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1 **Pectin degradation accounts for apple tissue fragmentation during thermomechanical-**  
2 **mediated puree production**

3  
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16

17 **Abstract**

18 The relationship between fruit and puree's characteristics is still poorly understood. In  
19 particular, it is not understood how pectin solubilisation and degradation alter the texture of  
20 plant-cell dispersions and how a targeted application of processing conditions can be used to  
21 design naturally textured food products. Systematic combinations of thermal and mechanical  
22 treatments with three different temperatures (70, 83, 95 °C) and grinding speeds (300, 1000,  
23 3000 rpm), applied on one-month (T1) and six-months stored (T6) apples, were used to  
24 generate apple purees with contrasted structural and textural characteristics. For T1, serum  
25 viscosity increased with increasing temperature (8 to 104 mPa.s) with a marked increase in  
26 pectin solubilisation (1 to 6 mg/g serum). Pectin macromolecular size and

27 (arabinose+galactose)/rhamnose ratio, estimating pectin side chain branching, decreased with  
28 temperature. For T6, pectin showed decreased galactose, leading to facilitated cell separation,  
29 low serum viscosity (~16 mPa.s) and restricted impact of process conditions on pectin  
30 composition and structure. Grinding had limited impact on pectin solubilisation for T1 and T6  
31 but strongly impacted particle size (498–1096  $\mu\text{m}$  for T1 or 320–1068  $\mu\text{m}$  for T6) and puree's  
32 viscosity (871–1475 mPa.s for T1 or 853–1453 mPa.s for T6). Tissue fragmentation was  
33 favoured by temperature increase for T1 and by the maturation of raw apples. Process  
34 parameters induced differences in the puree's structure and texture depending on the  
35 maturation level of raw apples. The observed changes were linked to pectin degradation and  
36 substantial side chain loss.

37

38 *Malus x domestica* Borkh.; Texture; Rheology; Particle size; Cell separation; Polysaccharide

39

## 40 **1. Introduction**

41

42 Plant-based purees are suspensions of individual cells and cell clusters (pulp) dispersed in an  
43 aqueous phase (serum) (Rao, 1992). The texture is an important quality characteristic of plant-  
44 based dispersions (Szczesniak & Kahn, 1971) driven by particle size distribution, pulp content  
45 and serum viscosity (Espinosa et al., 2011; Leverrier, Almeida, Espinosa-Munoz, & Cuvelier,  
46 2016; Rao, 1992). These factors can be modulated by applying particular thermal and  
47 mechanical treatments on raw material in order to optimise pureed food's textural  
48 characteristics without the addition of texture-controlling agents such as starches, gums, and  
49 stabilisers. Many studies focused either on the effect of thermal (Anthon, Diaz, & Barrett,  
50 2008; Christiaens et al., 2012) or mechanical (Espinosa et al., 2011; Moelants et al., 2012;  
51 Moelants et al., 2014) treatments. However, both heating and grinding are complementary  
52 during processing since both treatments alter the puree's structure.

53 The structural characteristics are linked to modifications in pectin structure and composition  
54 (Sila et al., 2009). Pectins contribute to intercellular adhesion and cell wall porosity and  
55 strength (Carpita & Gibeaut, 1993; Jarvis, 1984). However, these plant cell wall  
56 polysaccharides are especially vulnerable to enzymatic and chemical degradation. Indeed,  
57 pectin solubilisation during processing induces tissue softening during processing (Van  
58 Buren, 1979), thus facilitating subsequent tissue disruption via mechanical treatments.

59 Pectins are complex groups of polysaccharides, comprising homogalacturonan (HG),  
60 rhamnogalacturonan I (RG I) and II (RG II). HG is composed of a linear chain of  $\alpha$ -1,4-linked  
61 galacturonic acids, which can be methyl-esterified at the C6 position. The degree of  
62 methylation (DM) and the distribution of ester groups over the HG backbone are crucial since  
63 sequences of several consecutive non-esterified galacturonic acids can induce cross-links with  
64 calcium ions (Kohn & Luknár, 1977). This mechanism may contribute to cell-cell adhesion in  
65 the middle lamella. Endogenous pectin methylesterase (PME) can demethoxylate the HG  
66 backbone, which can be cross-linked by calcium ions and cause an increase in cell adhesion  
67 and texture (Waldron, Smith, Parr, Ng, & Parker, 1997) or be further degraded by  
68 polygalacturonase (PG) (Sila et al., 2009), resulting in texture loss. While enzyme activity is  
69 rapidly inactivated by heat, chemical reactions are generally promoted. Pectins are susceptible  
70 to acid hydrolysis due to the acidic pH of apples (around 3.7) and other fruits. On the other  
71 hand,  $\beta$ -elimination is less critical during fruit-based food production (Waldron, Parker, &  
72 Smith, 2003).

73 Although the most studied changes are reported for HG pectins, RG I domains are also  
74 sensitive to heat treatment. RG I consists of a backbone with alternating rhamnose and  
75 galacturonic acid residues. Rhamnose residues can be decorated by neutral sugar side chains  
76 composed of galactose and/or arabinose (Ridley, O'Neill, & Mohnen, 2001). Arabinans are  
77 particularly susceptible to acid hydrolysis, followed by galactans (Green, 1967; Thibault,  
78 Renard, Axelos, Roger, & Crepeau, 1993). Interestingly, "debranching" (i.e., loss of arabinans

79 and galactans) was associated with attenuated cell adhesion in apple fruits (Nara, Kato, &  
80 Motomura, 2001; Pena & Carpita, 2004). Additionally, RG II, which accounts for less than  
81 10% of the pectin content, has a galacturonic acid backbone with several complex  
82 oligosaccharide side chains attached to it (Mohnen, 2008; Ndeh et al., 2017).  
83 Only a few studies have systematically analysed the combined effect of thermal and  
84 mechanical processes on puree's texture (Day, Xu, Oiseth, Lundin, & Hemar, 2010; Lopez-  
85 Sanchez et al., 2011). Furthermore, no extensive studies have investigated the consequences  
86 of pectin degradation (thermal degradation) on mechanical processing. However,  
87 modifications in pectin composition and structure are crucial in understanding the impact of  
88 processing on plant-based food structure and texture. Herein, we evaluated particle size, pulp  
89 wet mass and serum viscosity following the application of different temperature and grinding  
90 regimes and elucidated the process-structure-function relationship of pectins and their  
91 influence on apple puree texture. As pectin modifications in the raw material linked to post-  
92 harvest storage alter puree's texture (Buegy, Rolland-Sabaté, Leca, & Renard, 2020), the  
93 impact of processing conditions was compared between apples stored for one and six months.

94

## 95 **2. Material and Methods**

96

### 97 *2.1. Plant material*

98

99 Apple (*Malus x domestica* Borkh.) cultivar Golden Delicious was grown in Mallemort,  
100 France and harvested in September 2018 and 2019, corresponding to the commercial harvest  
101 dates. In 2018, apples were chemically thinned using 3 L/ha PRM 12® RP (ethephon, 17  
102 April 2018), 2 L/ha PRM 12® RP (24 April 2018), 3.5 L/ha MaxCel® (6-  
103 Benzylaminopurine) and 1.5 kg/ha Rhodofix® (1-naphthalenacetic acid, 30 April 2018) and  
104 3.5 L/ha MaxCel® (4 May 2018). In 2019, apples were thinned with 18 L/ha ATS

105 (ammonium thiosulphate, 20 April 2019), 0.15 kg/hL Rhodofix® (27 April 2019) and 5 L/ha  
106 MaxCel® (4 May 2019).

107 Apples harvested in 2018 and 2019 were stored for six months (T6) and one month (T1),  
108 respectively, at 4 °C in a normal atmosphere. One day before processing, apples were divided  
109 into two equal groups. The first batch was used to isolate the alcohol-insoluble solids (AIS)  
110 (see section 2.4.1), the second batch was processed into a puree.

111

## 112 *2.2. Puree preparation*

113

114 Approximately 3 kg of apples were cored, cut into 12 pieces and processed under vacuum  
115 using a cooker-cutter (RoboQbo Qb8-3, RoboQbo, Bentivoglio, Italy), equipped with two  
116 micro-serrated cutting blades and a mixing blade. T1 and T6 apples were processed under  
117 identical conditions (Supplementary Fig. S1). Each temperature (70, 83, 95 °C) was combined  
118 with a blade rotation speed (300, 1000, 3000 rpm), resulting in nine different treatments. Each  
119 process was conducted in triplicate, and each repetition was spaced by one week due to the  
120 time needed for sample characterisation.

121 Since the temperature increase was longer for higher temperatures (difference of 140 s  
122 between 70 and 95 °C), a low blade rotation speed of 100 rpm was applied during this period.

123 This step was employed to mix the apples without grinding them. Upon reaching the working  
124 temperature, we increased the blade rotation speed and maintained this speed for 30 min. **The**  
125 **heating effects caused by blending were counteracted by the cooling system of the cooker-**  
126 **cutter, guaranteeing a constant value ( $\pm 1$  °C) around the indicated process temperature.**

127 Purees were not refined, and physicochemical characterisations were carried out directly after  
128 processing once the purees reached room temperature. Analytical measurements were  
129 conducted on thawed samples previously stored at -20 °C.

130

131 *2.3. Physico-chemical characterisation*

132

133 *2.3.1. Rheology of the purees and sera*

134 Rheological analyses of both purees and sera were conducted at 22.5 °C as reported  
135 previously (Buergy et al., 2020), a stress-controlled rheometer (Physica MCR301) equipped  
136 with a Peltier cell (CPTD-200) and a measuring cylinder (CC27/S) from Anton Paar (Graz,  
137 Austria). For purees, the flow curve and amplitude sweep were measured using a vane  
138 measuring system (FL100/6W). Rheological analyses were not theoretically adapted for  
139 purees containing particles larger than 1 mm, since the measuring system gap was 3.46 mm.  
140 Thus, rheological values obtained for purees prepared at 70 and 83 °C and 300 rpm should be  
141 treated cautiously.

142 Serum viscosity was measured with a flow curve using a double gap cylinder geometry set  
143 (DG27).

144

145 *2.3.2. Particle size distribution*

146 Laser granulometry (Mastersizer 2000, Malvern Instruments, Malvern, UK) was employed to  
147 measure the particle size distribution in the puree as described by Buergy et al. (2020). The  
148 Malvern's software was used to calculate the averaged size distribution over three repeated  
149 measurements on the same sample. Each puree was analysed twice.

150

151 *2.3.3. Pulp wet mass (PWM) and water retention capacity (WRC)*

152 **Each puree (3 repetitions × 9 processes) was** centrifuged (7690 × g, 15 min at 15 °C) and  
153 separated into pulp and serum. The ratio of the pulp weight to the initial weight of the puree  
154 was defined as the PWM and expressed in g/100 g (Espinosa et al., 2011). The WRC of the  
155 pulp was defined as the amount of water retained by the pulp's cell wall polysaccharides and  
156 expressed in g/g dry weight (Robertson et al., 2000). The ratio of the PWM to the pulp's dry

157 weight was estimated after AIS isolation. The WRC was only analysed for samples processed  
158 at 3000 rpm since these purees represented individual cells. One sample at each temperature  
159 was measured, both for T1 and T6.

160

## 161 *2.4. Analytical*

162

### 163 *2.4.1. Cell wall isolation and serum precipitation*

164 Cell wall polysaccharides were extracted as AIS, using the protocol established by Le  
165 Bourvellec et al. (2011). This method involves adding 700 mL of ethanol per litre of raw  
166 apples to extract the AIS. **Three replicates using 10 representative apples** were performed, and  
167 AIS yields were expressed in mg/g fresh weight (FW).

168 The pulp was washed with water and the AIS were isolated as previously described by Buergy  
169 et al. (2020). The pulp's AIS yield was calculated by dividing the dry pulp weight by the  
170 initial pulp weight after water-washing and expressed as mg/g FW.

171 The serum AIS were isolated by alcohol precipitation according to the method of Buergy et  
172 al. (2020). The dry sample weight and the initial serum weight ratio provides a rough estimate  
173 of the soluble pectin content in the continuous phase. Serum AIS yields are expressed in mg/g  
174 serum FW. Since the present study focused on apples stored for one month, the AIS extraction  
175 was conducted with all three T1 replicates, but only one T6 sample.

176

### 177 *2.4.2. Cell wall polysaccharide analysis*

178 After the acid hydrolysis of neutral sugars and the internal standard myo-inositol (Saeman,  
179 Moore, Mitchell, & Millett, 1954), the free sugars were derivatised to volatile alditol acetates  
180 (Englyst, Wiggins, & Cummings, 1982). Then the samples were applied to a Clarus 500 gas  
181 chromatograph (PerkinElmer, Waltham, USA) equipped with a flame ionisation detector  
182 (FID) and an OPTIMA® capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness,



183 Macherey-Nagel, Düren, Germany), and analysed at 230 °C. Helium was used as the carrier  
184 gas.

185 Galacturonic acid (GalA) content in the acid hydrolysate was determined  
186 spectrophotometrically using the *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen,  
187 1973).

188 Methanol was quantified using the stable isotope dilution assay after saponification (Renard  
189 & Ginies, 2009). Samples were analysed on a Trace 1300 gas chromatograph (Thermo  
190 Scientific, Waltham, USA), equipped with a TG-WaxMS capillary column (30 m × 0.25 mm  
191 i.d., 0.5 µm film thickness, Thermo Scientific, Waltham, USA) and coupled to a ISQ LT  
192 single quadrupole mass spectrometer (Thermo Scientific, Waltham, USA).

193 The molar ratio of methanol to GalA gave the degree of methylation (DM) is expressed as a  
194 percentage (%).

195

#### 196 *2.4.3. Starch determination*

197 We used the total starch assay kit K-TSTA (Megazyme, Wicklow, Ireland) to quantify the  
198 starch content in the AIS of serum and raw apples. The specified enzyme concentrations were  
199 doubled since residual polyphenols in the AIS might reduce enzyme activity. Each sample  
200 was analysed in duplicate. All values for AIS, neutral sugars, GalA and methanol were  
201 adjusted according to the respective starch content.

202

#### 203 *2.4.4. High-performance size-exclusion chromatography coupled to multi-angle laser light* 204 *scattering (HPSEC-MALLS) and online viscometry*

205 As described by Buergy et al. (2020), the molar mass and size distribution of soluble pectins  
206 were determined on a high-performance size-exclusion chromatography (HPSEC) system  
207 coupled to a multi-angle laser light scattering detector (DAWN HELEOS 8+ (Wyatt  
208 Technology, Santa Barbara, USA) fitted with a K5 flow cell and a GaAs laser,  $\lambda = 660$  nm), a

209 differential refractive index detector (Shimadzu, Tokyo, Japan), an online viscometer  
210 (Viscostar III, Wyatt Technology) and a prominence diode array detector (DAD). Initial  
211 concentrations of 2.5 mg AIS/mL (serum) or 10 mg AIS/mL (raw apples) were used to extract  
212 soluble pectins directly into the eluent (acetate buffer, 0.2 M, pH 3.6). Samples were eluted at  
213 a flow rate of 0.6 mL/min at 40 °C and separated by three PolySep-GFC columns (P3000,  
214 P5000 and P6000, 300 × 7.8 mm) and a guard column, all from Phenomenex (Le Pecq,  
215 France). The ASTRA® software (Wyatt Technology, version 7.3.2.19 for PC) was used for  
216 data treatment as described by Buergy et al. (2020). The weight-average molar mass  $\bar{M}_w$ , the  
217 z-average intrinsic viscosity  $[\eta]_z$  and the z-average viscometric hydrodynamic radius  $\bar{R}_{hz}(v)$   
218 were obtained at the summit of the main peak. Each sample was injected once. For T1,  
219 samples of the first repetition were not considered since molar mass distribution was  
220 significantly different from the other repetitions. This discrepancy was likely due to  
221 incomplete starch degradation in these samples.

222

#### 223 2.4.5. Nuclear magnetic resonance spectroscopy (NMR)

224 <sup>1</sup>H-NMR was employed to visualise β-elimination products in the serum AIS and was  
225 performed according to Tjan, Voragen, and Pilnik (1974). At 4 °C, AIS (5 mg/mL) were  
226 solubilised for 1 h in distilled water before adjusting the pH to 6 with NaOH. The samples  
227 were centrifuged (4272 × g, 15 min at 4 °C) to remove possible cell wall remnants. The  
228 supernatant was freeze-dried before the sample was suspended in D<sub>2</sub>O (0.75 mL) over night at  
229 4 °C. The following day, the sample was freeze-dried and suspended in D<sub>2</sub>O as before. The  
230 samples obtained were analysed at all of the temperatures (3000 rpm, T1). As a positive  
231 control, serum AIS, obtained at 95 °C, 3000 rpm and diluted to 5 mg/mL with distilled water,  
232 was heated for 30 min at 100 °C at pH 6. Afterwards, the control sample's pH was readjusted  
233 to pH 6 and treated in the same way as the samples. Samples and the control were analysed in  
234 D<sub>2</sub>O on an NMR spectrometer (Avance III 400 NB, Bruker, Wissembourg, France) equipped

235 with a BBO 5 mm probe. We recorded 1D  $^1\text{H}$  spectra at 70 °C. The most significant  
236 acquisition parameters included a  $^1\text{H}$  90° pulse of 10.2  $\mu\text{s}$ , 128 scans and a recycle delay of 10  
237 s. Water signal presaturation was used to minimise the residual HDO signal.  
238 NMR relaxation was used to monitor the interactions between the  $^1\text{H}$  of water and the AIS in  
239 samples ground at 3000 rpm for all temperatures. These interactions can be related to the  
240 porosity of the system. For these experiments, samples from both T1 and T6 were utilised  
241 since they all presented individual cells.  
242 Transverse relaxation ( $T_2$ ) of pulp AIS (100 mg) rehydrated in distilled water and excess  
243 water gently removed, was measured at 4 °C on a Bruker Minispec mq20 (0.47 T), equipped  
244 with a thermostated  $^1\text{H}$  probe, using the Carr-Purcell-Meiboom-Gill pulse sequence. Echo  
245 time was 1 ms, 2000 even echoes were collected, and 128 scans were acquired with a recycle  
246 delay of 7 s. An inverse Laplace transformation (ILT) was applied to convert the relaxation  
247 signals into a continuous distribution of the  $T_2$  relaxation components (Lahaye, Bouin,  
248 Barbacci, Le Gall, & Foucat, 2018; Saunders, Bunggyoo, Maes, Akle, & Zahr, 2012).

249

## 250 *2.5. Statistical analysis*

251

252 The technological repetitions were spaced by one week, and a complete protocol (temperature  
253  $\times$  grinding speed) was carried out each week. Analyses were generally conducted once for  
254 each of the three repetitions. Exceptions are stated in the text. The Shapiro-Wilk test was used  
255 to determine if the results were normally distributed. The Kruskal-Wallis non-parametric test  
256 (Kruskal & Wallis, 1952) was employed to assess differences between the samples at the 95%  
257 level of significance, using the Microsoft Excel XLSTAT package (Addinsoft, 2020). Pooled  
258 standard deviations (PSD) were calculated for each series of repeated measurements using the  
259 sum of individual variances weighted by the individual degrees of freedom (Box, Hunter, &  
260 Hunter, 1978). The R statistical software (R Core Team, 2018) was used to perform principal

261 component analysis (PCA) and linear regression, using the "FactoMineR" (Lê, Josse, &  
262 Husson, 2008) and "stats" packages included in the R statistical software, respectively.

263

### 264 **3. Results and discussion**

265

266 In a preliminary test (data not shown), purees were prepared at 50 and 70 °C and yielded  
267 purees with equivalent textures. It was concluded that the apple enzymes did not sufficiently  
268 degrade pectins during processing. Indeed, PG is only present in minimal amounts in apples  
269 (Wu, Szakacsdoenzi, Hemmat, & Hrazdina, 1993) and is unlikely to impact apple puree's  
270 texture. Additionally, apple PME has low activity at pH 3.7 and a D-value of 0.7 min at 60°C  
271 ( $Z = 9.2^\circ$ ) (Denes, Baron, & Drilleau, 2000), which means that it is inactivated during the  
272 come-up time, and at least partially accounts for the unaltered textures of the purees in the  
273 preliminary test. Notably, 70 °C was the lowest processing temperature at which we could  
274 unequivocally evaluate the impact of the chemical reactions on apple purees. Moreover, since  
275 the heat increase speed was identical for all samples during puree processing, PME would  
276 have been able to demethylate pectins equally in all purees.

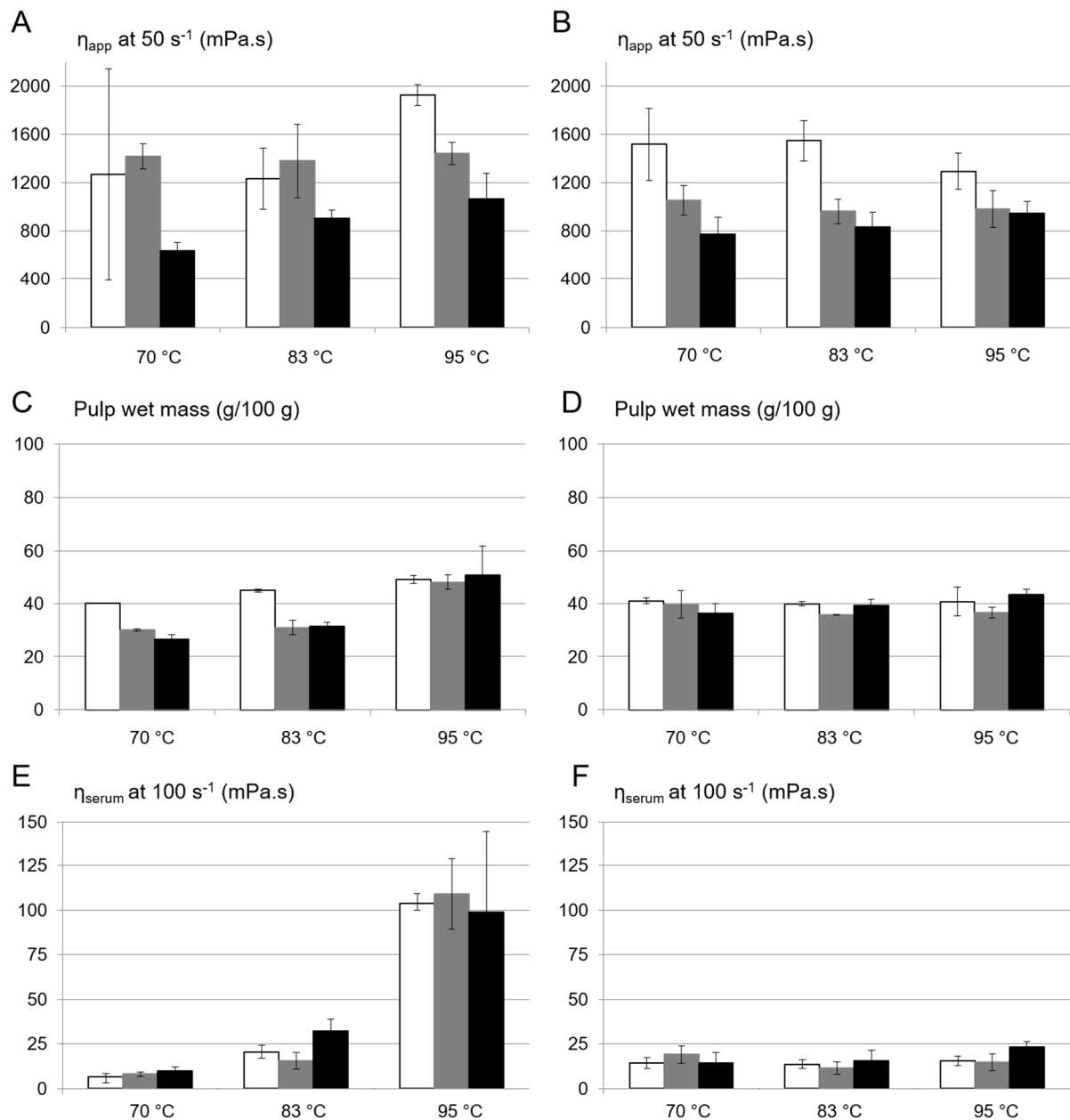
277

#### 278 *3.1. Textural characteristics of apple purees*

279

280 Different process conditions led to contrasted textural characteristics of apple purees.  
281 Apparent viscosity (Fig. 1A) obtained at 70 °C and 300 rpm had a high standard deviation.  
282 This result was due to large apple cell clusters (Supplementary Fig. S2) that were not  
283 sufficiently separated by slow grinding. Consequently, the altered rheological values for the  
284 purees obtained under these conditions have limited reliability. When these results were not  
285 considered, the data show that increasing the grinding speed significantly reduced the puree's  
286 viscosity (Supplementary Table S1). While temperature did not have a significant impact,

287 high temperatures increased viscosity, especially at 3000 rpm. Other textural characteristics  
288 such as yield stress,  $G'$  and  $G''$  (Supplementary Table S1) were also significantly attenuated  
289 with increased grinding but were not influenced by temperature. The same trend was observed  
290 for T6 (Supplementary Table S1), and the effect of grinding on apparent viscosity was more  
291 pronounced since even slow grinding speeds led to homogenous puree textures (Fig. 1B).  
292 Hence, the range of apparent viscosity values was slightly reduced compared to T1. However,  
293 the storage duration did not significantly alter textural characteristics in this experiment.  
294



295  
 296 **Fig. 1.** Apparent puree viscosity at 50 s<sup>-1</sup> (A, B), pulp wet mass (C, D) and serum viscosity at  
 297 100 s<sup>-1</sup> (E, F) for purees obtained with different temperatures and grinding speeds for T1 (A,  
 298 C, E) and T6 (B, D, F). White bars represent purees ground at 300 rpm, grey bars purees  
 299 ground at 1000 rpm and black bars purees ground at 3000 rpm.

300

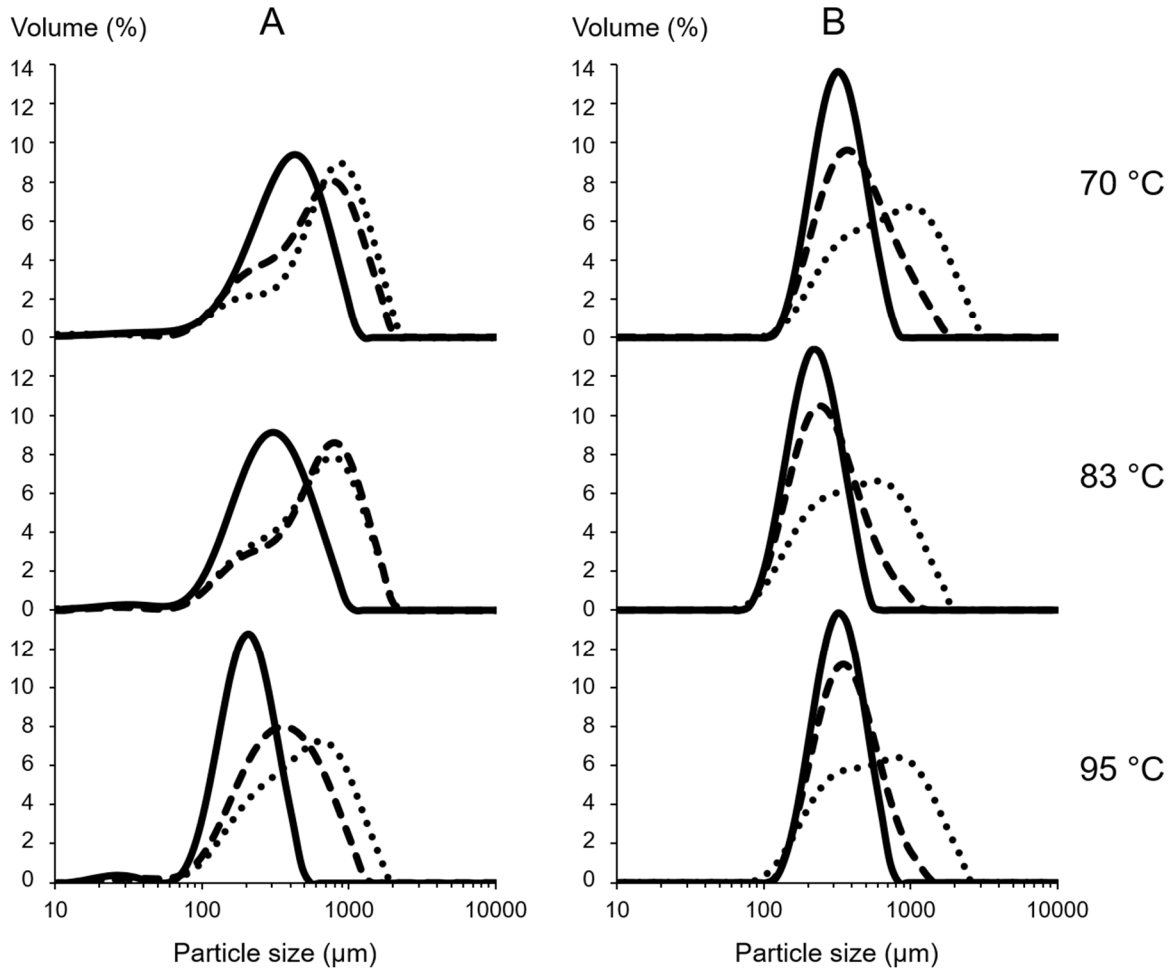
### 301 3.2. Determinants of apple puree's texture

302

#### 303 3.2.1. Particle size distribution

304 For T1 and T6, particle size decreased with increased grinding speed (Fig. 2). This  
305 observation was correlated with puree's viscosity (Fig. 3A), which also decreased with  
306 grinding.

307



308

309 **Fig. 2.** Particle size distribution of purees obtained with different temperatures and grinding  
310 speeds for T1 (A) and T6 (B). Dotted lines represent purees ground at 300 rpm, dashed lines  
311 purees ground at 1000 rpm, and continuous lines purees ground at 3000 rpm.

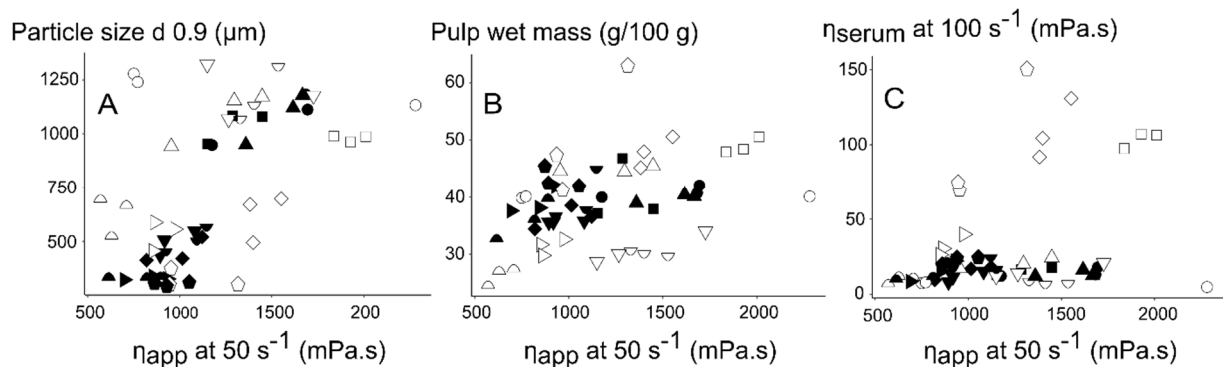
312

313 For T1, particle size was also influenced by temperature (Fig. 2A). Purees prepared at low  
314 grinding speeds (300 and 1000 rpm) and moderate temperatures (70 and 83 °C) showed a  
315 bimodal distribution with the main peak around 950 µm and a shoulder, indicative of smaller  
316 particles. After grinding at 3000 rpm, particles showed a monomodal distribution around 480

317  $\mu\text{m}$  at  $70\text{ }^\circ\text{C}$  and  $360\text{ }\mu\text{m}$  at  $83\text{ }^\circ\text{C}$ . Particle size distributions for purees heated at  $95\text{ }^\circ\text{C}$  were  
 318 more narrow and exhibited smaller sizes at all grinding speeds:  $630\text{ }\mu\text{m}$ ,  $315\text{ }\mu\text{m}$  and  $210\text{ }\mu\text{m}$   
 319 for purees ground at 300, 1000 and 3000 rpm, respectively. Since apple cells are around  $200\text{ }\mu\text{m}$   
 320  $\mu\text{m}$  in size (Khan & Vincent, 1990; Leverrier et al., 2016), only purees produced at  $95\text{ }^\circ\text{C}$  and  
 321 3000 rpm led to individualised cells for T1. Although these purees showed the smallest  
 322 particle sizes, they were more viscous than purees prepared at lower temperatures, even at the  
 323 same grinding speed (Fig. 1A). In this sense, grinding alone cannot account for the puree's  
 324 texture.

325 For T6 (Fig. 2B), particle size depended only on grinding speed (Supplementary Table S1).  
 326 Whereas purees ground at 300 rpm showed a prominent peak with a small shoulder, purees  
 327 prepared with grinding speeds of 1000 and 3000 rpm presented large quantities of individual  
 328 cells at all temperatures tested. Even though the same procedures produced smaller particles  
 329 than T1, this did not always result in less viscous purees (Fig. 1B).

330



331

332 **Fig. 3.** Scatterplots of particle size ( $d_{0.9}$ ) in the puree (A), pulp wet mass (B) and serum  
 333 viscosity at  $100\text{ s}^{-1}$  (C) as a function of apparent puree viscosity at  $50\text{ s}^{-1}$ . Empty symbols  
 334 represent samples for T1 and filled symbols samples for T6.  $70\text{ }^\circ\text{C}$ , 300 rpm (circle);  $70\text{ }^\circ\text{C}$ ,  
 335 1000 rpm (half-circle downwards);  $70\text{ }^\circ\text{C}$ , 3000 rpm (half-circle upwards);  $83\text{ }^\circ\text{C}$ , 300 rpm  
 336 (triangle);  $83\text{ }^\circ\text{C}$ , 1000 rpm (triangle with the apex pointing downwards);  $83\text{ }^\circ\text{C}$ , 3000 rpm



337 (triangle with the apex pointing to the right); 95 °C, 300 rpm (square); 95 °C, 1000 rpm  
338 (diamond); 95 °C, 3000 rpm (pentagon).

339

### 340 3.2.2. *Pulp wet mass, water retention capacity and cell wall porosity*

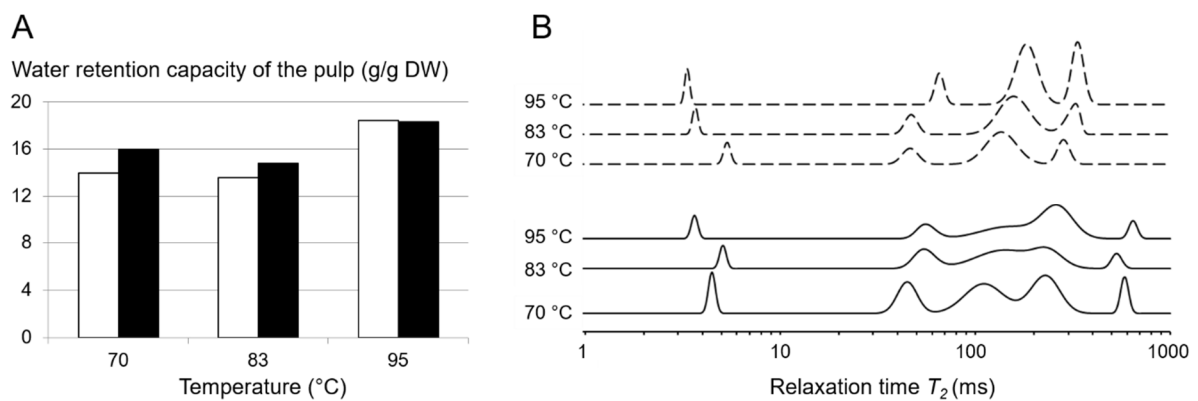
341 PWM for T1 (Fig. 1C) was similar for purees obtained at 70 and 83 °C. On the other hand,  
342 this value was significantly increased in purees prepared at 95 °C. Since the pulp's WRC  
343 (Fig. 4A) were also higher for purees heated at 95 °C, higher temperatures probably increased  
344 cell wall degradation, suggesting that the cell wall was retaining water. A similar observation  
345 was reported previously with heat-treated dietary fibres (Guillon, Barry, & Thibault, 1992)  
346 and apple cell wall material (Müller & Kunzek, 1998).

347 Grinding speed did not induce significant differences (Supplementary Table S1). The higher  
348 PWM of the purees obtained at 300 rpm (70 and 83 °C) resulted from intact cell clusters,  
349 which did not pack during centrifugation. These cell clusters are more rigid and occupy more  
350 volume (Leverrier et al., 2016; Lopez-Sanchez, Chapara, Schumm, & Farr, 2012).

351 For T6, the temperature did not affect the PWM values (Fig. 1D). It is plausible that the fruit  
352 maturation damaged the cell wall structure, limiting the impact of temperature. Grinding  
353 showed a slightly significant impact on PWM for T6 but no trend could be observed. Overall,  
354 the PWM was not significantly altered with storage. However, the PWM of aged cells seemed  
355 to be higher when moderate temperatures (70 and 83 °C) and higher grinding speeds (1000  
356 and 3000 rpm) were applied. The results obtained under these conditions were similar to T1  
357 subjected to low grinding (300 rpm) or high temperature (95 °C). This result is supported by  
358 the pulp's WRC (Fig. 4A) and could be explained by cell walls' porosity.

359 The  $T_2$  relaxation times (Fig. 4B) showed water mobility distribution in the rehydrated pulp  
360 AIS. For these types of experiments, longer  $T_2$  relaxation values are indicative of greater  
361 water mobility. This relationship can be roughly linked to the meso-porosity of the material  
362 since an increase in  $T_2$  relaxation times involve, among others, an increase in average pore

363 size (Meng & Ragauskas, 2014). For T1, five  $T_2$  relaxation domains were identified. In  
 364 contrast, only four domains were detected in T6. These domains were slightly more narrow,  
 365 particularly at the two highest temperatures, including a relative T6 sample "homogenisation".  
 366 The slowest water mobility centred on a  $T_2$  value of about 4 ms, corresponding to pore sizes  
 367 ranging between 5 and 7.5 nm (Barron et al., 2021), could be associated with water molecules  
 368 involved in strong interaction with macromolecules. These values tended to decrease with  
 369 increased temperature, indicating reduced pore size with higher temperatures. Environments  
 370 with higher relaxation times (45–340 ms) all tended to increase with temperature. This  
 371 observation is partly due to an increase in pore size of 15-120 nm with temperature and  
 372 accounts for augmented WRC and PWM values. In general, higher  $T_2$  relaxation times were  
 373 observed in aged cells, indicating more porous cell walls and higher PWM. The exact values  
 374 are presented in Supplementary Table S2.



375  
 376 **Fig. 4.** Water retention capacity of the pulp (A) and relaxation times  $T_2$  (B) for purees  
 377 obtained at different temperatures and ground at 3000 rpm for T1 (white bars or continuous  
 378 lines) and T6 (black bars or dashed lines).

379  
 380 Similar results were obtained with fresh purees (data not shown). However, other  
 381 measurements, such as Simons' stain or NMR cryoporometry (Meng & Ragauskas, 2014),  
 382 should be conducted to confirm the impact of temperature and storage on cell wall porosity.

383 Although the T1 purees prepared at higher temperatures exhibited smaller particle sizes, the  
384 purees were slightly more viscous (Fig. 1A). This result was probably due to the higher PWM  
385 values being correlated with more viscous purees (Fig. 3B). Additionally, while the T6  
386 particles were significantly smaller (Supplementary Table S1), the purees' textural  
387 characteristics (apparent viscosity, yield stress,  $G'$  and  $G''$ ) were similar for T1 and T6. The  
388 higher PWM values in the older apples may have a smoothing effect on particle size. These  
389 results demonstrate the complex interactions between different texture determinants.  
390 Furthermore, altering these interactions by mechanical or thermal treatments can influence the  
391 puree texture.

392

### 393 3.2.3. Serum viscosity

394 Serum viscosity was significantly altered by temperature increase for T1 (Fig. 1E) but not for  
395 T6 (Fig. 1F). This effect was especially apparent for T1, in which the colour of the sera was  
396 more intense with increased grinding speed (Supplementary Fig. S3). This result might be due  
397 to small particle fragments in the serum. However, this did not alter serum viscosity since the  
398 grinding speed had no significant effect on this parameter. Overall, the storage duration did  
399 not significantly modify serum viscosity (Supplementary Table S1). It should be pointed out  
400 that the interaction between storage duration and temperature had a major impact, with T1  
401 displaying lower serum viscosity values at 70 °C and impressively higher values at 95 °C than  
402 T6.

403 The purees' serum viscosity was found to be poorly correlated with their apparent viscosity  
404 (Fig. 3C). Additionally, serum viscosity did not vary significantly between the samples,  
405 except for T1 purees heated at 95 °C. These high serum viscosities might modify the sensory  
406 perception of consistency, resulting in a smoother texture in the mouth (Espinosa-Munoz,  
407 Symoneaux, Renard, Biau, & Cuvelier, 2012).

408

409 *3.3. Relation between process conditions and puree's texture*

410

411 Principal component analysis (PCA, Fig. 5) was conducted with textural characteristics  
412 (apparent viscosity; yield stress;  $G'$ ;  $G''$ ), and possible determinants of puree's texture (particle  
413 size; PWM, i.e. the amount of liquid phase bound by insoluble particles; serum viscosity) for  
414 T1 and T6 were evaluated. Together, the first two principal components (PC1 and PC2)  
415 explained more than 80% of the total variance. Yield stress,  $G'$  and  $G''$  were grouped at PC1,  
416 together with particle size, indicating a strong dependence. T1 samples (Fig. 5B) were more  
417 variable than T6 since they were more dispersed in the sample map. In conclusion, the impact  
418 of processing on the puree's texture and its determinants was reduced by prolonged post-  
419 harvest storage. Indeed, NMR relaxation experiments (Fig. 4B) and previous results (Buegry  
420 et al., 2020) confirmed this conclusion.

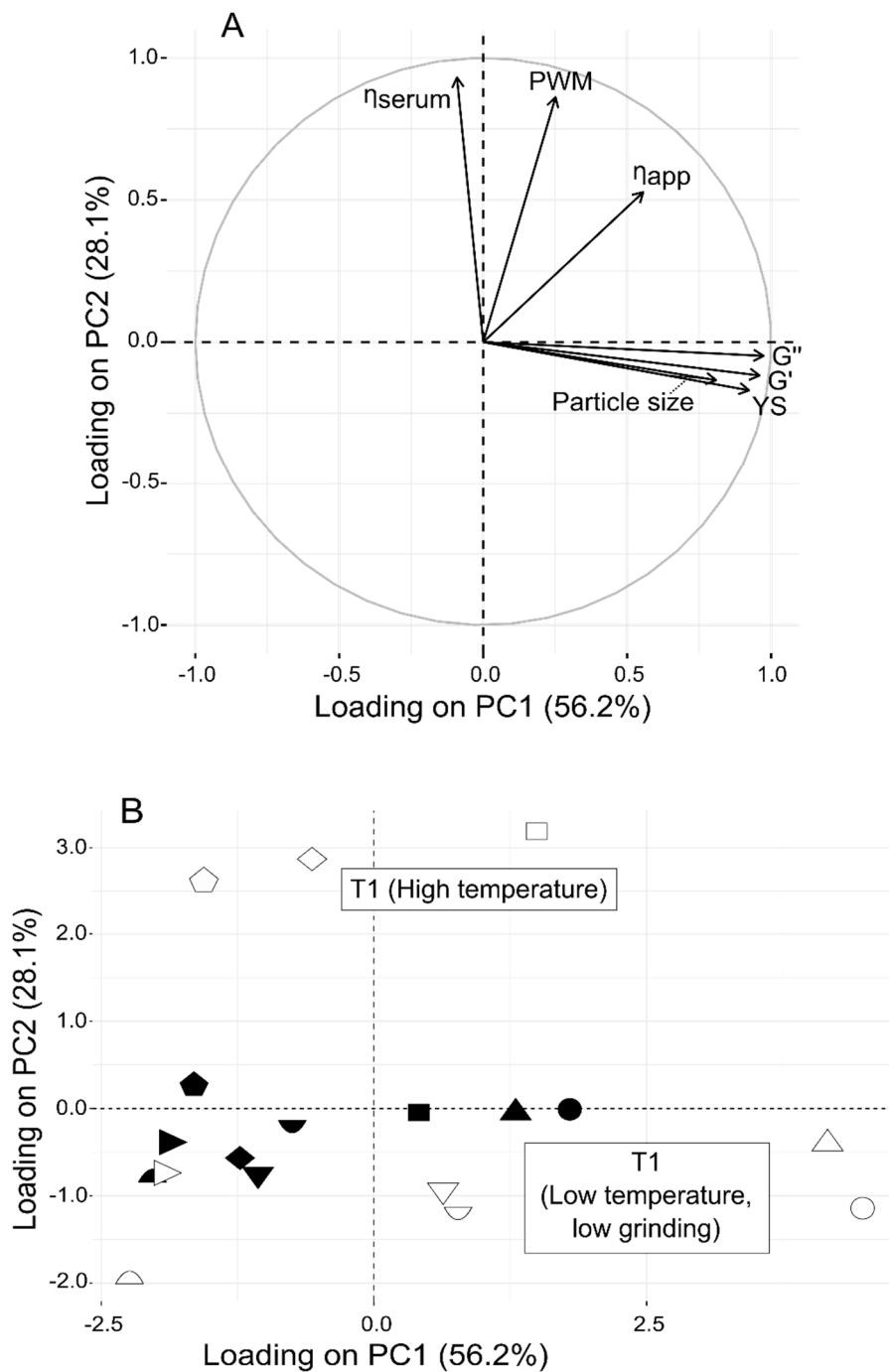
421 T1 samples produced at 70 and 83 °C were placed together at PC1, while purees prepared at  
422 95 °C were explained by PC2. This result led to two extremes, one at PC1 that accounted for  
423 samples ground at 300 rpm and heated at moderate temperatures (70 and 83 °C) and one at  
424 PC2, samples heated at 95 °C.

425 Samples were separated according to the grinding speed along PC1 (Fig. 5B). Thus, large  
426 particles were related to high yield stress,  $G'$  and  $G''$ . Cell clusters deform less easily than  
427 individual cells and occupy more volume in the puree, consequently increasing the  
428 rheological characteristics (Leverrier et al., 2016; Lopez-Sanchez et al., 2012). Moreover,  
429 PWM and serum viscosity were placed near PC2 and perpendicular to the parameters  
430 mentioned above. These results suggest that the two input variables temperature and grinding,  
431 produce different effects.

432 Indeed, in the sample map (Fig. 5B), PC2 separated T1 purees produced at 95 °C, which were  
433 characterised by high PWM and high serum viscosities (Fig. 1C and E), from T1 purees  
434 produced at moderate temperatures (70 °C and 83 °C). On the other hand, the T6 purees were

435 intermediate. Apparent viscosity was less well explained in the PCA and stood alone between  
436 PC1 and PC2. This observation indicated some co-dependence with particle size (along PC1)  
437 and PWM and serum viscosity (along PC2). Hence, texture formation was influenced by  
438 grinding, a step that highly affected particle size, and temperature, which facilitated tissue  
439 separation in synergy with grinding, increased PWM, and serum viscosity.

440



441

442 **Fig. 5.** Principal component analysis (PCA) of rheological characteristics ( $\eta_{\text{app}}$ : apparent  
443 puree viscosity at  $50 \text{ s}^{-1}$ , YS: yield stress,  $G'$  and  $G''$ : puree's storage and loss modulus,  
444 respectively, at an angular frequency of  $10 \text{ rad/s}$ ) and texture determinants (Particle size:  
445 particle size  $d_{0.9}$ , PWM: pulp wet mass,  $\eta_{\text{serum}}$ : serum viscosity at  $100 \text{ s}^{-1}$ ) of purees prepared  
446 with apples stored for one and six months before processing. Correlation circle of variables  
447 loadings on PC1 and PC2 (A). Sample maps of scores on PC1 and PC2 (B). For more  
448 legibility, PCA was conducted on the mean value of three replications as the same patterns  
449 were obtained as for PCA with all values. Empty symbols represent samples for T1 and filled  
450 symbols samples for T6.  $70 \text{ }^{\circ}\text{C}$ , 300 rpm (circle);  $70 \text{ }^{\circ}\text{C}$ , 1000 rpm (half-circle downwards);  
451  $70 \text{ }^{\circ}\text{C}$ , 3000 rpm (half-circle upwards);  $83 \text{ }^{\circ}\text{C}$ , 300 rpm (triangle);  $83 \text{ }^{\circ}\text{C}$ , 1000 rpm (triangle  
452 with the apex pointing downwards);  $83 \text{ }^{\circ}\text{C}$ , 3000 rpm (triangle with the apex pointing to the  
453 right);  $95 \text{ }^{\circ}\text{C}$ , 300 rpm (square);  $95 \text{ }^{\circ}\text{C}$ , 1000 rpm (diamond);  $95 \text{ }^{\circ}\text{C}$ , 3000 rpm (pentagon).

454

#### 455 *3.4. Serum pectins can help to explain texture determinants*

456

457 Mechanical and thermal treatment act synergistically during puree processing, affecting  
458 texture determinants to different extents because temperature favours pectin degradation and  
459 solubilisation (Sila et al., 2009). The detailed structural and compositional characterisation of  
460 the pectins solubilised during processing (serum pectins) could provide insight into the  
461 chemical processes that occur during apple processing. This knowledge could help elucidate  
462 the complex interactions between texture determinants and lead to a specific puree's texture,  
463 depending on post-harvest storage. Serum pectins were solubilised from the middle lamella or  
464 the primary cell wall (Sila et al., 2009), thus facilitating tissue fragmentation (i.e., reduced  
465 particle size) or increasing porosity and consequently the pulp's WRC (i.e., increased PWM).  
466 The amount of solubilised pectins would, in turn, explain serum viscosity.

467

468 *3.4.1. Chemical composition of serum pectins*

469 For T1, the amount and chemical composition of serum pectins was significantly altered by  
470 temperature but not by grinding (Table 1). Serum AIS values were significantly increased  
471 with elevated temperature, indicating improved pectin solubilisation from the cell wall and  
472 middle lamella. This effect might weaken the cell wall structure and intercellular adhesion,  
473 facilitating cell separation during grinding at elevated temperatures. Previous studies showed  
474 that heating raw apple, carrot or broccoli tissue before grinding aided separation (Day et al.,  
475 2010; Lopez-Sanchez et al., 2011; Müller & Kunzek, 1998) but the results were not  
476 corroborated with pectin analysis. Increased pectin solubilisation might also increase the pore  
477 sizes in the cell wall (Fig. 4B), leading to improved WRC of the pulp (Fig. 4A) and PWM  
478 (Fig. 1C). The amount of solubilised pectins was also correlated with the temperature-  
479 dependent increase in serum viscosity (Fig. 6A). Starch solubilisation might additionally  
480 increase serum viscosity. The amount of starch in the serum significantly increased with  
481 temperature and slightly with grinding speed until reaching a concentration of about 13 g/L at  
482 95 °C and 3000 rpm. Considering that the gelatinisation temperature of starch in excess water  
483 is around 60 °C, temperatures above 70 °C might solubilise starch better (Singh, Inouchi, &  
484 Nishinari, 2005). Increased grinding also seemed to enhance starch solubilisation and was  
485 probably due to increased cell wall rupture. Although amylose gels at less than 10 g/L  
486 (Doublier & Choplin, 1989) and a 20 g/L amylopectin solution provides a viscosity increase  
487 of 5 mPa.s (Matignon et al., 2014), these relatively low concentrations cannot account for the  
488 high serum viscosity increase.

489 The GalA content in serum AIS also increased with temperature, confirming improved pectin  
490 solubilisation at high temperatures. The GalA/rhamnose ratio estimated the proportion of HG  
491 to RG I pectins. It was not altered either by temperature or by grinding, indicating that HG  
492 and RG I pectins were similarly solubilised. Although arabinan side chains are generally more  
493 susceptible to temperature than galactans (Green, 1967; Thibault et al., 1993), the

494 galactan/arabinan ratio decreased with increasing temperature. Pectin RG I fractions rich in  
495 arabinans thus seemed to be more tightly bound to the cell wall and required more  
496 degradation for solubilisation. The ratio of (arabinose+galactose)/rhamnose also decreased  
497 with increasing temperature, revealing attenuated RG I side chain branching. Since RG I side  
498 chains were shown to be linked to the cellulose-xyloglucan network in the cell wall (Popper &  
499 Fry, 2005; Zykwiniska, Ralet, Garnier, & Thibault, 2005), pectins seemed to be less attached  
500 and thus more susceptible to solubilisation during processing.

501 All serum pectins were highly methylated, and the DM was not affected by temperature  
502 (results not shown), attesting limited impact of PME activity during apple processing.

503 Compared to T1, the amount and composition of serum pectins for T6 were less affected by  
504 thermomechanical processes (Table 1) during puree production. This observation could be  
505 because endogenous enzymes already degraded pectins. The serum AIS content at 70° C was  
506 higher for T6 than T1 but remained constant at any temperature, thus accounting for T6's  
507 constant serum viscosity regardless of the processing conditions (Fig. 1F). Starch was not  
508 detected. The AIS composition of serum pectins was stable for T6, whatever the grinding or  
509 temperature. As an exception, the galactan/arabinan ratio was slightly decreased with  
510 increased temperature, but the values were less dispersed than for T1 and the overall  
511 (arabinose+galactose)/rhamnose ratio was not modified. Compared to T1, the latter ratio was  
512 less critical and indicated reduced RG I side chain branching in T6 serum pectins. This  
513 substantial decrease in RG I side chains (i.e., arabinose and galactose) probably contributed to  
514 the higher relative GalA content in the T6 samples. Concerning T1, the HG to RG I ratio,  
515 expressed as GalA/rhamnose, was not altered by temperature or grinding. However, this ratio  
516 showed that, although the GalA content was higher for T6, more RG I was extracted,  
517 especially with grinding. This result may be due to reduced RG I side chains, leading to a  
518 weakened cell wall structure, as explained before. As observed by Nara et al. (2001) or Pena  
519 and Carpita (2004), reduced RG I side chains were probably linked to reduced cell adhesion.



520 Indeed, cell separation was easy for T6 and purees prepared with aged apples showed  
521 significantly smaller particles (Supplementary Table S1). Additionally, grinding generated  
522 similar particle sizes independent of temperature. As previously stated by Buergy et al.  
523 (2020), ageing facilitated cell separation for T6. As hypothesised before, the weakening of the  
524 cell wall in aged apples probably increased cell wall porosity (Fig. 4B), leading to increased  
525 WRC (Fig. 4A) and PWM (Fig. 1D).

526 Since raw apples also displayed RG I side chain loss, GalA increase and more RG I during  
527 post-harvest storage (Supplementary Table S3), it is plausible that storage could be exploited  
528 for producing a desired puree's texture.

529

530 **Table 1.** AIS yield, composition, and macromolecular characteristics of serum pectins, followed by Kruskal-Wallis *H*- and *P*-values.

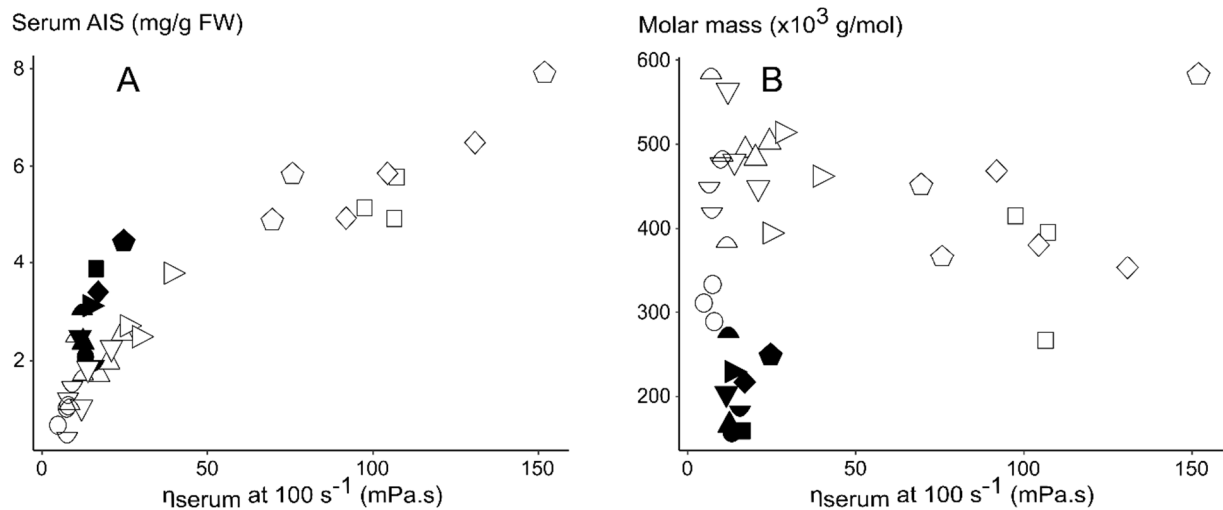
Storage (months)	Temp. (°C)	Grinding (rpm)	AIS (mg/g FW)	Starch (mg/g FW)	GalA (mg/g AIS)	GalA/Rha	Ara + Gal/Rha	Gal/Ara	$\bar{M}_w \times 10^3$ (g/mol)	$[\bar{\eta}]_z$ (mL/g)	$d_{happ}$ (g nm <sup>3</sup> /mol)
1	70	300	0.9	0.1	527	73	24	1.4	311	1903	0.8
		1000	1.1	0.2	556	94	24	1.4	545	1869	0.8
		3000	1.6	0.5	470	60	26	1.3	480	1811	0.8
	83	300	2.1	0.4	554	117	22	1.2	493	1609	0.9
		1000	1.7	0.4	539	88	25	1.2	522	1760	0.9
		3000	3.0	0.6	543	93	25	1.0	455	1783	0.8
	95	300	5.3	0.5	646	74	17	0.8	406	1229	1.2
		1000	5.8	1.0	602	82	21	0.8	425	1272	1.2
		3000	6.2	1.3	577	81	22	0.8	409	1268	1.2
6*	70	300	2.1	ND	683	52	8	1.1	157	510	3.0
		1000	1.8	ND	593	26	6	1.2	188	488	3.1
		3000	3.0	ND	605	30	7	1.0	273	579	2.6
	83	300	2.4	ND	663	48	7	1.0	166	583	2.6
		1000	2.5	ND	601	26	6	0.9	204	650	2.3
		3000	3.1	ND	605	27	6	1.0	229	620	2.4
	95	300	3.9	ND	689	67	10	0.8	159	507	3.0
		1000	3.4	ND	636	31	6	0.8	217	607	2.5
		3000	4.5	ND	619	28	6	0.8	250	636	2.4
PSD			0.8	0.5	49	28	3	0.1	50	162	0.1

1	Temperature H	20.9	10.6	9.6	3.4	7.5	22.6	5.5	18.6	18.3
	Temperature P	<b>&lt;0.001</b>	<b>0.005</b>	<b>0.008</b>	0.184	<b>0.023</b>	<b>&lt;0.001</b>	0.064	<b>&lt;0.001</b>	<b>0.000</b>
	Grinding H	1.1	3.3	2.0	0.4	3.0	0.5	4.8	1.0	1.2
	Grinding P	0.572	0.190	0.361	0.804	0.220	0.798	0.089	0.614	0.556
6*	Temperature H	6.0	-	1.7	1.2	1.2	7.2	2.4	3.2	4.6
	Temperature P	0.051	-	0.430	0.561	0.561	<b>0.027</b>	0.301	0.202	0.099
	Grinding H	1.7	-	5.6	5.6	5.6	0.1	2.4	2.5	0.3
	Grinding P	0.430	-	0.061	0.061	0.061	0.957	0.301	0.288	0.875
	Storage H	0.3	-	10.7	15.7	19.7	2.4	19.1	19.7	11.9
	Storage P	0.596	-	<b>0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.121	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.001</b>

531 \* Only one repetition per sample.

532 The ratio GalA/Rha estimated the relative amount of HG versus RG I, (Ara+Gal)/Rha estimated RG I branching and Gal/Ara the proportion of  
533 RG I side chains. Ratios were calculated using the yields of galacturonic acid (GalA) and neutral sugars arabinose (Ara), galactose (Gal) and  
534 rhamnose (Rha) content, expressed in mg/g AIS (Supplementary Table S4). AIS and chemical composition of serum pectins were corrected for  
535 the starch content. PSD: Pooled standard deviations; ND: Not detected; FW: Fresh weight (serum); AIS: Alcohol insoluble solids;  $\bar{M}_w$ : Weight-  
536 average molar mass;  $[\eta]_z$ : z-average intrinsic viscosity;  $d_{happ}$ : Apparent molecular density, calculated as  $\bar{M}_w / (\frac{4}{3} \cdot \pi \cdot \bar{R}_{hz}(v)^3)$  with  $\bar{R}_{hz}(v)$ : z-  
537 average viscometric hydrodynamic radius. Macromolecular characteristics were taken at the apex of the main peak.

538



539

540 **Fig. 6.** Scatterplots of serum viscosity at  $100 \text{ s}^{-1}$  against serum AIS (A) and molar mass of  
 541 serum pectins at the apex of the main peak (B). Empty symbols represent samples for T1 and  
 542 filled symbols samples for T6.  $70 \text{ }^{\circ}\text{C}$ , 300 rpm (circle);  $70 \text{ }^{\circ}\text{C}$ , 1000 rpm (half-circle  
 543 downwards);  $70 \text{ }^{\circ}\text{C}$ , 3000 rpm (half-circle upwards);  $83 \text{ }^{\circ}\text{C}$ , 300 rpm (triangle);  $83 \text{ }^{\circ}\text{C}$ , 1000  
 544 rpm (triangle with the apex pointing downwards);  $83 \text{ }^{\circ}\text{C}$ , 3000 rpm (triangle with the apex  
 545 pointing to the right);  $95 \text{ }^{\circ}\text{C}$ , 300 rpm (square);  $95 \text{ }^{\circ}\text{C}$ , 1000 rpm (diamond);  $95 \text{ }^{\circ}\text{C}$ , 3000 rpm  
 546 (pentagon).

547

#### 548 3.4.2. Macromolecular characteristics of serum pectins

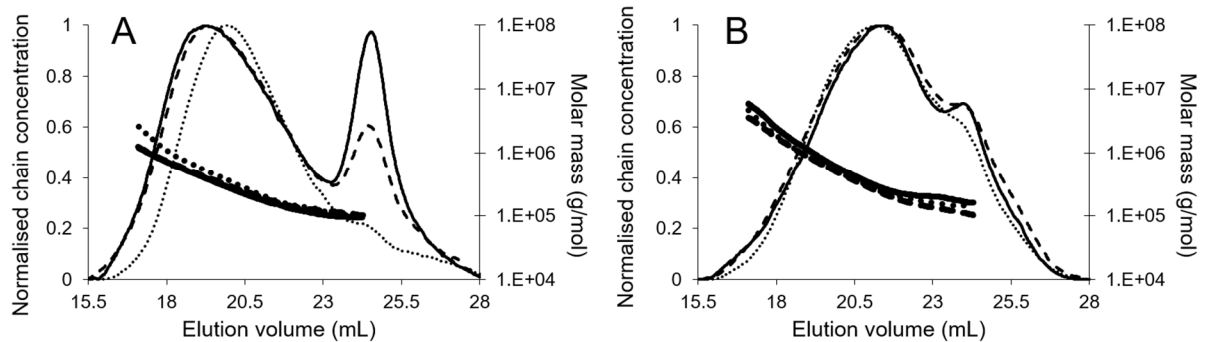
549 Macromolecular characteristics of soluble serum pectins give information about their  
 550 molecular structure and density. For T1, molar mass (Table 1) was not significantly altered by  
 551 temperature but showed an overall decrease when higher temperatures were applied. For T6,  
 552 molar mass was not altered by processing, but values were significantly lower than for T1.  
 553 The molar mass distributions of serum pectins (Fig. 7) are presented for samples prepared at  
 554 3000 rpm and different temperatures. For T1 purees (Fig. 7A) processed at moderate  
 555 temperatures ( $70 \text{ }^{\circ}\text{C}$  and  $83 \text{ }^{\circ}\text{C}$ ), the main fraction of serum pectin eluted at the same elution  
 556 volume ( $\sim 19 \text{ mL}$ ), whereas the main peak was shifted to higher elution volumes after heating  
 557 at  $95 \text{ }^{\circ}\text{C}$ . This result indicated a smaller pectin size for the samples obtained at  $95 \text{ }^{\circ}\text{C}$ . A

558 second peak eluting at higher elution volumes (~25 mL), which represented smaller pectin  
559 fractions, was also present, and its proportion decreased with increased temperature.  
560 However,  $\bar{M}_w$  (90–105 x 10<sup>3</sup> g/mol) and  $\bar{R}_{hz}(v)$  (10–16 nm) were similar in all samples and  
561 could not be differentiated. For T6 (Fig. 7B), processing temperature did not affect the serum  
562 pectins' macromolecular size. Compared with T1, the main peak of all pectin chromatograms  
563 was shifted to higher elution volumes, and the second peak was smaller, indicating pectin  
564 degradation during storage as observed before (Buergy et al., 2020). Hence, both increased  
565 temperature (95 °C, for T1) and post-harvest storage induced pectin degradation, thus  
566 favouring pectin solubilisation. A reduction in RG I side chains weakened the cell wall  
567 structure, leading to reduced cell adhesion and more porous cell walls. However, no trend was  
568 observed between serum viscosity and molar mass (Fig. 6B).

569 It has been reported that pectin conformation can significantly impact serum viscosity (Diaz,  
570 Anthon, & Barrett, 2009). Herein, intrinsic viscosity was significantly affected by temperature  
571 increase (Table 1), leading to reduced values at 95 °C despite higher pectin concentrations.  
572 Lower intrinsic viscosities at 95 °C for comparable molar mass at 70 and 83 °C indicted a  
573 more compact pectin structure. This observation was confirmed by  $\bar{R}_{hz}(v)$  conformation plots  
574 (Supplementary Fig. S4) and the apparent polymer density  $d_{happ}$  (Table 1). The latter was  
575 similar at 70 and 83 °C but significantly higher for 95 °C. Intrinsic viscosity was significantly  
576 lower for T6, roughly half that of T1, whereas  $d_{happ}$  increased by more than 2-fold. Since  
577 serum pectins in purees from aged apples have more GalA and less arabinose and galactose  
578 (Table 1), this higher density could result from more properly folded pectins because of  
579 reduced steric hindrance. Although the conformation of serum pectins was altered, it could  
580 not be linked to serum viscosity (data not shown).

581 Grinding had no impact on the macromolecular characteristics of the T1 serum pectins. In  
582 contrast, molar mass and intrinsic viscosity of T6 pectins increased with grinding, although

583 this trend was not statistically significant. Macromolecular characteristics of soluble pectins  
 584 extracted from raw apples showed the same trend as serum pectins during post-harvest  
 585 storage (i.e., smaller molar mass and intrinsic viscosity but higher  $d_{happ}$ ) (Supplementary  
 586 Table S3).  
 587



588 **Fig. 7.** Normalised chain concentration and molar mass versus elution volume of serum  
 589 pectins for T1 (A) and T6 (B) for purees prepared at 70 °C (continuous lines), 83 °C (dashed  
 590 lines) and 95 °C (dotted lines) and a grinding speed of 3000 rpm. The signal (mV) obtained  
 591 by the differential refractive index detector was normalised by dividing all data points by the  
 592 signal at the peak's apex.  
 593

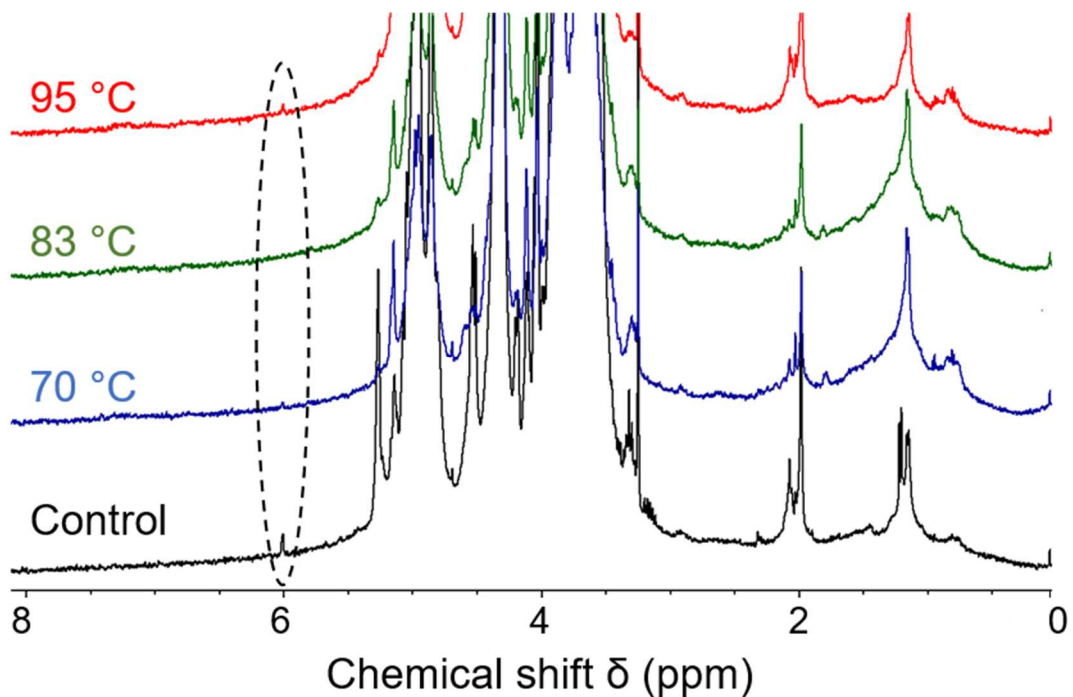
594

### 595 3.4.3. Mechanisms of pectin degradation during processing

596 At the natural pH of apples (~3.7), pectin degradation is minimal and might be due to either  
 597 acid hydrolysis or  $\beta$ -elimination (Liu, Renard, Rolland-Sabaté, Bureau, & Le Bourvellec,  
 598 2021). For T1 (Table 1), serum pectins showed higher amounts of GalA and the RG I side  
 599 chains arabinan and galactan were hydrolysed by temperature increase, which probably  
 600 favoured the extractability of pectins from the cell wall. Moreover, T1 serum pectins showed  
 601 reduced molar mass, macromolecular size and intrinsic viscosity (Table 1 and Fig. 7A) when  
 602 temperature increased from 83 to 95 °C, attesting pectin degradation. To investigate the  
 603 mechanisms leading to pectin degradation at 95 °C, NMR was used to detect  $\beta$ -elimination

604 products in serum pectins, since the 235 nm wavelength in the DAD signal after sample  
605 separation by HPSEC was not enough to detect  $\beta$ -elimination (data not shown). A small  
606 signal in the  $^1\text{H}$ -NMR spectrum around 6 ppm indicated the presence of  $\beta$ -elimination  
607 products (Tjan et al., 1974). This signal was visible in the positive control and, slightly, in the  
608 serum AIS obtained from purees heated at 95 °C (Fig. 8). In conclusion, only little  $\beta$ -  
609 elimination occurred, even at 95 °C, and acid hydrolysis seemed to be the predominant  
610 reaction causing pectin degradation during apple processing.

611



612

613 **Fig. 8.**  $^1\text{H}$ -NMR spectra of control and serum pectins extracted from the purees at 3000 rpm  
614 with different temperatures for T1. The control (serum pectin 95 °C, 3000 rpm) was heated at  
615 100 °C for 30 min at pH 6 to enhance  $\beta$ -elimination.

616

## 617 5. Conclusions

618

619 Both elevated temperature (95 °C) and apple maturation led to pectin degradation, resulting in  
620 facilitated tissue fragmentation during processing. This treatment affected particle size, an

621 important determinant of puree's texture, and other parameters such as PWM and serum  
622 viscosity. Since pectin and puree's structures were significantly altered by increasing  
623 temperature in fresh (T1) but not in older (T6) apples, the combination of storage duration and  
624 temperature had a significant impact. Although mechanical treatments were the predominant  
625 factor for texture formation, they did not alter the pectin composition or structure, in T1 or  
626 T6. The temperature had only a limited impact on the apple puree's texture. However, it  
627 strongly affected PWM and serum viscosity, which counteracted the effect of smaller  
628 particles produced by grinding. This study highlighted the importance of pectin analysis in  
629 explaining the impact of thermomechanical processes on puree's texture. A detailed  
630 characterisation of the pulp's cell wall composition and structure should be performed in  
631 future work. It would also be interesting to conduct detailed kinetics, over short periods of 5  
632 to 15 min, at different temperatures and grinding speeds to precisely identify the moment  
633 structural alterations occur.

634

#### 635 **CRedit authorship contribution statement**

636

637 **Alexandra Buergy:** Conceptualization, Investigation, Formal analysis, Data curation,  
638 Writing - original draft. **Agnès Rolland-Sabaté:** Conceptualization, Supervision, Validation,  
639 Writing - review & editing. **Alexandre Leca:** Conceptualization, Investigation, Supervision,  
640 Writing - review & editing. **Xavier Falourd:** Investigation, Formal analysis, Writing - review  
641 & editing. **Loïc Foucat:** Investigation, Formal analysis, Writing - review & editing.  
642 **Catherine M. G. C. Renard:** Conceptualization, Funding acquisition, Project administration,  
643 Validation, Writing - review & editing.

644

#### 645 **Declaration of competing interest**



646

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648

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