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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
34 applied on fresh (NF), freeze-dried (FD) and cell wall materials (AIS) of raw and
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**

51 Sample preparation is a key point for quality of analytical data. Infrared
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
56 preparation and a rapid data acquisition. However, this questions the balance between
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.

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

72 Direct ATR-FTIR estimations on fruit fresh homogenates have obtained good
73 results to predict soluble solids content, dry matter content, titratable acidity, some
74 individual sugars and organic acids (Bureau, Ścibisz, Le Bourvellec, & Renard, 2012;
75 Ayvaz et al., 2016). As infrared spectroscopy is extremely sensitive to changes of
76 hydrogen bonding (Jackson & Mantsch, 1995), the main drawback of spectral
77 measurements is the low sensitivity and limited specific signals of chemical
78 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).

102 ATR-FTIR applications to assess fruit textural properties (mainly focus on cell
103 wall compositions) are always performed on their cell wall materials (AIS) (Canteri,
104 Renard, Le Bourvellec, & Bureau, 2019; Ćzymanska-Chargot, Chylinska, Kruk, &
105 Zdunek, 2015). However, extracting the cell wall requires a large consumption of
106 chemical solvents if starting from fresh samples (up to 1 L ethanol and 0.4 L acetone/
107 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).

119 In this study, ATR-FTIR spectroscopy was applied on the corresponding raw
120 apples and processed purees. Spectra were acquired on different kinds of
121 homogeneous samples such as fresh (NF for non-freeze-dried), freeze-dried (FD) and
122 cell wall extracts (AIS for alcohol insoluble solids) in order to: i) evaluate how much
123 sample preparation improved the prediction of chemical, textural and rheological
124 characteristics of purees (number of quality traits and their precision) and ii) identify
125 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
131 practices (Th+ to 50 to 100 fruits/tree) and no thinning (Th- to 150-200 fruits/tree)
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 puree 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, textural 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 × 4 storage times × 2 refining levels ×
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⁺/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
183 by 5 minutes at rest. The viscosity was then measured at a controlled shear rate range
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 (η_{50} and η_{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
192 linear viscoelastic region as determined by the AS test (0.05%) in the angular
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
206 attenuated total reflectance (ATR) sampling accessory and a deuterated triglycine
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
219 collected from 4000 cm^{-1} to 650 cm^{-1} and were corrected against the background
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

224 experimental dataset expressed as standard deviation values (SD). Analysis of
225 variance (ANOVA) was carried out to determine the significant differences due to the
226 controlled factors (thinning, storage and puree mechanical refining) on both apples
227 (**Table S-1**) and purees of each variety (**Table S-2 and Table S-3**) using XLSTAT
228 (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^{-1} , 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^{-1} , 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 differentiation. For PLS (Partial least square) modelling (in
243 **Part 3.3**), the baseline correction coupled with SNV pre-processing had the best
244 performances to correct multiplicative interferences and variations in baseline shift,
245 and reached the best prediction results.

246 Leave-one-out PLS models were developed using spectra of fresh (NF),
247 freeze-dried (FD) and AIS of puree samples, for which the three spectral matrices (NF,
248 FD and AIS) corresponded to the same reference dataset. A total number of 72
249 averaged spectra for each puree form (NF, FD and AIS) corresponding to 3 apple
250 groups (GS, GD Th+ and GD Th-) \times 4 storage times \times 2 puree refining modalities \times 3
251 biological replicates was used as modelling dataset. PLS model performance was
252 assessed using the determination coefficient of cross-validation (R_{cv}^2), the

253 root-mean-square error of cross-validation (RMSECV), the number of latent variables
254 (LVs), the ratio of the standard deviation values (RPD) and the linkable spectral
255 regions (**Tables 1 and 2**). The linearity correlation plots between measured and
256 predicted values of all PLS models were showed in supplementary materials (**Figure**
257 **S-5 and Figure S-6**).

258

259 **3. Results and discussions**

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

261 PCA and FDA applied on the spectra of NF puree samples successfully allowed
262 to detect puree differences coming from the raw apple variabilities (cultivar, fruit
263 thinning and storage period) (**Figure 2**). They also highlighted the modifications of
264 puree structure by the mechanical refining over several months of apple storage
265 (**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
268 delicious (Th- and Th+), in relation with the fructose variation followed at 1061 cm^{-1}
269 (Bureau, Cozzolino, & Clark, 2019). Moreover, the peak at 1022 cm^{-1} , reported as a
270 peak specific to sucrose in apple juices (Leopold, Leopold, Diehl, & Socaciu, 2011),
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^{-1}) and the increase of fructose (1065 cm^{-1}) 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
279 fruits based on the specific C-C and C-O-C bonds of carbohydrates, such as 1022 cm^{-1} ,
280 1061 cm^{-1} , 1065 cm^{-1} .

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 (η_{50} and η_{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
298 macroscopic images showed above (**Figure 3**). According to the third factorial
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
302 spectral variations were related to the decrease of soluble organic acids (1718 cm^{-1}
303 and 1709 cm^{-1}), soluble polysaccharides, pectins and absorbed water (1740 cm^{-1} , 1695
304 cm^{-1} , 1682 cm^{-1} , 1668 cm^{-1} , 1655 cm^{-1} and 1468 cm^{-1}) between the two refining
305 conditions (**Figure S-3**). Although the peaks of carbohydrates at 1019 cm^{-1} and 1049
306 cm^{-1} (glucose/fructose) and 1155 cm^{-1} (the glycosidic linkage) are known to
307 successfully monitor the consistency of tomato juice (Ayvaz et al., 2016), the region
308 between 1750 and 1450 cm^{-1} highly contributed to the discrimination of apple purees
309 according to their particle size and their rheological behavior after mechanical

310 refining treatments. These differences between tomato and apple might be due to the
311 nature of the datasets and in particular the impact of post-harvest storage on chemical
312 compositions (sugars and acids) and textural properties (pectins degradations) as
313 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
329 and 1500 cm^{-1} , this region giving overlapped information related to pectins, proteins,
330 phenolics and absorbed water (Kačuráková et al., 1999), detailed in the following
331 section:

332 - The increase of the bands at 1750 cm^{-1} in NF (**Figure 4b**), 1788 cm^{-1} and 949
333 cm^{-1} in FD (**Figure 4d**) were specific of C=O, C-O and C-C stretching vibrations of
334 carboxylic acids and polysaccharides (Canteri, Renard, Le Bourvellec, & Bureau,
335 2019; Kyomugasho et al., 2015). These observations were in accordance with the
336 increase of soluble fiber fractions and total polysaccharide contents after apple
337 cooking (Colin-Henrion, Mehinagic, Renard, Richomme, & Jourjon, 2009).

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

339 reported to correspond to the vibration of C=O from protein or pectic acid ester
340 (Abidi, Cabrales, & Haigler, 2014). These peaks were consistent with the
341 aforementioned pectic absorption peaks (1750 cm^{-1} and 1788 cm^{-1}), in accordance
342 with the increase of pectin content in purees. In the same way, this absorbance
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).

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

353 - the specific bands of soluble acids (1712 cm^{-1} in NF, 1718 cm^{-1} in FD) (Clark,
354 2016) and of sugars (fructose at 1084 cm^{-1} and 1061 cm^{-1} ; sucrose at 1113 cm^{-1})
355 (Bureau, Cozzolino, & Clark, 2019), which have been validated with standard
356 chemicals in ATR-FTIR, could partially contribute to the dynamics of puree changes.
357 These spectral variations relating the decreases of acid contents and increases of
358 fructose at 1712 cm^{-1} were also in line with the results of chemical measurements
359 (Table S1 and Table S2).

360 In cell wall materials (AIS), two negative peaks at 1100 cm^{-1} and 984 cm^{-1}
361 (Figure 4f), could be attributed to the solubilization of the cell wall pectins after
362 thermal processing (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998;
363 Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000), consistent with the
364 acid hydrolysis and β -elimination of pectins depolymerization while apple processing
365 (Le Bourvellec et al., 2011). Conversely, two positive peaks at 1595 cm^{-1} and 1030
366 cm^{-1} could be linked to the increase of lignin (Garside & Wyeth, 2003) and cellulose
367 contents (Fasoli, et al., 2016; Chulz & Baranska, 2007) in cell wall materials. A

368 possible explanation is the depolymerization of cell wall polysaccharides (mainly
369 pectins) during maturation resulting in a relative enrichment of lignin and cellulose in
370 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; Ćila et al., 2009).

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

380 Acceptable to good predictions of SSC, TA, DMC, fructose and malic acid could
381 be obtained on fresh (NF) and/or freeze-dried (FD) purees by ATR-FTIR, giving RPD
382 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 cm^{-1})
385 (Bureau, Cozzolino, & Clark, 2019) and to the acids in FD (1724 cm^{-1}) (Clark, 2016).
386 In purees, the prediction accuracy of these two quality traits was similar in NF and FD
387 samples with a R_{cv}^2 higher than 0.94 for SSC and higher than 0.89 for DMC. A good
388 correlation between SSC and DMC in purees ($R^2=0.78$) and the similar related
389 spectral signals used in models (mainly 1724 cm^{-1} and 1061 cm^{-1}) explained the good
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.
392 A particularly strong absorption at 1718 cm^{-1} was used in the TA models in both NF
393 and FD samples.

394 Concerning the main individual sugars and acids (sucrose, fructose and malic
395 acid), ATR-FTIR on FD samples provided more accurate prediction results ($R_{cv}^2>0.87$
396 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
398 peaks in the region 1150-900 cm^{-1} in FD samples. But, despite the similar typical
399 peaks, specific peaks such as 1034 cm^{-1} for sucrose and 1084 cm^{-1} for fructose were
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
404 cm^{-1} specific to soluble substances, was useful. In fresh samples, the linear models for
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^+ /kg FW). After
408 freeze-drying, the specific spectral area (1725-1710 cm^{-1}) corresponding to acidity
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
415 purees. The significant signals at 985 cm^{-1} corresponding to CH stretching of cellulose
416 (Fahey, Nieuwoudt, & Harris, 2017) and at 1147 cm^{-1} for C-O-C vibration of
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).

420 Briefly, ATR-FTIR technique worked well to evaluate global quality traits of
421 interest in apple purees: SSC, TA and DMC. The prediction of cell wall contents (AIS)
422 was possibility only on freeze-dried apple purees. Concerning the detailed
423 composition including the individual components, the prediction was possible directly
424 on fresh puree for malic acid whereas the prediction of the main individual sugars
425 (fructose and sucrose) required the puree freeze-drying. The prediction of glucose was

426 not acceptable in apple purees whatever the tested conditions.

427 Surprisingly, prediction was acceptable ($R_{cv}^2 > 0.87$, RPD > 3.1) for rheological
428 parameters such as puree viscosity (η_{50} or η_{100}) and visco-elasticity (G' , G'' in both
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 (η_{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 η_{50} and η_{100} , predictions were better in FD samples than in NF and AIS
434 samples. Particularly, two main spectral areas (1718 cm^{-1} and $1620\text{-}1595\text{ cm}^{-1}$) in NF
435 and FD samples appeared to be highly relevant to predict the puree viscosity.
436 Differently, in AIS samples, the two major peaks (1018 cm^{-1} and 1110 cm^{-1}) linked to
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
443 purees with the better RPD and RMSEC_v than on FD samples. From frequency sweep
444 tests (FS), the gel-like behaviors (FS- $G' > \text{FS-}G''$) of all purees could be well
445 estimated in FD samples ($R_{cv}^2 > 0.90$), even with a large variation of FS- G' and FS- G''
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).

449 Although acceptable results of PLS regression were obtained on the three sample
450 types for the prediction of puree rheological properties (viscosity and viscoelasticity)
451 and particle information (sizes and volume), it is worth signaling the differences of
452 their fingerprint peaks: i) for fresh NF samples, the major region between 1750 and
453 1500 cm^{-1} was attributed to the absorbed water and complex soluble substances
454 (pectins, polyphenols and proteins); ii) for cell wall AI□ extracts, the typical peaks

455 (1018 cm^{-1} , 1083 cm^{-1}) were mainly related to their pectic and phenolic variations; iii)
456 for freeze-dried FD samples, the specific peaks, 1500-1750 cm^{-1} and 1200-900 cm^{-1} ,
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 ($\text{RPD}>3.0$), 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.

480 Briefly, ATR-FTIR associated with suitable sample pre-treatments in fruit
481 processing could offer sufficient information for the industrial and research demands.
482 Balancing the pre-treated methods to stabilize samples and knowing the potential
483 ability of infrared spectroscopy are both crucial for rapid and accurate analyses in

484 fruit processing. Based on our results, future works could be extended to a wide span
485 of complex processing strategies (drying, juicing, fermentation etc.) and/or
486 operational units.

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495

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622

623 **Figure captions**

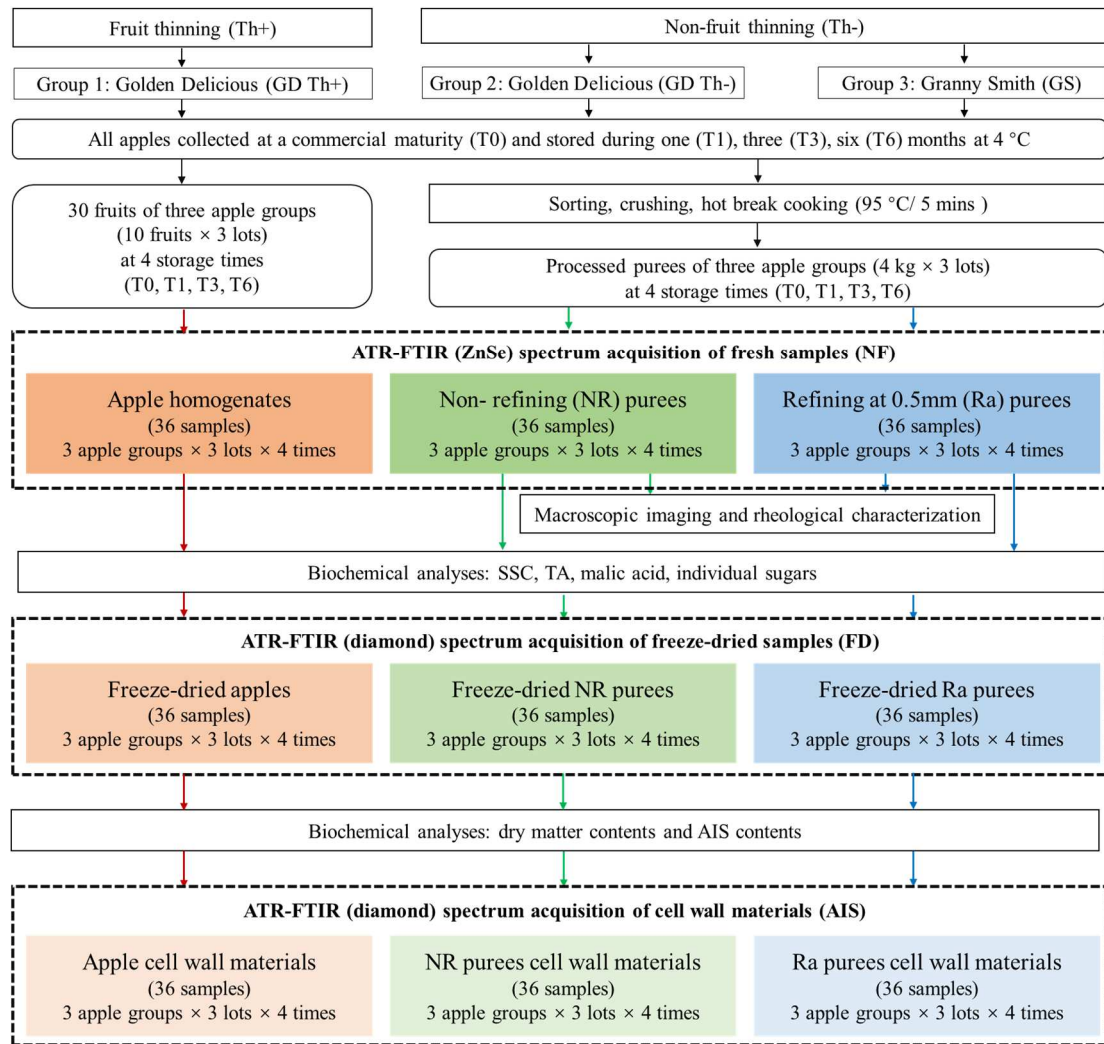
624 **Figure 1.** Experimental scheme for apple and puree samples preparation,
625 characterization using ATR-FTIR and reference analyses.

626 **Figure 2.** PCA on the SNV pre-treated ATR-FTIR spectra ($900\text{-}1800\text{ cm}^{-1}$) of purees
627 (NF samples) prepared with normal thinned ‘Granny Smith’ apples (GS marked with
628 Δ), thinned (Th+) ‘Golden Delicious’ apples (GD Th+ marked with \bigcirc) and
629 non-thinned ‘Golden Delicious’ apples (GD Th- marked with \square) stored in cold
630 storage room (4°C) during 0, 1, 3 and 6 months (T0, T1, T3 and T6): (a) the scores
631 plot of the two first components (PC1 and PC2); (b) the loading plot of PC1; (c) the
632 loading plot of PC2.

633 **Figure 3.** FDA on the SNV pre-treated ATR-FTIR spectra ($900\text{-}1800\text{ cm}^{-1}$) of
634 non-refined (* with 95% confidence ellipse circles) and refined (Δ with 95%
635 confidence ellipse circles) ‘Golden Delicious’ and ‘Granny Smith’ purees at harvest
636 (T0), after one-month (T1), three months (T3) and six months (T6) of storage at 4°C .
637 Macroscopic laser scanning images of puree particle distributions at harvest (T0) and
638 after six-month storage (T6).

639 **Figure 4.** Maps of Factorial Discriminant Analysis (FDA) performed on the
640 SNV-pre-treated ATR-FTIR spectra ($900\text{-}1800\text{ cm}^{-1}$) of all fresh apple homogenates
641 (named ‘Ho’) and the corresponding processed purees (named ‘Pu’) with: (a) fresh
642 samples (‘NF’), (c) freeze-dried samples (‘FD’), (e) cell wall samples (‘AIS’); (b) the
643 second factorial score (‘F2’) of fresh samples, (d) the second factorial score (‘F2’) of
644 freeze-dried samples (‘FD’); (f) the first factorial score (‘F1’) of cell wall samples.

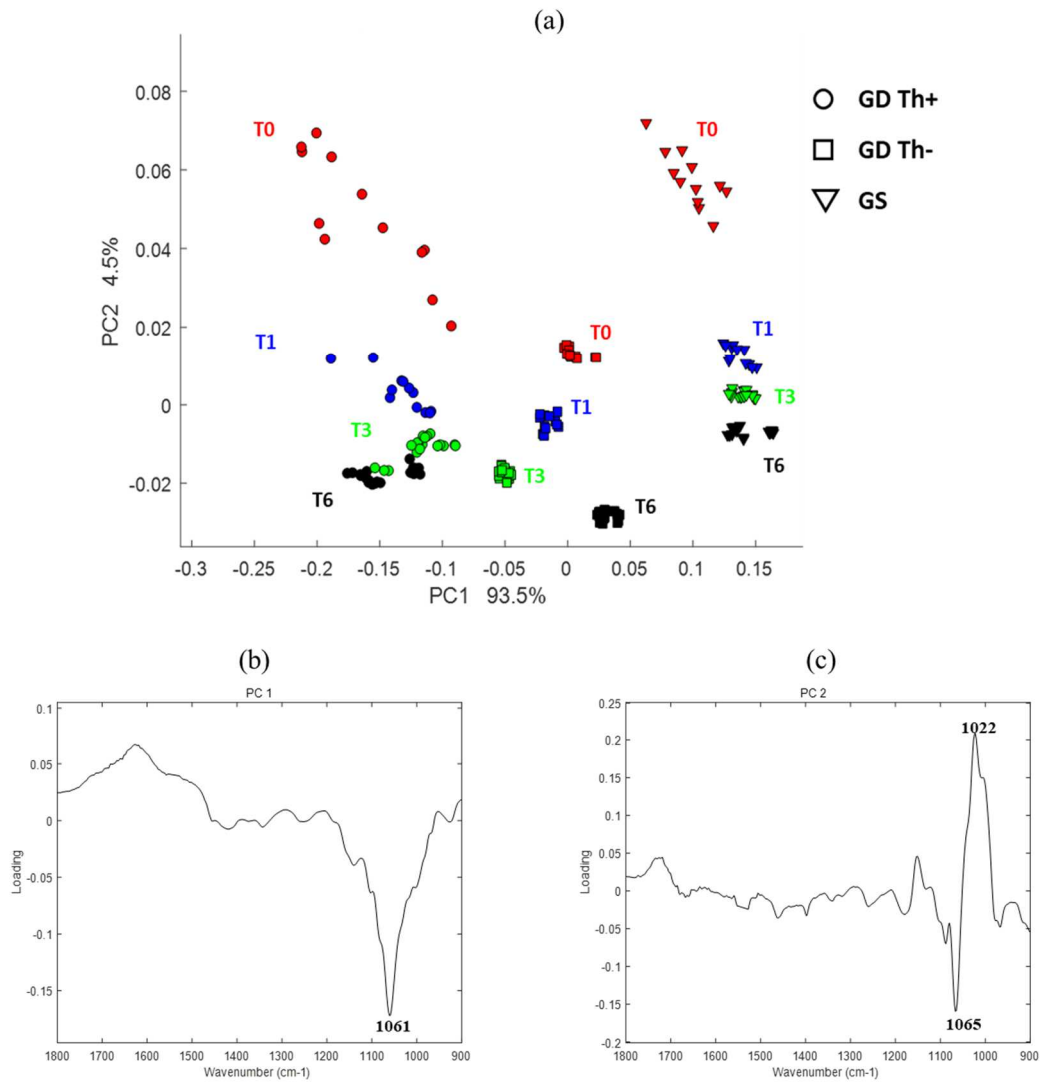
645 **Figures**



646

647 **Figure 1**

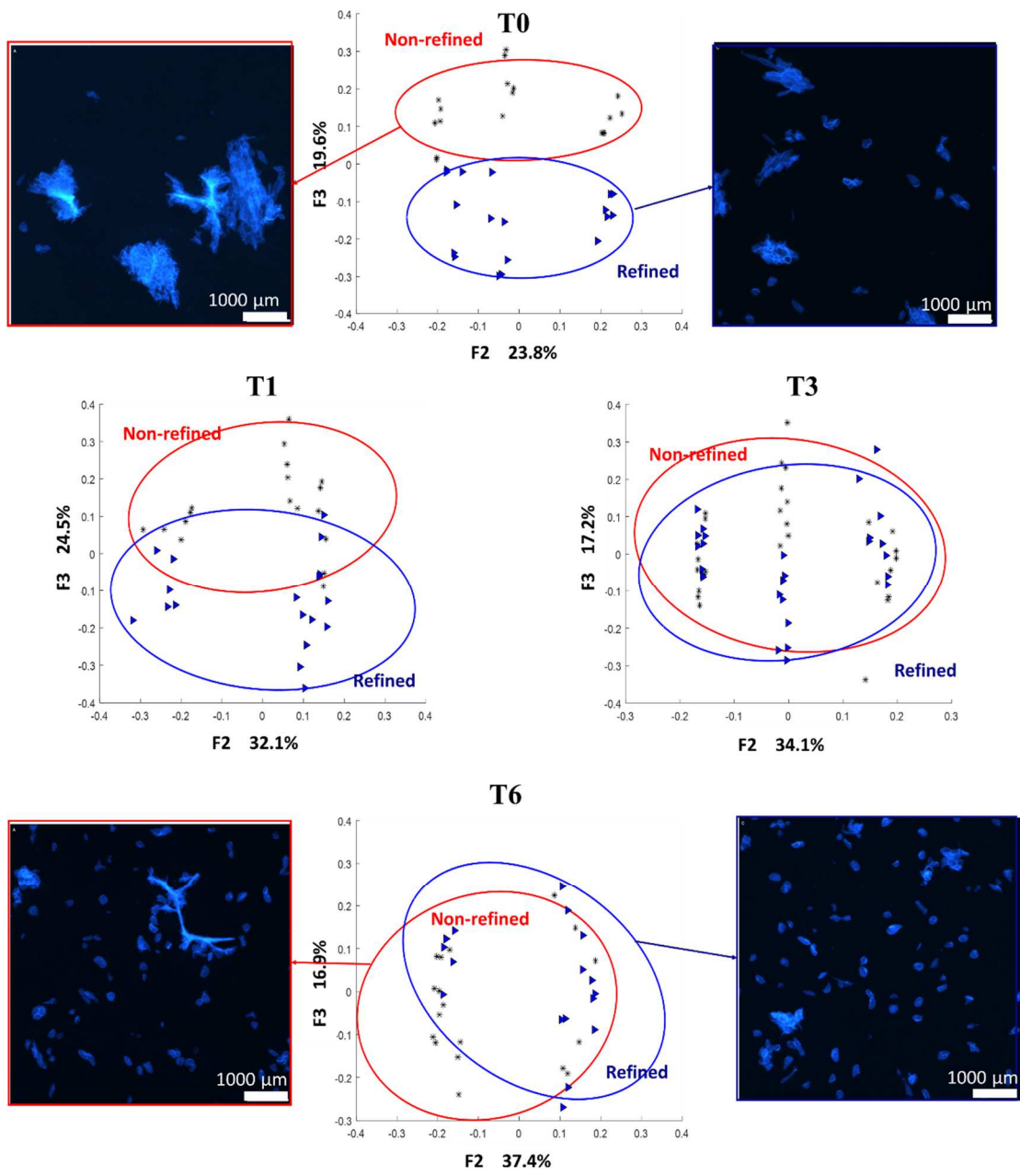
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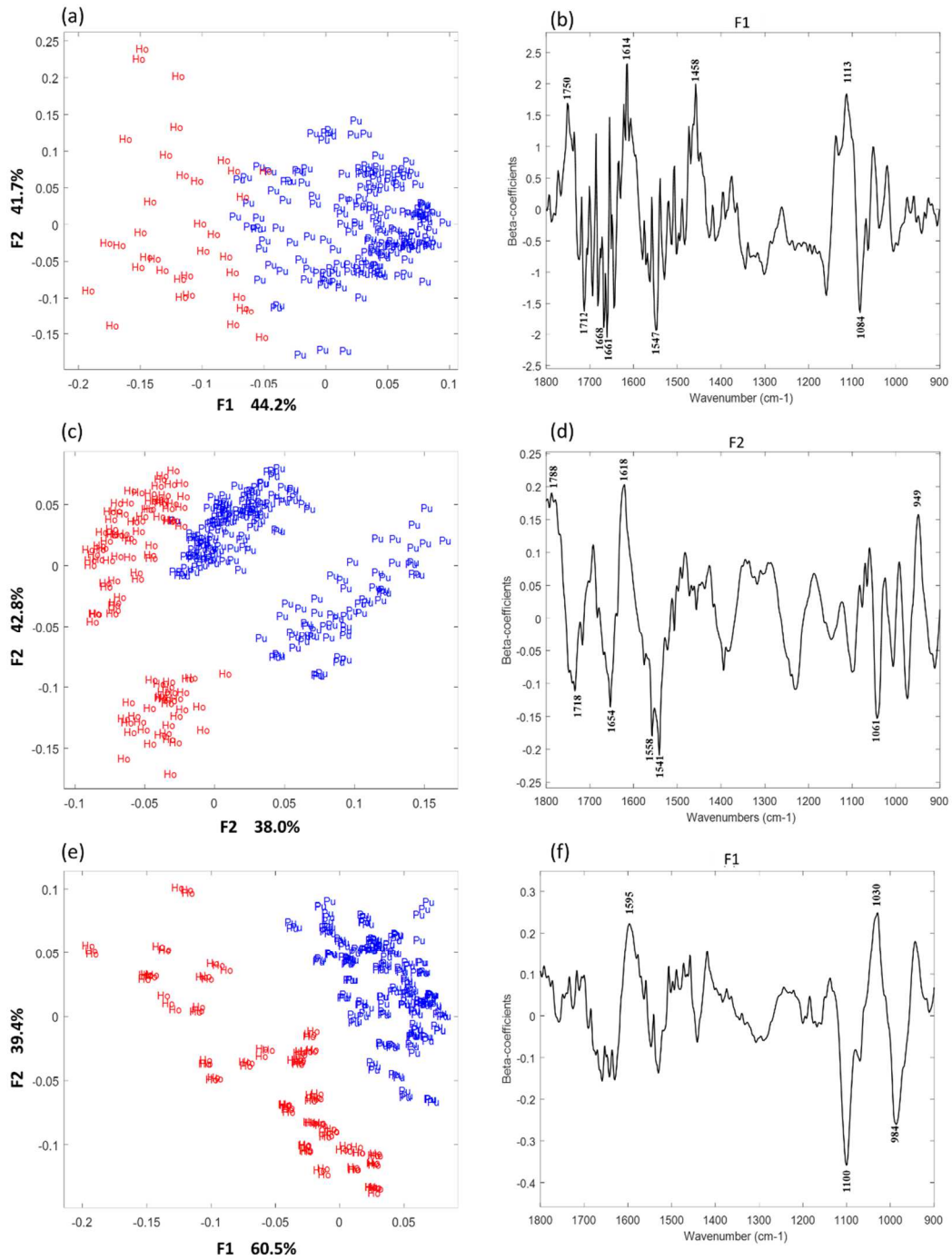
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Figure 2



651

652 **Figure 3**



653

654

Figure 4

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

Parameter	Sample	Range	SD	Leave-one-out PLS (n=72)				Linkable regions (cm ⁻¹)
				R _c ²	RMSECV	LVs	RPD	
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			0.95	0.5	3	4.9	1058-1065, 1724-1735, 998-1001
Sucrose (g/kg FW)	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
	FD			0.87	7.8	7	3.2	998-1001, 1080-1084, 1030-1034, 1124-1137, 998-1102
Glucose (g/kg FW)	NF	13.5-25.7	3.4	0.65	2.0	9	1.7	1720-1715, 1656-1645, 1539-1562, 1886-1753, 1163, 1067, 1015
	FD			0.70	1.8	6	1.9	1028-1034, 1578-1570, 1010-1015, 1420- 1397, 1079, 985-998
Fructose (g/kg FW)	NF	40.0-99.9	18.9	0.88	6.0	8	3.1	1635-1655, 1078-1086, 1028-1034, 987-998, 1137-1142
	FD			0.90	5.3	6	3.6	1082-1090, 1030-1034, 987-989, 926-928, 1061-1665, 1035-1046
TA (meq/kg FW)	NF	25.0-109.1	22.8	0.97	3.8	4	6.0	985-998, 1084-1095, 1715-1730, 1695-1701
	FD			0.98	3.0	3	7.6	1716-1724, 987-989, 962-968
Malic acid (g/kg FW)	NF	2.35-8.97	1.63	0.91	0.5	4	3.3	1082-1095, 995-1001, 1715-1730, 1539
	FD			0.94	0.4	5	4.3	1716-1733, 1541-1558, 1695-1705, 1022-1024
DMC (g/g FW)	NF	0.16-0.24	0.03	0.89	0.01	6	3.1	1055-1068, 1443-1430, 1113-1135, 965-978, 1741-1730
	FD			0.92	0.01	5	3.6	1710-1728, 1541-1558, 1514-1507
AIS content (mg/g DW)	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
	FD			0.88	10.1	7	3.3	1142-1150, 985-995, 1058-1065, 1058, 995-1005, 1650-1665
AIS content (mg/g FW)	NF	16.5-48.9	6.1	0.76	3.5	9	2.0	1655-1685, 1605-1620, 1665-1685, 1700-1722, 965-985, 1094-1105
	FD			0.83	2.3	8	2.7	1055-1065, 985-995, 1030-1035, 1142-1150, 1165-1193, 1096-1101

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⁻¹ and spectrum pre-processing: baseline-correction and SNV.

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

Parameter	Sample	Range	SD	Samples (n=72)				Linkable regions (cm ⁻¹)
				R _{cv} ²	RMSECV	LVs	RPD	
η ₅₀	NF			0.84	0.18	8	2.5	1620-1635, 1662-1670, 1718-1726, 1110-1122, 1080-1109, 1450-1456
	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
η ₁₀₀	NF			0.83	0.09	8	2.5	1610-1620, 1718-1726, 1560-1584, 1080-1110, 1450-1456
	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
AS-G' (Pa)	NF			0.82	425	10	2.4	1645-1665, 1047-1055, 1082-1088, 1450-1456, 1530-1547, 925-932,
	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
AS-G'' (Pa)	NF			0.83	98	9	2.5	1530-1547, 1456-1464, 1645-1665, 1080-1088, 1610-1618, 925-932
	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
yield stress	NF			0.86	4.4	9	2.9	1082-1088, 1530-1547, 1686-1699, 1030-1043, 1610-1618, 1090-1111,
	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
FS-G' (Pa)	NF			0.84	303.5	8	2.6	1645-1665, 1530-1549, 1456-1464, 1610-1620, 1058-1063
	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
FS-G'' (Pa)	NF			0.82	63.3	10	2.5	1645-1665, 1456-1464, 1530-1549, 1685-1695, 1058-1063, 1610-1618,
	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

d (4:3)	NF			0.90	59	9	3.3	1701-1710, 1655-1668, 1034-1038, 1718-1726, 986-995, 1534-1541, 1145-1152
	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
d (3:2)	NF			0.86	21	10	3.0	1146-1158, 1034-1038, 1405-1412, 1082-1119, 1560-1597, 986-995, 1730-1742
	FD	132-422	64	0.85	23	10	2.8	1027-1039, 1056-1065, 1110-1124, 915-939, 1008-1015, 1625-1648
	AIS			0.81	26	9	2.3	974-995, 1018-1023, 1235-1256, 1045-1083, 1727-1735, 1605-1615

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
662 area: 1800-900 cm⁻¹ and spectrum pre-processing: baseline-correction and SNV.

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