

# Fresh, freeze-dried or cell wall samples: Which is the most appropriate to determine chemical, structural and rheological variations during apple processing using ATR-FTIR spectroscopy?

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1	Fresh, Freeze-dried or Cell Wall Samples: Which is the Most Appropriate to
2	Determine Chemical, Structural and Rheological Variations During Apple
3	Processing Using ATR-FTIR Spectroscopy?
4	
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# 25 Highlights:

- 26 Proposition of puree sample preparation according to the expected quality traits.
- 27 Similar spectral fingerprints due to processing in fresh and freeze-dried samples.
- 28 ATR-FTIR on fresh purees could predict particle size and volume affecting texture.
- 29 ATR-FTIR on freeze-dried purees could assess viscosity and viscoelasticity.
- 30 ATR-FTIR on cell walls could highlight their changes during processing.
- 31

#### 32 Abstract

33 Attenuated total reflectance Fourier transform spectroscopy (ATR-FTIR) was applied on fresh (NF), freeze-dried (FD) and cell wall materials (AIS) of raw and 34 35 processed apples. These samples prepared from 36 apple sets and the corresponding 36 72 purees, issued from different varieties, agricultural practices, storage periods and 37 processing conditions, were used to build models including exploratory analysis, 38 supervised classification and multivariate calibration. Fresh and freeze-dried samples 39 presented similar fingerprint spectral variations due to processing. ATR-FTIR directly 40 on fresh purees satisfactorily predicted textural properties such as particle average 41 size and volume (RPD> 3.0), while freeze-drying improved assessment of chemical 42 (RPD> 3.2) and rheological (RPD> 3.1) parameters using partial least-squares 43 regression. The assessment of texture and macrocomponents of purees can be 44 obtained with a limited sample preparation. For research applications because of a 45 need of sample preparation, changes of cell wall composition during fruit processing 46 could be assessed in relationship with pectin degradation.

47 Keywords: Malus domestica Borkh., Mid infrared spectroscopy, apple processing,

48 Partial Least-Squares Regression (PLSR), discrimination

49

# 50 **1. Introduction**

Sample preparation is a key point for quality of analytical data. Infrared 51 52 spectroscopy (near or mid-infrared), because of its integrative nature, is one of the 53 main candidates for a rapid qualification of agricultural commodities and processed 54 food, especially in the view of process analytical technology (PAT). Advanced 55 techniques based on infrared spectroscopy offer the advantages of a minimal sample preparation and a rapid data acquisition. However, this questions the balance between 56 57 data intensity and required sample preparation hence man-power: are the data 58 acquired on "raw" samples sufficient for process monitoring, quality control or 59 process comprehension? A specific point is also that foods are frequently highly 60 hydrated and not stable, so that appropriate steps must be taken to preserve samples 61 for later quality control. As the time consumption and cost of sample preparation are 62 generally barriers to a rapid and precise determination by spectroscopy, knowing the 63 most efficient sample pretreatments could contribute to improve analytical results as 64 well as to provide informative options at both, laboratory and industrial scales.

Different methods for the reference data acquisition such as HPLC, GC-MS or NMR (Bureau et al., 2013), types of spectroscopy or related hyperspectral images (NIR, MIR, Raman) (Baranska, Schütze, & Schulz, 2006) and modeling algorithms (Van Boekel, 2008) have been intensively compared on fruits. It seems also crucial to compare and determine the optimal sample form (fresh, freeze-dried or cell wall extracts) and the associated changes occurring during fruit processing, notably using infrared spectroscopy which has the potential to be applied both, on-line and off-line.

Direct ATR-FTIR estimations on fruit fresh homogenates have obtained good results to predict soluble solids content, dry matter content, titratable acidity, some individual sugars and organic acids (Bureau, Ścibisz, Le Bourvellec, & Renard, 2012; Ayvaz et al., 2016). As infrared spectroscopy is extremely sensitive to changes of hydrogen bonding (Jackson & Mantsch, 1995), the main drawback of spectral measurements is the low sensitivity and limited specific signals of chemical compositions under strong water interactions in fresh fruit suspensions, such as citric

79 acid in apples (Bureau, Ścibisz, Le Bourvellec, & Renard, 2012), lycopene and 80 β-carotene in tomato (Baranska, Schütze, & Schulz, 2006). Moreover, classical 81 measurements of rheological properties and particle size distribution of fruit products 82 require costly rheometer, particle sizing equipment and experienced staffs. Therefore, 83 one of the challenging works is to investigate the possibility of ATR-FTIR to estimate 84 the specific rheological modifications (viscosity and viscoelastic parameters) and then 85 to monitor textural changes (particle size and volume) for both, accurate 86 determinations in scientific research or rapid and direct assessment in industrial 87 processing.

88 Much more information can be extracted from dry food commodities, such as the 89 structural changes of cereals (Georget & Belton, 2006), micronutrients in fruits (Lu et 90 al., 2011) and even cell wall content variations (Canteri, Renard, Le Bourvellec, & 91 Bureau, 2019). To overcome the limitations observed on highly hydrated products, 92 such as fruits, drying methods with as limited as possible alteration of composition 93 and structure are needed. Thus, freeze-drying prevents evolution of samples under the 94 action of endogenous enzymes (notably oxidation and hydrolysis). It also carries out a 95 concentration due to water elimination, so that specific components present in low 96 concentrations can have significant spectral absorptions. But freeze-drying is 97 expensive and time-consuming, needing at least 24-48 hours. It allowed to obtain 98 similar predictions of chemical compositions than those in fresh samples (de Oliveira, 99 de Castilhos, Renard, & Bureau, 2014; Oliveira-Folador et al., 2018). Few detailed 100 studies compared the differences and limitations of ATR-FTIR fingerprint regions on 101 fresh and corresponding freeze-dried plant leaves (Durak & Depciuch, 2020).

ATR-FTIR applications to assess fruit textural properties (mainly focus on cell
wall compositions) are always performed on their cell wall materials (AIS) (Canteri,
Renard, Le Bourvellec, & Bureau, 2019; Zymanska-Chargot, Chylinska, Kruk, &
Zdunek, 2015). However, extracting the cell wall requires a large consumption of
chemical solvents if starting from fresh samples (up to 1 L ethanol and 0.4 L acetone/
1.0 - 1.5 g cell wall). The accelerated or pressurized solvent extractors (ASE, PSE)

108 can allow multiplexing and thus a faster and less solvent-consuming cell wall 109 preparation, but only from already freeze-dried samples. After removing all soluble 110 components (mainly sugars and acids), specific signals related to pectins, cellulose 111 and hemicelluloses have proven to be useful for the fast evaluation of cell wall 112 polysaccharides during fruit growth and subsequent storage (Szymanska-Chargot, 113 Chylinska, Kruk, & Zdunek, 2015). Although some cell wall modifications in plants 114 (Femenia, García-Pascual, Simal, & Rosselló, 2003) and fruits (Cardoso et al., 2009) 115 under heating and dehydration have been investigated by ATR-FTIR. However, for 116 fruit processed purees, little work has been done on ATR-FTIR to detect their cell wall 117 changes during processing and monitor rheological and mechanical properties 118 (Ferreira, Barros, Coimbra, & Delgadillo, 2001).

In this study, ATR-FTIR spectroscopy was applied on the corresponding raw apples and processed purees. Spectra were acquired on different kinds of homogeneous samples such as fresh (NF for non-freeze-dried), freeze-dried (FD) and cell wall extracts (AIS for alcohol insoluble solids) in order to: i) evaluate how much sample preparation improved the prediction of chemical, textural and rheological characteristics of purees (number of quality traits and their precision) and ii) identify signals specific of the variations which occur during apple processing.

## 126 **2. Materials and methods**

# 127 2.1 Plant Material

128 Apples of two cultivars: 'Golden Delicious' (GD) and 'Granny Smith' (GS) were 129 harvested at commercial maturity in 2017 in an experimental orchard named La 130 Pugère (Mallemort, Bouches-du-Rhône, France). Standard commercial fruit thinning practices (Th+ to 50 to 100 fruits/tree) and no thinning (Th- to 150-200 fruits/tree) 131 132 were compared during the ripening of 'Golden Delicious'. The three obtained apple 133 groups (Th+ GD, Th- GD and GS) were stored in a cold chamber at 4°C and at around 134 90% of humidity during one, three and six months (respectively T1, T3 and T6), 135 except the first batch (T0) were analyzed and processed the day after harvest without 136 any storage time.

137 Each apple batch (T0, T1, T3 and T6) was divided into two subsets (**Figure 1**):

138 i) the first subset was dedicated to apples characterization: 3 replicates of 10 139 apples were selected and separated into two aggregate samples as described by 140 Bureau (Bureau, Ścibisz, Le Bourvellec, & Renard, 2012). One sample corresponding 141 to the NF sample was stored at -80°C and then homogenized at 11000 rpm with an 142 Ultraturrax T-25 (IKA, Labortechnik, GmbH, Staufen, Germany) after 1.5 h of 143 thawing at 22.5 °C for biochemical and spectral characterizations. The other sample 144 corresponding to the freeze-dried (FD) was used to extract cell wall materials (AIS). 145 Finally, 36 NF, FD and AIS samples (3 apple groups × 4 storage times × 3 biological 146 replicates) of raw apple fruits were obtained.

147 ii) the second sub-set was dedicated to pure processing: 3 replicates of apples (4 148 kg each) were used to produce three puree lots. After sorting and washing, apples 149 were cored and cut in 8 portions, then processed in a multi-functional processing 150 system (Roboqbo, Qb8-3, Bentivoglio, Italy). Half of the each puree (2 kg) was 151 refined with a 0.5 mm (Ra) sieve (Robot Coupe C80 automatic refiner, Robot Coupe 152 SNC, Vincennes, France) whereas the other half was not refined (NR). Finally, fresh 153 puree samples (NF) were conditioned in two hermetically sealing cans: one was 154 cooled at room temperature (22.5 °C) before the next-day measurements of 155 rheological, textual and some chemical (soluble solids and titratable acidity) 156 properties, while the other was freeze-dried (FD) and stored at -20 °C for AIS 157 extraction. Thus, in total 72 NF, FD and AIS samples of purees were prepared and 158 characterized, corresponding to 3 apple groups  $\times$  4 storage times  $\times$  2 refining levels  $\times$ 159 3 biological replicates.

# 160 2.2 Biochemical Analyses

161 Soluble solids content (SSC) was determined with a digital refractometer 162 (PR-101 ATAGO, Norfolk, VA, USA) and expressed in °Brix at 20°C. Titratable 163 acidity (TA) was determined by titration up to pH 8.1 with 0.1 mol/L NaOH and 164 expressed in mmol H<sup>+</sup>/kg of fresh weight (FW) using an autotitrator (Methrom, 165 Herisau, Switzerland). Sugars (glucose, fructose and sucrose) and malic acid were 166 quantified using an enzymatic method with kits for food analysis (Sigma-Aldrich, 167 Deisenhofen, Germany) and expressed in g/kg FW. These measurements were 168 performed with a SAFAS flx-Xenius XM spectrofluorimeter (SAFAS, Monaco). The 169 dry matter content (DMC) was estimated with the weight of freeze-dried samples 170 upon reaching a constant weight (freeze-dryer, 5 days). Cell wall materials (AIS) were 171 isolated using the method proposed by Renard (Renard, 2005). and the cell wall 172 contents (AIS contents) were expressed in both, fresh weight (FW) and dry matter 173 weight (DW). Three biological replicates were characterized for each biochemical 174 trait and each sample.

# 175 2.3 Rheological Analyses

176 The puree rheological measurements consisted in one rotational (flow curve) and 177 two oscillatory (amplitude and frequency sweeps) tests, carried out using a Physica 178 MCR-301 controlled stress rheometer (Anton Paar, Graz, Austria) at 22.5 °C. 50 mL 179 of each puree sample was placed in a C-CC27 with an inner radius of 14.46 mm 180 measuring cup (Anton Paar, Graz, Austria). All tests were performed by a six blade 181 vane geometry FL 100/6W with a radius of 11 mm (Anton Paar, Graz, Austria). The 182 flow curves were performed after a pre-shearing period of 1 minute at 50/s followed by 5 minutes at rest. The viscosity was then measured at a controlled shear rate range 183 184 of [10; 250]/s on a logarithmic ramp, at a rate of 1 point every 15 seconds. The values 185 of the viscosity at 50/s and 100/s ( $\eta_{50}$  and  $\eta_{100}$  respectively) were kept as indicators of 186 the sensorial puree texture (Engelen & de Wijk, 2012; Espinosa-Muñoz et al. 2012) 187 during consumption. Amplitude Sweep (AS) tests were performed at an angular 188 frequency of 10 rad./s in the deformation range of [0.01; 100] %, in order to 189 determine the linear viscoelastic range of the purees and the yield stress, defined as 190 the crossing point between the storage modulus (AS-G') and the loss modulus 191 (AS-G") curves. Frequency Sweep (FS) measurements were operated within the linear viscoelastic region as determined by the AS test (0.05%) in the angular 192 193 frequency range of [0.1; 100] rad./s. For means of comparison the storage and loss 194 moduli (FS-G' and FS-G") were taken at 1 rad./s to evaluate the viscoelastic

195 properties of the studied purees. Puree samples were diluted in distilled water to 196 separate particles and stained with calcofluor white at 0.1 g/L and highlighted with a 197 365 nm UV lamp (Soukup, 2014). A high-resolution digital video camera (Baumer 198 VCXU31C, Baumer SAS, France) with a macro lens (VSTech 0513, VS Technology 199 Corporation, Japan.) was used to visualize the distribution and dispersion of puree 200 particles. The particle sizes averaged over volume d(4:3) (de Brouckere mean) and 201 over surface area d(3:2) (Sauter mean) were measured with a laser granulometer 202 (Rawle, 2003) (Mastersizer 2000, Malvern Instruments, Malvern, UK).

203

# 2.4 ATR-FTIR spectrum acquisition

204 ATR-FTIR spectra were collected at room temperature using a Tensor 27 FTIR 205 spectrometer (Bruker Optics, Wissembourg, France) equipped with a horizontal attenuated total reflectance (ATR) sampling accessory and a deuterated triglycine 206 207 sulphate (DTGS) detector. Three replications of spectral measurement were 208 performed on all raw and processed apples for fresh (NF for non freeze-dried), 209 freeze-dried (FD) and cell wall (AIS) samples. The spectra of all samples were 210 acquired in random order. The instrument adjustment and spectral acquisition were 211 controlled by OPUS software Version 5.0 (Bruker Optics®). The spectra of raw and 212 processed apples were acquired using two different crystals. A big zinc selenide 213 (ATR-ZnSe) crystal with dimensions of 6 cm x 1 cm and six internal reflections was 214 used for fresh samples (apple homogenates and purees) containing water. For the 215 freeze-dried and cell wall samples, a small crystal was used characterized by a 216 single-reflectance horizontal ATR-Diamond Cell (Golden Gate Bruker Optics) 217 equipped with a press tip flap system to press sample on the crystal always in the 218 same way. Spectra (32 scans for ATR-ZnSe and 16 scans for ATR-Diamond) were collected from 4000 cm<sup>-1</sup> to 650 cm<sup>-1</sup> and were corrected against the background 219 220 spectrum of air.

#### 221 **2.5 Statistical Analyses and Chemometrics**

222 After ensuring normal distribution with a Shapiro-Wilk test ( $\alpha$ =0.05), the 223 reference data were presented as mean values and the data dispersion within our experimental dataset expressed as standard deviation values (SD). Analysis of
variance (ANOVA) was carried out to determine the significant differences due to the
controlled factors (thinning, storage and puree mechanical refining) on both apples
(Table S-1) and purees of each variety (Table S-2 and Table S-3) using XLSTAT
(version 2018.5.52037, Addtionsoft SARL, Paris, France) data analysis toolbox.

229 Spectral pre-processing and multivariate data analysis were performed with 230 Matlab 7.5 (Mathworks Inc. Natick, MA) software using the SAISIR package 231 (Bertrand & Cordella, 2008). The absorption band between 2400-2300 cm<sup>-1</sup>, due to 232 carbon dioxide, was discarded prior to the calculation. All FT-IR data were 233 pre-processed with baseline correction, standard normal variate (SNV) and a 234 derivative transform calculation Savitzky–Golay method, gap size = 11, 21, 31) of 235 first or second order. After pretests of these pre-processing treatments applied on 236 several different spectral regions, the best results of prediction and discrimination 237 were obtained on the range 1800-900 cm<sup>-1</sup>, which has been already highlighted 238 (Bureau et al., 2009). Particularly, Principal Component Analysis (PCA) and Factorial 239 Discriminant Analysis (FDA) were applied on SNV pre-treated spectra (in Part 3.1 240 and Part 3.2). The specificity and sensitivity values of FDA discriminations were 241 calculated by the already reported method of Nargis (Nargis et al., 2019), in order to 242 better evaluate sample differentation. For PLS (Partial least square) modelling (in Part 3.3), the baseline correction coupled with SNV pre-processing had the best 243 244 performances to correct multiplicative interferences and variations in baseline shift, 245 and reached the best prediction results.

Leave-one-out PLS models were developed using spectra of fresh (NF), freeze-dried (FD) and AIS of puree samples, for which the three spectral matrices (NF, FD and AIS) corresponded to the same reference dataset. A total number of 72 averaged spectra for each puree form (NF, FD and AIS) corresponding to 3 apple groups (GS, GD Th+ and GD Th-) × 4 storage times × 2 puree refining modalities × 3 biological replicates was used as modelling dataset. PLS model performance was assessed using the determination coefficient of cross-validation ( $R_{cv}^2$ ), the root-mean-square error of cross-validation (RMSECv), the number of latent variables
(LVs), the ratio of the standard deviation values (RPD) and the linkable spectral
regions (Tables 1 and 2). The linearity correlation plots between measured and
predicted values of all PLS models were showed in supplementary materials (Figure
S-5 and Figure S-6).

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# 259 3. Results and discussions

# 260 3.1 Spectral characterization of NF (non-freeze-dried) apple purees

PCA and FDA applied on the spectra of NF puree samples successfully allowed to detect puree differences coming from the raw apple variabilities (cultivar, fruit thinning and storage period) (**Figure 2**). They also highlighted the modifications of puree structure by the mechanical refining over several months of apple storage (**Figure 3**).

266 In Figure 2, the first principal component (PC1) clearly discriminated the two 267 varieties ('Golden Delicious' and 'Granny Smith') and thinning practices for Golden delicious (Th- and Th+), in relation with the fructose variation followed at 1061 cm<sup>-1</sup> 268 269 (Bureau, Cozzolino, & Clark, 2019). Moreover, the peak at 1022 cm<sup>-1</sup>, reported as a peak specific to sucrose in apple juices (Leopold, Leopold, Diehl, & Socaciu, 2011), 270 271 appeared to be the main contributor of the second principal component (PC2), which 272 distinguished the storage times. Along the PC2 axis, the discrimination of storage 273 durations from T0 at the top to T6 at the bottom was in relation with the decrease of 274 sucrose (1022 cm<sup>-1</sup>) and the increase of fructose (1065 cm<sup>-1</sup>) in purees, in accordance 275 with the reference chemical dataset (Table S-2). Consequently, factors such as cultivar, 276 thinning practice and storage duration affecting raw apple characteristics induced 277 changes in the corresponding purees after processing. ATR-FTIR applied directly on 278 processed purees could then be useful for traceability of these effects impacting raw fruits based on the specific C-C and C-O-C bonds of carbohydrates, such as 1022 cm<sup>-1</sup>. 279 280 1061 cm<sup>-1</sup>, 1065 cm<sup>-1</sup>.

281 According to the reference data (Table S-2) and their PCA results (Figure S-1), 282 the mechanical refining resulted a clear reduction of cell wall contents (AIS in DW 283 and FW), viscosity ( $\eta_{50}$  and  $\eta_{100}$ ), viscoelasticity (yield stress, G' and G'' in both 284 oscillatory tests), particle size (d(4:3) and d(3:2)) in T0 purees prepared with apples at 285 harvest (T0). However, gradually over apple storage, less differences were detected 286 between the non-refined (NR) and refined (Ra) purees. The non-refined (NR) 'Golden 287 Delicious' and 'Granny Smith' purees were characterized by large apple particles and 288 only few small separated cells at the beginning of cold storage (T0) (Figure 3a). The 289 refining treatments mainly led to lower particle size by removing the big puree 290 particles (Figure 3a). However, at the end of storage (T6), both non-refined (NR) and 291 refined (Ra) purees were mostly composed of single cells and no clear difference was 292 observed between them (Figure 3d). This similar structure of NR and Ra purees at T6 293 could be due to an increase in cell separability linked to a decrease of the 294 intermolecular bonding between cell wall polymers and a notable increase of pectin 295 solubility during apple storage (Varela, Salvador, & Fiszman, 2007).

296 FDA performed on the spectra of all NR and Ra purees (NF samples) at each 297 apple storage time gave highly consistent observations with the reference data and macroscopic images showed above (Figure 3). According to the third factorial 298 299 components (F3) (F1 and F2 for cultivar and thinning discriminations, Figure S-2), 300 the two puree refining levels were well separated at T0, then appeared progressively 301 overlapped at T3 and T6 (Figure 3). Especially along the F3 axis, at T0, intensive spectral variations were related to the decrease of soluble organic acids (1718 cm<sup>-1</sup> 302 and 1709 cm<sup>-1</sup>), soluble polysaccharides, pectins and absorbed water (1740 cm<sup>-1</sup>, 1695 303 cm<sup>-1</sup>, 1682 cm<sup>-1</sup>, 1668 cm<sup>-1</sup>, 1655 cm<sup>-1</sup> and 1468 cm<sup>-1</sup>) between the two refining 304 305 conditions (Figure S-3). Although the peaks of carbohydrates at 1019 cm<sup>-1</sup> and 1049 cm<sup>-1</sup> (glucose/fructose) and 1155 cm<sup>-1</sup> (the glycosidic linkage) are known to 306 successfully monitor the consistency of tomato juice (Ayvaz et al., 2016), the region 307 308 between 1750 and 1450 cm<sup>-1</sup> highly contributed to the discrimination of apple purees 309 according to their particle size and their rheological behavior after mechanical

refining treatments. These differences between tomato and apple might be due to the nature of the datasets and in particular the impact of post-harvest storage on chemical compositions (sugars and acids) and textural properties (pectins degradations) as confounding factors in this apple processing experiment.

314

## 3.2 Spectral evaluation of the link between fresh and processed apples

315 FDA results showed a good ability to discriminate puree processing changes 316 (Figure 4) and cultivar differences (Figure S-4), according to the first two 317 discriminant factors (F1 and F2). Whatever the sample preparation (NF, FD and AIS), 318 a clear separation was observed between raw apples (homogenates) and processed 319 purees (Figures 4 a, c, e). The changes occurring during processing between raw 320 (homogenates) and processed (purees) products were illustrated on the first factorial 321 axis (F1) for the NF samples (with 97.2% specificity and 98.6% sensitivity) and AIS 322 materials (100% specificity and sensitivity), and on the second factorial axis (F2) for 323 FD samples (100% specificity and sensitivity).

324 Combining the main discriminant coefficients of the FDA models separating raw 325 and processed materials (F2 for NF and FD samples, F1 for AIS samples) (Figures 4 326 **b**, **d**, **f**) and using the absorption band assignments described in literature, allowed to 327 identify phenomena occurring during apple processing. In both NF and FD samples, 328 highly consistent variations of spectral intensity were commonly found between 1800 and 1500 cm<sup>-1</sup>, this region giving overlapped information related to pectins, proteins, 329 330 phenolics and absorbed water (Kačuráková et al., 1999), detailed in the following 331 section:

- The increase of the bands at 1750 cm<sup>-1</sup> in NF (**Figure 4b**), 1788 cm<sup>-1</sup> and 949 cm<sup>-1</sup> in FD (**Figure 4d**) were specific of C=O, C-O and C-C stretching vibrations of carboxylic acids and polysaccharides (Canteri, Renard, Le Bourvellec, & Bureau, 2019; Kyomugasho et al., 2015). These observations were in accordance with the increase of soluble fiber fractions and total polysaccharide contents after apple cooking (Colin-Henrion, Mehinagic, Renard, Richomme, & Jourjon, 2009).

- The bands at 1610-1620  $\text{cm}^{-1}$  (1614  $\text{cm}^{-1}$  in NF; 1618  $\text{cm}^{-1}$  in FD) have been

339 reported to correspond to the vibration of C=O from protein or pectic acid ester (Abidi, Cabrales, & Haigler, 2014). These peaks were consistent with the 340 aforementioned pectic absorption peaks (1750 cm<sup>-1</sup> and 1788 cm<sup>-1</sup>), in accordance 341 with the increase of pectin content in purees. In the same way, this absorbance 342 343 displays the same variations in a simplified experiment of apple cell wall (mainly 344 soluble pectins) submitted to similar puree processing conditions (100°C for 20 min at 345 pH 3.0) (Liu, 2019). In addition, the negligible concentration of proteins in fresh and 346 processed apples (0.17-0.57 g/100 g FW) limited the hypothesis concerning the 347 protein change during apple processing (U.S. Department of Agriculture, Agricultural 348 Research Service, 2019).

- the strong decrease of bands near 1630 cm<sup>-1</sup> and 1560 cm<sup>-1</sup> could be attributed
to the degradation of phenolic compounds during processing. These bands have been
already identified to quantify the polyphenol contents in freeze-dried apples (Bureau,
Ścibisz, Le Bourvellec, & Renard, 2012).

the specific bands of soluble acids (1712 cm<sup>-1</sup> in NF, 1718 cm<sup>-1</sup> in FD) (Clark,
2016) and of sugars (fructose at 1084 cm<sup>-1</sup> and 1061 cm<sup>-1</sup>; sucrose at 1113 cm<sup>-1</sup>)
(Bureau, Cozzolino, & Clark, 2019), which have been validated with standard
chemicals in ATR-FTIR, could partially contribute to the dynamics of puree changes.
These spectral variations relating the decreases of acid contents and increases of
fructose at 1712 cm<sup>-1</sup> were also in line with the results of chemical measurements
(Table S1 and Table S2).

In cell wall materials (AIS), two negative peaks at 1100 cm<sup>-1</sup> and 984 cm<sup>-1</sup> 360 361 (Figure 4f), could be attributed to the solubilization of the cell wall pectins after thermal processing (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998; 362 363 Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000), consistent with the 364 acid hydrolysis and  $\beta$ -elimination of pectins depolymerization while apple processing (Le Bourvellec et al., 2011). Conversely, two positive peaks at 1595 cm<sup>-1</sup> and 1030 365 366 cm<sup>-1</sup> could be linked to the increase of lignin (Garside & Wyeth, 2003) and cellulose 367 contents (Fasoli, et al., 2016; Cchulz & Baranska, 2007) in cell wall materials. A possible explanation is the depolymerization of cell wall polysaccharides (mainly
pectins) during maturation resulting in a relative enrichment of lignin and cellulose in
comparison with pectins after apple processing.

371 ATR-FTIR detected the processing changes from raw apples to purees by 372 scanning fresh, freeze-dried and cell wall samples. Particularly, spectra of fresh and 373 freeze-dried samples, i.e. with or without water, provided highly consistent 374 information on internal soluble matters (sugars, acids, pectins and phenolics). 375 Concerning the cell wall depolymerization (mainly pectin solubilization and galactose 376 loss), these change could be detected only by scanning the cell wall materials (AIS), 377 thus highlighting the solubilization of pectins diffusing from pulp to serum (Burgy et 378 al., 2018; 🗆 ila et al., 2009).

# 379 **3.3 Prediction of quality traits: comparison according to sample forms**

Acceptable to good predictions of SSC, TA. DMC, fructose and malic acid could be obtained on fresh (NF) and/or freeze-dried (FD) purees by ATR-FTIR, giving RPD from 3.1 to 5.2 (NF) and from 3.6 to 7.6 (FD) (**Table 1**).

383 The prediction of global fruit quality traits, such as SSC and DMC, depended on 384 two major spectral peaks, respectively, related to the sugars in NF (1061  $\text{cm}^{-1}$ ) (Bureau, Cozzolino, & Clark, 2019) and to the acids in FD (1724 cm<sup>-1</sup>) (Clark, 2016). 385 386 In purees, the prediction accuracy of these two quality traits was similar in NF and FD samples with a  $R_{cv}^2$  higher than 0.94 for SSC and higher than 0.89 for DMC. A good 387 388 correlation between SSC and DMC in purees ( $R^2=0.78$ ) and the similar related spectral signals used in models (mainly 1724 cm<sup>-1</sup> and 1061 cm<sup>-1</sup>) explained the good 389 390 prediction of both SSC and DMC in NF and FD samples. For the third global quality 391 trait, TA, its prediction was excellent with RPD higher than 6 in NF and FD samples. A particularly strong absorption at 1718 cm<sup>-1</sup> was used in the TA models in both NF 392 393 and FD samples.

Concerning the main individual sugars and acids (sucrose, fructose and malic acid), ATR-FTIR on FD samples provided more accurate prediction results ( $R_{cv}^2$ >0.87 and RPD>3.2) than on NF samples ( $R_{cv}^2$ >0.79 and RPD>2.3). For fructose and 397 sucrose, the regression coefficients of the models showed numerous characteristic peaks in the region 1150-900 cm<sup>-1</sup> in FD samples. But, despite the similar typical 398 peaks, specific peaks such as 1034 cm<sup>-1</sup> for sucrose and 1084 cm<sup>-1</sup> for fructose were 399 400 detected and used in their respective models. The lower RPD and the higher RMSECv 401 in NF than in FD samples were due to the presence of water leading to a lower 402 concentration of components and then a lower sensitivity to their variations. Moreover, 403 to obtain the best prediction of sugars in fresh samples, the spectral region 1700-1550 cm<sup>-1</sup> specific to soluble substances, was useful. In fresh samples, the linear models for 404 405 TA, SSC, DMC and malic acid prediction depended foremost on the sugar absorption 406 (fructose and sucrose), because of their relatively higher total concentrations 407 (99.4-228.9 g/kg FW) than those of acids (TA: 25-109.1 meq H<sup>+</sup>/kg FW). After freeze-drying, the specific spectral area (1725-1710 cm<sup>-1</sup>) corresponding to acidity 408 409 (Clark, 2016) became the main area of PLS models, due to their larger variations 410 during storage than those of individual sugars.

411 Another quality trait of interest is the AIS contents, which contributes to the 412 rheological properties of the processed apple purees products (Espinosa-Muñoz et al. 413 2012). The prediction of AIS contents is acceptable with RPD of 3.3 on FD purees, 414 when expressed in dry matter (DW). Its prediction was not possible directly on NF purees. The significant signals at 985 cm<sup>-1</sup> corresponding to CH stretching of cellulose 415 (Fahey, Nieuwoudt, & Harris, 2017) and at 1147 cm<sup>-1</sup> for C-O-C vibration of 416 417 glycosidic bound between uronic acids (Coimbra, Barros, Barros, Rutledge, & 418 Delgadillo, 1998) were in line with the previous PLS models built to predict AIS yield 419 in freeze-dried fruit and vegetables (Canteri, Renard, Le Bourvellec, & Bureau, 2019).

Briefly, ATR-FTIR technique worked well to evaluate global quality traits of interest in apple purees: SSC, TA and DMC. The prediction of cell wall contents (AIS) was possibility only on freeze-dried apple purees. Concerning the detailed composition including the individual components, the prediction was possible directly on fresh puree for malic acid whereas the prediction of the main individual sugars (fructose and sucrose) required the puree freeze-drying. The prediction of glucose was 426 not acceptable in apple purees whatever the tested conditions.

Surprisingly, prediction was acceptable ( $R_{cv}^2 > 0.87$ , RPD >3.1) for rheological 427 parameters such as pure viscosity ( $\eta_{50}$  or  $\eta_{100}$ ) and visco-elasticity (G', G'' in both 428 429 amplitude and frequency sweep tests and yield stress) on FD samples with less than 430 10 LVs and was better than on NF and AIS samples (Table 2). The single shear rate 431 value at 50/s ( $\eta_{50}$ ) has been described to be the best correlated with the in-mouth 432 texture perception of fluid foods (Chen & Engelen, 2012). For the two parameters 433 measured at  $\eta_{50}$  and  $\eta_{100}$ , predictions were better in FD samples than in NF and AIS 434 samples. Particularly, two main spectral areas (1718 cm<sup>-1</sup> and 1620-1595 cm<sup>-1</sup>) in NF 435 and FD samples appeared to be highly relevant to predict the puree viscosity. Differently, in AIS samples, the two major peaks (1018 cm<sup>-1</sup> and 1110 cm<sup>-1</sup>) linked to 436 437 the viscosity prediction have been conventionally attributed to the pectin changes in 438 fruit cell walls (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998). For the 439 specific viscoelastic parameters of purees (AS-G', AS-G" and yield stress) by 440 amplitude sweep tests, their prediction by ATR-FTIR was excellent in FD samples 441 with RPD values higher than 3.4. The yield stress, corresponding to the moment when 442 the puree starts to flow at the macroscopic level, could be predicted directly on NF purees with the better RPD and RMSECv than on FD samples. From frequency sweep 443 444 tests (FS), the gel-like behaviors (FS-G' > FS-G") of all purees could be well estimated in FD samples ( $R_{cv}^2 > 0.90$ ), even with a large variation of FS-G' and FS-G'' 445 446 (Table S-3). Surprisingly, fresh NF samples were the suitable sample type to evaluate 447 the particle size, both d(4:3) and d(3:2), with a good performance of the PLS models 448 (RPD>3.0).

Although acceptable results of PLS regression were obtained on the three sample types for the prediction of puree rheological properties (viscosity and viscoelasticity) and particle information (sizes and volume), it is worth signaling the differences of their fingerprint peaks: i) for fresh NF samples, the major region between 1750 and 1500 cm<sup>-1</sup> was attributed to the absorbed water and complex soluble substances (pectins, polyphenols and proteins); ii) for cell wall AI $\square$  extracts, the typical peaks 455 (1018 cm<sup>-1</sup>, 1083 cm<sup>-1</sup>) were mainly related to their pectic and phenolic variations; iii) for freeze-dried FD samples, the specific peaks, 1500-1750 cm<sup>-1</sup> and 1200-900 cm<sup>-1</sup>, 456 457 combining with those observed separately in NF and AIS samples were used. The 458 limited spectral sensitivity for the fresh suspensions (NF) and the restricted variations 459 for the cell wall extracts (AIS) resulted in a less accurate prediction of the rheological 460 behaviors than for freeze-dried FD samples. These results demonstrated the 461 possibility of ATR-FTIR technique to accurately estimate viscosity, elasticity and the 462 particle distributions directly on freeze-dried purees (FD). However, ATR-FTIR on 463 fresh purees (FD) had a good ability to directly evaluate the particle size and 464 properties (RPD>3.0), and also can probably to be used to evaluate the rheological 465 behaviors (viscosity and viscoelasticity) according the results of RPD values over 2.5 466 (Nicolai et al., 2007).

#### 467 **4. Conclusion**

468 As far as we know, this is the first report concerning the assessment of quality 469 variations in fruit products during processing depending on ATR-FTIR spectral 470 information of the same samples but characterized as fresh, freeze-dried and cell wall 471 extracts. Direct spectral measurements on fresh samples could provide a reliable 472 assessment of texture and major composition characteristics of purees. Thus, 473 ATR-FTIR technique can be adapted to routine analysis in fruit industries, a simple 474 method, using few steps for manufacturers. Long-time freeze-drying preparations still 475 keep the stability and consistency of the ATR-FTIR signals in comparison with those 476 of fresh samples, and provided more detailed assessments of rheological properties 477 and cell wall contents. ATR-FTIR on cell wall materials was the only way to identify 478 the variations of cell wall compositions, but not enough to overview the changes 479 during fruit processing.

Briefly, ATR-FTIR associated with suitable sample pre-treatments in fruit processing could offer sufficient information for the industrial and research demands. Balancing the pre-treated methods to stabilize samples and knowing the potential ability of infrared spectroscopy are both crucial for rapid and accurate analyses in fruit processing. Based on our results, future works could be extended to a wide span
of complex processing strategies (drying, juicing, fermentation etc.) and/or
operational units.

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622

# 623 Figure captions

624 Figure 1. Experimental scheme for apple and puree samples preparation,625 characterization using ATR-FTIR and reference analyses.

**Figure 2.** PCA on the SNV pre-treated ATR-FTIR spectra (900-1800 cm<sup>-1</sup>) of purees

(NF samples) prepared with normal thinned 'Granny Smith' apples (GS marked with  $\Delta$ ), thinned (Th+) 'Golden Delicious' apples (GD Th+ marked with O) and non-thinned 'Golden Delicious' apples (GD Th- marked with  $\Box$ ) stored in cold storage room (4°C) during 0, 1, 3 and 6 months (T0, T1, T3 and T6): (a) the scores plot of the two first components (PC1 and PC2); (b) the loading plot of PC1; (c) the loading plot of PC2.

**Figure 3.** FDA on the SNV pre-treated ATR-FTIR spectra (900-1800 cm<sup>-1</sup>) of non-refined (\* with 95% confidence ellipse circles) and refined ( $\triangle$  with 95% confidence ellipse circles) 'Golden Delicious' and 'Granny Smith' purees at harvest (T0), after one-month (T1), three months (T3) and six months (T6) of storage at 4°C. Macroscopic laser scanning images of puree particle distributions at harvest (T0) and after six-month storage (T6).

**Figure 4.** Maps of Factorial Discriminant Analysis (FDA) performed on the SNV-pre-treated ATR-FTIR spectra (900-1800 cm<sup>-1</sup>) of all fresh apple homogenates (named 'Ho') and the corresponding processed purees (named 'Pu') with: (a) fresh samples ('NF'), (c) freeze-dried samples ('FD'), (e) cell wall samples ('AIS'); (b) the second factorial score ('F2') of fresh samples, (d) the second factorial score ('F2') of freeze-dried samples ('FD'); (f) the first factorial score ('F1') of cell wall samples.

# 645 Figures











**T6** 









**Figure 4** 

					Leave-one-out P	LS (n=72)		
Parameter	Sample	Range	SD	$R_{cv}^{2}$	RMSECv	LVs	RPD	Linkable regions (cm <sup>-1</sup> )
SSC (°Brix)	NF	10.3-18.6	2.4	0.94	0.6	4	4.1	1055-1065, 1028-1030, 1558-1562, 1649-1653
	FD		2.4	0.95	0.5	3	4.9	1058-1065, 1724-1735, 998-1001
	NF	32.2-123.1	24.2	0.79	10.5	8	2.3	1084-1095, 1030-1034, 1574- 1583, 1225-1229, 916-920, 998-1102
Sucrose (g/kg Fw)	FD			0.87	7.8	7	3.2	998-1001, 1080-1084, 1030-1034, 1124-1137, 998-1102
Change (aller EW)	NF	10 5 05 7	3.4	0.65	2.0	9	1.7	1720-1715, 1656-1645, 1539-1562, 1886-1753, 1163, 1067, 1015
Glucose (g/kg Fw)	FD	13.3-23.7		0.70	1.8	6	1.9	1028-1034, 1578-1570, 1010-1015, 1420- 1397, 1079, 985-998
	NF	40.0-99.9	18.9	0.88	6.0	8	3.1	1635-1655, 1078-1086, 1028-1034, 987-998, 1137-1142
Fructose (g/kg F w)	FD			0.90	5.3	6	3.6	1082-1090, 1030-1034, 987-989, 926-928, 1061-1665, 1035-1046
$T \land (\dots, n \not h \to T W)$	NF	25.0-109.1	22.8	0.97	3.8	4	6.0	985-998, 1084-1095, 1715-1730, 1695-1701
TA (meq/kg Fw)	FD			0.98	3.0	3	7.6	1716-1724, 987-989, 962-968
	NF	NF 2.35-8.97 FD	1.63	0.91	0.5	4	3.3	1082-1095, 995-1001, 1715-1730, 1539
Malic acid (g/kg Fw)	FD			0.94	0.4	5	4.3	1716-1733, 1541-1558, 1695-1705, 1022-1024
	NF	0.16.0.24	0.02	0.89	0.01	6	3.1	1055-1068, 1443-1430, 1113-1135, 965-978, 1741-1730
DMC (g/g FW)	FD	0.16-0.24	0.03	0.92	0.01	5	3.6	1710-1728, 1541-1558, 1514-1507
	NF	100.4-271.7	33.3	0.75	16.9	10	1.9	1665-1685, 1701-1718, 1113-1128, 962-968, 1548-1560, 1605-1620
AIS content (mg/g Dw)	FD			0.88	10.1	7	3.3	1142-1150, 985-995, 1058-1065, 1058, 995-1005, 1650-1665
	NF	165 49.0	(1	0.76	3.5	9	2.0	1655-1685, 1605-1620, 1665-1685, 1700-1722, 965-985, 1094-1105
AIS content (mg/g FW)	FD	10.3-48.9 0.1		0.83	2.3	8	2.7	1055-1065, 985-995, 1030-1035, 1142-1150, 1165-1193, 1096-1101

Table 1. Prediction of apple processed purees composition using the leave-one-out PLS regression based on the fresh ('NF') and freeze-dried
 ('FD') ATR-FTIR spectra and reference data.

657 Puree spectra and reference data from two varieties ('Granny Smith', 'Golden Delicious') with different thinning conditions, a cold storage (during 0, 1, 3 and 6 months) and two puree refining conditions. Spectral

658 area: 1800-900 cm<sup>-1</sup> and spectrum pre-processing: baseline-correction and SNV.

					Samples (n=72)			
Parameter	Sample	Range	SD	$R_{cv}^2$	RMSECv	LVs	RPD	Linkable regions (cm <sup>-1</sup> )
	NF			0.84	0.18	8	2.5	1620-1635, 1662-1670, 1718-1726, 1110-1122, 1080-1109, 1450-1456
η50	FD	0.69-1.94	0.44	0.88	0.14	9	3.1	940-952, 1060-1065, 1455-1471, 925-935, 1078-1084, 1145-1150, 1718-1726
_	AIS			0.86	0.16	8	2.8	1018-1023, 1110-1115, 1160-1168, 1057-1083, 925- 935, 1618-1625
	NF			0.83	0.09	8	2.5	1610-1620, 1718-1726, 1560-1584, 1080-1110, 1450-1456
η100	FD	0.25-1.06	0.21	0.89	0.06	9	3.4	940-952, 1060-1065, 1150-1161, 1455-1471, 1020-1038, 983-995,
_	AIS			0.84	0.08	9	2.6	1018-1023, 1092-1110, 924- 935, 1057-1083, 1610-1625, 946-958
	NF			0.82	425	10	2.4	1645-1665, 1047-1055, 1082-1088, 1450-1456, 1530-1547, 925-932,
AS-G' (Pa)	FD	6-3612	1001	0.88	297	9	3.4	1020-1036, 1618-1635, 1060-1065, 1455-1471, 1084-1090, 983-995
_	AIS			0.85	332	9	3.0	1610-1625, 1078-1113, 1018-1023, 924- 935, 1039-1043, 1193-1216
	NF			0.83	98	9	2.5	1530-1547, 1456-1464, 1645-1665, 1080-1088, 1610-1618, 925-932
AS-G" (Pa)	FD	2-860	234	0.89	69	10	3.4	1015-1030, 1060-1068, 930-944, 1084-1090, 1465-1482, 1624-1643
_	AIS			0.86	72	9	3.1	1018-1023, 1078-1110, 1560-1584, 1610-1625, 924-935, 1193-1216
	NF			0.86	4.4	9	2.9	1082-1088, 1530-1547, 1686-1699, 1030-1043, 1610-1618, 1090-1111,
yield stress	FD	0.6-57.6	12.9	0.87	4.2	9	3.1	984-992, 1463-1470, 1048-1054, 935-944, 1142-1151, 1465-1482, 1090-1104
_	AIS			0.82	4.9	9	2.6	1039-1056, 1018-1023, 1078-1110, 946-958, 924- 935, 1610-1625
	NF			0.84	303.5	8	2.6	1645-1665, 1530-1549, 1456-1464, 1610-1620, 1058-1063
FS-G' (Pa)	FD	0.3-3105.6	798.2	0.90	217.6	10	3.3	946-955, 1015-1030, 1455-1471, 1090-1104, 1060-1068, 1612-1620
_	AIS			0.84	292.4	8	2.5	1018-1023, 1610-1625, 1092- 1110, 912-930, 1039-1056
	NF			0.82	63.3	10	2.5	1645-1665, 1456-1464, 1530-1549, 1685-1695, 1058-1063, 1610-1618,
FS-G" (Pa)	FD	0.3-511.1	158.7	0.91	48.1	8	3.3	937-949, 1060-1068, 1455-1471, 1011-1028, 1455-1462, 1092-1104
	AIS			0.87	56.1	10	2.9	1018-1023, 1570-1584, 1528-1542, 1092-1110, 1610-1625, 912-924

Table 2 Prediction of apple processed purees rheological parameters and textural properties using the leave-one-out PLS regression based on the
 fresh (NF), freeze-dried (FD) and cell wall (AIS) ATR-FTIR spectra and reference data.

	NF			0.90	59	9	3.3	1701-1710, 1655-1668, 1034-1038, 1718-1726, 986-995, 1534-1541,1145-1152
d (4:3)	FD	277-920	195	0.93	53	9	3.5	934-949, 1464-1482, 1540-1558, 1050-1056, 915-920, 1740-1765
_	AIS			0.87	65	8	3.0	1045-1083, 1502-1516, 1059-1067, 956-980, 1605-1615
-								
	NF			0.86	21	10	3.0	1146-1158, 1034-1038, 1405-1412, 1082-1119, 1560-1597, 986-995, 1730-1742
d (3:2)	NF FD	132-422	64	0.86 0.85	21 23	10 10	3.0 2.8	1146-1158, 1034-1038, 1405-1412, 1082-1119, 1560-1597, 986-995, 1730-1742 1027-1039, 1056-1065, 1110-1124, 915-939, 1008-1015, 1625-1648

661 Puree spectra and reference data from two varieties ('Granny Smith', 'Golden Delicious') with different thinning conditions, a cold storage (during 0, 1, 3 and 6 months) and two puree refining conditions. Spectral

area: 1800-900 cm<sup>-1</sup> and spectrum pre-processing: baseline-correction and SNV.

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