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▶ **To cite this version:**

Alexandra Buergy, Agnès Rolland-Sabaté, Alexandre Leca, Catherine M.G.C. Renard. Apple puree's texture is independent from fruit firmness. LWT - Food Science and Technology, 2021, 145, pp.111324. 10.1016/j.lwt.2021.111324 . hal-03189093

HAL Id: hal-03189093
https://hal.inrae.fr/hal-03189093v1

Submitted on 24 Apr 2023

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Version of Record: https://www.sciencedirect.com/science/article/pii/S0023643821004771
Manuscript_a1d83210847562a87ad5a13cd7c4a170

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 µm), 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

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1. Introduction

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

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

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

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

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

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

2. Material and Methods

2.1. Plant material

2.1.1. Raw apples

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

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

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

2.1.2. Puree preparation

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

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

2.2. Physico-chemical characterization

2.2.1. Texture of raw apples

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

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^{-1}), recording one point every 15 s. Apparent viscosity at 50 s^{-1} 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^{-1}, 8 min) using a double gap
142 cylinder geometry set (DG27). The value at a shear rate of 100 s^{-1} 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_3 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

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

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

2.3.5. Starch determination

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

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

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

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.

3.2. Cell wall composition of raw apples

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

3.3. Rheological characterization of apple purees

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

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

3.4. Analysis of texture determinants in purees

3.4.1. Particle size distribution

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

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

3.4.2. Pulp wet mass and water retention capacity

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

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

3.4.3. Serum viscosity, soluble pectin content and molar mass

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

Molar mass and molecular size were determined for soluble pectins of Process II. GS pectins exhibited the highest molecular size (lowest elution volume) and BRM a smaller size than BR, which eluted before (Fig. 2). All other cultivars had medium sizes between BR and BRM (not shown). GS had the highest molar mass at the main peak ($459x10^3$ g/mol), whereas molar masses of BR ($277x10^3$ g/mol) and BRM ($230x10^3$ g/mol) were not significantly different.

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

5. Conclusions

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

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

Declaration of competing interest

Declarations of interest: none.

Acknowledgements

This work was carried out as part of "Interfaces" flagship project, publicly funded through ANR (the French National Agency) under the "Investissements d´avenir" program with the reference ANR-10-LABX-001-01 Labex Agro and coordinated by Agropolis Fondation under the reference ID 1603-001. Studies conducted with the phytotron and the HPSEC-MALLS system were supported by the various CPER Platform 3A funders: European Union, European Regional Development Fund, the French Government, the Sud Provence-Alpes-Côte d'Azur Region, the Departmental Council of Vaucluse and the Urban Community of Greater Avignon.

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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^{-1} (G) as a function of apparent puree viscosity at 50 s^{-1}.
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, $AIS_{raw\ apple}$ (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: η_{app} (apparent viscosity of the puree at 50 s^{-1}), 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 η_{serum} (serum viscosity at 100 s^{-1}), AIS_{serum} (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 (%)	$\frac{Ara + Gal}{Rha}$
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
PSD	*0.4*	*0.9*	*0.5*	*2*	*19*	*5*	*2*
H value	*102*	*109*	*16*	*10*	*15*	*14*	*15*
P value	*< 0.001*	*< 0.001*	*0.007*	*0.066*	*0.012*	*0.013*	*0.011*

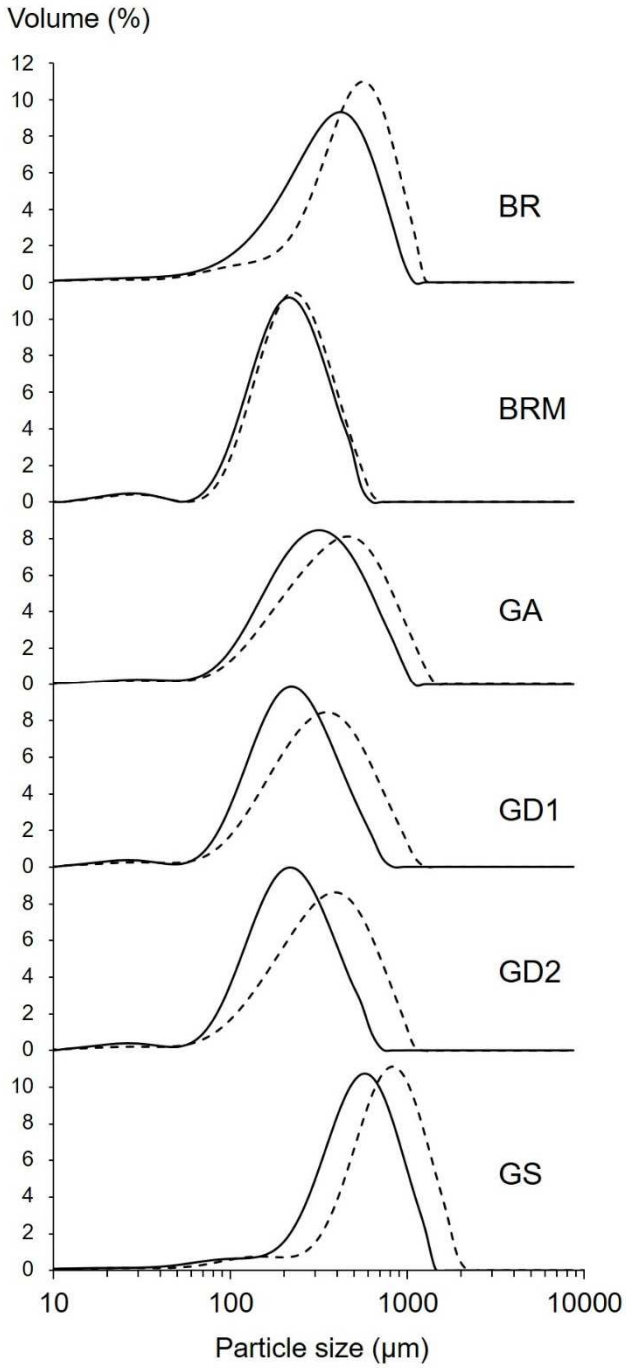
607 The ratio (Ara+Gal)/Rha estimated RG I branching and was calculated using the yields of
608 neutral sugars arabinose (Ara), galactose (Gal) and rhamnose (Rha), expressed in mg/g AIS

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

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

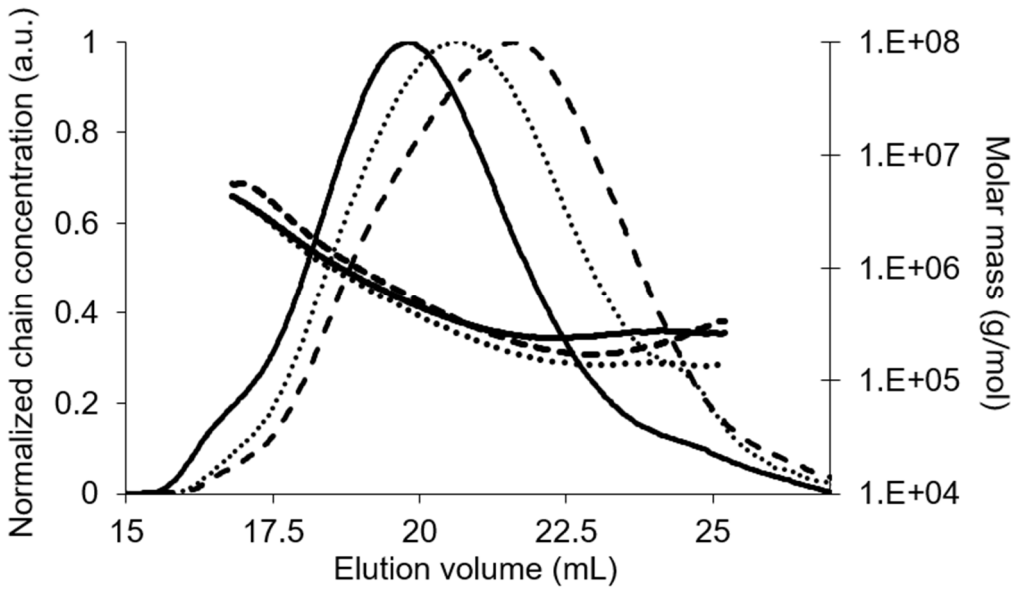
Cultivar	Process	η_{app} 50 s^{-1} (mPa.s)	G′ (Pa)	G″ (Pa)	PWM (g/100 g)	WRC (g/g DW)	η_{serum} 100 s^{-1} (mPa.s)	Serum AIS (mg/g FW)
BR	I	630	1080	216	26	14	8	0.5
	II	1088	1509	323	28	12	17	0.7
BRM	I	456	965	200	29	13	5	0.3
	II	680	1373	310	28	16	9	0.8
GA	I	480	720	138	24	14	7	0.2
	II	681	934	194	27	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
PSD		*55*	*121*	*31*	*1*	*1*	*3*	*0.2*
Cultivar	*H value*	*23*	*24*	*24*	*28*	*22*	*24*	*24*
	P value	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Process	*H value*	*10*	*7*	*8*	*2*	*2*	*6*	*4*
	P value	*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; η_{app}: Apparent viscosity at
621 50 s^{-1}; G′, G″: Storage and loss modulus, respectively, at an angular frequency of 10 rad/s;
622 PWM: Pulp wet mass; WRC: Water retention capacity of the pulp; η_{serum}: Serum viscosity at
623 100 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**


Normalized chain concentration (a.u.)
1
0.8
0.6
0.4
0.2
0
15
17.5
20
22.5
25
Elution volume (mL)
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
Molar mass (g/mol)

628

629 **Fig. 2**

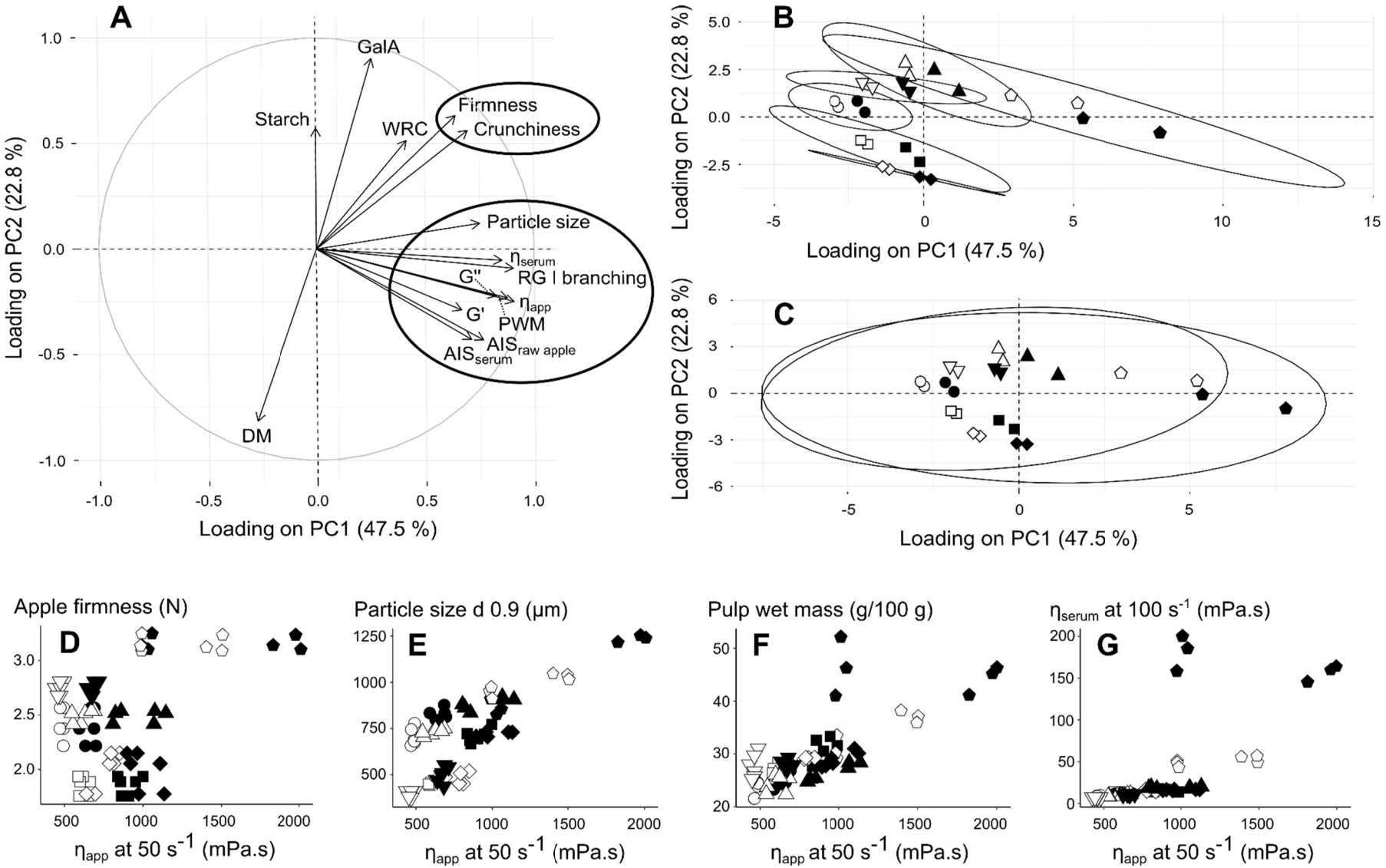

A
GalA
Starch
WRC
Firmness
Crunchiness
Particle size
η_{serum}
RG I branching
G''
η_{app}
G'
PWM
$AIS_{raw\ apple}$
AIS_{serum}
DM
Loading on PC2 (22.8 %)
Loading on PC1 (47.5 %)
B
Loading on PC2 (22.8 %)
Loading on PC1 (47.5 %)
C
Loading on PC2 (22.8 %)
Loading on PC1 (47.5 %)
Apple firmness (N)
D
η_{app} at 50 s^{-1} (mPa.s)
Particle size d 0.9 (µm)
E
η_{app} at 50 s^{-1} (mPa.s)
Pulp wet mass (g/100 g)
F
η_{app} at 50 s^{-1} (mPa.s)
η_{serum} at 100 s^{-1} (mPa.s)
G
η_{app} at 50 s^{-1} (mPa.s)

Fig. 3