclusion chromatography. This was achieved using an Akta Purifier (GE Healthcare,

262   Uppsala, Sweden) equipped with a mixed-bed column of TSK-GEL (GMPWXL, 300 mm x 7.8 mm, pore

263   size = 100-1000 A, particle size = 13 μm, theoretical plates/column ≥ 7000, pH range = 2-12, maximum

264   pressure = 300 psi; Tosoh Bioscience, Stuttgart, Germany) in combination with a TSK guard column

265   (PWXL). A 20 μL injection loop was used. Elution was executed with 0.05 M $NaNO_3$ at a flow rate of 0.7

266   mL/min for 25 min at 35 °C. A Shodex RI-101 detector (Showa Denko K.K., Kawazaki, Japan) was used to

267   monitor the eluent. In order to enhance the clarity of presentation, the signal (mV) obtained by the

268   detector was normalized through dividing all data points by the peak signal. Pullulan standards with MM

269   ranging from 180 to 788000 Da (Varian Inc, Palo Alto, California) were eluted to estimate the MM of the

270   fractions.

271

272   *2.8.6 Neutral sugar composition*

273   Cell wall fractions (0.005 g of freeze-dried WEP, CEP, NEP or HF) were hydrolyzed with 0.5 mL 4 M

274   trifluoroacetic acid at 110 °C for 1.5 h. The digested samples were cooled, dried under N_2 at 45 °C,

275   neutralized with 0.25 mL 1 M NH_4OH, and dried again under N_2 at 45 °C. Before analysis of neutral

276   sugars, the samples were diluted to 5 mL with demineralized water (organic free, 18 MΩ cm resistivity),

277   supplied by a Simplicity™ water purification system (Millipore, Billerica, USA).

278   The monosaccharides were analyzed with HPAEC using a Dionex system (DX600), equipped with a GS50

279   gradient pump, a CarboPac™ PA20 column (150 x 3 mm), a CarboPac™ PA20 guard column (30 x 3 mm).

280   An ED50 electrochemical detector (Dionex, Sunnyvale, USA) equipped with a reference pH electrode

281   (Ag/AgCl) and a gold electrode was used in the pulsed amperometric detection mode, performing a

282   quadruple potential waveform. Potentials E_1 = 0.1 V, E_2 = -2.0 V, E_3 = 0.6 V, and E_4 = -0.1 V were applied

283   for duration times t_1 = 400 ms, $t_2 + t_3$ = 40 ms, and t_4 = 60 ms. Samples (10 μl) were injected and eluted

284   at a flow rate of 0.5 ml/min at 30 °C. Two separate gradients for each sample enabled a complete

285   chromatographic separation of the analyzed monosaccharides (Fuc, Rha, Ara, Gal, Glc, Xyl, Man). After

286   equilibration with 100 mM NaOH for 5 min and another 5 min with 15 mM or 0.5 mM NaOH (for

287   gradient 1 and gradient 2 respectively), samples were isocratically eluted using 15 mM or 0.5 mM NaOH

288   (for gradient 1 and gradient 2 respectively) during 20 min. Afterwards, the column was regenerated

289   using 500 mM NaOH for 10 min. Mixtures of commercial sugar standards (Fuc, Rha, Ara, Gal, Glc, Xyl,

290   Man) at concentrations of 1 to 10 ppm were used daily as external standards for identification and

291   quantification.

292   To correct for degradation of the monosaccharides during the acid hydrolysis step, recovery values were

293   estimated. Hereto, mixtures of the sugar standards were subjected to the aforementioned hydrolysis

294   conditions, and the peak areas were compared to those of untreated standard mixtures.

295

296   <u>2.9   Statistical analysis</u>

297   All functionality parameters were measured in duplicate. The hypothesis test for the difference in the

298   means of the populations is the unpaired samples t test (Rees, 1997). The level of significance was set at

299     $P<0.05$.

300

301   ***3   Results and discussion***

302

303   <u>3.1   Effect of high-pressure homogenization on the microstructural and functional properties of</u>

304   <u>orange pulp</u>

305   As a result of juice extraction, orange pulp consists of ruptured juice vesicles. These juice vesicles mainly

306   contain parenchyma tissue cells with a polysaccharide-rich (cellulose, hemicelluloses and pectin) cell

307   wall. In terms of composition, orange pulp is thus an ideal functional ingredient. Although the swelling

308   volume (88 mL/g) and WHC (23.9 g/g_{anh}) are relatively high, the rheological properties of orange pulp

309   dispersions under flow conditions (Table 1) can however be improved towards higher viscosity and yield

310   stress values. Moreover, the product is very coarse and quite heterogeneous.

311   HPH of orange pulp resulted in a more homogeneous suspension with a smoother appearance

312   compared to the blended orange pulp suspension, particularly in case of the 800 bar treated pulp. The

313   swelling volume and WHC of the 200 bar homogenized sample remained similar to the initial raw

314   material (Table 1). However, both the Herschel-Bulkley yield stress and consistency index (which gives

315   an idea on the viscosity of a fluid) greatly increased. The 800 bar suspension demonstrated a non-

316   significant increase in both swelling volume and WHC, while the changes in flow properties were rather

317   limited compared to the 200 bar treated sample (Table 1). The values found for the different samples

318   were significantly higher than previously reported (Debon, Wallecan, & Mazoyer, 2012). However, the

319   samples from the mentioned study had been dried after HPH treatment. This most likely resulted in

320 partial shrinkage and structural collapse of the fibers, which would explain the observed differences.

321 Similar observations were made on other dried plant cell wall systems (Kunzek, 2005).

322 The effect of HPH on the particle size distribution of orange pulp is given in Fig. 1. As expected, a clear

323 reduction in particle size upon HPH could be noticed. This reduction was moreover depending on the

324 pressure level, and hence the shear forces, applied: the average particle size (the D [v, 0,5] value) of

325 blended orange pulp of 409 µm decreased to respectively 163 and 49 µm upon HPH at 200 and 800 bar.

326 The particle size reduction was also very clear from the microscopic observations (Fig. 2) showing large

327 tissue material in the blended sample going to completely distorted orange tissue material in the sample

328 homogenized at 800 bar. The observed changes are comparable to changes in particle size, linked to

329 changes in functional properties, for different other plant matrices (Chau et al., 2007; Bengtsson, &

330 Tornberg, 2011). It must however be emphasized that although particle size reduction during HPH can

331 be the main factor affecting the functional properties of plant-tissue based material, other changes like

332 for example changes in polysaccharides (see changes in pectin extractability in the second part of this

333 work), particle shape and particle rigidity might significantly contribute to the observed changes in

334 functional properties.

335

336 3.2   Effect of high-pressure homogenization on the cell wall polysaccharides in orange pulp

337 The cell wall material of blended and high-pressure homogenized orange pulp was extracted as AIR. As

338 expected, a high extraction yield (0.86 g AIR per g dry matter) was obtained (Table 2). The yield was

339 however shown to decrease upon HPH at elevated pressure (800 bar). Also the UA content of the

340 sample homogenized at 800 bar was lower (0.286 g UA per g dry matter) than the one obtained for the

341 blended sample (0.328 g UA per g dry matter) and the sample homogenized at 200 bar (0.322 g UA per g

342 dry matter) clearly indicating that HPH affects the amount of alcohol-insoluble material in orange pulp.

343 Hand in hand with these changes, changes in the pectin extractability properties upon HPH were

344 noticed: the AIR of the blended orange pulp only contained a small amount of WEP (14.2%) whereas the

345 AIR of the orange pulp homogenized at 800 bar contained more than 30% WEP. The data clearly show

346 that HPH at increasing pressure causes a gradual shift in the extractability properties of the pectic

347 polymers, making them less tightly bound to the orange pulp cell walls and thus more easily extractable.

348 The results moreover show that overall the amount of WEP gradually increases at the expense of the

349 NEP fraction, a fraction which is assumed to be rich in ester-bound pectin. This intriguing effect of HPH

350 on pectin extractability has been previously shown for carrot purées (Christiaens et al., 2012a) but was

351   not noticed for broccoli or tomato purée (Christiaens et al., 2012b; Christiaens et al. 2012c). In other

352   words, the effect is clearly matrix dependent but apparent in orange pulp.

353   In line with previous results on other matrices (Christiaens et al., 2012a/b/c), HPH did not affect the

354   DME of orange pulp AIR and the different fractions. Specifically, a DME of 62-69% was observed for

355   (homogenized) orange pulp AIR and CEP while a DME of 78-80% was noticed for (homogenized) orange

356   pulp WEP. On the other hand, the fractions' composition in terms of neutral sugars (Table 3) was

357   affected by the HPH treatment. Pectin polymers present in the WEP and CEP fraction of orange pulp

358   were shown to possess rather low amounts of neutral sugars compared to the amount of the backbone

359   UA units. A comparable ratio between neutral sugars and UA was found in tomato AIR which is known to

360   contain rather linear pectin polymers (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011). After HPH,

361   the ratio between UAs and neutral sugars was however changed for both the WEP and the CEP. Pectic

362   polymers in the WEP fraction of the high-pressure homogenized orange pulp were relatively richer in UA

363   compared to the blended sample, while the reverse effect was observed for the CEP. Also the

364   composition of the NEP, which is far more rich in rhamnogalacturonan-I-type neutral sugars, was

365   affected by HPH: due to HPH, the UA content in NEP is seriously decreased while the amount of neutral

366   sugars present only slightly decreased (for Rha and Ara) or even increased (Gal), indicating that HPH

367   affects the type of pectins (linear *versus* branched) present in the different fractions. After HPH, pectic

368   polymers in the WEP fraction are relatively richer in UA while the polymers in the CEP and the NEP

369   fractions become enriched in rhamnogalacturonan-I-type neutral sugars. These shifts are not surprising

370   as HPH changes the extractability characteristics of the pectic polymers in orange pulp and indicate that,

371   due to HPH, especially UA-rich pectic polymers are shifted from the CEP and/or NEP fractions to the

372   WEP fraction.

373   To investigate whether HPH also affected the size of the pectic polymers, the molar mass distribution of

374   the polymers in the different fractions was determined. HPH was clearly shown to decrease the size of

375   the pectic polymers in the WEP fraction: whereas the WEP of the blended sample has a molar mass

376   higher than 788 kDa, the WEP of the orange pulp high-pressure homogenized at 800 bar has a molar

377   mass around 404 kDa (Fig. 3). In addition, the molar mass distribution of the latter polymers was less

378   poly-disperse than the one of the blended sample. The molar mass and molar mass distribution of the

379   CEP (with an average molar mass higher than 788 kDa) and NEP (with a very broad molar mass

380   distribution between 47.3 and 788 kDa) were not affected by HPH. These results thus suggest a

381   mechanical, depolymerizing effect of HPH on the water-extractable pectic polymers in the AIR of orange

382 pulp or might point at a conformational change of the water-extractable pectic polymers upon HPH.
383 Similarly, molar mass changes of water-extractable carrot pectins due to HPH were also noticed by
384 Christiaens et al. (2012a). In this case, the effect of HPH was observed similar, but smaller, compared to
385 the effect of thermal processing on the water-extractable pectin. In addition, also the molar mass of
386 other polymers like β-glucan (Kivelä, Pitkänen, Laine, Aseyev, & Sontag-Strohm, 2010), guar gum,
387 hydroxyethylcellulose, sodium carboxymethylcellulose and sodium alginate (Villay et al., 2012) was
388 shown sensitive to HPH. In contrast, no effect on the molar mass of Arabic gum was found (Villay et al.,
389 2012). Apparently, the globular structure of polymers like Arabic gum prevents the depolymerizing or
390 conformational changing action of HPH.

391 In summary of this part of the current study, it can be concluded that HPH at elevated pressure clearly
392 affects the cell wall polymer properties in orange pulp. Through the mechanical action of HPH, orange
393 pulp cell wall polymers become more soluble resulting in less alcohol-insoluble material and resulting in
394 a duplication of the amount of WEP in the AIR. Due to HPH, the size of these water-extractable pectic
395 polymers is moreover decreased and the fraction becomes more enriched in UAs. As a consequence of
396 these observations, it is expected that the pectin concentration in the serum of the orange pulp
397 dispersion increases by HPH. As pectin concentration is known to influence the viscosity of plant-based
398 dispersions (Moelants et al., 2013b), this aspect might be important for the rheological properties of
399 high-pressure homogenized orange pulp dispersions.

400

401 3.3   The role of particle size on the functional properties of high-pressure homogenized orange pulp
402 In order to better understand the role of particle size on the functional properties of the orange pulp
403 samples, subsamples of different sizes were isolated from the blended and high-pressure homogenized
404 orange pulp dispersions. Subsamples that could be isolated in a substantial amount (Table 4) were
405 considered relevant and were further investigated in terms of microstructural and functional properties.
406 As HPH affects the particle size within the orange pulp dispersions, HPH obviously influenced the
407 presence of the different subsamples. Whereas the blended sample only contained less than 10% of
408 material in the smallest subsample (< 40 μm), more than 40% and 90% of the material turns up in this
409 fraction for the orange pulp homogenized at 200 and 800 bar respectively. This shift in particle fractions
410 confirms the fact that the degree of material disintegration is related to the pressure level, and hence
411 the shear forces applied, during HPH. It must hereby however be emphasized that the average size of
412 particles in subsamples (Table 5) is larger than the pore size of the sieves through which the particles

413   could pass and is thus larger than the number that is used to refer to the subsamples. Specifically, the

414   average particle size of the orange pulp subsamples can be presented as D(v, 0.5), D[4,3] and D[3,2].

415   Similar trends in these three diameters between the different subsamples can be observed. However,

416   due to the dominant contribution of large particles to the volume-based average diameters such as D(v,

417   0.5), D[4,3], their values were larger as compared to D[3,2], the surface-based average diameter. Also

418   the distribution of the particles within one subsample exceeds the difference between the pore sizes of

419   two consecutive sieves. These observations can be explained by the irregular shape and deformability of

420   the orange pulp particles and the inability of the laser diffraction technique used to capture these

421   properties as particle sizes were calculated assuming a spherical shape (Fig. 2 and Fig. 4). Similar

422   observations were made by Moelants et al. (2013a) who investigated the particle size distribution of

423   sieved carrot particles. During microscopic analysis of the different subsamples (Fig. 4), a remarkable

424   observation was done for the smallest subsample obtained from the orange pulp homogenized at 800

425   bar. The material of this subsample formed a kind of a hydrocluster or soft gel particle during the

426   analysis, i.e. while being 'squeezed' between the glass slide and the cover glass and thus while being

427   subjected to high local stresses. Conversely, this phenomenon was not apparent for the other

428   subsamples.

429   The determination of the UA content of the different subsamples (Table 4) indicated that part of the

430   pectic polymers were washed out during the wet sieving procedure that was used to produce the

431   subsamples. This washing-out effect is important to consider in the context of the functional properties

432   of the subsamples as interaction properties of particles might be affected.

433   The swelling properties of the fiber subsamples were all very high (Table 6). In general, values close to

434   100% were found for all the suspensions. This is significantly higher than the values measured on the

435   total samples (see Table 1). It might be concluded that the individual particle subsamples will arrange

436   differently than when blended together. Moreover, an increase of the homogenization pressure

437   resulted in particle subsamples with higher WHC, especially for the subsamples containing the smallest

438   material (< 40 µm) (Table 6). Furthermore, a clear trend was observed for the subsamples obtained from

439   orange pulp homogenized at 200 bar: the smaller the subsample particle size, the higher the WHC. In

440   particular, the particle fraction below 40 µm showed the highest WHC upon centrifugation. The higher

441   WHC of the smaller particle fractions may be related to the higher specific surface area of these small

442   particles. When comparing with the WHC value obtained for the entire 200 bar sample (Table 1), it

443 appears that the smallest particle subfraction has a dominant effect on the ability of the fiber to retain
444 water.

445 To investigate the rheological properties of the orange pulp samples and subsamples, oscillatory strain
446 tests were performed under controlled shear deformation on the packed material after centrifugation at
447 3500 g. Due to the limited amount of sample, a strain sweep but no additional frequency sweep was
448 measured. Since the samples are known to behave as gels, the values of the storage (G') and loss
449 modulus (G'') are independent of the angular frequency at which they are measured for strains within
450 the linear viscoelastic (LVE) region. Therefore, G' values were obtained from the strain sweep for a
451 particular strain within the LVE and reported in Table 7, for the original (total) fiber samples as well as
452 for the subsamples with a different particle size. A first interesting finding was that the large particle
453 subsamples had significantly higher G' values compared to the small particle ones. This observation is in
454 correspondence with results for jaboticaba and carrot pulp obtained by Sato and Cunha (2009) and
455 Moelants et al. (2013a) respectively, and was particularly evident for the subsamples isolated from the
456 orange pulp that was homogenized at 200 bar. In addition, G' of the total samples was smaller than the
457 moduli of the individual subfractions, with exception of G' of the smallest particle subsample isolated
458 from the orange pulp homogenized at 200 bar. This might indicate that even though some subsamples
459 have a much higher G' on their own, an antagonistic effect is observed when the different subfractions
460 are mixed. Similarly, Moelants et al. (2013a) observed lower G' values for carrot purée than G' of the
461 reconstituted subfractions. The larger width of the particle size distribution in carrot purée was
462 mentioned as possible explanation for this observation.

463 However, caution should be taken when analyzing the rheological data. The various subsamples were
464 not measured at the same moisture content due to their differences in WHC (Table 1 and 6). Therefore,
465 comparing the strain sweep curves of the samples can only be done on a relative basis. G' normalization
466 was applied by dividing the G' values with the linear viscoelastic range storage modulus as a function of
467 strain as applied by Adams, Frith, & Stokes (2004) for soft agar microgels. It is known that the maximum
468 strain within the linear viscoelastic range is inversely proportional to the particle size and the packed
469 material modulus. The greater ability of the softer particle network (as size decreases) to deform prior
470 to flow may be due to the interaction of the hydroclusters (3D packing and lubrication forces). Similar
471 behavior has been observed for soft agar microgels (Adams et al., 2004). Interestingly, the total orange
472 pulp sample homogenized at 200 bar had a very different behavior than its corresponding subsamples
473 (Fig. 5a). The overall sample turned out to be less deformable (indicated by the smaller critical strain)

474 than its constituent subfractions. Comparison between the normalized G' evolutions of the total

475 samples (Fig. 5b) showed that the starting material and the 200 bar sample behaved very similar. Visible

476 differences in smoothness could be observed. However, the 800 bar sample showed a more soft gel

477 behavior than the two other samples (indicated by the lower tan δ value for the sample homogenized at

478 800 bar in Table 7), which is in line with the microscopic evaluations (Fig. 4). When comparing the mass

479 balances of the fractions, it appeared that although about 40-45% of particles present in the 200 bar

480 sample were smaller than 40 μm (Table 4), this amount proved to be insufficient to significantly alter the

481 overall rigidity of the suspension.

482

483 ### *4   Conclusion*

484 Results obtained within the current work clearly suggest that the functional properties of orange pulp

485 suspensions are largely related to particle size. Particles smaller than 40 μm were proven to substantially

486 contribute to the swelling properties, the water holding capacity and the soft gel behavior of the fiber

487 suspensions. High-pressure homogenization (HPH) was shown to affect the particle size distribution,

488 and hence the functional properties of orange pulp whereby a pressure level higher than the one usually

489 applied in food industry (200 bar) was necessary to obtain orange pulp with desired functional

490 properties. By applying a homogenization step at 800 bar, a more homogenous orange pulp suspension

491 with a smooth appearance and with increased water holding capacity and improved swelling and flow

492 properties was obtained. This observation was related to the abundant presence of largely disintegrated

493 orange pulp material (particles smaller than 40 μm) that behaved like a softer particle network. In

494 addition to the particle reducing effect of HPH, also the effect of HPH on cell wall polymers might

495 contribute to the improved functional properties of high-pressure homogenized orange pulp. HPH was

496 found to mechanically affect the pectic polymers within the orange pulp cell walls thereby decreasing

497 their size or conformation and increasing their extractability properties and as a consequence also

498 increasing their presence in the serum of the orange pulp suspension. As pectin is a relatively rigid

499 polymer with lots of functional groups allowing interaction with water, these changes might indeed

500 contribute to the enhanced water holding and swelling capacity of high-pressure homogenized orange

501 pulp. The rheology of the subfractions did not correlate well with the behavior of the total sample, in

502 particular for the samples homogenized at 200 bar. Further research would be to study the packing

503 behavior of the various fractions in particular under shear, which might explain the observed

504 antagonistic effects.

505 Finally, it should be noted that the concentrations at which the functionalized fibers demonstrated high

506 swelling properties in water, which resulted in particle suspensions with a high yield stress, are too low

507 (around 2-4%) for enriching a food product with the fiber alone. However, it opens opportunities for

508 replacing label-unfriendly ingredients by natural alternatives.

509

510 ***5   Acknowledgements***

514

515 ***6   References***

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582

583 ***5   Figure captions***

584 **Fig. 1** Particle size distribution curves of blended orange pulp and orange pulp high-pressure
585 homogenized at 200 bar and 800 bar.

586 **Fig. 2** Representative micrographs (1st row: bright field; 2nd row: labelled with JIM7) of blended and high-
587 pressure homogenized (at 200 and 800 bar) orange pulp.

588 **Fig. 3** Molar mass distribution of the WEP fraction in blended and high-pressure homogenized (at 200
589 bar and at 800 bar) orange pulp. Elution times of pullulan standards are indicated to allow for a rough
590 estimation of the molar mass.

591 **Fig. 4** Representative pictures (1st row: bright field; 2nd row: labelled with JIM7) of the '<40 µm'
592 subsample of blended and high-pressure homogenized (at 200 bar and 800 bar) orange pulp.

593 **Fig. 5** Normalized G' as function of strain for the different particle fractions obtained after 200 bar
594 homogenization (a) and for the total samples at 0 bar, 200 bar and 800 bar (b).

595   **Table 1.** Swelling volume, water holding capacity and Herschel-Bulkley parameters ($\pm$ standard deviation; n = 2, from 2 separate pouches) of

596   blended and high-pressure homogenized (at 200 bar and 800 bar) orange pulp fibers in standardised tap water.

	blended	200 bar	800 bar
Swelling volume (mL/g, dry weight basis) 1 *g*	88.0 ($\pm$ 2.0)[a]	89.0 ($\pm$ 2.4)[a]	94.0 ($\pm$ 3.0)[a]
Water holding capacity (g water/g dry weight fiber) 3500 *g*	23.9 ($\pm$ 0.9)[a]	23.6 ($\pm$ 0.9)[a]	25.5 ($\pm$ 0.1)[a]
Hershel-Bulkley fit at 4% concentration (dry weight basis)			
Yield stress (Pa)	99.3 ($\pm$ 0.1)[b]	160.9 ($\pm$ 10.6)[a]	183.7 ($\pm$ 16.6)[a]
Consistency index (Pa.s)	70.8 ($\pm$ 2.1)[b]	89.9 ($\pm$ 1.4)[a]	66.4 ($\pm$ 15.2)[a,b]
Pseudoplasticity Index (-)	0.275 ($\pm$ 0.003)[a]	0.220 ($\pm$ 0.001)[b]	0.237 ($\pm$ 0.029)[a,b]
r^2 of fit of each replicate	0.99625 0.99530	0.99993 0.99993	0.99957 0.99980

597   [a] measurements within a row with the same superscript letter are not significantly different (95% confidence limit).

598

599 **Table 2.** Alcohol-insoluble residue (AIR) content, uronic acid (UA) content ($\pm$ standard deviation; n = 2) and the relative amount of the different

600 fractions (UA in the fraction relative to UA in the sum of fractions $\pm$ standard deviation; n = 2) in blended and high-pressure homogenized (at 200

601 and 800 bar) orange pulp.

	AIR content	UA content	Pectin fraction (%)		
	(g AIR/g DM)	(g UA/g DM)	WEP	CEP	NEP
Blended	0.86 ± 0.02	0.328 ± 0.006	14.2 ± 0.3	37.0 ± 0.4	45.7 ± 0.8
200 bar	0.87 ± 0.02	0.322 ± 0.017	22.2 ± 0.5	36.8 ± 1.8	38.6 ± 1.4
800 bar	0.78 ± 0.03	0.286 ± 0.011	30.4 ± 1.2	36.3 ± 0.5	31.8 ± 0.7

602 *DM: dry matter*

603

604

Table 3. Neutral sugar content (mg/g AIR) (± standard deviation; n = 2) of pectic fractions, compared to the uronic acid (UA) content, in blended and high-pressure homogenized (at 200 and 800 bar) orange pulp (Fuc = fucose, Rha = rhamnose, Ara = arabinose, Gal = galactose, Glc = glucose, Xyl = xylose, Man = mannose).

		Fuc	Rha	Ara	Gal	Glc	Xyl	Man	UA*
Blended	WEP	0.20 ± 0.01	1.75 ± 0.14	6.10 ± 0.30	5.18 ± 0.26	2.99 ± 0.15	1.21 ± 0.02	1.62 ± 0.08	46 ± 1
	CEP	0.24 ± 0.03	1.39 ± 0.25	9.20 ± 0.48	2.85 ± 0.12	0.49 ± 0.03	0.42 ± 0.05	0.32 ± 0.02	119 ± 3
	NEP	0.84 ± 0.12	8.47 ± 0.02	55.18 ± 5.28	23.00 ± 4.07	3.01 ± 2.64	13.30 ± 16.05	4.98 ± 5.98	147 ± 3
200 bar	WEP	0.29 ± 0.01	2.35 ± 0.08	8.77 ± 0.19	7.94 ± 0.54	3.36 ± 0.39	1.48 ± 0.06	1.73 ± 0.03	65 ± 2
	CEP	0.23 ± nd	1.45 ± nd	9.36 ± nd	3.00 ± nd	0.46 ± nd	0.46 ± nd	0.31 ± nd	108 ± 6
	NEP	0.71 ± 0.04	8.02 ± 0.00	49.11 ± 0.47	19.12 ± 0.12	0.87 ± 0.05	2.07 ± 0.12	0.40 ± 0.08	113 ± 3
800 bar	WEP	0.25 ± 0.05	1.96 ± 0.55	8.81 ± 1.61	6.25 ± 1.58	1.74 ± 0.34	0.94 ± 0.34	0.87 ± 0.25	81 ± 10
	CEP	0.26 ± 0.01	2.05 ± 0.03	12.80 ± 0.11	3.97 ± 0.01	0.06 ± 0.00	0.47 ± 0.00	0.44 ± 0.02	97 ± 5
	NEP	1.38 ± 0.00	6.58 ± 0.78	46.55 ± 0.98	24.60 ± 2.69	2.03 ± nd	6.94 ± nd	1.28 ± nd	85 ± 2

* Colorimetrically determined

nd: not determined

611 **Table 4.** Approximate relative presence (weight %) of subsamples in blended and high-pressure homogenized (at 200 bar and 800 bar) orange
612 pulp and uronic acid content ($\pm$ standard deviation; n =2) in relevant subsamples.

		< 40 µm	40-80 µm	80-125 µm	125-250 µm
Blended	Relative presence (w %)	< 10%	< 10%	< 10%	$\pm$ 15%
	UA content (g UA/g DM)	nd	nd	nd	0.251 ± 0.008
200 bar	Relative presence (w %)	> 40%	$\pm$ 20%	$\pm$ 20%	$\pm$ 15%
	UA content (g UA/g DM)	0.238 ± 0.005	0.269 ± 0.007	0.271 ± 0.006	0.289 ± 0.002
800 bar	Relative presence (w %)	> 90%	$\pm$ 8%	$\pm$ 2%	-
	UA content (g UA/g DM)	0.250 ± 0.006	nd	nd	nd

613 DM: dry matter
614 nd: not determined

615

616   **Table 5.** Overview of particle size distribution parameters (and their standard deviation; n=2) for relevant subsamples obtained for the
617   blended and high-pressure homogenized (at 200 bar and 800 bar) orange pulp.

618

		D(v, 0.1)	D(v, 0.5)	D(v, 0.9)	D[4,3]	D[3,2]	Spread
Blended	**125-250 µm**	**137.3**	**317.6**	**591.1**	**341.9**	**176.4**	**2.6**
		2.5	*4.7*	*3.3*	*3.7*	*1.9*	*0.0*
200 bar	**< 40 µm**	**16.9**	**51.2**	**123.7**	**63.3**	**26.0**	**4.1**
		0.4	*1.1*	*10.9*	*4.7*	*0.0*	*0.4*
200 bar	**40-80 µm**	**42.9**	**101.4**	**209.3**	**115.2**	**64.6**	**2.6**
		0.3	*0.1*	*5.9*	*1.9*	*0.1*	*0.1*
200 bar	**80-125 µm**	**61.1**	**152.4**	**314.6**	**172.9**	**92.0**	**2.8**
		0.1	*0.6*	*3.8*	*1.4*	*0.4*	*0.0*
200 bar	**125-250 µm**	**106.8**	**279.8**	**582.9**	**314.1**	**153.6**	**3.1**
		0.7	*1.5*	*12.4*	*3.6*	*0.0*	*0.1*
800 bar	**< 40 µm**	**13.3**	**39.4**	**100.9**	**52.3**	**21.7**	**4.0**
		0.7	*1.8*	*17.7*	*8.7*	*0.2*	*0.7*

619

620

621

622   **Table 6.** Swelling volume and water holding capacity ($\pm$ standard deviation; n = 2 from 2 separate pouches) of orange pulp fractions at 0 bar, 200
623   bar and 800 bar in standardised tap water. Values in bold were obtained from a single measurement due to insufficient sample quantity to
624   perform the evaluations in duplicate.

| Batches orange pulp	blended	200 bar	200 bar	200 bar	200 bar	800 bar
	125-250 µm	125-250 µm	80-125 µm	40-80 µm	<40 µm	<40 µm
Swelling volume (mL/g, dry weight basis) 1 *g*	**nps**	**95**	nps	99 (± 1)	nps	nps
Water holding capacity (g water/g dry weight fiber) 3500 *g*	**21.7**	**21.4**	21.9 (± 0.2)[c]	22.6 (± 0.1)[c]	24.0 (± 0.2)[b]	27.3 (± 0.2)[a]

625   nps: no phase separation

626   [a] measurements within a row with the same superscript letter are not significantly different (95% confidence limit).

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629   **Table 7.** Linear viscoelastic range moduli of the packed phase material after centrifugation at 3500 g.

Batches orange pulp	blended	blended	200 bar	200 bar	200 bar	200 bar	200 bar	800 bar	800 bar
	Total	125-250 µm	Total	125-250 µm	80-125 µm	40-80 µm	<40 µm	Total	<40 µm
G* (Pa)	9977	13 640	5020	20 000	10 597	8 329	4 387	3212	4 367
G' (Pa)	9758	13 373	4930	19 807	10 499	8 245	4 337	3170	4 335
G'' (Pa)	2078	2 755	942	2 812	1 433	1 175	664	517	535
tan(δ)	0.21	0.21	0.19	0.14	0.14	0.14	0.15	0.16	0.12
LVR strain (%)	0.44	1.19	0.76	0.60	0.80	1.03	1.22	1.08	1.18

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632
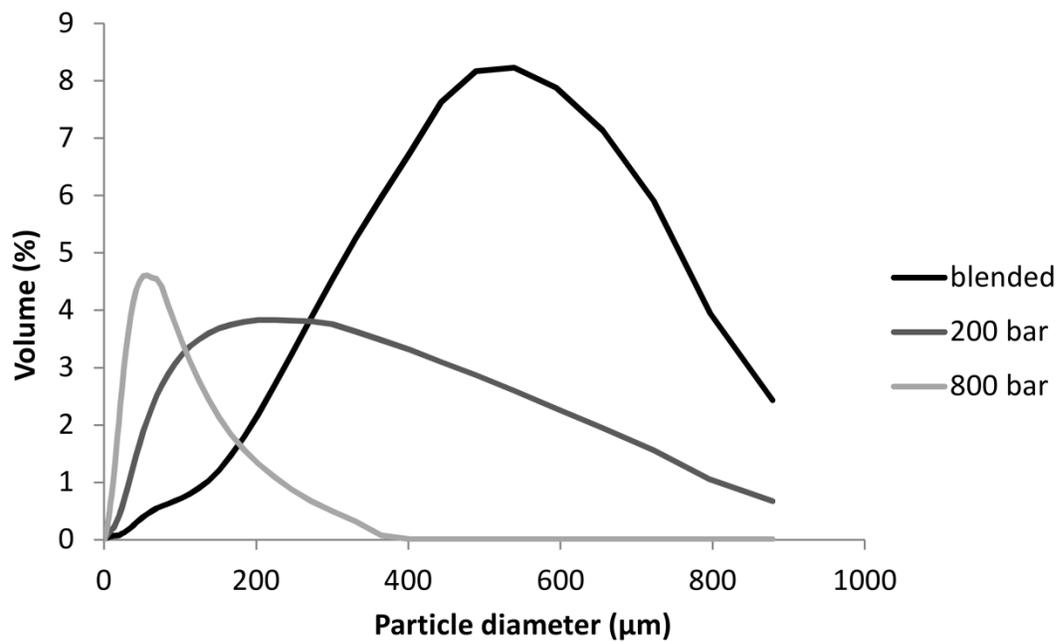
633

634   Figure 1

635

636

637   Figure 2

638

788000   404000   212000   112000   47300   22800   11800
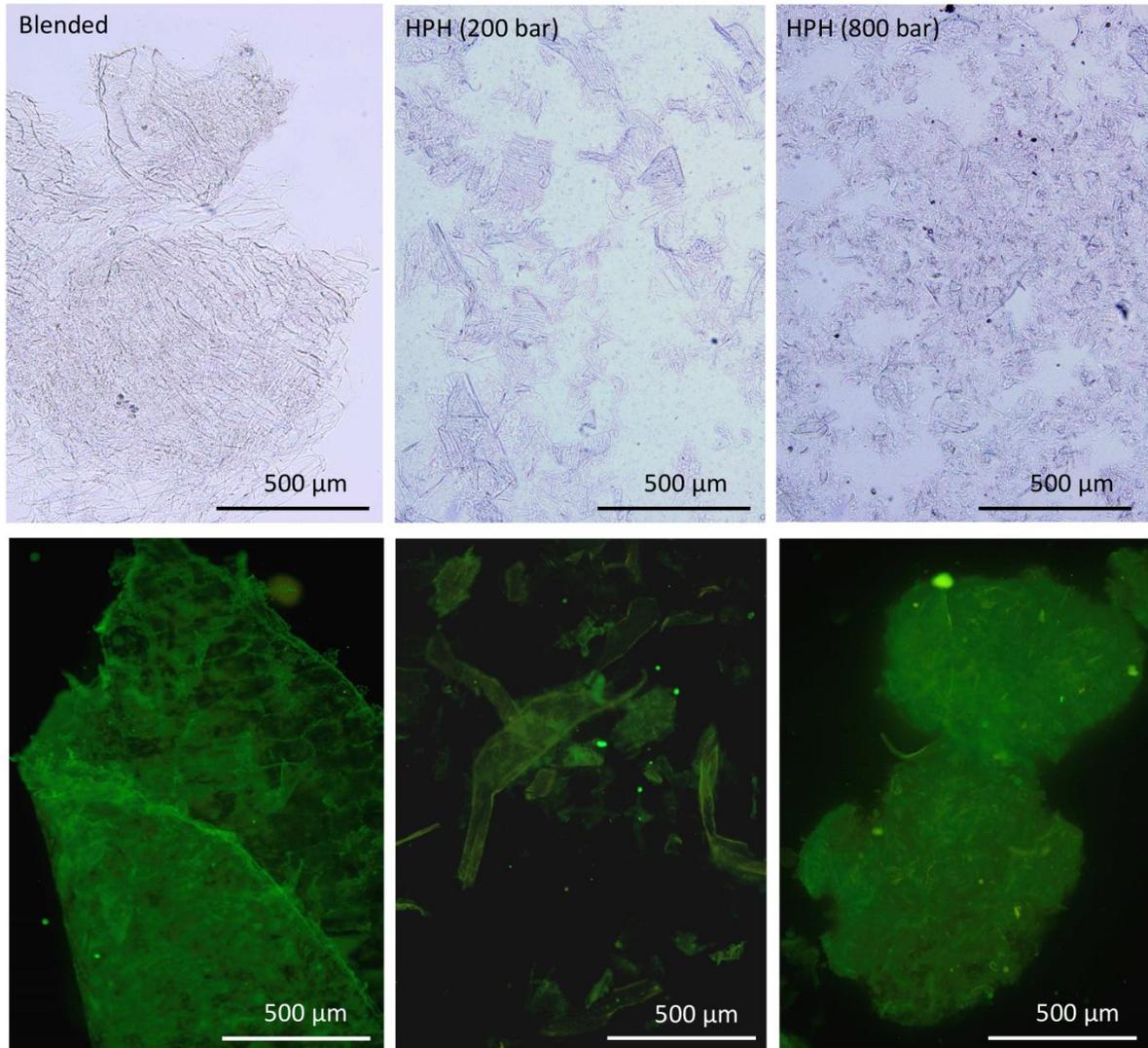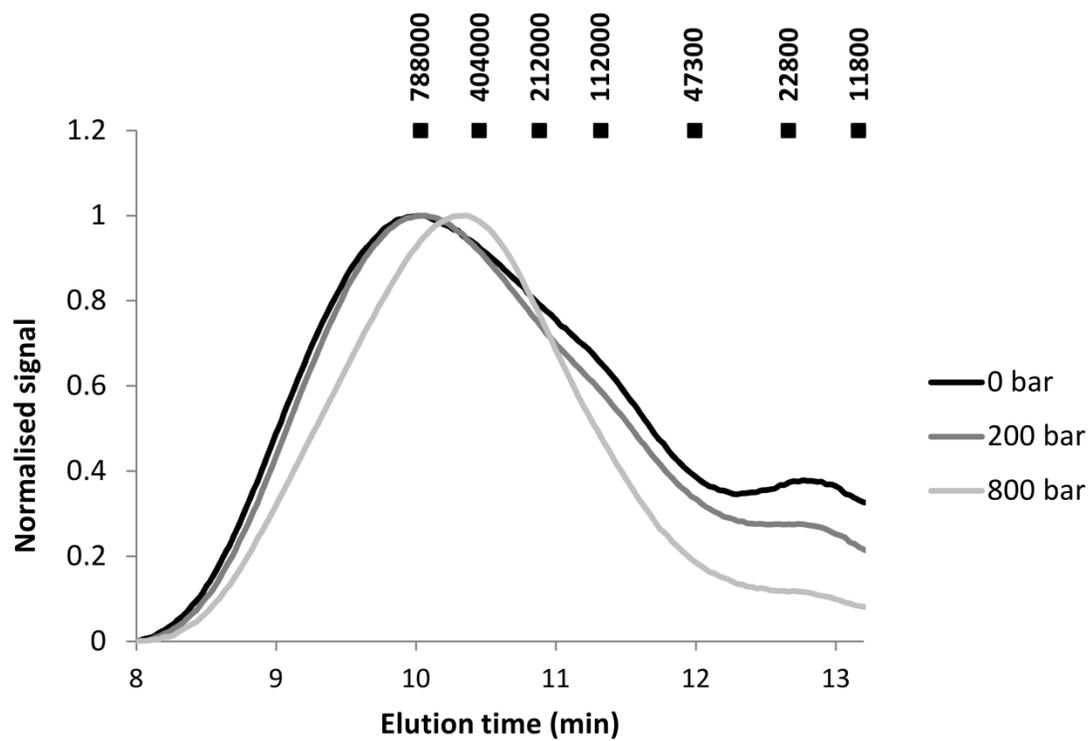
639

640   Figure 3

641

| | Blended | 200 bar | 800 bar |
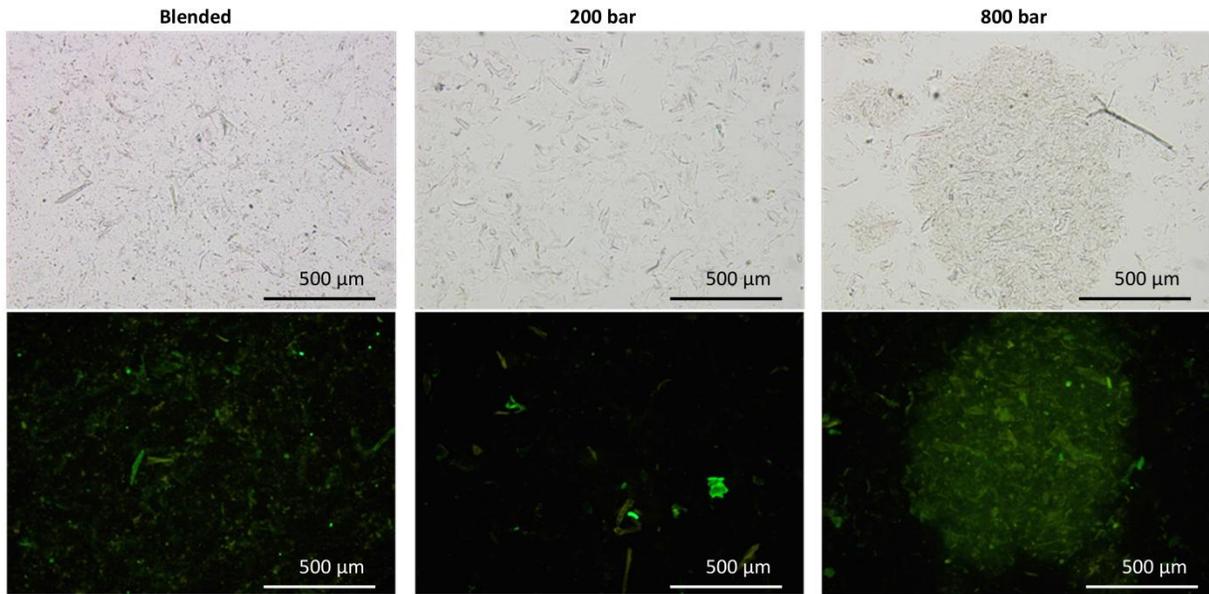
642

643   Figure 4

644

a

b

645

646   Figure 5

647