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Abstract

The texture of pureed fruits and vegetables depends primarily on the original tissue structure and cell wall (CW) properties. However, how variations in the raw fruits' cellular and molecular structure determine the rheological behaviour of the purees is little understood, though pectin degradation appears to play a key role. Cultivars, fruit load and post-harvest storage were used to obtain raw apples with different tissue structures, which were processed into purees under simulation of an industrial process. The rheological behaviour of the purees was then compared to particle size, pulp wet mass and serum viscosity. The polysaccharide composition of soluble and insoluble CW material were determined after preparation of alcohol insoluble residue. Macromolecular size and molar mass distributions of soluble pectins were analysed using high performance size-exclusion chromatography coupled to multi-angle laser light scattering and online viscometry. Variations in the raw material, especially induced by post-harvest storage, generated a wide range of different puree's textures. Rheological behaviour of apple purees was driven by particle size, which decreased

during prolonged post-harvest storage due to reduced cell adhesion. This was correlated with pectic side chain hydrolysis and modifications in pectin molar mass and structure. Similar trends during storage were observed with different apple cultivars and agricultural practices.

Fruit processing; Apple; Puree; Rheology; Particle size; Polysaccharide

1. Introduction

Purees are manufactured from the edible parts of fruits and vegetables, which are mainly composed of parenchyma cells. They are dispersions of soft and deformable insoluble particles (pulp) in an aqueous medium (serum) composed of sugars, organic acids and pectic polysaccharides (Rao, 1992). The pulp is primarily composed of cell wall clusters, individual cells whose content was emptied during puree processing or cell fragments from the parenchyma of the original fruit, ranging between some mm and some hundred µm in size (Espinosa et al., 2011; Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016). In apple fruits, parenchyma cells are polyhedral in shape and between 50 and 300 µm in diameter (Khan & Vincent, 1990).

In reconstituted model systems, the amount, size and shape of individual cells or cell clusters as well as the viscosity of the serum are reported to be key factors in determining the puree's texture (Espinosa-Munoz, Renard, Symoneaux, Biau, & Cuvelier, 2013; Espinosa et al., 2011; Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016; Rao, 1992), and are all closely linked to the quality of pectic polysaccharides. However, to the best of our knowledge, no extensive study investigated the relationship between quality variations of raw apples, linked to pectins, and texture of corresponding purees. In contrast, the evolution of pectic substances in raw apples is well documented. For example, fruit cultivar as well as maturity stage modify

51 the chemical and structural properties of the cell wall (Billy et al., 2008; Fischer & Amado,
52 1994; Le Bourvellec et al., 2011).
53 The main compartment of parenchyma cells is the large central vacuole, surrounded by the
54 gel-like cytoplasm. The thin (0.1 to 10 μm) and semi-rigid cell wall, containing cellulose,
55 hemicellulose and pectins, is located around this complex and plays an important role as it
56 assures structural support to the plant cell (Darvill, McNeil, Albersheim, & Delmer, 1980).
57 The colloidal middle lamella is situated between the primary cell walls of adjacent cells,
58 holding them together and thus, forming the tissue (Thakur, Singh, & Handa, 1997). The
59 middle lamella is almost only composed of pectic polysaccharides, accompanied by proteins
60 (Carpita & Gibeaut, 1993) but without cellulose (Guillemin et al., 2005).
61 Pectin molecules are a group of different complex polysaccharides rich in covalently linked
62 D-galacturonic acid that form a negatively charged backbone (Albersheim, Darvill, Oneill,
63 Schols, & Voragen, 1996). The homogalacturonans (HG) are composed of a long, linear chain
64 of α-D-(1 → 4)-galacturonic acids. These regions do not carry any neutral sugars as side
65 chains but the galacturonic acid residues can be methyl esterified to a varying degree at C6
66 positions, defining the degree of methylation (DM) (Thakur, Singh, & Handa 1997).
67 Depending on the DM, HG chains may self-associate; an unmethylated C-6 position of the
68 HG backbone is negatively charged and may ionically interact with calcium-ions to form a
69 stable gel with other HG molecules if more than 10 consecutive unmethyl-esterified
70 galacturonic acid residues are coordinated (Kohn & Luknár, 1977). In the
71 rhamnogalacturonan I (RG I) backbone, α-D-(1 → 4)-galacturonic acid and α-L-(1 → 2)-
72 linked rhamnose molecules residues alternate, whereas the rhamnosyl residues can be
73 substituted with neutral sugar chains. These lateral chains can be linear or branched and are
74 mainly composed of α-L-arabinofuranosyl and/or β-D-galactopyranosyl forming arabinans,
75 galactans and arabinogalactans (Ridley, O'Neill, & Mohnen, 2001). Rhamnogalacturonan II

(RG II) molecules consist of a galacturonic acid backbone, carrying four complex oligosaccharide chains consisting of 12 different glycosyl residues in over 20 different linkages (Mohnen, 2008). *In muro*, RG II exists predominantly as a dimer that is cross-linked by a borate-diol ester (O'Neill et al., 1996).

During processing into puree, rising temperatures destabilize cellular membranes and favour both enzymatic and chemical reactions, resulting in degradation and solubilization of pectic polysaccharides that are involved in cell-to-cell adhesion (Massiot & Renard, 1997). The tissue is then softened due to increased cell separation. In addition, mechanical treatment partly disrupts the parenchyma cells that release their cell content.

Due to the increasing number of consumers and purchased quantities of fruit purees, food companies are highly interested in predicting and controlling the puree's texture since this is a major quality attribute and thus a key for the liking and, in turn, repeated purchase of the products (Waldron, Smith, Parr, Ng, & Parker, 1997). However, fruit purees may need to be rectified by concentration, addition of sugar or hydrocolloids, which may be perceived negatively by consumers. Thus, there is a global trend towards the production of healthier and more natural fruit products and the reduction of artificial stabilizers or texturizing agents (Market Research Future, 2019). This could be reached by playing on the structural factors of the raw fruit, as it is known, but not mastered by industry, that texture of plant-based products highly depends on the cell wall structure of the raw material (Waldron, Smith, Parr, Ng, & Parker, 1997).

The objective of this study was thus to identify the impact of pectin structure in raw apples on texture variations of the corresponding purees. To reach this aim, two apple cultivars, four post-harvest storage durations and two growing conditions were chosen to generate contrasted raw apples. Their impact on the structural factors as well as the cell wall composition of the apple purees was studied in order to relate microstructure to the puree's texture.

2. Material and Methods

2.1. Plant material

Apple (*Malus domestica* Borkh.) cultivars, namely 'Granny Smith' and 'Golden Delicious' were cultivated in Mallemort, France and harvested in September 2017 according to the optimal harvest dates. 'Granny Smith' (GS) and half of the trees of the cultivar 'Golden Delicious' (GD) were thinned chemically (GD1), leading to less but bigger fruits (201 ± 7 g per apple). GS trees showed regular fruit growth and were thus thinned with 600 g/ha Amid-Thin® W (2-(1-naphthyl)acetamide) and 1 kg/hL Rhodofix® (1-naphthalenacetic acid). GD1 trees exhibited low fruit charge and were thus treated with 15 L/ha ammonium thiosulfate and 0.15 kg/hL Rhodofix®. Additionally, 3.5 L/ha MaxCel® (6-Benzylaminopurine) were applied on small fruits of both varieties. The other half of GD trees was not thinned (GD2), resulting in more but smaller fruits (176 ± 4 g per apple). The raw apples used in this article were characterized in detail (e.g. fruits' texture) in another study (Lan, Jaillais, Leca, Renard, & Bureau, 2020).

A first series of samples were processed directly after harvest (T0). The apples were then stored for one (T1), three (T3) and six (T6) months at 4 °C prior to processing. The atmosphere was not controlled to allow post-harvest storage to have significant impact.

2.2. Puree preparation

For each cultivar, growth condition and storage time, processing was conducted in triplicate. Approximately 3 kg of apple fruits per batch were cored, sliced into 12 equal portions and

126 processed into puree. A cooker-cutter (RoboQbo Qb8-3, RoboQbo, Bentivoglio, Italy) was
127 used for apple processing, imitating a hot break process at 95 °C, with stirring at 105 rad/s
128 during five minutes under vacuum. Each batch of apple puree was divided into two portions:
129 one was refined by an automatic sieve (Cobot Coupe C80, Robot Coupe SNC, Vincennes,
130 France) of 0.5 mm (R), removing skin and other larger particles, and the other one was not
131 refined (NR). At T0, only NR samples were produced. Rheological measurements, particle
132 size analysis and determination of pulp wet mass were conducted in the same week as puree
133 preparation. Pulp and serum were then frozen separately (-20 °C) until cell wall extraction
134 and analysis.
135
136 *2.3. Rheological measurements*
137
138 All tests were performed using a stress-controlled rheometer (Physica MCR301, Anton Paar,
139 Graz, Austria) equipped with a Peltier cell (CPTD-200, Anton Paar) and an external cylinder
140 (CC27/S, Anton Paar). All rheological measurements were performed at 22.5 °C and samples
141 were changed for each test.
142
143 *2.3.1. Puree*
144 A vane measuring system with a 3.46 mm gap (CC27/S + FL100/6W, Anton Paar) was used
145 to analyse the rheological behaviour of the purees. The flow curve was recorded by measuring
146 the viscosity over a logarithmically distributed range of shear rate values $\dot{\gamma}$ between 10 and
147 250 s^{-1}. One point was recorded every 15 seconds. Homogenous dispersion of the product in
148 the measurement cell was ensured by application of a pre-shear of 50 s^{-1} during two minutes,
149 followed by a five minute rest to let the puree return to a rheological equilibrium prior to
150 analysis. The texture of the purees was compared by means of the apparent viscosity (η_{app}) at

a shear rate of 50 s^{-1} as this value represents an approximation of the shear rate to which a soft food product is subjected in the mouth during mastication (Stokes, 2012).

Amplitude sweep tests were performed at deformation values γ between 0.01 and 100 % at a fixed angular frequency ω of 10 rad/s. Five points were measured per decade and the time required to measure each point was set by the software. The yield stress obtained at the intersection of G' and G" was used as a characteristic point to estimate the minimum shear stress that must be applied to the puree to initiate flow (Espinosa-Munoz, Renard, Symoneaux, Biau, & Cuvelier, 2013). The values of G' and G" were averaged in the linear viscoelastic range. The end of the linear domain was estimated as the strain inducing a decrease of G' values exceeding 10 % of its value in the linear domain.

The method used for rheological measurements of the purees was theoretically not adapted for particles larger than 1 mm as the gap of the measuring cell was approximately 3 mm large. The only samples containing particles (skin fragments) larger than 1 mm were not refined purees. However, rheological measurements were repeatable for these samples and the method was thus considered valid.

2.3.2. Serum

The viscosity of the continuous phase obtained by centrifugation of the puree was measured using a double gap cylinder geometry (DG27, Anton Paar). Before each measurement, a resting step of one minute was applied. Viscosity values (η_{serum}) were taken at a shear rate of 100 s^{-1} from a flow curve, assuming the in-mouth perception of serum requires a higher shear rate than purees.

2.4. Pulp wet mass

The pulp wet mass represented the fraction of humid particles after separation of the puree into pulp and serum by centrifugation at 7690 x g for 15 minutes at 15 °C. It was calculated as the ratio of the pulp weight to the initial weight of the puree and expressed in % as described in literature (Espinosa-Munoz, Renard, Symoneaux, Biau, & Cuvelier, 2013).

2.5. Particle size distribution

The particle size distribution of the pulp was measured by laser granulometry (Mastersizer 2000, Malvern Instruments, Malvern, UK). The samples were dispersed in distilled water (refractive index: 1.33) and analysed by the Mie theory. The refractive index of the sample was set at 1.52 (Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016) and the absorptive index was chosen to be 0.00 due to the translucent character of apple cells. Each sample was analysed in duplicate and the Malvern's software averaged the size distribution over three repeated measurements on the same sample.

2.6. Alcohol insoluble solids (AIS)

AIS of raw apples and purees were extracted as described by Le Bourvellec et al. (2011). AIS of serum and pulp were prepared based on Renard (2005) and Le Bourvellec et al. (2011). Prior to cell wall extraction, pulp was water-washed to remove soluble pectins. Approximately 25 g of fresh pulp were suspended in ultrapure water (130 mL), then stirred one night (10 rad/s, 4 °C) and centrifuged (7690 x g, 10 min, 15 °C). The residue was rinsed once again with water before 10 g of the sediment were stirred (10 rad/s, 4 °C) one night in ethanol (96 % v/v, 50 mL). The suspension was filtered on a 75 mL Sep-pack column (Interchim, Montluçon, France) equipped with a 20 µm filter. The extraction was continued

with ethanol (70 % v/v) till absence of sugars as shown by negative reaction in the phenol sulphuric test (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Subsequently, the samples were washed twice with acetone (60 % v/v), once with acetone (80 % v/v) and three times with acetone (100 % v/v). The residue was dried at 40 °C during 48 hours in a drying oven and weighed. The cell wall content of the pulp was estimated as the relation between the dry pulp weight and the initial pulp weight after water-washing, expressed in %.

For serum, 100 mL were stirred (31 rad/s, 4 °C) overnight in 250 mL ethanol (96 % v/v), then centrifuged (7690 x g, 15 °C, 10 minutes). The residue was washed several times with ethanol (70 % v/v) until all free sugars were removed. Afterwards, samples were dissolved in water (50 mL) overnight (31 rad/s, 4 °C) to redissolve the pectins, then freeze-dried and ground with an *A11 basic* batch mill (IKA, Staufen, Germany). A rough estimation of the soluble pectin content can be calculated by the relation between the samples' weight after freeze-drying and the initial weight of 100 mL serum, expressed in %.

2.7. Water retention capacity

The water retention capacity of the pulp was calculated as the amount of water retained by the mass of the pulp's AIS (g/g dry weight) according to Robertson et al. (2000). In this study, it was estimated as the relation between the pulp wet mass and the mass of the pulp's AIS. The mass of the fibre is generally negligible and was thus not considered in this equation.

2.8. Sugar composition of AIS

2.8.1. Cell wall analysis

AIS (10 mg) were subjected to prehydrolysis with sulphuric acid (72 % v/v) for one hour at room temperature. Afterwards, the samples were diluted to 1 M sulphuric acid by addition of water and internal standard (inositol) and heated at 100 °C for 3 hours (Saeman, Moore, Mitchell, & Millett, 1954). For neutral sugars analysis, the free sugars were derivatized to volatile alditol acetates according to the method described by Englyst, Wiggins, and Cummings (1982). They were injected on a Clarus 500 gas chromatograph (PerkinElmer, Waltham, USA) equipped with a flame ionization detector (FID) and a OPTIMA® capillary column of 30 m × 0.25 mm i.d. and 0.25 µm film thickness (Macherey-Nagel, Düren, Germany). Helium was used as carrier gas at 1.5 mL/min and the injector temperature was set at 250 °C in split mode (ratio 1:8). The oven temperature was maintained at 230 °C.

Galacturonic acid was measured spectrophotometrically by the m-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973) in the acid hydrolysates.

Methanol was quantified via stable isotope dilution assay after saponification (Renard & Ginies, 2009) on a *Trace 1300* gas chromatograph (Thermo Scientific, Waltham, USA) and a coupled *ISQ LT* single quadrupole mass spectrometer (Thermo Scientific, Waltham, USA). Capillary column was a TG-WaxMS of 30 m × 0.25 mm i.d. and 0.5 µm film thickness. Samples' headspace was injected in split injector (ratio 15:1) at 220 °C. Helium (70 kPa) was used as carrier gas. The oven temperature was set at 40 °C. Electron ionization (70 eV) was used at 250 °C.

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

2.8.2. Starch determination

Starch was quantified in the AIS of the pulp, serum and raw apples using the total starch assay kit K-TSTA (Megazyme, Wicklow, Ireland) according to the manufacturer's instructions

except that the amount of enzymes was doubled to counteract enzyme inhibition by the interaction of remaining polyphenols in the AIS with the enzymes. The test was performed in duplicate. All values for AIS, neutral sugars, galacturonic acid and methanol were corrected by the respective starch content in the sample.

2.9. High performance size-exclusion chromatography coupled to multi-angle laser light scattering (HPSEC-MALLS) and online viscometry

Molar mass and size distributions of soluble pectins was obtained by HPSEC-MALLS coupled to viscometric detection. An Ultra Fast Liquid Chromatography Prominence system (Shimadzu, Kyoto, Japan) including a LC-2OAD pump, a DGU-20A5 degasser, a SIL-20ACHT autosampler, a CTO-20 AC column oven, a SPD-M20A diode array detector and a RID-10A refractive index detector from Shimadzu (Tokyo, Japan) was employed together with a multi-angle laser light scattering detector (DAWN HELEOS 8+ fitted with a K5 flow cell and a GaAs laser, λ = 660 nm) and a ViscostarIII viscometer from Wyatt Technology Corporation (Santa Barbara, CA). Three HPSEC columns (PolySep-GFC-P3000, P5000 and P6000 300 × 7.8 mm) and a guard column from Phenomenex (Le Pecq, France) were used for the separation and all the HPSEC-MALLS system was maintained at 40 °C. The eluent, an acetate buffer (0.2 M) at pH 3.6, was carefully filtered through a 0.1 µm omnipore™ membrane from Millipore (Milford, USA) and degassed before elution at a flow rate of 0.6 mL/min for 130 minutes. Pectins were solubilized overnight under magnetic stirring directly in the filtered eluent at 4 °C to a theoretical concentration of 10 mg AIS/mL (raw apples) and 2.5 mg AIS/mL (serum). The samples were centrifuged (7690 x g, 4 °C, 10 minutes) and the supernatant was filtered through 0.45 µm hydrophilic PTFE syringe filter (Macherey-Nagel, Düren, Germany) before injection (100 µL). ASTRA® software from

Wyatt Technology Corporation (version 7.1.4 for PC) was used to establish M_i and R_{gi}, the molar mass and the radius of gyration at the i^{th} slice of the chromatogram using the light scattering signal from six angles (20.4 °-110 °) and the Zimm formalism with a one order polynomial fit to extrapolate the data to zero angle (Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004). ASTRA® software was also used to calculate the viscometric hydrodynamic radius for the equivalent sphere by combining viscosity and molar mass measurements using the following equation derived from the Einstein and Simha relation (Einstein, 1906, 1911; Simha, 1940):

$$[\eta]_i M_i = \gamma N_A V_{hi} = \frac{10\pi}{3} N_A R_{hi}^3 \quad (1)$$

where $[\eta]_i$, R_{hi} and V_{hi} are the intrinsic viscosity, the hydrodynamic radius and the hydrodynamic volume at the i^{th} slice of the chromatogram, $\gamma = 2.5$ for spheres and N_A is the Avogadro number. The weight-average molar mass $\overline{M}_w$, the z-average radius of gyration $\overline{R}_{gz}$, the z-average intrinsic viscosity $\overline{[\eta]}_z$ and the z-average viscometric hydrodynamic radius $\overline{R}_{hz}(v)$ were obtained by taking the summations over the whole peaks. The refractive index increment (dn/dc) for glycans was fixed at 0.146 mL/g and the normalization of photodiodes was achieved using a low molar mass pullulan standard (P20) from Showa Denko K.K. (Tokyo, Japan).

2.10. Statistical analysis

The Shapiro-Wilk test showed that not all data were normally distributed. Therefore, they were assessed by Kruskal-Wallis non-parametric test (Kruskal & Wallis, 1952) using R statistical software (R Core Team, 2018). Here, the H value is the test statistic that is used to

calculate the P values. Differences were considered to be significant at $P < 0.05$. Standard deviations were calculated for each series of replicated measurement using the sum of individual variances weighted by the individual degrees of freedom (Box, Hunter, & Hunter, 1978). Principal Component Analysis (PCA) was performed using the package "FactoMineR" (Lê, Josse, & Husson, 2008) for R statistical software.

3. Results and discussion

3.1. Rheological parameters of apple purees

All apple purees showed a shear-thinning behaviour (data not shown) as already described (Espinosa et al., 2011; Rao, Cooley, Nogueira, & McLellan, 1986). Apple cultivar and tree thinning practice (summarized as "raw material", Table 1) led to significantly different puree's viscosities. The highest values were obtained for GD1 and the lowest for GD2 (Fig. 1A). The apparent viscosity of the purees (η_{app}) also changed significantly with post-harvest storage (Table 1). However, this change did not follow a monotonous tendency, as for GD (GD1 and GD2) the viscosity decreased during the first months of post-harvest storage before increasing again between three and six months. For GS, however, η_{app} decreased monotonously for not refined samples during storage whereas viscosity of refined samples increased (Fig. 1A). The refined purees showed significantly lower viscosity values than not refined samples which might be explained by particle size.

Different apple cultivars and fruit loads also generated significantly different yield stress values (Table 1), the highest for GD2 and the lowest for GS (Fig. 1B). For GD apples, yield stress decreased abruptly between purees prepared from directly harvested apples and purees made from apples stored for one month. Interestingly, yield stress values from purees

obtained from GD at the two fruit loads converged between three and six months. During post-harvest storage, the yield stress showed similar tendencies as apparent viscosity as a minimum was observed after three months for GD1, GD2 and not refined GS samples. Refined GS purees showed continuously increasing yield stress values. Unlike apparent viscosity, yield stress was not significantly affected by refining (Table 1).

3.2. Structural parameters of apple purees

3.2.1. Serum viscosity

Highest values of serum viscosity (Fig. 1C) were obtained with GS and the lowest with GD2, except at T6. Even if serum viscosity of GS decreased markedly between T0 and T1, no significant variations were detected over post-harvest storage (Table 1). Interestingly, the purees showing the highest serum viscosities revealed the lowest yield stress values. This might be due to the lubricant effect of the serum as stated by Espinosa-Munoz, Renard, Symoneaux, Biau, and Cuvelier (2013), even if this trend was only observed for purees prepared with freshly harvested apples. It was hypothesized that pectins' characteristics, such as chemical structure and molar mass, were modified over storage so that the lubricant effect of the serum became less important. Refining did not influence the viscosity of the soluble phase.

3.2.2. Pulp wet mass

For GD apples, pulp wet mass (Fig. 1D) increased significantly over post-harvest storage. However, for GS, the pulp wet mass decreased strongly during the first month of post-harvest storage, then increased again. The contrasted trends between the two cultivars could be due to differences in the cell wall structure, leading to different water retention capacities. Generally,

refining did not induce differences in pulp wet mass, though the not refined samples contained skin particles.

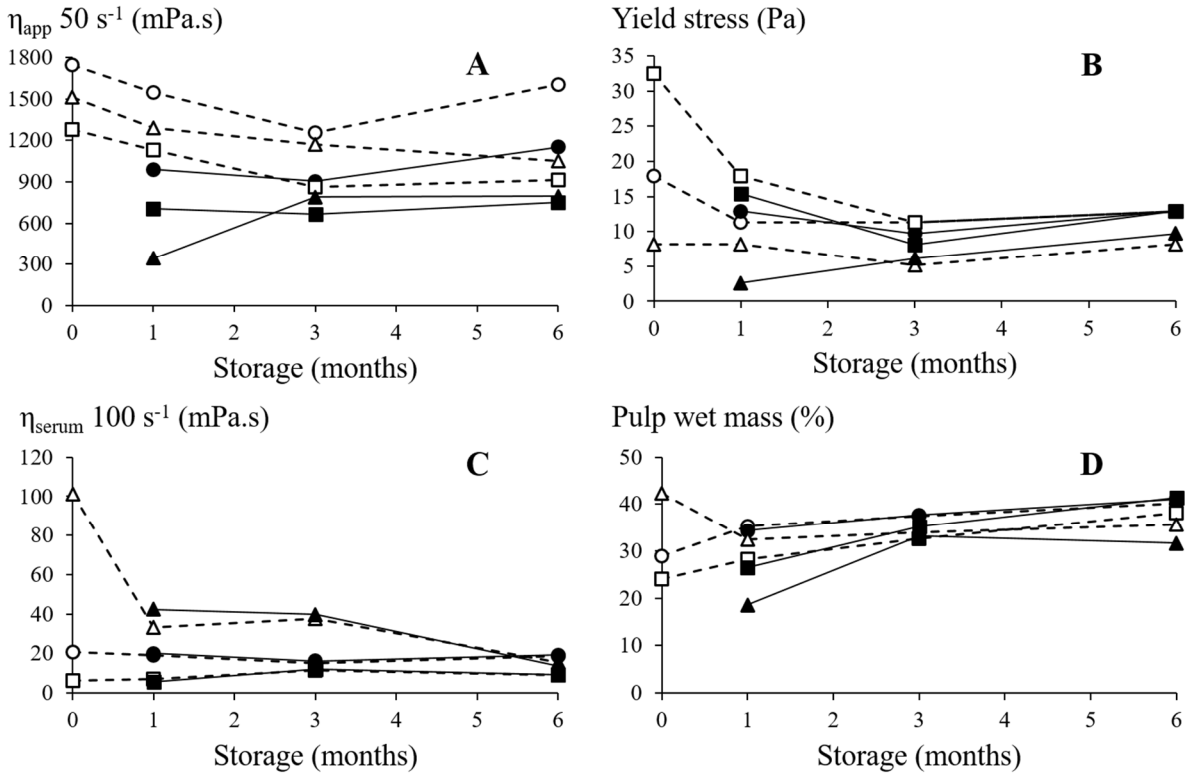


Fig. 1. Rheological and structural parameters of apple purees in function of post-harvest storage duration: Apparent viscosity at 50 s^{-1} (A), yield stress (B), serum viscosity at 100 s^{-1} (C), pulp wet mass (D).

Round symbols represent GD1, rectangular symbols GD2 and triangular symbols GS. Empty symbols display not refined and filled symbols refined (0.5 mm) samples.

Table 1

Kruskal-Wallis *H*-values and *P*-values performed on apparent viscosity at 50 s^{-1} (η_{app}), yield stress, serum viscosity at 100 s^{-1} (η_{serum}) and pulp wet mass (% Pulp).

	η_{app} 50 s^{-1} (mPa.s)	Yield stress (Pa)	η_{serum} 100 s^{-1} (mPa.s)	% Pulp (%)
Raw material *H*	16	34	45	7
Raw material *P*	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**
Storage *H*	16	12	3	20
Storage *P*	**< 0.05**	**< 0.05**	0.41	**< 0.05**
Refining *H*	34	2	1	0
Refining *P*	**< 0.05**	0.17	0.31	0.93

3.2.3. Particle size distribution

As a representative example, Fig. 2 shows the evolution of particle size distribution during post-harvest storage for GD2. Directly after harvest (T0), purees showed a single peak around 1000 µm representing cell clusters (Espinosa-Munoz, Renard, Symoneaux, Biau, & Cuvelier 2013; Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016). Refining eliminated some large particles and the peak shifted to particle sizes close to 800 µm. Although a screen opening of 0.5 mm was used for refining, particles bigger than 0.5 mm were detected, probably because these flexible and deformable particles (Leverrier, Moulin, Cuvelier, & Almeida, 2017) could be pressed through the openings.

After one month of post-harvest storage, a second peak around 200 µm appeared for not refined samples. Espinosa-Munoz, Renard, Symoneaux, Biau, and Cuvelier (2013) and Leverrier, Almeida, Espinosa-Munoz, and Cuvelier (2016) attributed this peak to individual cells by confocal and light microscopy, respectively. Refined samples still showed a monomodal size distribution with a broader peak compared to T0, near 500 µm. Smaller particles in refined purees were in line with lower viscosity values (Fig. 1A) as described in literature (Espinosa et al., 2011; Schijvens, van Vliet, & van Dijk, 1998).

385 At three and six months of post-harvest storage, the particle size remained stable and both the
386 refined and not refined purees showed a similar size distribution with a main peak around
387 200 µm. The size and amount of cell clusters in apple purees thus decreased over post-harvest
388 storage to individual cells. This could be explained by reduced cell adhesion due to pectin
389 solubilization in the middle lamella by pectinase activity (Koziol, Cybulska, Pieczywek, &
390 Zdunek, 2017). Thus, a prolonged storage period led to softer tissues, which could be
391 fragmented more easily during processing.
392 A remarkable result was the convergence of the particle size of not refined and refined purees
393 over storage time. Consequently, refining of apple purees had limited effect after three months
394 of post-harvest storage as the particles were already very small after processing. The same
395 results were obtained with GD1 and GS (data not shown). The texture of refined and not
396 refined purees was, however, still different after three or six months of post-harvest storage
397 (Fig. 1A). Fragments of apple skin remained in not refined samples, and thus increased the
398 apparent viscosity of these samples, but were too big to be detected by laser granulometry.
399 Particle size analysis also revealed differences depending on the apple cultivar and fruit load
400 (Fig. 2). Between three and six months of post-harvest storage, particle size was stable for GD
401 purees but still decreased slightly for GS samples (data not shown). Therefore, a plateau
402 seemed to be reached after six months of post-harvest storage, which was used to compare the
403 particle size of different apple cultivars and fruit loads. Fruit thinning enhances cell division
404 (Goffinet, Robinson, & Lakso, 1995) and, depending on apple cultivar and thinning treatment,
405 cell expansion (Milić et al., 2017; Wismer, Proctor, & Elfving, 1995). Purees of thinned GD1
406 apples revealed bigger particles than GD2, being in accordance with literature (Milić et al.,
407 2017). GS apples were also thinned but showed a similar particle size distribution as GD2.
408 This could be due to varietal effects, which do not respond in the same way to fruit thinning.

409 It is also pointed out that GS and GD1 apples were not thinned with the same chemicals and
410 the same dose as the trees of these varieties initially possessed different fruit loads.
411 Interestingly, GD1 purees, revealing the biggest particles, also showed the highest viscosity
412 value after six months of post-harvest storage (Fig. 1A). For GD1 and GD2, apparent
413 viscosity of the purees decreased until three months of post-harvest storage, which was in line
414 with a decreasing particle size. Apparent viscosity then increased from three to six months of
415 post-harvest storage, while particle size remained unchanged. In this case, the puree's texture
416 was determined by pulp wet mass as it was the only structural factor that increased during
417 post-harvest storage (Fig. 1D). The apparent viscosity of not refined GS samples decreased
418 monotonously during post-harvest storage, which was in line with continuously decreasing
419 particle sizes. Consequently, particle size was the most important factor determining puree's
420 texture in this experiment. Pulp wet mass appeared to be the second most important factor as
421 it affected texture only when particle size between two or more purees were the same.

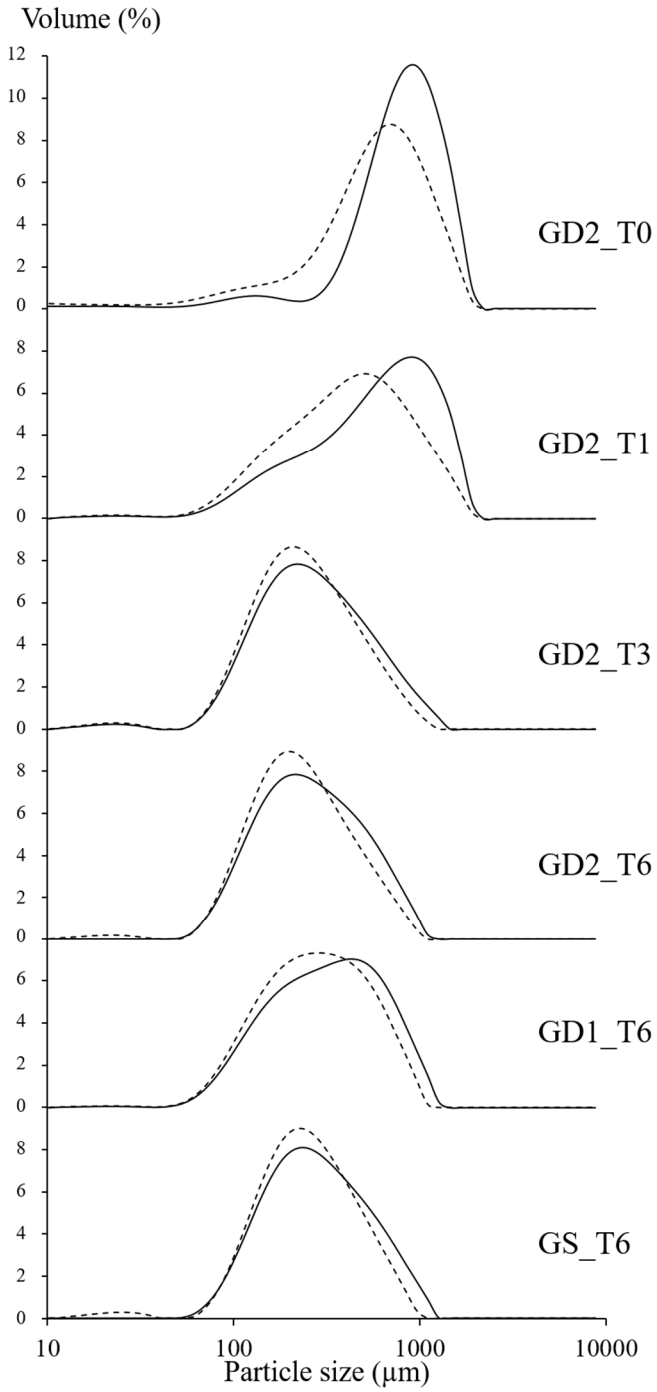


Fig. 2. Particle size distribution of the purees issued from different apple cultivars and at different post-harvest maturity stages. GD1: Golden Delicious, reduced fruit load; GD2: Golden Delicious, high fruit load; GS: Granny Smith; T0: Directly after harvest; T1: After 1 month of post-harvest storage; T3: After 3 months of post-harvest storage; T6: After 6 months of post-harvest storage; Continuous lines represent not refined purees and dashed lines refined (0.5 mm) purees.

The key factors determining puree's texture are particle size, insoluble cell wall content and serum viscosity and are all strongly linked to the cell wall (CW) composition and tissular structure. Hence, the composition of CW polysaccharides as well as pectins' macromolecular characteristics in raw fruits and purees were examined in the following sections.

3.3. Composition of cell wall polysaccharides

Table 2 summarizes the yields and compositions of the AIS of raw apples (FR), pulp (PL) and serum (SE). Puree results are not shown given that Le Bourvellec et al. (2011) described only minor modifications in CW polysaccharides from raw apples into puree during processing. Post-harvest storage was identified as the factor inducing the most significant changes in CW polysaccharides compared to raw material or refining. Consequently, for legibility, Table 2 only shows the evolution of cell wall polysaccharides and starch during post-harvest storage for not refined samples, averaged for GS, GD1 and GD2. The whole dataset can be found in Supplementary Table S1.

AIS composition of raw apples (Table 2) is consistent with previous studies (Fischer & Amado, 1994; Le Bourvellec et al., 2011; Massiot & Renard, 1997; Renard, 2005), i.e. presence of cellulose, highly methylated pectins rich in xylogalacturonans and hemicelluloses fucogalactoxyloglucans and mannans. The AIS content of raw apples decreased over post-harvest storage. The same trend was observed by Fischer and Amado (1994) and the values were similar to those found in literature (15-27 mg/g FW) (Colin-Henrion, Mehinagic, Renard, Richomme, & Jourjon, 2009; Le Bourvellec et al., 2011; Massiot & Renard, 1997; Renard, 2005). The main sugars found in the AIS of raw apples were glucose and galacturonic acid, corresponding to cellulose and pectins, respectively. The glucose content (corrected for starch) was not altered by storage duration, whereas galacturonic acid slightly increased and

454 arabinose and galactose decreased. Arabinose and galactose are part of the side chains of RG I
455 and showed the highest values after glucose and galacturonic acid. A decrease of pectin side
456 chains is a common feature in stored fruits, observed for apples and many other fruits such as
457 tomatoes, pears and strawberries (Fischer & Amado, 1994; Gross & Sams, 1984; Gwanpua et
458 al., 2014). Rhamnose did not evolve during post-harvest storage. Xylose showed a slight but
459 significant increase that has already been observed by Fischer and Amado (1994) and that
460 might be attributed to the decrease of the neutral sugars arabinose and galactose, resulting in a
461 relatively higher amount of xylose. Together with fucose and some galactose and glucose,
462 xylose forms fucogalactoxyloglucan, which is the main hemicellulose of apple CWs (Aspinall
463 & Fanous, 1984; Renard, Voragen, Thibault, & Pilnik, 1991). Mannose, the main element of
464 the hemicellulose mannan (Voragen, Schols, & Pilnik, 1986), increased slightly during apple
465 fruit storage. Pectins in the AIS of raw apples were highly methylated (> 50 %), in accordance
466 with previously published values (65-80 %) (Billy et al., 2008; De Vries, Voragen, Rombouts,
467 & Pilnik, 1981; Le Bourvellec et al., 2011). The DM remained unchanged during storage.
468 Billy et al. (2008), De Vries, Voragen, Rombouts, and Pilnik (1981) and Gwanpua et al.
469 (2014) also observe no change in the DM of raw apple CW during post-harvest storage.
470 The AIS content of the pulp (Table 2) was between three to four times higher than AIS of raw
471 apples. This reflected the concentration of insoluble fibres, namely cellulose, hemicellulose
472 and insoluble pectins, in the pulp. Major trends of CW composition found in FR were
473 maintained during apple processing and could be detected in the pulps' polysaccharides. The
474 main sugar was glucose, coming primarily from cellulose, followed by galacturonic acid. The
475 starch content was lower than in raw fruit, decreasing to not detectable values at T6. The DM
476 of pectins occurring in the pulp remained stable over post-harvest storage and was slightly
477 higher than the DM of raw apple pectins. The sugars constituting the side chains of RG I,
478 arabinose and galactose, decreased significantly during storage. Sugars associated with

hemicelluloses (xylose, mannose and fucose) showed similar or slightly higher values, all increasing (by balance) during post-harvest storage. Interestingly, AIS of the pulp decreased significantly over post-harvest storage. However, the pulp wet mass (Fig. 1D) generally increased. This demonstrated improved water retention capacities of aged cells as calculated in Table 2, resulting in an alleged higher pulp wet mass. The water retention capacity of plant cells is thought to be affected by the porosity of the CW induced by structural changes of CW polysaccharides and their association (Bidhendi & Geitmann, 2016). Lopez-Sanchez et al. (2020) showed that calcium cross-linking of HG pectins impedes interaction of pectin chains with cellulose and thus reduces densification of the CW. As a result, water retention increases. However, this effect can be excluded in this study: the CW charge, necessary to initiate calcium cross-linking, did not change over post-harvest storage as the DM of the pectins in the pulp remained constant. Another study reported an interaction between RG I side chains and the cellulose-xyloglucan network in the CW (Zykwinska, Ralet, Garnier, & Thibault, 2005). The decreasing amounts of arabinose and galactose during storage (Table 2) might thus be responsible for CW loosening. Hence, CW porosity could increase and would be able to retain a higher amount of water.

Serum samples contained low concentrations of soluble polysaccharides, in accordance with the values (1-5 mg/g FW) found by Le Bourvellec et al. (2011). At T0, the serum AIS contained high values of glucose and relatively low galacturonic acid. The abnormal values at T0 could be explained by presence of gelatinized starch in the serum at T0, accounting for more than half of the AIS. From T1, glucose content was more than five times less and galacturonic acid became the main sugar. This confirmed that pectins were the main constituent in serum, as expected (Le Bourvellec et al., 2011). Soluble pectins were highly methylated and contained arabinose and galactose, probably from RG I side chains, which decreased significantly until T3. Interestingly, values for arabinose and galactose increased

again between three and six months of post-harvest storage. Xylose and fucose, both neutral sugars deriving from hemicelluloses, showed lower values compared to FR or pulp AIS and no obvious trend during storage could be observed. No mannose was detected in the serum AIS. Colin-Henrion, Mehinagic, Renard, Richomme, and Jourjon (2009) and Le Bourvellec et al. (2011) found similar trends regarding the amount and composition of the AIS in the pulp and the serum.

The CW composition showed a clear decrease of RG I side chains during post-harvest storage. This might be related to decreasing particle sizes in the puree (see section 3.2.3.) as a reduction of cell adhesion in apples through RG I debranching has already been described in literature (Pena & Carpita, 2004). Another study correlated apple fruit softening to a decreasing number of HG-calcium complexes in the middle lamella (Ng et al., 2013). The DM of firmer apples should then be less important to initiate cross-linking with calcium ions. Nevertheless, the latter study showed an increase of firmness without demethylation, leading to the conclusion that calcium cross-linking does not always induce firmer fruits. As the calcium content in the fruit is determined at harvest and will not alter significantly during post-harvest storage (slight changes may only occur due to altered dry matter) and as the pectins analysed in this study were highly methylated (Table 2), and thus unlikely to form calcium bridges, these complexes were not considered in this study.

Table 2

Yields AIS from fresh weight (mg/g fresh weight), water retention capacity of the pulp (g/g dry weight) and compositions (mg/g AIS) of the AIS of raw apples (FR), pulp (PL) and serum (SE). Data are averaged from compositions of GS, GD1 and GD2 at a given storage time and puree results are those of not refined (NR) samples. For detailed dataset, see Supplementary Table S1.

Storage (months)	Type	Yields AIS	Water retention	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	MeOH (DM%)	Starch
0	FR	29		13	12	138	52	22	127	344	259	34 (70)	220
	PL	103	10	13	11	130	63	24	135	339	249	36 (80)	72
	SE	3		23	9	96	28	0	122	222	426	73 (92)	554
1	FR	26		12	15	125	61	22	93	313	319	40 (70)	54
	PL	89	11	16	13	130	69	27	101	361	248	36 (81)	27
	SE	2		13	6	71	16	0	79	38	670	107 (86)	176
3	FR	25		13	19	106	68	23	74	325	329	44 (74)	5
	PL	87	12	16	14	106	79	28	84	379	258	35 (76)	4
	SE	1		12	7	55	19	0	55	32	709	111 (89)	12
6	FR	23		15	15	95	69	26	74	347	319	41 (73)	2
	PL	76	13	16	15	101	82	29	84	406	232	36 (86)	0
	SE	2		18	14	69	27	0	64	39	649	120 (100)	9
	FR	*4*		*3*	*5*	*14*	*6*	*4*	*18*	*31*	*34*	*7 (14)*	*39*
SD	*PL*	*11*	*1*	*2*	*1*	*13*	*5*	*2*	*20*	*24*	*22*	*3 (10)*	*25*
	SE	*1*		*3*	*4*	*18*	*10*	*0*	*26*	*135*	*100*	*19 (10)*	*71*
Kruskal-Wallis	FR *H*	*7*		*4*	*6*	*23*	*21*	*11*	*21*	*8*	*15*	*7 (0.8)*	*33*
	FR *P*	*0.07*		*0.25*	*0.10*	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	*0.05*	***< 0.05***	*0.08 (0.84)*	***< 0.05***
	PL *H*	*16*	*16*	*14*	*23*	*18*	*27*	*12*	*18*	*20*	*8*	*0.4 (5)*	*29*

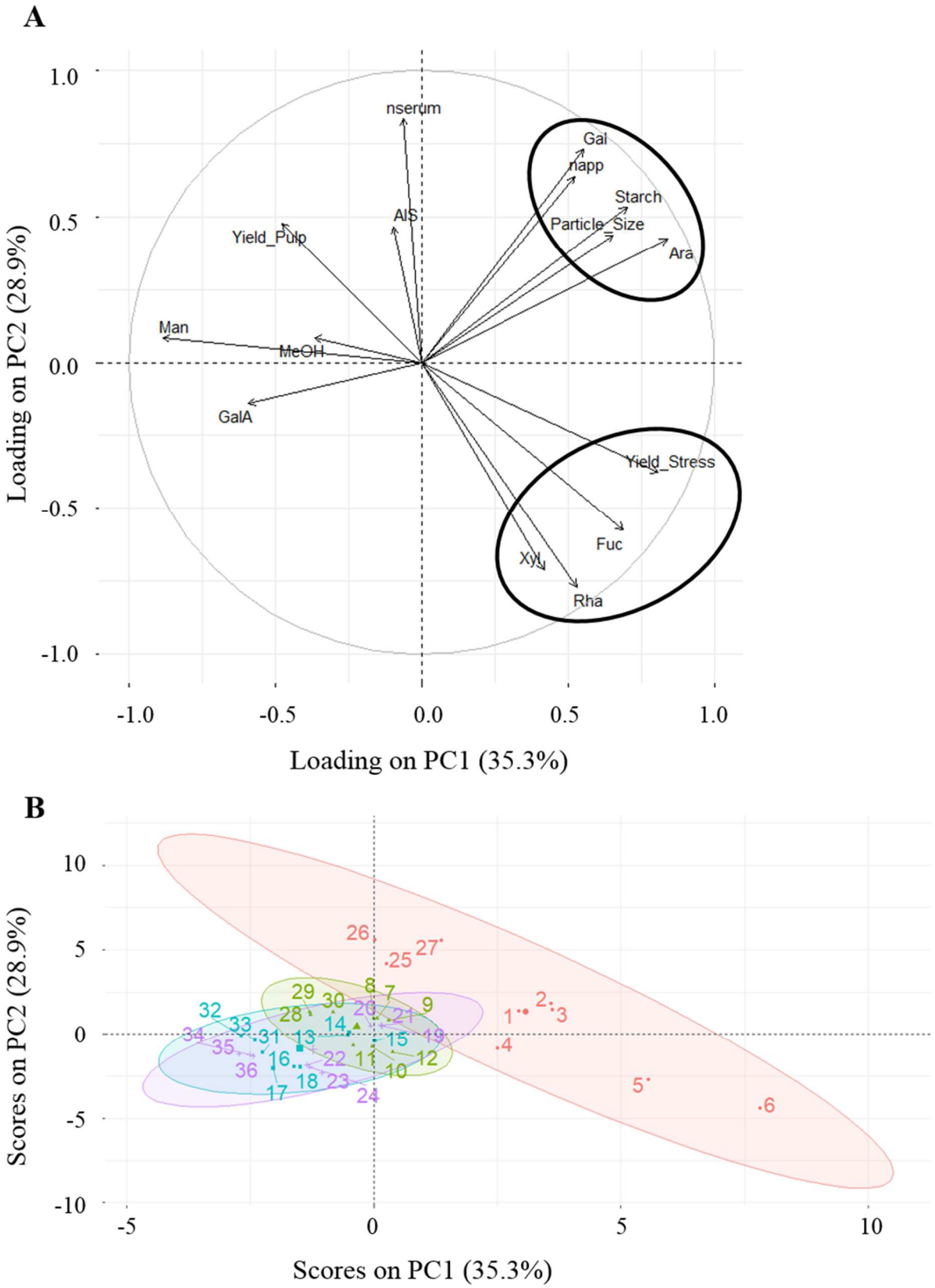
PL *P*	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	*0.05*	*0.94 (0.21)*	***< 0.05***
SE *H*	*10*		*25*	*15*	*18*	*9*	-	*27*	*3*	*23*	*14 (9)*	*30*
SE *P*	***< 0.05***		***< 0.05***	***< 0.05***	***< 0.05***	***< 0.05***	-	***< 0.05***	*0.34*	***< 0.05***	***< 0.05 (<0.05)***	***< 0.05***

Rha: Rhamnose; Fuc: Fucose; Ara: Arabinose; Xyl: Xylose; Man: Mannose; Gal: Galactose; Glc: Glucose without starch; GalA: Galacturonic acid; MeOH: Methanol; DM%: Degree of methylation; SD: Standard deviation of the mean (degrees of freedom: 32). All values were corrected for the starch content. Yields of AIS in serum and pulp are expressed relative to the weight of serum and pulp, respectively, collected after centrifugation.

3.4. Relationship between pectin composition and the purees' texture

In order to visualize the relation between the puree's viscosity, its determining factors (CW content, particle size, serum viscosity) and pectin composition of the puree, a principal component analysis (PCA) was performed (Fig. 3). The first two principal components (PC1 and PC2) explained together nearly 65 % of the total variance. A group of correlated variables (Fig. 3A) was that comprising puree's viscosity (napp), starch content in the puree (Starch, deduced from starch analysis of raw apples), particle size (Particle_Size) and the pectic sugars arabinose (Ara) and galactose (Gal). All these parameters decreased during post-harvest storage and allowed differentiation of the purees by post-harvest storage duration (Fig. 3B). Although the studied apple cultivars and agricultural practices showed significant differences for both textural characteristics and pectin composition (Supplementary Table S1), their CW structure failed to explain puree´s texture. It was thus decided to not further consider these parameters and to group the samples by post-harvest storage. "Starch" was most modified between T0 and T1, which could be linked with individuation of the T0 samples on the sample map. Mannose (Man) contributed highly to PC1, which was probably linked to the higher mannose content in GS apples (Supplementary Table S1), as all GS samples were shifted to the negative side of PC1 relative to GD. Serum viscosity (nserum) was highly accounted for on PC2 (Fig. 3A). A second group of correlated variables was that of xylose (Xyl), fucose (Fuc) (both key components of fucogalactoxyloglucans), rhamnose (Rha) and to a lesser extent yield stress (Yield_Stress). Puree's AIS (AIS), methanol (MeOH), galacturonic acid content (GalA) and pulp wet mass (Yield_Pulp) were all poorly represented on plane 1x2. Interestingly, purees at T0 showed a high dispersion, whereas the other purees were less dispersed. According to these results, puree's viscosity was mainly affected by starch content and particle size, both parameters that decreased during post-harvest storage of apples.

In model systems, the apple puree´s texture was shown to be determined by the volume occupied by the particles, that is to say their quantity and size (Espinosa-Munoz, Renard, Symoneaux, Biau, & Cuvelier, 2013; Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016). In these reconstituted systems, the amount of insoluble cell walls (AIS) and the size of the particles could be varied independently, so that the insoluble solids content has a first-order effect on the rheological behaviour of apple purees. In real systems, however, particle size seemed to be the most important factor determining the puree´s texture, given the fact that the amount of AIS did not change sufficiently to have an impact on texture.



564

565 **Fig. 3.** Principal component analysis (PCA) of neutral sugars, rheological and structural
566 characteristics of apple purees over post-harvest storage. Correlation circle of variables

loadings on PC1 (33.8 %) and PC2 (27.4 %) (A). Sample map of scores on PC1 (33.8 %) and PC2 (27.4 %) as function of post-harvest storage (B). The red ellipse represents samples at T0, the green ellipse samples at T1, the blue ellipse samples at T3 and the violet ellipse samples at T6. All correlation ellipses correspond to the 95 % confidence interval around the barycentre. Only not refined (NR) samples were considered and the values of AIS and neutral sugars were corrected for the starch content (deduced from starch analysis of raw apples). napp: Apparent viscosity of the puree at 50 s^{-1}; Yield_Stress: Yield stress of the puree; nserum: Serum viscosity at 100 s^{-1}; Yield_Pulp: Pulp wet mass; Particle_Size: Particle size in the puree (d 0.9); AIS: Alcohol Insoluble Solids; Rha: Rhamnose; Fuc: Fucose; Ara: Arabinose; Xyl: Xylose; Man: Mannose; Gal: Galactose; GalA: Galacturonic acid; MeOH: Methanol; Starch: Starch in the puree (deduced from starch analysis of raw apples). 1-3: T0_GD1; 4-6: T0_GD2; 7-9: T1_GD1; 10-12: T1_GD2; 13-15: T3_GD1; 16-18: T3_GD2; 19-21: T6_GD1; 22-24: T6_GD2; 25-27: T0_GS; 28-30: T1_GS; 31-33: T3_GS; 34-36: T6_GS (GD1: Golden Delicious, reduced fruit load; GD2: Golden Delicious, high fruit load; GS: Granny Smith; T0: Directly after harvest; T1: After 1 month of post-harvest storage; T3: After 3 months of post-harvest storage; T6: After 6 months of post-harvest storage).

3.5. Macromolecular characteristics of soluble pectins

The decrease of particle size over post-harvest storage could be further explained by analysis of the macromolecular characteristics of soluble pectins. As they followed common trends over post-harvest storage and processing, they were averaged from the samples of GD1, GD2 and GS (Table 3). Although the structural features of soluble pectins varied according to different cultivars and agricultural practices (Supplementary Table S2), no clear trend could be detected.

592 GD2 was chosen as a representative example to illustrate the molar mass distribution of
593 soluble pectins in raw apples (Fig. 4A) and sera (Fig. 4B). Both showed a shift of the main
594 peak to higher elution volumes, i.e. polysaccharides of smaller macromolecular sizes, during
595 storage. This shift was the most drastic during the first month of post-harvest storage. A
596 second, narrower peak was detected at high elution volumes. Interestingly, its concentration
597 diminished from T0 to T6. However, starch could be excluded to contribute to this peak as the
598 determined molar mass was too small compared to values reported in literature (1.0×10^8–
599 4.8×10^8 g/mol) (Rolland-Sabate, Guilois, Jaillais, & Colonna, 2011). Hence, this peak was
600 assigned to some oligosaccharides remaining in the AIS. Molar mass of soluble pectins
601 (Table 3) in raw apples decreased significantly during post-harvest storage. The viscometric
602 hydrodynamic radius also decreased for raw apple pectins, further confirming a decrease in
603 pectin size during apple storage. Gwanpua et al. (2014) also showed a decrease in molar mass
604 of water soluble pectins during post-harvest storage of apples and ascribed this change to
605 either pectin depolymerisation, an extensive loss of RG I side chains or both. Molar mass of
606 serum pectins only decreased during the first month of post-harvest storage, before a slight
607 increase was observed at T6. The same trend was observed for the hydrodynamic radius.
608 Apparently, high molar mass pectins could be extracted during puree processing at T6.
609 During the first month of post-harvest storage, $\overline{[\eta]}_z$ of both raw apple and serum pectins
610 increased even if molar mass decreased (Table 3). This was due to structural changes in
611 soluble pectins, showing more extended and less dense conformations during storage.
612 However, $\overline{[\eta]}_z$ decreased for raw apples and sera at T6 while molar mass values kept
613 decreasing (raw fruit pectins) or remained similar (serum pectins). This indicated presence of
614 pectins with less extended and denser conformations. The shape and structure of
615 polysaccharides can also be assessed by calculating the ρ-parameter ($\rho = \overline{R}_{gz}/\overline{R}_{hz}$) which has
616 theoretical values of ~1.0 for soft spheres, values higher than 1.5 for linear polymers and

617 increasing values with the linearity and the stiffness of the system (Burchard, 1983). This
618 ratio increased for soluble pectins of raw apples over post-harvest storage, indicating a
619 process of shape extension of soluble raw apple pectins that corresponds well to the
620 linearization of the pectins. In contrast, the ρ-parameter increased for serum pectins until T1,
621 then decreased slightly. Thus, serum pectins seemed to be the most linear at T1.
622 In summary, macromolecular sizes and molar masses of raw apple pectins decreased during
623 prolonged post-harvest storage. In addition, they became more linear, probably due to RG I
624 side chain hydrolysis. The decrease of neutral sugars arabinose and galactose during storage,
625 as detected in section 3.3., supported this hypothesis. Serum pectins revealed the same trend
626 during the first month of post-harvest storage. At T6, in contrast, high molar mass pectins
627 could be extracted during puree processing. They also showed less linear characteristics, in
628 line with increased arabinose and galactose contents of serum pectins at T6 (section 3.3.).
629 RG I side chains were found to interact with the cellulose-xyloglucan network in the CW
630 (Zykwinska, Ralet, Garnier, & Thibault, 2005). We thus think that RG I side chain hydrolysis
631 as well as a decrease in macromolecular size and molar mass of raw apple pectins during
632 storage reduced pectin entanglement in the CW structure. This might enhance extractability of
633 high molar mass pectins from RG I regions during processing.
634 Interestingly, soluble pectins in raw apples and sera showed the same macromolecular size
635 but different molar masses both at T0 (Fig. 5A) and T6 (Fig. 5B). At T0 and T1, soluble
636 pectins in raw fruits showed higher molar masses than serum pectins (Table 3). Processing at
637 T0 and T1 did thus not lead to degradation of the pectic main chain (same macromolecular
638 size) but to loss of RG I side chains (decreased molar mass). In addition, serum pectins
639 exhibited similar $\overline{R}_{hz}(v)$ but higher $\overline{[\eta]}_z$ and ρ values at T0 and T1, further confirming
640 existence of more linear and thus less branched pectins in the serum. However, at T6, serum
641 pectins showed higher molar masses, higher $\overline{R}_{hz}(v)$ and $\overline{[\eta]}_z$ but lower ρ values at same

642 macromolecular sizes compared to raw fruit pectins. It thus seemed that, after prolonged post-
643 harvest storage, less linear and more branched pectins could be solubilized in the serum,
644 probably because they were less attached to the CW. However, no simple links were found
645 between serum viscosity and the molar mass of soluble pectins.

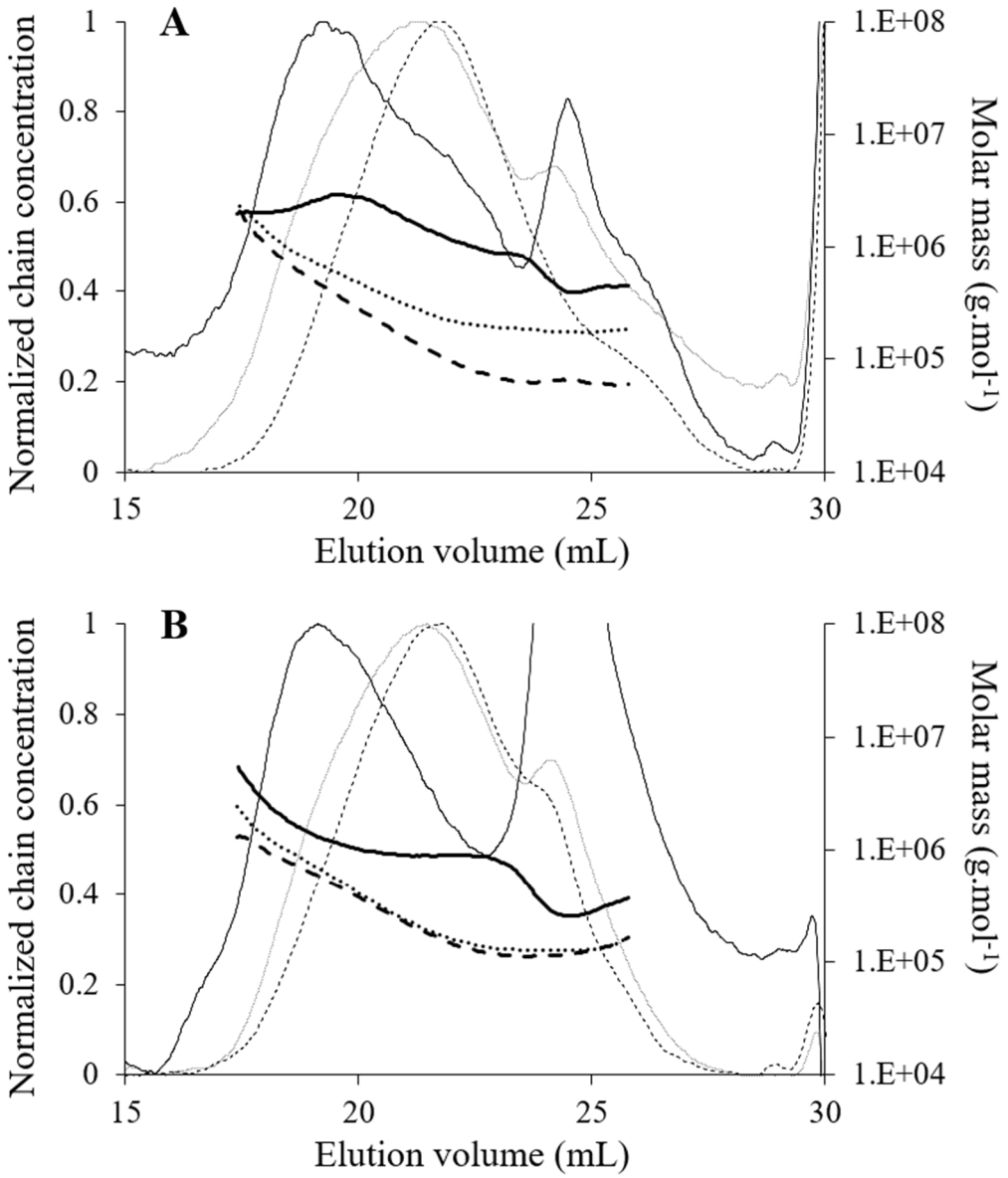
646

647 **Table 3**

648 Evolution of macromolecular features of soluble pectins in raw apples (FR) and
649 corresponding sera (SE) during post-harvest storage measured by HPSEC-MALLS coupled to
650 online viscometry. Data are averaged from compositions of GS, GD1 and GD2 at a given
651 storage time and serum results are those of not refined (NR) samples. For detailed dataset, see
652 Supplementary Table S2.

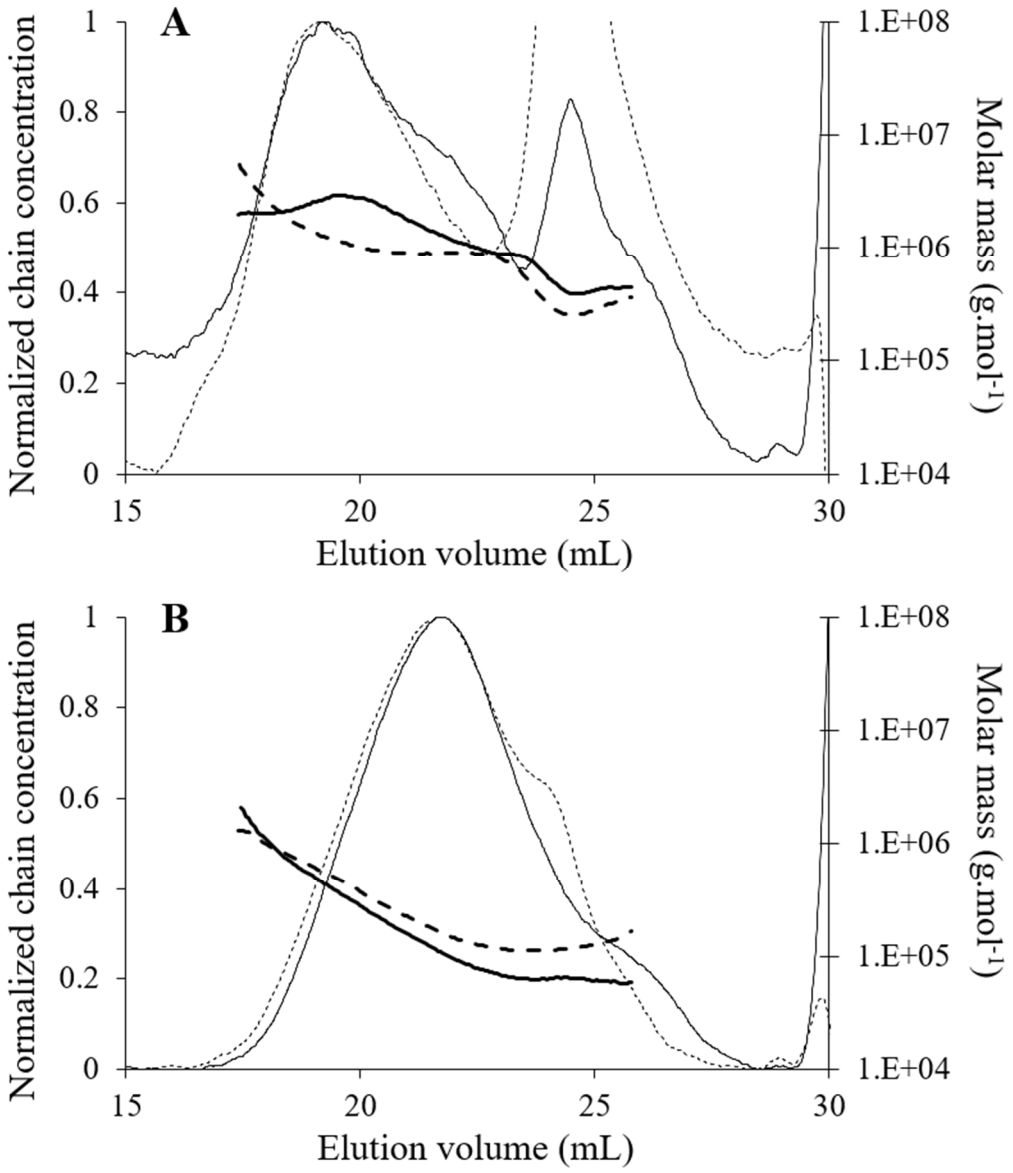
Storage (months)	Type	$\overline{M}_w$ x 10^3 (g/mol)	$\overline{R}_{hz}(v)$ (nm)	$\overline{[\eta]}_z$ (mL/g)	$\rho = \overline{R}_{gz}/\overline{R}_{hz}(v)$
0	FR	1374	66	1162	0.9
	SE	924	68	1619	1.0
1	FR	430	50	1496	1.3
	SE	361	51	1721	1.5
6	FR	181	40	1396	1.6
	SE	410	52	1502	1.4
SD	*FR*	*215*	*5*	*210*	0.1
	SE	*157*	*8*	*212*	0.2
Kruskal-Wallis	FR *H*	*23*	*16*	*11*	*21*
	FR *P*	***<0.05***	***<0.05***	***<0.05***	***<0.05***
	SE *H*	*17*	*8*	*4*	*14*
	SE *P*	***<0.05***	***<0.05***	*0.17*	***<0.05***

653 FR: Raw fruit; SE: Serum; $\overline{M}_w$: weight-average molar mass; $\overline{R}_{hz}(v)$: z-average viscometric
654 hydrodynamic radius; $\overline{[\eta]}_z$: z-average intrinsic viscosity; $\overline{R}_{gz}$: z-average radius of gyration;
655 SD: Standard deviation of the mean (degrees of freedom: 22).



656

657 **Fig. 4.** Representative chromatograms for the cultivar GD2 in the function of storage.
658 Normalized chain concentration and molar mass versus elution volume of soluble pectins in
659 raw apples (A) and sera (B). Continuous lines represent samples at T0, dotted lines samples at
660 T1 and dashed lines samples at T6. The signal (mV) obtained by the RID detector was
661 normalised through dividing all data points by the peak signal.



662

663 **Fig. 5.** Representative chromatograms for the cultivar GD2 in the function of the type (raw
664 apple versus serum pectins). Normalized chain concentration and molar mass versus elution
665 volume of soluble pectins at T0 (A) and T6 (B). Continuous lines represent raw apple and
666 dashed lines serum pectins. The signal (mV) obtained by the RID detector was normalised
667 through dividing all data points by the peak signal.

668

669

670

4. Conclusions

Post-harvest storage duration influenced puree's viscosity the most, whereas the CW composition of apple cultivars and agricultural practices failed to explain the puree´s texture. However, they all showed similar trends during storage. Particle size decreased during prolonged post-harvest storage and seemed to be the most important factor determining puree's texture as smaller particles generated less viscous purees. When two purees showed similar particle sizes, pulp wet mass determined texture. In raw apples, prolonged storage let to pectin RG I side chain hydrolysis as well as to a reduction of soluble pectins' macromolecular size and molar mass. This promoted softening of apple tissue due to reduced cell adhesion, leading to facilitated fragmentation during processing. In addition, water retention capacities of the cells increased when aged, probably due to elevated porosity as the pectins were less entangled in the CW structure. During puree processing, prolonged post-harvest storage facilitated extractability of high molar mass pectins that were less linear and more branched. However, the pectic main chain was not degraded during processing.

CRediT authorship contribution statement

Alexandra Buergy: Investigation, Formal analysis, Data curation, Writing - original draft. **Agnès Rolland-Sabaté:** Supervision, Validation, Writing - review & editing. **Alexandre Leca:** Investigation, Supervision, Writing - review & editing. **Catherine M. G. C. Renard:** Conceptualization, Funding acquisition, Project administration, Validation, Writing - review & editing.

Declaration of competing interest

Declarations of interest: none.

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Appendix A. Supplementary data

Supplementary Table S1

Supplementary Table S2

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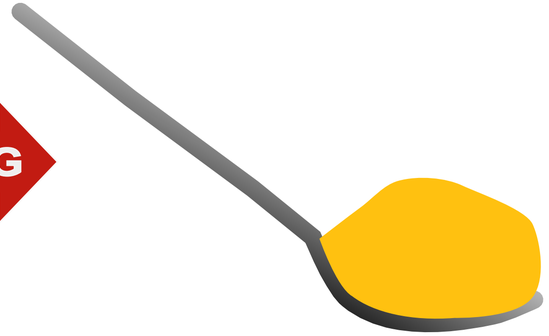
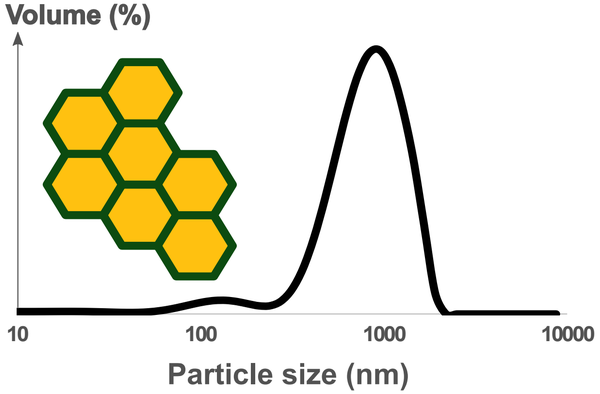
At Harvest

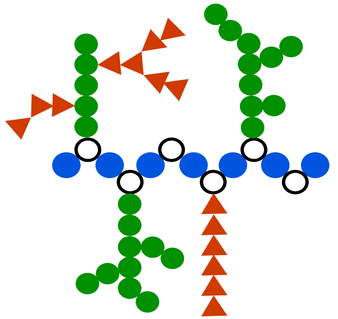
PROCESSING



High viscosity
of the apple puree



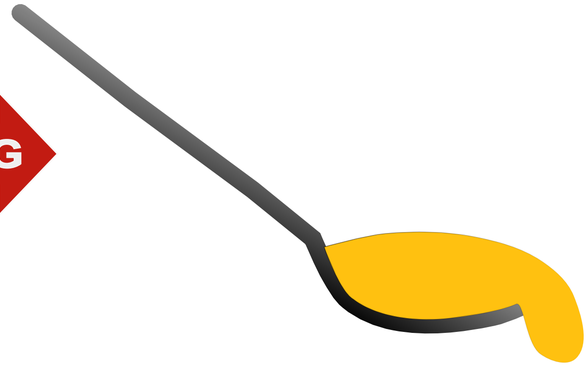
Large cell fragments



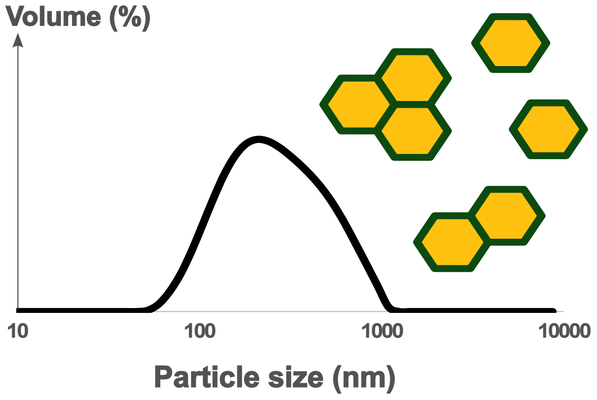
Highly branched pectins

Prolonged
storage time

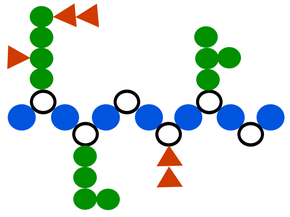
PROCESSING



Low viscosity
of the apple puree



Small cell fragments



Less branched pectins