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# Apple puree's texture is independent from fruit firmness

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## Abstract

How cellular and molecular structure of raw fruits impact puree's texture is still an unresolved question. Texture variations of purees obtained from four apple cultivars of contrasted texture (Braeburn, Gala, Golden Delicious, Granny Smith) and two modalities (mealiness, fruit load) after two contrasted processes were investigated. Although puree's viscosity strongly varied between cultivars (562–1368 mPa.s), it did not correlate with apple firmness, except for Granny Smith. This cultivar had the firmest fruits (3.2 N) and the most viscous purees (1368 mPa.s), in accordance with large particles (around 650  $\mu$ m), high pulp wet mass and serum viscosity. Mealy Braeburn apples showed lower puree's viscosity (562 mPa.s) than their not-mealy homologues (779 mPa.s). This was due to reduced cell adhesion, maybe because of lower (arabinose+galactose)/rhamnose ratio, leading to smaller particles during processing. Process also impacted puree's viscosity (692–939 mPa.s), with more viscous purees obtained with the high temperature-low shear process.

*Malus domestica* Borkh.; Processing; Cell adhesion; Pectin; Mealiness

## 26 **1. Introduction**

27

28 Plant-based purees are concentrated suspensions of soft particles, composed of individual  
29 parenchyma cells and cell clusters (pulp) of the original fruit. These are dispersed in the  
30 aqueous content (serum) of the cells' vacuoles, which are emptied during processing (Rao,  
31 1992). In plant-based purees, texture is an important quality characteristic (Szczesniak &  
32 Kahn, 1971). It is determined by particle size distribution, pulp content and serum viscosity  
33 (Espinosa et al., 2011; Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016; Rao, 1992)  
34 and depends on the original cell wall structure (Waldron, Smith, Parr, Ng, & Parker, 1997).

35 Pectins are known to be the polysaccharides in the plant cell wall, which are the most affected  
36 by enzymatic and chemical degradation during processing (Van Buren, 1979). In raw fruits,  
37 pectins contribute to the mechanical strength of the tissue, among others because they  
38 associate individual cells through the middle lamella (Carpita & Gibeaut, 1993; Jarvis, 1984).  
39 Pectin solubilisation during heat treatment leads to softening of the apple tissue, which can  
40 consequently be disrupted by mechanical treatment. The magnitude of cell disruption and  
41 pectin solubilisation thus defines the puree's texture.

42 However to this day, cell wall properties defining the firmness of raw apples were never  
43 correlated to the puree's texture. Bourles, Mehinagic, Courthaudon, and Jourjon (2009) found  
44 no strict correlation between the firmness of raw apples and the texture of cooked apple slices.  
45 However, they did not analyse cell wall polysaccharides although pectins seem to play a key  
46 role in understanding texture deterioration during cooking (Ella Missang, Maingonnat,  
47 Renard, & Audergon, 2012).

48 Pectins' homogalacturonan (HG) consists of  $\alpha$ -1,4-linked galacturonic acids that can be  
49 methyl-esterified at the C6 positions. Depending on the degree of methylation (DM) and the  
50 distribution of ester groups, HG molecules can form cross-links with calcium ions (Kohn &

51 Luknár, 1977), contributing to intercellular adhesion in the middle lamella. Although  
52 degradation of pectic HGs are the most studied, other pectin domains may also affect fruit  
53 texture, especially rhamnogalacturonan I (RG I). RG I possesses a backbone with alternating  
54 rhamnose molecules in addition to galacturonic acid. Neutral sugar side chains, composed of  
55 galactose and/or arabinose are attached to the rhamnosyl residues (Ridley, O'Neill, &  
56 Mohnen, 2001), and are associated with firm texture in apple fruits (Nara, Kato, & Motomura,  
57 2001; Pena & Carpita, 2004). Recently, a loss of RG I side chains during post-harvest storage  
58 of raw apples was correlated to less viscous purees: because of RG I loss, cell adhesion in the  
59 raw fruits decreased and fragmentation during processing was facilitated (Buergy, Rolland-  
60 Sabaté, Leca, & Renard, 2020).

61 In this study, the impact of fruit firmness on puree's texture was investigated using four apple  
62 cultivars and two modalities (mealiness, fruit load). The cultivars (Braeburn, Gala, Golden  
63 Delicious, Granny Smith) were selected as they were expected to show contrasted fruit  
64 firmness and might respond differently to heat treatment (Kim, Smith, & Lee, 1993; Rao,  
65 Cooley, Nogueira, & McLellan, 1986). Two different processes (high temperature-low shear  
66 process and low temperature-high shear process), chosen to generate contrasted puree's  
67 textures, were applied. Cell wall composition and firmness of raw fruits were compared to the  
68 puree's texture and macromolecular characteristics of soluble pectins.

69

## 70 **2. Material and Methods**

71

### 72 *2.1. Plant material*

73

#### 74 *2.1.1. Raw apples*

75 Four apple (*Malus domestica* Borkh.) cultivars, namely Braeburn (BR), Gala (GA), Golden  
76 Delicious (GD) and Granny Smith (GS) were grown in Mallemort, France and harvested in  
77 August and September 2018 corresponding to the commercial harvest dates. BR, GA, GS and  
78 half of the GD trees (GD1) were thinned chemically to reduce fruit load (Supplementary  
79 Table S1). The other half of the GD trees were not thinned (GD2), resulting in more but  
80 smaller fruits.

81 Apples were stored for one month at 4 °C in normal atmosphere in order to reduce the starch  
82 content. Half of the BR apples (BRM) were stored for 11 days at 24 °C and a relative  
83 humidity between 90% and 100% (customised phytotron, Froid et Mesures, Beaucozé,  
84 France) prior to processing in order to accelerate development of mealiness (Barreiro et al.,  
85 1998).

86 The day before processing, apples were separated into two equivalent groups, one determined  
87 for puree processing, one for raw apple characterization. For analysis of raw fruits, three  
88 replicates of ten representative apples were chosen per cultivar. After determination of fruit  
89 texture at 23 °C, apples were cored and cut in 12 equal portions. The pieces were separated  
90 equally into three groups and cut vertically. Only four pieces per apple, systematically spread  
91 over sides and height, were retained in order to obtain a batch of 40 apple pieces that were  
92 immediately frozen in liquid nitrogen and then stored at -20 °C to isolate the alcohol insoluble  
93 solids (AIS) (Renard, 2005).

94

#### 95 *2.1.2. Puree preparation*

96 For all cultivars, each process was conducted in triplicate. Apples (3 kg) were cored, sliced  
97 into 12 equal pieces and processed under vacuum into puree by a cooker-cutter (RoboQbo  
98 Qb8-3, RoboQbo, Bentivoglio, Italy). To inactivate apple pectin methylesterase and only  
99 consider chemical pectin degradation, temperatures higher than 59 °C (Denes, Baron, &

100 Drilleau, 2000) were applied in both processes (Supplementary Fig. S1). Process I consisted  
101 in grinding at 3000 rpm during temperature increase (202 s) and the following 15 min at  
102 70 °C. The purees were then pasteurized (95 °C, 2 min). Process II comprised grinding at  
103 3000 rpm during temperature increase (360 s), followed by 400 rpm for 17 min at 95 °C. Half  
104 part of each puree was refined by an automatic sieve (Robot Coupe C80, Robot Coupe SNC,  
105 Vincennes, France) of 0.5 mm, removing skin and particles larger than the sieve opening.  
106 Once purees reached room temperature, rheology, particle size and pulp wet mass were  
107 analysed. Pulp and serum were separated by centrifugation of the puree (7690 x g, 15 min,  
108 15 °C) and then frozen separately (-20 °C) until AIS extraction.

109

## 110 *2.2. Physico-chemical characterization*

111

### 112 *2.2.1. Texture of raw apples*

113 Texture of raw apples was determined by a puncture test via a multipurpose texture analyser  
114 (TAPlus, Lloyd Instruments, Farenham, UK), using a punch probe of a diameter of 2 mm.  
115 This allowed to penetrate into a peeled apple section without compression of the tissue. The  
116 puncture probe penetrated up to a depth of 17 mm in order to assess a large range of the  
117 parenchyma and thus to be representative of the whole fruit. Firmness of apple flesh was  
118 calculated as the ratio of penetration energy, averaged in the plateau region of the load-  
119 deflection curve, to the height of testing, giving the puncture mean load. Crunchiness was  
120 estimated by calculating the linear distance between consecutive points from the force-  
121 distance curve in a range of 10 mm at the load plateau (Gregson & Lee, 2003). The test was  
122 repeated 10 times on three different apples of the same cultivar.

123

### 124 *2.2.2. Rheology of the purees and sera*

125 Samples were analysed at 22.5 °C using a stress-controlled rheometer (Physica MCR301),  
126 equipped with a Peltier cell (CPTD-200) and a measuring cylinder (CC27/S), all from Anton  
127 Paar (Graz, Austria).

128 A vane measuring system (FL100/6W) with a 3.46 mm gap was used for purees. For flow  
129 curves, viscosity was followed over a logarithmically distributed range (shear rate values  
130 between 10 and 250 s<sup>-1</sup>), recording one point every 15 s. Apparent viscosity at 50 s<sup>-1</sup> was  
131 chosen to compare the puree's textures as it represents the approximate shear rate in mouth  
132 (Shama & Sherman, 1973).

133 Amplitude sweep tests were measured from 0.01 to 100% at a constant angular frequency  
134 (10 rad/s), recording five points per decade. The time required to measure each point was  
135 defined by the software. The values of G' and G'' were averaged in the linear viscoelastic  
136 range.

137 Considering the gap of the measuring system, rheological analysis was theoretically not  
138 adapted for purees containing particles larger than 1 mm. However, NR purees, the only  
139 samples containing particles (skin fragments) larger than 1 mm, showed high repeatability.  
140 The method was thus considered valid for internal comparison.

141 Serum viscosity was analysed by a flow curve (10 to 1000 s<sup>-1</sup>, 8 min) using a double gap  
142 cylinder geometry set (DG27). The value at a shear rate of 100 s<sup>-1</sup> was retained, as it was  
143 expected that oral perception of serum would require a higher shear rate than purees.

144

### 145 *2.2.3. Particle size distribution*

146 The particle size distribution was analysed by laser granulometry (Mastersizer 2000, Malvern  
147 Instruments, Malvern, UK) as described previously (Buergy et al., 2020). Each sample was  
148 analysed twice and the Malvern's software averaged the size distribution over three repeated  
149 measurements on the same sample.

150

#### 151 2.2.4. *Pulp wet mass (PWM) and water retention capacity (WRC)*

152 The PWM was calculated as the ratio of the pulp weight after centrifugation to the initial  
153 weight of the puree and expressed in *g/100 g* (Espinosa et al., 2011). The amount of water  
154 retained by the mass of the pulp's cell wall polysaccharides (*g/g* dry weight) was defined as  
155 the WRC (Robertson et al., 2000). It was estimated as the relation between the PWM and the  
156 pulp's dry weight. The fibres' mass in the wet mass is generally negligible and was thus not  
157 considered.

158

### 159 2.3. *Analytical*

160

#### 161 2.3.1. *Chemicals*

162 Acetic anhydride, methanol-d<sub>3</sub> and *o*-methylimidazole were from Acros Organics (Geel,  
163 Belgium). Acetone (pure) was from Carlo Erba Reagents (Val-de-Reuil, France). Acetic acid  
164 (glacial), ammonia solution (35 *g/100 mL*), methanol (LC-MS grade), sodium sulphate  
165 (anhydrous) and sulphuric acid were from Fisher Scientific (Loughborough, UK). Sodium  
166 hydroxide and phenol were from Merck KGaA (Darmstadt, Germany). Calcium chloride,  
167 galacturonic acid, myo-inositol, *m*-hydroxydiphenyl, potassium hydroxide, sodium acetate,  
168 sodium borohydride, sodium hydrogen phosphate, sodium tetraborate and sugars (arabinose,  
169 fucose, galactose, glucose, mannose, rhamnose, xylose) were from Sigma-Aldrich (Steinheim,  
170 Germany). Dichloromethane and ethanol (96 *g/100 mL*) were from VWR Chemicals  
171 (Fontenay-sous-Bois, France). All reagents were used without further purification.

172

#### 173 2.3.2. *Cell wall isolation*

174 Cell wall polysaccharides were isolated as alcohol insoluble solids (AIS).



175 The frozen apple pieces were freeze-dried and finely ground before AIS were extracted with  
176 ethanol (700 mL/L) as described by Le Bourvellec et al. (2011). AIS were expressed in mg/g  
177 fresh weight (FW).

178 Pulp was water-washed and AIS were prepared according to Buergy et al. (2020). Once free  
179 sugars were absent (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), samples were dried by  
180 solvent exchange, followed by 48 h in a drying oven at 40 °C. The AIS of the pulp was  
181 determined as the relation between the dry pulp weight and the initial pulp weight after water-  
182 washing and expressed in mg/g FW.

183

#### 184 2.3.3. Serum precipitation

185 AIS of the serum was alcohol-precipitated as described by Buergy et al. (2020). The pectin  
186 content in the serum was roughly estimated by calculating the ratio between the weight of the  
187 sample after freeze-drying and the initial weight of serum (100 mL), expressed in mg/g FW.

188

#### 189 2.3.4. Cell wall polysaccharide analysis

190 Neutral sugars and myo-inositol (internal standard) were analysed after acid hydrolysis  
191 (Saeman, Moore, Mitchell, & Millett, 1954). The free sugars were derivatised to volatile  
192 alditol acetates (Englyst, Wiggins, & Cummings, 1982) and analysed using a Clarus 500 gas  
193 chromatograph (PerkinElmer, Waltham, USA), equipped with a flame ionization detector  
194 (FID) and a OPTIMA® capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness,  
195 Macherey-Nagel, Düren, Germany) at 230 °C, using helium as carrier gas.

196 The acid hydrolysates were tested spectrophotometrically for galacturonic acid (GalA) using  
197 the *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973).

198 Methanol content was determined by stable isotope dilution assay after saponification  
199 (Renard & Ginies, 2009). It was analysed on a Trace 1300 gas chromatograph (Thermo

200 Scientific, Waltham, USA) with a TG-WaxMS capillary column (30 m × 0.25 mm i.d.,  
201 0.5 µm film thickness, Thermo Scientific, Waltham, USA), coupled to a ISQ LT single  
202 quadrupole mass spectrometer (Thermo Scientific, Waltham, USA).

203 The degree of methylation (DM) was calculated as molar ratio of methanol to GalA and  
204 expressed in %.

205

#### 206 *2.3.5. Starch determination*

207 Starch was quantified in the AIS of raw apples using the total starch assay kit K-TSTA  
208 (Megazyme, Wicklow, Ireland). As residual polyphenols in the AIS might inactivate the  
209 enzymes, the enzyme concentrations were doubled compared to the manufacturer's  
210 instructions. Each sample was analysed twice. All values for AIS, neutral sugars, GalA and  
211 methanol were corrected by the respective starch content in each sample.

212

#### 213 *2.3.7. High performance size-exclusion chromatography coupled to multi-angle laser light* 214 *scattering (HPSEC-MALLS)*

215

216 Molar mass and size distribution of soluble pectins were determined by HPSEC-MALLS,  
217 coupled to a differential refractive index detector (Shimadzu, Tokyo, Japan). The equipment,  
218 detectors and sample preparation were the same as reported previously (Buegy et al., 2020).  
219 Serum pectins (2.5 mg AIS/mL eluent, 100 µL) were eluted at 40 °C with an acetate buffer  
220 (0.2 mol/L, pH 3.6) at a flow rate of 0.6 mL/min and separated by three PolySep-GFC  
221 columns (P3000, P5000 and P6000, 300 × 7.8 mm) and a guard column, all from  
222 Phenomenex (Le Pecq, France). Data were treated by ASTRA<sup>®</sup> software (Wyatt Technology  
223 Corporation, version 7.3.2.19 for PC) as described before (Buegy et al., 2020).

224

225 *2.4. Statistical analysis*

226

227 Fruit texture was determined on thirty apples. All other measurements were conducted once  
228 for each of the three replications, except starch determination and particle size distribution  
229 that were analysed twice for each of the three replications. At least two of three serum pectins  
230 were injected on the HPSEC-MALLS system. As the Shapiro-Wilk test indicated that not all  
231 results were normally distributed, they were compared with Kruskal-Wallis non-parametric  
232 test (Kruskal & Wallis, 1952) at the 95% level of significance. The XLSTAT package  
233 (Addinsoft, 2020) for Microsoft Excel was used. Pooled standard deviations were calculated  
234 for each series of replicated measurement using the sum of individual variances weighted by  
235 the individual degrees of freedom (Box, Hunter, & Hunter, 1978). Principal component  
236 analysis (PCA) was performed using the package “FactoMineR” (Lê, Josse, & Husson, 2008),  
237 linear regression using the package “ggplot2” (Wickham, 2016), both for R statistical  
238 software (R Core Team, 2018).

239

240 **3. Results**

241

242 *3.1. Apple texture*

243

244 Determination of apple firmness and crunchiness (Table 1) confirmed significant differences  
245 in apple texture, depending on the cultivar. GS showed the highest firmness and crunchiness  
246 values, whereas GD1 and GD2 showed the lowest values. Here, fruit thinning of GD apples  
247 did not lead to different fruit textures. BR was firm and crunchy, while BRM was firm but not  
248 crunchy, confirming acquisition of the mealy texture of BRM apples (Barreiro et al., 1998).  
249 GA revealed intermediate firmness and crunchiness values.

250

### 251 *3.2. Cell wall composition of raw apples*

252

253 Apple fruit texture might be influenced by the cell wall structure and composition (Table 1).  
254 AIS of raw apples were in a usual range of 15-27 mg/g FW (Le Bourvellec et al., 2011;  
255 Massiot & Renard, 1997; Renard, 2005), with GS showing the highest values. Except BR,  
256 with noticeably high starch contents, all cultivars were nearly starch-free. Amounts of GalA  
257 in the AIS varied between cultivars. DM were also significantly different between cultivars  
258 but all pectins were highly methylated (> 50%). The pectic RG I branching was estimated as  
259 the ratio of neutral sugars (arabinose+galactose)/rhamnose. *Since the AIS of the whole fruit*  
260 *was used to calculate this ratio, some galactose and arabinose residues of apple*  
261 *hemicelluloses xyloglucan and arabinoxylan, respectively, were included in this equation.*  
262 *However, their amounts are quite less important than arabinose and galactose of RG I side*  
263 *chains (Fügel, Carle, & Schieber, 2004), leading only to a slight overestimation of the actual*  
264 *ratio.* It was highest in GS and lowest in GA pectins. Pectins of BRM apples were slightly less  
265 branched than BR apples.

266

### 267 *3.3. Rheological characterization of apple purees*

268

269 Although the refining step generated less viscous purees, refined and not refined purees  
270 showed the same trends between cultivars and the two processes. Therefore, only not refined  
271 purees are detailed in the results section. The data of refined purees are reported in  
272 Supplementary Table S3.

273

274 The chosen cultivars and processes generated a wide range of textures (Table 2), with  
275 “cultivar” being the major parameter. The highest viscosities were obtained with GS and the  
276 lowest with GA and BRM. Purees prepared with BR apples were more viscous than purees of  
277 their mealy homologue. Purees of GD2 showed slightly higher viscosity values than GD1.  
278 Process II produced systematically slightly more viscous purees and higher  $G'$  and  $G''$ . All  
279 samples showed higher  $G'$  than  $G''$  values, corresponding to a structured viscoelastic product  
280 with soft solid-like behaviour, in accordance with another study on apple purees (Espinosa et  
281 al., 2011).

282

### 283 *3.4. Analysis of texture determinants in purees*

284

#### 285 *3.4.1. Particle size distribution*

286 Particle size was demonstrated to be the most important factor influencing puree’s texture in  
287 “real” systems (Buergy et al., 2020), i.e. without dilution or concentration of the purees. Here,  
288 particle size showed a monomodal distribution for all cultivars and both process conditions  
289 (Fig. 1).

290

291 Generally, Process I generated smaller particles. An exception was BRM. While BR showed  
292 cell clusters around 400  $\mu\text{m}$  (Process I) and 650  $\mu\text{m}$  (Process II), apple flesh of BRM was  
293 ground to individual cells around 200  $\mu\text{m}$  with both processes. Among all cultivars, GS  
294 showed the largest particles (500–800  $\mu\text{m}$ ). GD2 showed slightly larger particles than GD1.

295

#### 296 *3.4.2. Pulp wet mass and water retention capacity*

297 In “real” systems, PWM (Table 2) is considered to have a second order impact on puree’s  
298 texture (Buergy et al., 2020). It differed significantly between cultivars but not between the

299 two processes. However, purees prepared by Process II showed slightly higher PWM for all  
300 cultivars, except BRM. WRC (Table 2) was also not significantly influenced by the process.  
301 GS had notably high PWM and WRC.

302

### 303 *3.4.3. Serum viscosity, soluble pectin content and molar mass*

304 Process II increased serum viscosity and soluble pectin content in the sera of BR, BRM, GD1  
305 and GS but not of GA and GD2 (Table 2). GS purees had remarkably high serum viscosities.  
306 Serum viscosity of BR was similar to BRM for Process I but higher for Process II.

307 Molar mass and molecular size were determined for soluble pectins of Process II. GS pectins  
308 exhibited the highest molecular size (lowest elution volume) and BRM a smaller size than  
309 BR, which eluted before (Fig. 2). All other cultivars had medium sizes between BR and BRM  
310 (not shown). GS had the highest molar mass at the main peak ( $459 \times 10^3$  g/mol), whereas molar  
311 masses of BR ( $277 \times 10^3$  g/mol) and BRM ( $230 \times 10^3$  g/mol) were not significantly different.

312

## 313 **4. Discussion**

314

315 A PCA was conducted on raw apples' and purees' characteristics. The first two principal  
316 components (PC1 and PC2) explained together more than 70% of the total variance. Variables  
317 related to apple texture (firmness and crunchiness) were grouped in the correlation circle  
318 (Fig. 3A). Neither starch nor GalA contents could be correlated to fruit texture, whereas the  
319 DM showed a slightly negative correlation. Calcium crosslinks can be formed between HG  
320 pectins in the middle lamella of parenchyma cells if pectin molecules exhibit more than 10  
321 consecutive unmethyl-esterified GalA residues (Kohn & Luknár, 1977). They are associated  
322 with improved cell adhesion (Gwanpua, et al., 2016; Jarvis, Briggs, & Knox, 2003) but cannot  
323 always be linked to firmer fruits (Li, et al., 2020; Ng, et al., 2013). **Some recent findings**

324 indicate that calcium crosslinks between HG molecules limit their association with cellulose,  
325 leading to reduced cell wall mechanical properties (Lopez-Sanchez, et al., 2020). In apple,  
326 decreased RG I branching correlates with a loss of fruit texture due to decreased cell adhesion  
327 (Pena & Carpita, 2004) and their possible ability to control turgor pressure in complement to  
328 xyloglucan and cellulose (Lahaye, Bouin, Barbacci, Le Gall, & Foucat, 2018). A decrease in  
329 RG I side chains might also loosen the cell wall structure due to decreased interactions with  
330 cellulose (Zykwinska, Ralet, Garnier, & Thibault, 2005), xyloglucan (Popper & Fry, 2008)  
331 and between RG I galactan side chains (Makshakova, Gorshkova, Mikshina, Zuev, & Perez,  
332 2017). This might facilitate the access of pectinolytic enzymes to their substrates, resulting in  
333 reduced fruit firmness. RG I branching was the highest in pectins from GS (Table 1), which  
334 also had the highest apple firmness and crunchiness. Pectins of BRM apples were slightly less  
335 branched than BR apples due to a loss of arabinose and galactose side chains in BRM, also  
336 associated with mealiness in apples (Nara et al., 2001). Nevertheless, no simple relation  
337 linking RG I branching to apple texture was found, so that no correlation was detected in the  
338 PCA. Cell shape and packing (Lapsley, Escher, & Hoehn, 1992; McAtee, Hallett, Johnston, &  
339 Schaffer, 2009) as well as structural organization of plant cell wall polysaccharides and  
340 cellular water partition (Lahaye et al., 2018; Lahaye, Falourd, Laillet, & Le Gall, 2020) might  
341 also be considered to fully explain textural differences but were not analysed here.

342 Both apple firmness and crunchiness were orthogonal to rheological properties of the puree  
343 (apparent viscosity,  $G'$  and  $G''$ ) in Fig. 3A and thus varied independently, as confirmed in  
344 Fig. 3D. Bourles et al. (2009) reached the same conclusion for the texture of cooked apple  
345 slices. As an exception, GS had the firmest and crunchiest apples (Table 1) and showed the  
346 highest puree's texture (Table 2). Fruit thinning did not induce different fruit textures. GD2  
347 purees were, however, slightly more viscous than GD1, linked to particle size, while Buergy

348 et al. (2020) described higher viscosity and bigger particles for GD1 purees. The harvest year  
349 or, most probably, the late fruit thinning in this study might explain this difference.

350 Rheological properties of the puree formed a group in the PCA's correlation circle (Fig. 3A)  
351 with several factors (particle size, PWM and serum viscosity) that are known to alter puree's  
352 texture (Espinosa et al., 2011; Leverrier et al., 2016; Rao, 1992). Interestingly, RG I  
353 branching and AIS of raw apples were also part of this group, whereas WRC of the pulp was  
354 not. Correlation of particle size, PWM and serum viscosity to apparent viscosity of the purees  
355 could be confirmed with Fig. 3E-G. However, several slopes were visible, indicating that  
356 cultivars responded differently to processing. Especially GS showed particularly high particle  
357 sizes, PWM and serum viscosities, resulting in highly viscous purees.

358 Cultivars could be clearly distinguished on the sample map (Fig. 3B) and thus strongly  
359 affected puree's viscosity. Process I and II could not be differentiated on the sample map (Fig.  
360 3C), indicating limited impact of process. However, all purees prepared with Process II were  
361 slightly shifted to positive values on PC1. This was in accordance with higher viscosity,  $G'$   
362 and  $G''$ , linked to increased particle size, PWM and serum viscosity. [Studies on both apple](#)  
363 [\(Espinosa-Munoz, Symoneaux, Renard, Biau, & Cuvelier, 2012\)](#) and [carrot \(Appelqvist,](#)  
364 [Cochet-Broch, Poelman, & Day, 2015\)](#) dispersions revealed that large particles are perceived  
365 as grainy and crunchy, whereas high PWM leads to more consistent and dry textures in  
366 mouth. In addition, high serum viscosities increase the perception of consistency and reduce  
367 the sensation of graininess due to elevated pectin content. Hence, GS purees were expected to  
368 show higher consistency than other cultivars, whereas Process II might increase consistency  
369 perception for all cultivars.

370 Process II generally induced larger particles (Fig. 1) because of lower grinding speed, leading  
371 to more viscous purees. An exception was BRM. While BR purees showed cell clusters, apple  
372 flesh of BRM was ground to individual cells with both processes. Cell adhesion appeared



373 reduced in mealy apples, as even a low grinding speed was sufficient to induce tissue  
374 fragmentation during processing. Reduced cell adhesion in BRM apples might be correlated  
375 to decreased RG I branching (Table 1) as had been hypothesized before (Nara et al., 2001;  
376 Pena & Carpita, 2004). Due to smaller particles, BRM purees were less viscous than BR  
377 purees. Among all cultivars, GS showed the largest particles (Fig. 1), in accordance with the  
378 highest puree's texture. Cell adhesion in GS apples seemed particularly high, maybe due to  
379 higher RG I branching (Table 1). Initially larger cells in GS could be excluded as Buergy et  
380 al. (2020) showed similar particle sizes for individualised cells in GS and GD apples.

381 In several cultivars, pectin solubilisation was favoured by high temperatures of Process II  
382 (Table 2), as already reported in tomato and carrot purees (Lin et al., 2005; Moelants et al.,  
383 2013). This increased serum viscosity. However, soluble pectin content alone could not  
384 explain the remarkably high serum viscosities of GS. The high macromolecular size and  
385 molar mass of soluble GS pectins also contributed to viscosity increase.

386 BR showed higher serum viscosities than BRM for Process II, although the AIS content of  
387 both modalities was similar (Table 2). Molar mass was also similar but BRM pectins were  
388 smaller (Fig. 2) and thus contributed less to serum viscosity.

389 BRM purees showed similar particle sizes and similar PWM for both processes but different  
390 puree's textures. Serum viscosity, higher for Process II, coincided with the higher puree's  
391 texture for this process. Serum viscosity could thus have a significant impact on puree's  
392 texture when particle size and PWM were identical.

393

## 394 **5. Conclusions**

395

396 Apple texture could not predict rheological properties of purees, although the cultivars  
397 strongly affected puree's viscosity. Remarkably high puree's texture obtained for GS was in

398 accordance with the biggest particles, highest PWM and serum viscosity. Mealy apples  
399 showed reduced cell adhesion and were thus more susceptible to cell fragmentation during  
400 puree processing, resulting in less viscous purees. The process had a limited effect, although  
401 the high temperature-low shear process (Process II) produced more viscous purees. The lower  
402 grinding speed generated bigger particles and the higher temperature increased both the PWM  
403 and the serum viscosity because of facilitated pectin solubilisation. Combined effects of  
404 temperature regime and shear intensities could modulate puree's texture and thus mitigate  
405 poor performance of some cultivars.

406

#### 407 **Declaration of competing interest**

408

409 Declarations of interest: none.

410

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412

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421

422

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568

569 **Figure captions**

570

571 **Fig. 1.** Particle size distribution of purees issued from different apple cultivars and modalities.  
572 Continuous lines represent purees obtained by Process I (70 °C, 3000 rpm) and dashed lines  
573 purees obtained by Process II (95 °C, 400 rpm). BR: Braeburn; BRM: Braeburn, mealy; GA:  
574 Gala; GD1: Golden Delicious, reduced fruit load; GD2: Golden Delicious, high fruit load;  
575 GS: Granny Smith. One representative sample was chosen out of six replications to illustrate  
576 particle size distribution.

577

578 **Fig. 2.** Normalized chain concentration and molar mass versus elution volume of soluble  
579 pectins for GS (continuous lines), BR (dotted lines) and BRM (dashed lines) for Process II  
580 (95 °C, 400 rpm). The signal (mV) obtained by the differential refractive index detector was  
581 normalised through dividing all data points by the signal at the summit of the peak.

582

583 **Fig. 3.** Principal component analysis (PCA) (A-C) and scatterplots (D-G) of textural  
584 characteristics and cell wall composition of raw apples and rheological characteristics and  
585 texture determinants of 0.5 mm refined and not refined apple purees. Correlation circle of  
586 variables loadings on PC1 and PC2 (A). Sample maps of scores on PC1 and PC2 as a function  
587 of the cultivar (B) and the process (C). For more legibility, PCA was conducted on the mean  
588 value of three replications as the same sample patterns were obtained as for PCA with all  
589 values. All correlation ellipses correspond to the 95% confidence interval around the  
590 barycentre. Scatterplots of apple firmness (D), particle size (d 0.9) in the puree (E), pulp wet  
591 mass (F) and serum viscosity at 100 s<sup>-1</sup> (G) as a function of apparent puree viscosity at 50 s<sup>-1</sup>.  
592 The values of AIS and cell wall composition of raw apples were corrected for the starch

593 content. Raw apple's characteristics in the PCA: Firmness, Crunchiness, AIS<sub>raw apple</sub> (alcohol  
594 insoluble solids of the raw apples) Starch, GalA (galacturonic acid), DM (degree of  
595 methylation), RG I branching (ratio of neutral sugars (arabinose+galactose)/rhamnose).  
596 Puree's characteristics in the PCA:  $\eta_{app}$  (apparent viscosity of the puree at 50 s<sup>-1</sup>), G', G''  
597 (puree's storage and loss modulus, respectively, at an angular frequency of 10 rad/s), Particle  
598 size (particle size d 0.9), PWM (pulp wet mass), WRC (water retention capacity of the pulp),  
599  $\eta_{serum}$  (serum viscosity at 100 s<sup>-1</sup>), AIS<sub>serum</sub> (alcohol insoluble solids of the serum). Braeburn  
600 (triangle); Braeburn, mealy (inverted triangle); Gala (circle); Golden Delicious, reduced fruit  
601 load (square); Golden Delicious, high fruit load (lozenge); Granny Smith (pentagon). Empty  
602 symbols represent Process I (70 °C, 3000 rpm) and filled symbols represent Process II (95 °C,  
603 400 rpm).

604

605 **Table 1.** Textural characteristics and cell wall composition of raw apples depending on the  
606 cultivar and modalities (mealiness, fruit load), followed by Kruskal-Wallis *H* and *P* values.

Cultivar	Firmness (N)	Crunchiness (dimensionless)	AIS (mg/g FW)	Starch (mg/g AIS)	GalA (mg/g AIS)	DM (%)	$\frac{\text{Ara} + \text{Gal}}{\text{Rha}}$
BR	2.5	14	22	52	377	67	19
BRM	2.7	11	21	5	383	59	15
GA	2.4	12	20	4	328	76	12
GD1	1.9	11	23	3	318	82	18
GD2	2.1	11	26	3	268	82	19
GS	3.2	14	27	3	363	67	28
<i>PSD</i>	<i>0.4</i>	<i>0.9</i>	<i>0.5</i>	<i>2</i>	<i>19</i>	<i>5</i>	<i>2</i>
<i>H value</i>	<i>102</i>	<i>109</i>	<i>16</i>	<i>10</i>	<i>15</i>	<i>14</i>	<i>15</i>
<i>P value</i>	<i>&lt; 0.001</i>	<i>&lt; 0.001</i>	<i>0.007</i>	<i>0.066</i>	<i>0.012</i>	<i>0.013</i>	<i>0.011</i>

607 The ratio (Ara+Gal)/Rha estimated RG I branching and was calculated using the yields of  
608 neutral sugars arabinose (Ara), galactose (Gal) and rhamnose (Rha), expressed in mg/g AIS

609 (Supplementary Table S2). BR: Braeburn; BRM: Braeburn, mealy; GA: Gala; GD1: Golden  
 610 Delicious, reduced fruit load; GD2: Golden Delicious, high fruit load; GS: Granny Smith;  
 611 AIS: Alcohol insoluble solids; GalA: Galacturonic acid; DM: Degree of methylation; FW:  
 612 Fresh weight; PSD: Pooled standard deviation (degrees of freedom: 174 for firmness and  
 613 crunchiness (n for each cultivar = 30), 30 for starch (n for each cultivar = 6) and 12 for other  
 614 analyses (n for each cultivar = 3)).

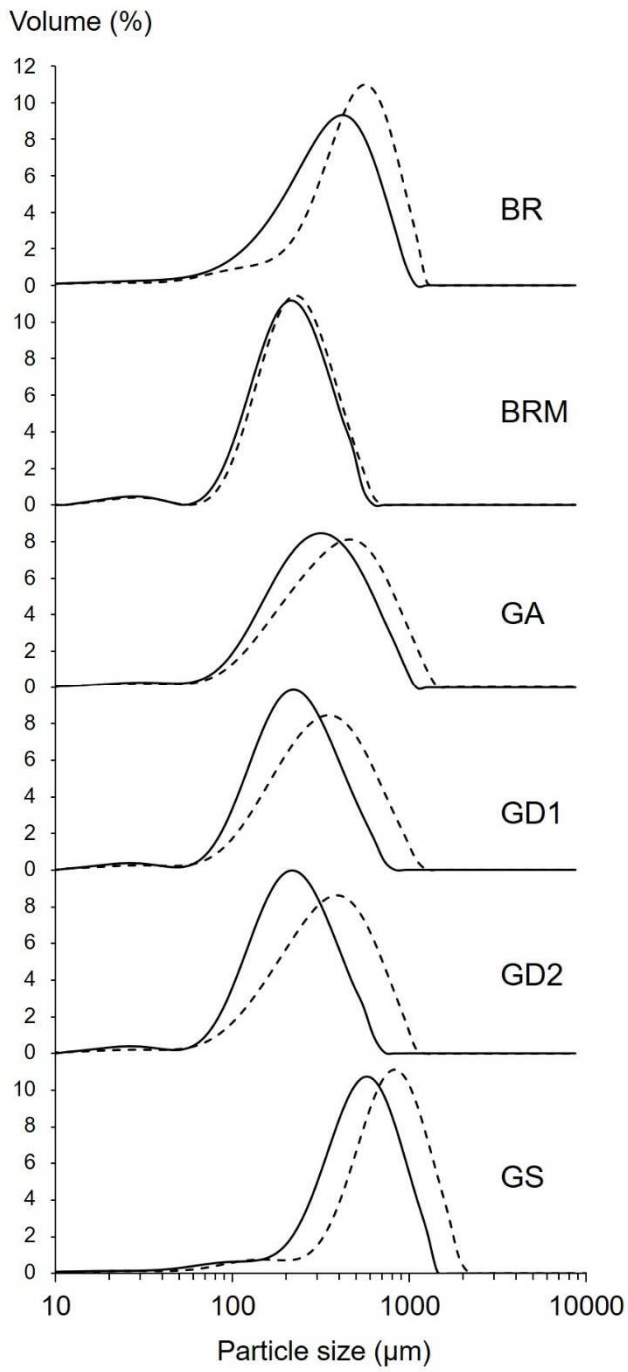
615

616 **Table 2.** Rheological characteristics and texture determinants of not refined apple purees  
 617 depending on the cultivar, modalities (mealiness, fruit load) and the process (Process I: 70 °C,  
 618 3000 rpm; Process II: 95 °C, 400 rpm), followed by Kruskal-Wallis *H* and *P* values.

Cultivar	Process	$\eta_{app} 50 \text{ s}^{-1}$ (mPa.s)	$G'$ (Pa)	$G''$ (Pa)	PWM (g/100 g)	WRC (g/g DW)	$\eta_{serum} 100 \text{ s}^{-1}$ (mPa.s)	Serum AIS (mg/g FW)
BR	I	630	1080	216	26	14	8	0.5
	II	1088	1509	323	28	12	17	0.7
BRM	I	456	965	200	29	13	5	0.3
	II	680	1373	310	28	16	9	0.8
GA	I	480	720	138	24	14	7	0.2
	II	681	934	194	27	14	10	0.1
GD1	I	632	984	191	28	15	11	0.5
	II	943	1391	291	32	12	14	2.6
GD2	I	776	1246	247	29	12	12	0.9
	II	1060	1601	342	30	11	14	1.0
GS	I	1467	1836	386	37	23	52	1.4
	II	1937	1794	543	44	17	155	3.9
<i>PSD</i>		55	121	31	1	1	3	0.2
<i>Cultivar</i>	<i>H value</i>	23	24	24	28	22	24	24
	<i>P value</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
<i>Process</i>	<i>H value</i>	10	7	8	2	2	6	4
	<i>P value</i>	0.002	0.010	0.004	0.137	0.206	0.012	0.049

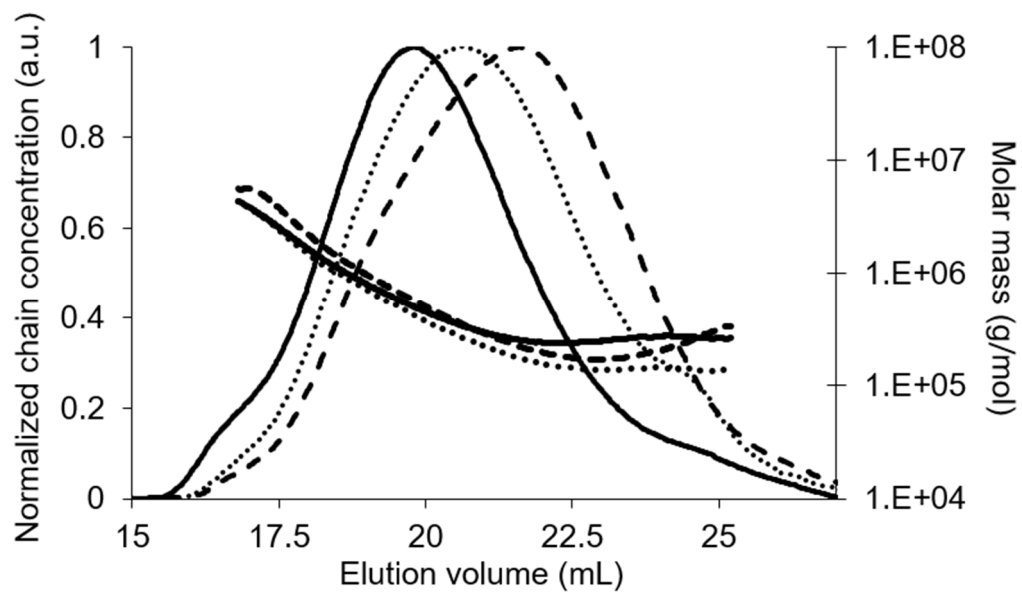
619 BR: Braeburn; BRM: Braeburn, mealy; GA: Gala; GD1: Golden Delicious, reduced fruit  
620 load; GD2: Golden Delicious, high fruit load; GS: Granny Smith;  $\eta_{app}$ : Apparent viscosity at  
621  $50 \text{ s}^{-1}$ ;  $G'$ ,  $G''$ : Storage and loss modulus, respectively, at an angular frequency of  $10 \text{ rad/s}$ ;  
622 PWM: Pulp wet mass; WRC: Water retention capacity of the pulp;  $\eta_{serum}$ : Serum viscosity at  
623  $100 \text{ s}^{-1}$ ; AIS: Alcohol insoluble solids; DW: Dry weight; FW: Fresh weight; PSD: Pooled  
624 standard deviation (degrees of freedom: 12 (n for each cultivar = 3)).

625



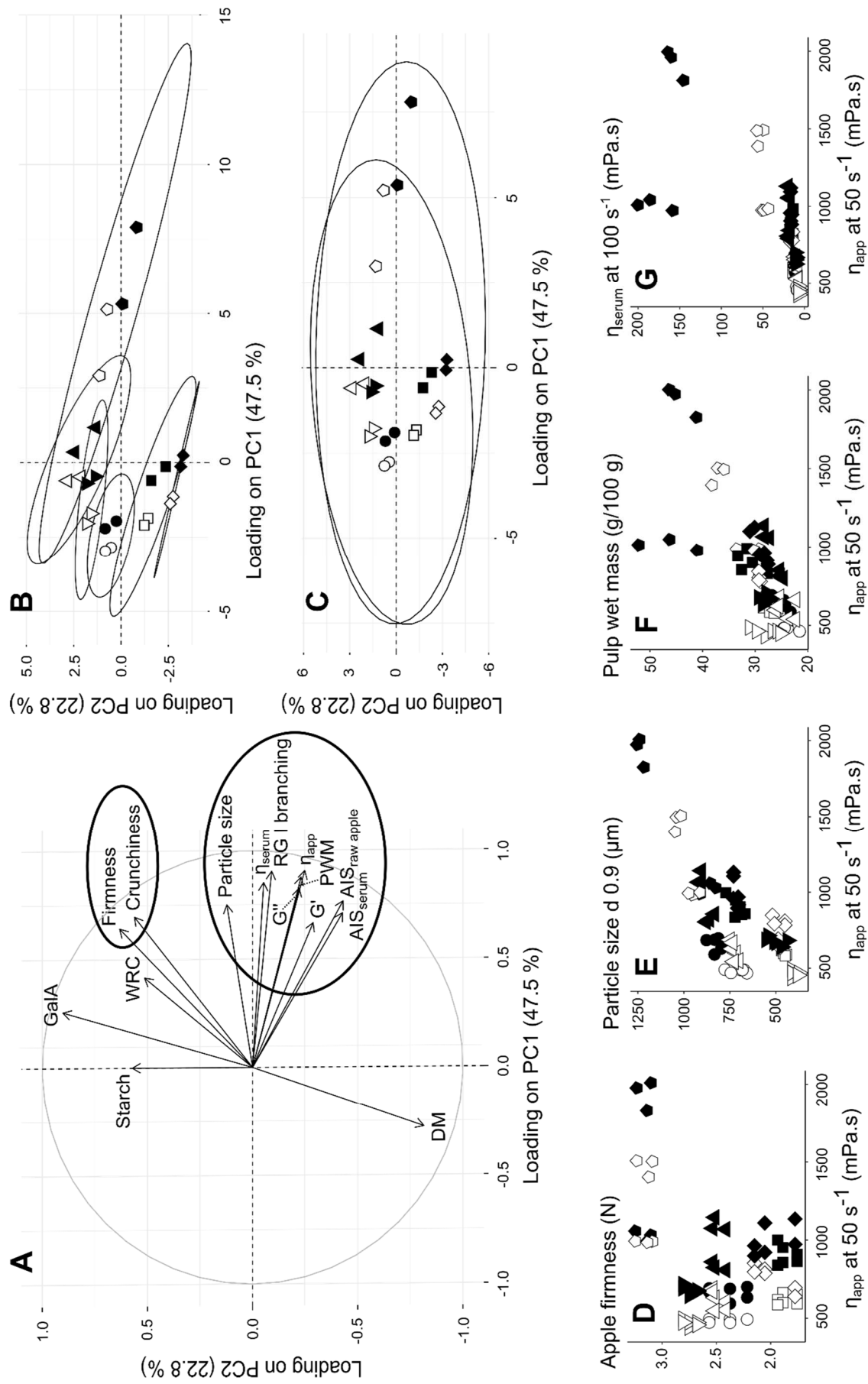
626

627 **Fig. 1**



628

629 **Fig. 2**



630

631 **Fig. 3**