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## Changes in cell wall neutral sugar composition related to pectinolytic enzyme activities and intra-flesh textural property during ripening of ten apricot clones

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1 **Changes in cell wall neutral sugar composition related to pectinolytic enzyme**  
2 **activities and intra-flesh textural property during ripening of ten apricot**  
3 **clones**

4  
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17  
18 **Abstract**

19 The changes of texture and cell wall characteristics of apricot were investigated in ten clones  
20 at two maturity stages. Fruit firmness, cell wall composition and enzyme activity of three  
21 apricot flesh zones were analysed. The AIS (alcohol-insoluble solids) were characterised by  
22 high amounts of uronic acid (179-300 mg g<sup>-1</sup> AIS) and relatively high amounts of cellulosic  
23 glucose (118-214 mg g<sup>-1</sup> AIS). The methylesterification degree varied significantly among the  
24 different clones ranging from 58 to 97 in Ab 5 and Mans 15 respectively. Conversely to zones  
25 firmness, enzymatic activity was higher in pistil followed by equatorial and peduncle zones.  
26 The ripening effect has been observed in firmness evolution according to enzymatic activity.  
27 This correlation allowed a classification of clones depending on softening. Among studied  
28 clones, Ab 5, Marouch 16, Mans 15 and Cg 2 were less influenced by softening and have the  
29 advantage of a technological valorisation for the processing industry.

30  
31 **Keywords:** *Prunus armeniaca* L.; sugar; firmness; pectinmethylesterase;  $\beta$ -galactosidase;  
32 maturity

33 **1. Introduction**

34

35 The Marrakesh region is considered the most important apricot growing area in Morocco.  
36 However, the traditional varieties are almost the only source of production. Indeed, various  
37 renovations have occurred in recent years to introduce new cultivars to meet market  
38 requirements and satisfy consumers and industry demands. The National Institute for  
39 Agricultural Research in Marrakesh (INRA) has undertaken a research program targeting  
40 areas of traditional cultivation of several fruit species including creating a collection of apricot  
41 clones. Some of these clones have already been the subject of characterization studies of  
42 quality agronomic performance (Ayour et al., 2017).

43 Beside aroma and sweetness, texture is one of the important quality attributes of apricot  
44 fruit; it influences consumer acceptability, postharvest manipulation, shelf-life, microbial  
45 susceptibility and suitability for processing into different products (Gatti et al., 2009).  
46 Although only little information is available specifically on apricot, texture is often related to  
47 cell wall structure and composition. Cell walls are important elements of plant cells and are  
48 implicated in evolution of fruit firmness and texture during ripening. The softening of apricot  
49 fruit during ripening has been associated with alterations in the cell wall materials (De  
50 Martino et al., 2002). Moreover, the soft fruits are easily damaged, including latent damage,  
51 but little is known of the mechanism of softening in these fruits. Many studies on fruit texture  
52 focused on cell walls since remarkable changes occur in their polysaccharide's composition,  
53 structure and organization consecutive to the induction of enzymes and proteins expression on  
54 ripening (Brummell, 2006; Goulao & Oliveira, 2008; Renard & Ginies, 2009; Ella Missang et  
55 al., 2012; Lahaye et al., 2014; Brahem et al., 2017; Liu et al., 2017). Apricot fruit was  
56 characterized by high heterogeneity in its tissue structure (Archibald & Melton, 1987). Ella  
57 Missang et al. (2012) reported that in fresh apricot fruit, firmness decreased gradually from  
58 the external to the internal tissues. From the peduncle to the pistil, firmness was higher in the  
59 peduncle zone, followed by the pistil zone and at last the equatorial zone.

60 The heterogeneity of apricot texture depends on genetic (Ruiz & Egea, 2008) and tissue  
61 type within the fruit (Ella Missang et al., 2011). The latter variations were due to different  
62 tissue histology and cell wall polysaccharide characteristics in the different fruit regions. To  
63 highlight the importance of the heterogeneity and its influence on the quality of apricot,  
64 research work has been carried out in recent years on the relationship between the texture of  
65 the fruit and industrial processing (Ella Missang et al., 2012 ; Ayour et al., 2016; Deng et al.,

66 2018; Deng et al., 2019). Indeed, the rapid loss of texture of the apricot fruit during storage  
67 and processing is a limiting factor for its commercialisation and use. To better understand the  
68 variability of apricot texture, we have already studied the impact of heat treatment according  
69 to the stage of harvesting fruit in a wide range of cultivars. In fact, the tested cultivars had  
70 different degrees of sensitivity to heat treatment, probably in relation to their textural  
71 characteristics of the fruit. Consequently, we have identified physical criteria for the selection  
72 of apricots intended for processing (Ayour et al., 2016).

73       Recently, physical and chemical analyses conducted by Deng et al. (2018) indicated a  
74 strong correlation between the texture loss, the decomposition and the degradation of the  
75 pectin polysaccharides of the cell wall in relation to the reduction of the drying time of  
76 apricots. In another study, Deng et al. (2019) indicated that this drying time of apricot was  
77 influenced by the ripening stage, which is related to changes in the physicochemical and  
78 pectic properties in the tissue nanostructure of the fruit. Indeed, it is necessary to choose fruits  
79 with an appropriate degree of ripeness to achieve a high drying rate. Lahaye et al. (2014) were  
80 able to exploit the cell wall composition heterogeneity in the inner and outer parenchyma  
81 tissue to discriminate fruit of nine apricot varieties. Phenotyping and chemotyping of  
82 structural characteristics known to contribute to fleshy fruit texture showed several  
83 discriminating variables, among which methyl and acetyl groups esterifying cell wall  
84 polysaccharides were particularly the better discriminants. Cell walls can be modified by  
85 pectinolytic enzymes. In research on texture engineering of fruits and vegetables through  
86 pectin modification by endogenous enzymes, most attention has been paid to  
87 pectinmethylesterase (PME) and polygalacturonase (PG). PME is one of the key enzymes  
88 involved in plant carbohydrate metabolism (Hubbard & Pharr, 1992). It catalyzes the  
89 demethylation of the pectin homogalacturonan domain, favoring, if present, the action of PG,  
90 which subsequently depolymerizes pectin by cleaving the galacturonan linkages (Duvetter et  
91 al., 2009). Further, pectin solubilization by  $\beta$ -galactosidase ( $\beta$ -Gal) is actively involved in  
92 fruit softening (Brummell et al., 2001). Rose & Bennet (1999) have correlated the cell wall  
93 degradation with  $\beta$ -Gal and other glycosidases, which activities consist in hydrolyzing the  
94 glycosidic side chains of pectin. These activities increase during ripening of apricots  
95 (Cardarelli et al., 2002). Brummell et al. (2004) have reported that all these enzyme activities  
96 lead to the depolymerization and solubilisation of pectin, which are correlated with a decrease  
97 in fruit firmness. However, Ribas-Agustí et al. (2017) reported that texture loss of apricots  
98 was more correlated to the increased acidity compared to other biochemical parameters

99 include pectinolytic enzyme activities (Pectinmethylesterase, Polygalacturonase,  $\beta$ -  
100 Galactosidase and  $\alpha$ -Arabinosidase). Recently, Fan et al. (2019) studied the effect of parietal  
101 enzymes on the apricot texture, in particular the degradation of polysaccharides with  
102 ultrastructure modification in its cell wall, the main contribution to the softening. To avoid  
103 this degradation, they reported the advantages of near freezing temperature (NFT) storage in  
104 the inhibition of cell wall modifying enzymes ( $\beta$ -galactosidase, pectin methyl esterase,  
105 polygalacturonase and cellulase) and related genes expressions.

106 Most texture studies on cell wall composition and enzymatic activity are related to  
107 whole fruit, mainly concerning physiological and ripening aspects. In terms of food quality,  
108 special attention has been given to changes in the cell wall during ripening, in order to  
109 optimize the textural attributes of the plant, as well as other quality characteristics dependent  
110 on these modifications (Waldron et al., 2003). However, studies on the fine characterization  
111 of the intra-flesh texture associated to the cell wall biochemistry, in particular as quality  
112 control, are scarce. Fines studies of tissue structure are needed to better understand the  
113 underlying biochemical and microstructural phenomena involved in the texture loss, thus  
114 making it possible to identify the selection criteria for apricots intended for processing as we  
115 reported in a previous study on apricot texture (Ayour et al., 2016).

116 Therefore, this study concerned to ten apricot clones aimed to: **i)** Characterize the  
117 texture changes in relation to cell wall neutral sugar composition and the pectinolytic enzyme  
118 activities on three tissue zones of fruit flesh (peduncle zone, equatorial zone, and pistil zone)  
119 in order to determine the cell wall composition and its potential variability. **ii)** Investigate the  
120 variation in firmness through the different flesh tissue zones according to the pectinolytic  
121 enzyme activity during ripening. **iii)** Assess the suitability of apricots for technological  
122 purposes, especially for processing to canned halves, an important Agri-food industry in  
123 Marrakesh region.

124 **2. Material and methods**

125

126 *2.1. Standards and chemicals*

127

128 Liquid nitrogen, ethanol and acetone were from Fisher Scientific (Strasbourg, France).  
129 Phenol, sulfuric acid, meta-hydroxyphenyl (MHDP) and methanol were from Merck  
130 (Darmstadt, Germany). Acetic acid, phosphate buffer, citrate buffer, borate buffer, sodium  
131 chloride (NaCl), apple pectin, p-nitrophenyl- $\beta$ -D-galactopyranoside and p-nitrophenol  
132 (Sigma-Aldrich (Darmstadt, Germany). Sugars (glucose, arabinose, fucose, galactose, xylose,  
133 mannose and rhamnose) were from Fluka (Buchs, Switzerland). Sodium hydroxide,  
134 Methanol-d3 (CD3OH), Potassium hydroxide (KOH) and inositol were from Sigma-Aldrich  
135 (Deisenhofen, Germany). Acetonitrile of HPLC grade was obtained from VWR International  
136 (Radnor, USA). Polyvinylpyrrolidone (PVPP), Triton X-100 were from Sigma-Aldrich,  
137 (Saint-Quentin-Fallavier, France).

138

139 *2.2. Fruit and sampling*

140

141 Apricot fruits from ten clones (Boum A2, Agdez LG1, Marouch 4, Ab 5, Marouch 16,  
142 Rtil 4, Clone C, Mans 15, Agdez C2 and Cg 2) were from the apricot collection in Saâda  
143 experimental field of the Regional Center for Agricultural Research in Marrakesh, National  
144 Institute for Agricultural Research (INRA), Morocco (30°21\_08.4\_N 9°30\_29.0\_W). All the  
145 studied clones were harvested at two stages of maturity (M1: commercially ripe and M2:  
146 consumption ripe) based on fruit firmness and surface color.

147 The fruit is considered commercially ripe when it reached complete size and an intense  
148 color (light, orange or red depending on genotype), but still firm to withstand handling,  
149 transport and storage conditions. At the consumption stage, the fruit is soft and could easily be  
150 detached from the tree. It is the fruit intended for the local market and for consumption almost  
151 immediate (ready to be eaten) (Ayour et al., 2017).

152 Batches of harvested apricots (n=30 per maturity stage) was divided into two groups  
153 (n=10 and n=20). The first group was used for the analysis of the firmness by an electronic  
154 penetrometer (Petrotest PNR 10, Anton Paar, Villeneuve la Garenne, France) at three tissue  
155 zones (peduncle, equatorial and pistil) on the fruit flesh. The second group was used for cell  
156 wall composition and enzymatic analysis by corresponding tissue zone (2 replicates of 10

157 units per zone). These apricots were cut into small pieces per tissue zone using a sharp knife  
158 and immediately crushed in liquid nitrogen (A11 basic analytic, Ika, Staufen, Germany) and  
159 stored at -80 °C until analyses. Samples were identified by repeat number, tissue zone, stage  
160 of maturity and clone name. Cell wall composition and enzymatic activities were analysed in  
161 duplicate. However, analysis of cell wall composition was performed only on commercial  
162 stage fruits (M1).

163

### 164 2.3. *Texture test of the different fruit zones*

165

166 Sampling was carried out as described in Ribas-Agusti et al. (2017). A tissue slice of  
167 about 1.5 cm thick was cut longitudinally using a sharp knife. The three tissue zones defined  
168 on the surface section (peduncle, equatorial and pistil) are presented in Figure 1. Firmness at  
169 each tissue zone was measured using an electronic penetrometer (Petrotest PNR 10)  
170 corresponding to the maximum pressure (Pa).

171 One measurement was made per tissue zone and per fruit; the average value and standard  
172 deviation of the 10 fruits were calculated by corresponding zone.

173

174

175

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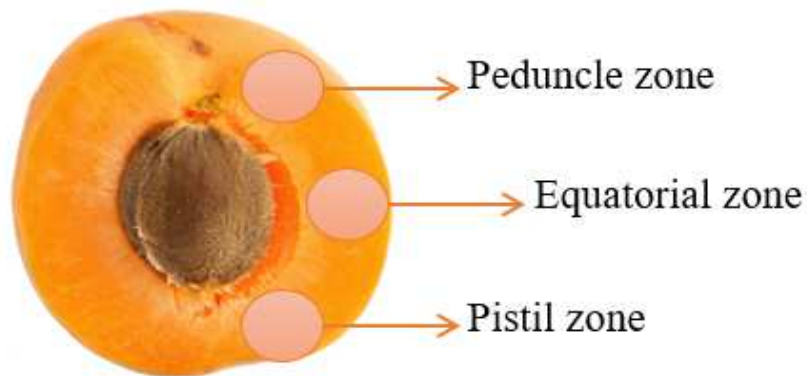
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182 **Fig. 1.** Apricot slice showing the three tissue zones included in the experimentation and the  
183 penetration points of the texture test.

184 2.4. Cell wall composition

185

186 2.4.1. Cell wall isolation and extraction

187 Cell wall materials were prepared as alcohol-insoluble solids (AIS) from the different  
188 tissue zones according to Renard et al. (1990) and Renard (2005) using hot rinsing method,  
189 briefly described below. About 500 mg of apricot homogenate frozen were weighed and  
190 suspended in 100 mL of 70 % ethanol and left for 25 min at boiling (temperature: 82–85 °C).  
191 The suspension was vigorously mixed and stabilized at room temperature for 1 hour and then  
192 filtered with a filtration system using the columns (75 ml empty SPE column (Interchim)  
193 equipped with a sinter of 20 µm) and a vacuum system Restek (Restek Corporation,  
194 Bellefonte, PA, USA). Subsequently, the insoluble material was resuspended in 70% alcohol.  
195 The washings were continued till absence of sugars as shown by absence of color in the  
196 phenol sulfuric test (Dubois et al., 1956). Samples were then washed twice with acetone:  
197 water: acetic acid (v/v/v 60:39:1) to extract polyphenols (Guyot et al., 1998), afterwards twice  
198 with acetone: water solution (v/v 80:20), then with pure acetone until discoloration of the  
199 substrate. This solid residue (AIS) was dried at 40 °C overnight and weighed.

200

201 2.4.2. Analytical methods

202 Neutral sugars were analysed as alditol acetates after acid hydrolysis by two ways. For  
203 glucose from cellulose analysis, the samples (c.a. 10 mg of AIS) were subjected to pre  
204 hydrolysis with 250 µL 72% sulfuric acid for 1 h at room temperature (Saeman et al., 1954),  
205 then diluted to sulfuric acid (1 mol L<sup>-1</sup>) by addition of water and internal standard (inositol).  
206 However, no pre hydrolysis was carried out for the analysis of neutral sugars (simple  
207 hydrolysis), including non-cellulosic glucose, the samples (10 mg) were dissolved in sulfuric  
208 acid (1 mol L<sup>-1</sup>) with internal standard. All samples were placed in an oven at 100 °C for 3 h  
209 for hydrolysis, after that they were derivatised to volatile alditol acetates (Englyst et al.,  
210 1982). They were injected onto a GC-FID HP 5890 Series II (Agilent, Palo Alto, CA)  
211 containing a DB225-MS capillary column (30 m x 0.25 mm i.d. coated with, 0.25 µm film  
212 thickness; Agilent). The conditions were: injection temperature 250 °C in split mode (ratio  
213 1:25); hydrogen as carrier gas at 45 cm/s (1.3 mL min<sup>-1</sup>); oven temperature isothermal at 215  
214 °C. Uronic acids were measured spectrophotometrically by the *m*-hydroxydiphenyl assay as  
215 described by Blumenkrantz & Asboe-Hansen (1973) with galacturonic acid as external  
216 standard, expressed as anhydrouronic acids (AUA). Uronic acids were measured after acid



217 hydrolysis by Saeman procedure as described above. Methanol was determined in AIS as  
218 described by Renard and Ginies (2009) by stable isotope dilution assay using headspace-GC–  
219 MS after saponification. Degree of methylation (DM) was calculated as the molar ratio of  
220 methanol to uronic acids.

221

## 222 2.5. Pectinolytic enzyme activities

223

### 224 2.5.1. Determination of Pectinmethylesterase activity

225 Pectinmethylesterase (PME) activities were determined by titration as described in  
226 Ribas-Agustí et al. (2017) with minor modifications. The extract from apricot powder (1 g)  
227 was produced with 1 mL 0.2 mol L<sup>-1</sup> phosphate buffer, pH 7.0, containing 1 mol L<sup>-1</sup> NaCl.  
228 The mixture was stirred for 2 h at 4 °C and centrifuged for 30 min at 16,000×g and 4 °C to  
229 obtain the PME crude extract (clear supernatant). An aliquot of the extract (250 µL) was  
230 added to 1 mL of apple pectin (3.5 g L<sup>-1</sup>) solution (70–75% esterification, Sigma-Aldrich), in  
231 0.12 mol L<sup>-1</sup> NaCl. The mixture was kept for 30 min at pH 7.0 and 35 °C under stirring in an  
232 automatic pH titrator (Metrohm, Herisau, Switzerland). The carboxylic groups liberated by  
233 de-esterification of the pectin methyl groups were measured by titration with 0.1 mol L<sup>-1</sup>  
234 NaOH. The amount of carboxylic groups liberated at pH 7.0 and 35 °C was used to define the  
235 PME activity. The PME activity was expressed as nkat g<sup>-1</sup> fruit fresh weight.

236

### 237 2.5.2. Determination of β-Galactosidase activity

238 β-Galactosidase activities were measured as described by Ribas-Agustí et al. (2017)  
239 with some modifications. The enzyme was extracted from 1 g of samples with 1 mL 0.1 mol  
240 L<sup>-1</sup> borate buffer, pH 9.0, containing 1.2 mol L<sup>-1</sup> NaCl, 10 mL L<sup>-1</sup> Triton X-100, and 10 g L<sup>-1</sup>  
241 PVPP. The mixture was stirred for 2 h at 4 °C and centrifuged for 30 min at 16,000×g and 4  
242 °C to extract β-galactosidase (clear supernatant).

243 The incubations were performed in a microplate spectrophotometer (FLX-Xenius, Safas,  
244 Monaco), at 37 °C for 60 min. We used 1 mmol L<sup>-1</sup> *p*-nitrophenyl-β-D-galactopyranoside  
245 (Sigma- Aldrich) in 50 mmol L<sup>-1</sup> citrate buffer (pH 4.4) as substrate. An aliquot of the  
246 extracts (10 µL) was added to 190 µL of substrate, and the absorbance was recorded at 400  
247 nm every 1 min to measure the liberation of nitrophenol. The rate of nitrophenol release from  
248 the glycosides, at pH 4.4 and 37 °C, was used to define the glycosidase activities. Its  
249 quantification was performed using a *p*-nitrophenol standard curve at 0.4 - 1.6 mmol L<sup>-1</sup>

250 range (Sigma-Aldrich). The  $\beta$ -galactosidase activity was expressed as nkat g<sup>-1</sup> fruit fresh  
251 weight.

252

## 253 2.6. Statistical analysis

254

255 The firmness, cell wall composition and enzymatic activities (dependent variables) data  
256 of each apricot clone were statistically evaluated by three-way analysis of variance (ANOVA)  
257 using the three independent variables: tissue zone, clone and maturity stage. Cell wall  
258 composition was analyzed in duplicate : 2 replicates of 10 plant material units per zone per  
259 clone. Firmness was analyzed per zone for 10 fruits per clone and per maturity stage. PME  
260 and  $\beta$ -Gal activities were analyzed in duplicate: 2 replicates of 10 plant material units per  
261 zone, per maturity stage and per clone. The analytical reproducibility of the results was  
262 determined as pooled standard deviations (Pooled SD). This parameter was calculated for  
263 each series of replicates per variable using the sum of individual variances weighted by the  
264 individual degrees of freedoms described by Kosmala et al. (2013).

265 Data of measured firmness, cell wall composition and enzyme activities of flesh tissue  
266 zones for M1 stage were subjected to multivariate analysis. The objective was to investigate  
267 the relationships between apricot texture, cell wall composition and enzyme activities. The  
268 Pearson's correlation coefficients between all parameters were calculated. Principal  
269 component analysis (PCA) was performed on datasets obtained from apricots using XLSTAT  
270 statistical software version 2018.

271

## 272 3. Results and discussion

273

### 274 3.1. Cell wall composition of apricot tissues

275

276 Significant differences ( $P \leq 0.005$ ) were observed between apricot clones for the three  
277 tissue zones (peduncle, equatorial and pistil). However, less significant difference was  
278 observed between the three zones within the same clone. All tissues zones showed relatively  
279 high amounts of alcohol insoluble solids expressed by their yields (Table 1). Indeed, the AIS  
280 yields were higher than those reported by Ella Missang et al. (2012) which varied between 16  
281 and 17 mg g<sup>-1</sup> FW for cell wall materials in apricot median tissues. All other data available on  
282 apricot cell walls concern only the whole fruit, without distinguishing the different tissue

283 zones. AIS yields found in the present work were close to those reported by Kurz et al. (2008)  
284 for some apricot varieties (24.9–25.9 mg g<sup>-1</sup> FW) and lower than obtained cell wall yields  
285 (30.5–52.4 mg g<sup>-1</sup>) for Canino apricot variety (Femenia et al., 1998). The compositions of the  
286 AIS (Table 1) were characterised by high amounts of uronic acid (179 – 300 mg g<sup>-1</sup> AIS).  
287 Glucose (mostly cellulosic, 118-214 mg g<sup>-1</sup> AIS) was the main neutral sugar in the AIS from  
288 apricot flesh, followed by arabinose. This result was in line with previous studies: Ella  
289 Missang et al. (2012) report that for all nine fresh tissues, uronic acids (268–338 mg g<sup>-1</sup> AIS)  
290 from pectin was the predominant sugar, followed by glucose (197–217 mg g<sup>-1</sup> AIS). Lahaye et  
291 al. (2014) and Femenia et al. (1998) describe that glucose and uronic acid as the main  
292 monosaccharides in apricot AIS. Arabinose, galactose, commonly found in pectin side-chains,  
293 and mannose, which is a hemicellulose sugar (mannan), were all present in higher  
294 concentrations than reported by previous studies (Ella Missang et al., 2012; Lahaye et al.,  
295 2014). The presence of fucose, in addition to relatively high amounts of xylose, suggested the  
296 presence of hemicelluloses composed of xyloglucan polymers as found recently in apricot  
297 (Liu et al., 2017) and in other *Prunus* species, such as plum (Nunes et al., 2008; Renard and  
298 Ginies, 2009) or peach (Brummell et al., 2004; Yoshioka et al., 2011). This monosaccharide  
299 was in the ranges reported by other authors (Femenia et al., 1998; Ella Missang et al., 2012;  
300 Lahaye et al., 2014) but lower than in Kurz et al. (2008). Recently, in other fruit specie, Ren  
301 et al. (2020) report that glucose was the most abundant component followed by arabinose and  
302 galactose in composition of cell-wall polysaccharides of custard apple (*Annona squamosa*).  
303 Besides, a small proportion of rhamnose, xylose and mannose occurs .

304 Pectin methyl esterification varied significantly among the different clones ranging from  
305 DM = 58 to 97 in Ab 5 and Mans 15 respectively for equatorial zone. These DM were  
306 comparable to those reported by Lahaye et al. (2014) (from 46 to 81). Femenia et al. (1998)  
307 describe a decrease in methyl esterification of pectin during apricot ripening (from 87 to 60)  
308 according to the observed increase in pectin methylesterases gene expression and enzyme  
309 activity observed in a large number of ripening fleshy fruits (Brummell, 2006; Goulao &  
310 Oliveira, 2008).

311 The cell wall composition depends strongly on apricot structural tissues. In this study  
312 the measures were collected in the median tissue of the three zones, which appears quite  
313 homogeneous with typical parenchymal cells. These cells are organised relative to the  
314 vascular bundles, as illustrated by Archibald & Melton (1987), and have no preferential  
315 orientation relative to the fruit structure. Archibald & Melton (1987) report that apricot is

316 characterized by high heterogeneity in its tissue structure, with five layers: an epidermis, a  
317 hypodermal layer, a middle layer of thin walled parenchyma cells, a layer of radially  
318 elongated cells and a layer of small cells adjacent to the pit. This heterogeneity is already  
319 studied (Ayour et al., 2016; Ella Missang et al., 2011) and these five layers have different  
320 responses during thermal treatment. The median equatorial zone appears the most  
321 representative for texture characterization in the apricot intended for processing. Indeed, this  
322 study was carried out on the three median zones in peduncle, equatorial and pistil parts  
323 (Figure 1). Meanwhile, changes in the tissue structure, involved in the extensive solubilization  
324 and depolymerization of pectin in the middle lamella of cell walls, and the modification of  
325 pectin, are related to the ability to bind water and organic molecules (Simal et al., 2005).  
326 Moreover, not only the chemical compositions but also the structural polymerization of  
327 neutral sugars impact the AIS characteristics. Previous studies have indicated that apricot  
328 softening is caused by solubilization of cell wall polysaccharides, which has therefore  
329 decreased cell wall strength and intercellular adhesion (Ella Missang et al., 2012; Fan et al.,  
330 2017; Deng, et al., 2019; Ren et al., 2020). The ripening induced a softening of the texture,  
331 associated with the depolymerized and solubilized pectin. Since then, water-soluble pectin  
332 (WSP) content has increased considerably, coupled with the decrease in chelator-soluble  
333 pectin (CSP) and Na<sub>2</sub>CO<sub>3</sub>-soluble pectin (NSP) content. During this time, the structure of  
334 pectin is strongly altered during maturation, as observed by AFM. The length of the polymer,  
335 the amounts of branching and the size of the aggregates are reduced during the development  
336 of maturity (Deng, et al., 2019). Ren et al. (2020) report that molecular weight distribution  
337 and monosaccharide composition of cell-wall polysaccharides changed greatly during storage,  
338 especially the degradation of pectin polysaccharide. In addition to storage, the genotype  
339 represents one of the most influencing factors on the chemical composition of the cell wall. In  
340 this study, sugar composition was able to distinguish the different apricot clones and slightly  
341 between the flesh zones. Xylose, arabinose, mannose, rhamnose, and uronic acid showed  
342 significant differences between the flesh zones belonging in the same clone. The (zone\*clone)  
343 interaction shows that the difference between the monosaccharide concentrations depend on  
344 clone and flesh zone. The apricot clones were found to be relatively rich in AIS according to  
345 the literature data, except for Clone C and Agdez C2.

**Table 1.** Yields, monosaccharide compositions and DM of alcohol-insoluble solids (AIS) isolated from different tissue zones of fresh apricot fruits of ten apricot clone.

Zone	Clone	Yield (mg g <sup>-1</sup> FW)	Monosaccharide composition (mg g <sup>-1</sup> AIS)											
			Rha	Fuc	Ara	Xyl	Man	Gal	AUA	Non-cellulosic Glc	Cellulose	Total NS + AUA	MeOH (mg/g)	DM
Peduncle	Boum A2	24	11	4.1	90	39	20	40	241	9	169	612	28	64
	Agdez LG1	15	12	5.4	87	41	18	51	255	14	182	664	32	70
	Marouch 4	20	12	4.2	73	32	19	38	243	16	165	602	31	70
	Ab 5	26	13	3.1	94	35	16	50	229	10	160	610	32	77
	Marouch 16	17	10	4.8	72	34	19	37	274	10	175	634	32	64
	RTil 4	20	12	4.3	82	46	21	37	225	14	193	634	27	66
	Clone C	18	10	4.7	92	34	18	39	204	12	164	577	26	70
	Mans 15	19	12	4.6	83	37	22	38	213	14	194	617	33	86
	Agdez C2	22	14	4.3	77	30	24	40	203	21	182	595	30	82
	Cg 2	27	12	4.5	106	34	18	43	179	10	175	582	26	79
Equatorial	Boum A2	20	12	4.1	82	37	21	40	267	9	180	652	29	59
	Agdez LG1	17	11	4.2	75	37	17	47	262	12	172	637	32	66
	Marouch 4	17	11	4.0	67	35	21	38	276	10	173	635	31	63
	Ab 5	28	10	4.2	95	40	16	48	295	10	154	673	31	58
	Marouch 16	16	14	4.3	68	32	21	39	253	11	189	631	32	70
	RTil 4	20	14	4.1	108	35	20	45	215	9	195	645	27	69
	Clone C	25	11	3.5	83	28	18	39	184	14	118	498	28	84
	Mans 15	18	11	4.8	77	34	21	38	187	12	187	571	33	97
	Agdez C2	23	13	4.4	68	32	26	38	183	24	175	563	27	80
	Cg 2	26	15	4.0	78	41	24	41	199	11	214	627	30	83
Pistil	Boum A2	23	13	4.7	82	34	21	41	251	10	178	634	29	64
	Agdez LG1	19	9	4.3	72	38	17	43	268	11	157	618	31	64
	Marouch 4	19	12	4.2	63	32	20	35	294	14	169	642	32	60
	Ab 5	27	10	4.0	88	35	16	45	300	9	149	655	33	61
	Marouch 16	20	12	4.0	64	32	21	36	285	14	175	643	34	67
	RTil 4	21	15	4.2	72	37	23	37	262	12	187	649	33	68
	Clone C	20	12	4.3	92	21	6	39	220	11	169	574	32	80
	Mans 15	21	13	4.9	85	37	22	41	215	14	196	627	33	85
	Agdez C2	26	12	4.5	81	33	26	45	183	20	170	574	29	86
	Cg 2	28	10	4.2	107	32	17	40	197	11	165	582	27	76
Pooled SD	4	1.6	0.3	13	4	4	4	38	6	18	72	7	11	
ANOVA	Clone	15***	6***	6***	19***	20***	36***	10***	15***	6***	9***	35**	23**	16***
	Tissue Zone	6**	4*	1	6**	13***	7**	1	5*	2*	1	9*	8**	1
	Clone*Zone	5*	5*	2	6**	9**	7**	2	1	1	3*	4*	7**	1

348 Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Cellulose: calculated by glucose from Saeman hydrolysis – glucose (No-cellulosic Glc)  
349 from simple hydrolysis, AUA: anhydrouronic acids, MeOH: methanol, DM: degree of methylation. Pooled SD: pooled standard deviation, Fisher's Value F value,  $P \leq 0.05$ : \*,  $P \leq 0.01$ : \*\*,  $P \leq$   
350 0.001: \*\*\*.

### 351 3.2.Changes of apricot flesh firmness

352

353 Firmness varied significantly (Table 2 and Table 3) between apricot clones according to  
354 flesh zones and maturity stages. The fruit firmness was significantly higher in apricots harvested  
355 at the commercial stage (M1) than in those harvested at the consumption stage (M2). The same  
356 pattern of maturity evolution was found among all apricot clones, as found by Sharma et al.  
357 (2011) who reported that harvest maturity significantly influences the firmness of some Japanese  
358 prunus. Deng et al. (2019) reported that texture of apricot was strongly dependent on the  
359 maturation stages, the hardness decreased considerably ( $P \leq 0.05$ ) with advance in the maturity  
360 of apricots. Indeed, softening during ripening has been confirmed in other reports on apricots  
361 (Ayour et al., 2016; Fan et al., 2017; Amoriello et al., 2018;), mango (Cárdenas-Pérez et al.,  
362 2018) and bananas (Yap et al., 2017).

363 Agdez C2 and Ab 5 were the firmest and the least firm clones respectively. Our results  
364 were consistent with the study by Caliskan et al. (2012) which reported values from  $11 \cdot 10^4$  to  $47$   
365  $10^4$  Pa (exactly from 107873 to 470719 Pa) in firmness measurements on whole apricots.  
366 Firmness was monitored from the peduncle to the pistil zone (Table 2). For most clones, the  
367 firmness was higher in the peduncle, followed by the equatorial and at last the pistil zone. All  
368 differences were significant, in agreement with Ella Missang et al. (2012) who report higher  
369 firmness in the peduncle tissue followed by the equatorial and pistil zones. However, the  
370 equatorial zone is the most homogeneous flesh part among the clones, such that low differences  
371 in firmness values were observed between studied apricots compared to both other zones. The  
372 apricot is also characterized by a great texture heterogeneity of these tissues (Ella Missang et al.,  
373 2012).

374 Recently, Zhang et al. (2018) indicated that weight loss and firmness were the major  
375 factors affecting the quality of apricots. To cope with this physiological problem related to the  
376 maturation of the fruit, the authors studied the advantages of a biological treatment which  
377 prevented the decrease in firmness and benefited the textural properties of the tissue, namely: An  
378 edible coating of protein isolate soybeans (SPI) and chitosan which has been used to extend the  
379 shelf life of apricots stored at 2 °C.

380

### 381 3.3.Changes in Pectinmethylesterase activity

382

383 Significant effects were found for clone, ripening stage, tissue zone and their interactions

384 on PME activity (Table 2 and Table 3). Significant differences in enzyme activities were  
385 observed between the apricot clones in both ripening stages. The highest enzyme activity was  
386 found in Agdez LG1 and the lowest level was determined for Cg 2. Higher PME activity was  
387 observed with ripening for all studied clones, except for Agdez LG1 and Marouch 16 and more  
388 pronounced in Ab 5, Mans 15 and Cg 2. These results highlight the importance of cultivar and  
389 ripening stage for PME activity in apricot fruit. Similar results were found in other works with  
390 different apricot cultivars, who also reported that PME activity increased during the maturity  
391 stages (Arancibia & Motsenbocker, 2004; Lohani et al., 2004; Deng et al., 2005; Abaci & Asma,  
392 2014). The apricot clones were characterized by low PME activity values, compared to Abaci &  
393 Asma (2014) who found a significant difference in enzyme activity between the unripe and ripe  
394 fruits of six apricot cultivars. Even higher activities were reported in other apricot cultivars  
395 (Cardarelli et al., 2002; Botondi et al., 2003). The effect of the tissue zone was different  
396 concerning the different clones and the ripening stages: For most clones, PME activity was  
397 higher in the pistil and equatorial zones. Similar result was found by Ribas-Agustí et al. (2017)  
398 who reported lowest PME activity in the peduncle, followed by the equatorial and highest  
399 activity in the pistil zone.

400 Fan, et al. (2019) reported that the degradation of polysaccharides in the cell wall is a  
401 consequence of the synergy between several key enzymes that modify the cell wall, including  
402 PME, PG,  $\beta$ -GAL and CEL. In accordance with our study and other reports (Carvajal et al.,  
403 2015; Liu et al., 2016) the parietal enzymatic activities of apricot increased during the fruit  
404 ripening process. The use of NFT storage suppressed increases in these enzymes activities  
405 according to Fan et al. (2019). Indeed, PME activity in apricots increased rapidly at the start of  
406 storage at 5 °C, while the increase in this enzymatic activity was effectively inhibited by storage  
407 at 0 °C and NFT. Compared to storage at 0 °C, NFT storage suppressed the activity of the apricot  
408 enzyme at lower levels. The activity of the PME in the apricots stored at the NFT were  
409 respectively 88% of that of the apricots stored at 0 °C on the 60<sup>th</sup> day.

410

#### 411 *3.4.Changes in $\beta$ -Galactosidase activity*

412

413 Significant effects were found for clone, ripening stage, tissue zone and their interactions  
414 on  $\beta$ -galactosidase activity (Table 2 and Table 3). Agdez LG1 and Boum A2 had the highest  
415 levels of  $\beta$ -Gal activities while the lowest level was determined for Cg 2. Increased activity was  
416 observed with ripening for most studied clones, this agreed with various studies which have



417 indicated that activity of  $\beta$ -galactosidase increased during ripening of many fruits (Golden et al.,  
418 1993; Lazan et al., 2004).

419 However, the effect of maturity stage was not significant on the  $\beta$ -Galactosidase activity,  
420 in fact few differences have been found between M1 and M2, compared to PME activity (Table  
421 3). For most clones,  $\beta$ -Gal activity was higher in the pistil zone followed by both other zones  
422 (equatorial and peduncle), with relative order depending on clone. Ribas-Agustí et al. (2017) also  
423 reported an increasing trend of  $\beta$ -galactosidase activity with ripening, and from peduncle to pistil  
424 zones. These results highlight the important implication of genotype and tissue zone in evolution  
425 of  $\beta$ -Gal activity in apricot fruit. Indeed, earlier reports (Gulzar & Amin, 2012; Ribas-Agustí et  
426 al., 2017) indicate that the  $\beta$ -galactosidases may be involved in the modification of the tissues  
427 structure and cell wall polysaccharides of apricot fruits, suggesting its important role in  
428 softening.

429 As for the PME activity, Fan et al. (2019) reported that  $\beta$ -GAL activity in apricots  
430 increased rapidly at the start of storage at 5 °C, while the increase in this enzymatic activity was  
431 effectively inhibited by storage at 0 °C and NFT. Compared to storage at 0 °C, NFT storage  
432 suppressed the activity of the apricot enzyme at lower levels. The activity of  $\beta$ -GAL in apricots  
433 stored at NFT was 74% respectively of that of apricots stored at 0 °C on the 60th day. Moreover,  
434 changes in enzymatic activities in apricots have shown similar trends for all cell wall enzymes  
435 (Fan, et al., 2019).

Clone	Firmness (kPa)						PME (nkat g <sup>-1</sup> )						β-Gal (nkat g <sup>-1</sup> )					
	M1			M2			M1			M2			M1			M2		
	Ped	Equ	Pis	Ped	Equ	Pis	Ped	Equ	Pis	Ped	Equ	Pis	Ped	Equ	Pis	Ped	Equ	Pis
Boum A2	183	162	113	159	153	99	10	27	25	10	27	27	12	12	14	12	8	20
Agdez LG1	173	162	110	164	146	109	33	39	45	33	40	43	9	11	13	10	15	21
Marouch 4	215	164	167	157	152	105	4	8	10	5	6	12	6	4	9	6	8	7
Ab 5	154	124	41	108	101	88	7	18	20	23	18	24	4	7	7	10	7	7
Marouch 16	209	206	195	186	177	119	4	16	8	4	9	6	4	8	7	4	8	7
RTil 4	158	116	117	98	67	37	5	18	18	5	23	22	4	3	14	6	12	11
Clone C	263	270	161	191	147	102	13	14	12	13	14	16	3	9	5	4	4	7
Mans 15	265	154	190	206	178	131	14	23	19	22	25	22	6	7	6	8	6	7
Agdez C2	295	217	186	179	165	140	22	38	40	25	38	42	6	6	17	8	10	15
Cg 2	259	201	192	202	192	142	0.1	3	3	1	6	6	4	3	5	5	5	6

436 **Table 2.** Changes of firmness, pectinmethylesterase (PME) and β-galactosidase (β-Gal) activities of apricots clones in different zones of flesh during ripening.

437 PME: Pectinmethylesterase, BetaGal: β-Galactosidase.

438 M1: commercial stage; M2: consumption stage.

439 Ped: Peduncle, Equ: Equatorial, Pis: Pistil.

440

441 **Table 3.** Three-way ANOVA analysis of firmness, pectinmethylesterase and β-galactosidase in studied apricot.

Variable	ANOVA									442
	Pooled SD	Clone	Maturity	Zone	Clone*Maturity	Clone*Zone	Maturity*Zone	Clone*Maturity*Zone	443	
Firmness	19	10***	17***	14***	19***	19***	10**	16**	444	
PME	10	7***	8**	3***	11**	10*	8*	10*	445	
BetaGal	7	2***	5*	3***	4**	5**	7	5*	446	
									447	
									448	

449 Pooled SD: pooled standard deviation, Fisher's Value F value, P ≤ 0.05: \*, P ≤ 0.01: \*\*, P ≤ 0.001: \*\*\*.

450 PME: Pectinmethylesterase, BetaGal: β-Galactosidase.

451 *3.5. Multivariate analysis data of firmness and cell wall composition*

452

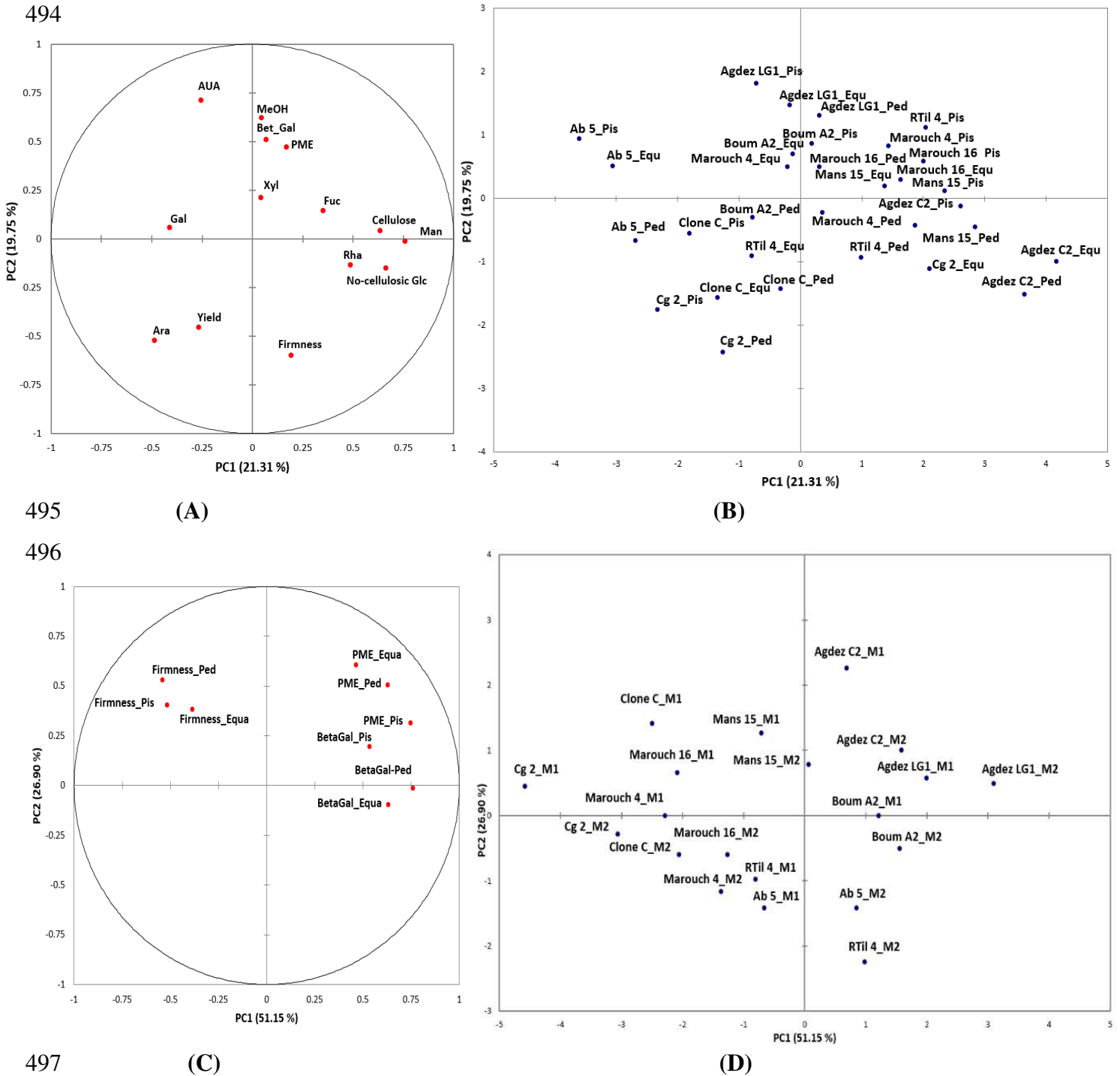
453 According to the Pearson correlation coefficients, the yields were not significantly  
454 correlated with neutral sugars, except for arabinose, which showed significant positive  
455 correlation coefficient (0.501). Among the positive correlations, cellulosic glucose was found  
456 correlated with rhamnose (0.624), non-cellulosic glucose with mannose (0.512) and arabinose  
457 with galactose (0.497). Uronic acid content was not correlated with neutral sugars but correlated  
458 with methanol (0.541) and firmness (-0.574). However, no significant correlation was found  
459 between cell wall AIS parameters and enzyme activities. The firmness was found negatively  
460 correlated with PME (-0.502) and  $\beta$ -Gal (-0.472). These correlations showed the implication of  
461 enzymes activities in degradation of the pectin and its participation in loss of apricot firmness.

462 The Principal Components Analysis (PCA) was used to illustrate the relationships among  
463 studied parameters, also to analyze the implication of cell wall composition and pectinolytic  
464 enzyme activities in the evolution of apricot texture considering the clone, tissue zone and  
465 ripening effects. The principal found correlations were plotted and shown in Figure 2.

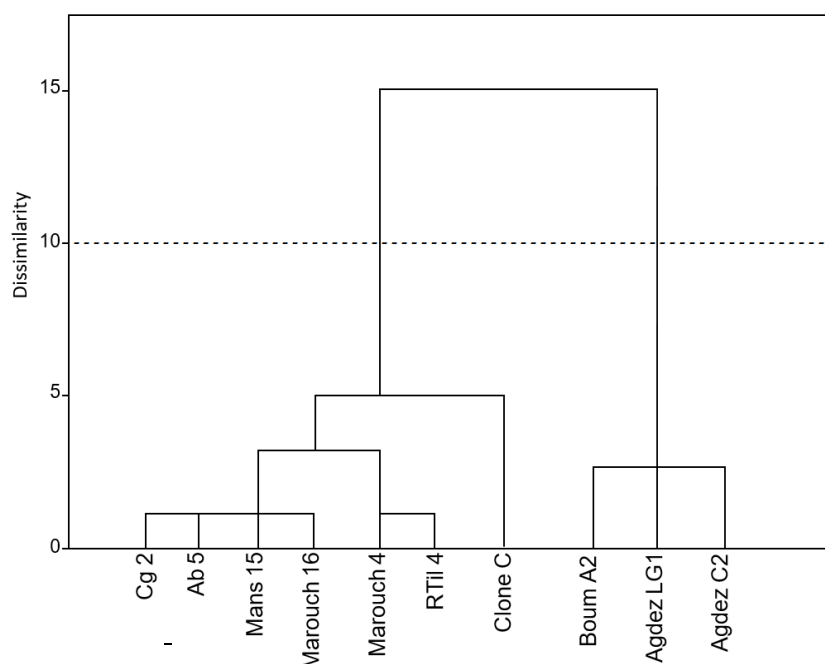
466 A first PCA was carried out using all studied parameters for M1 stage. More than 41 % of the  
467 total variance was explained by the first both principal components (Fig. 2A and 2B). The  
468 dispersion of the individuals (clone linked to zone) on the factorial map (Fig. 2B) showed the  
469 important impact of the genotype on cell wall composition and on enzyme activities compared to  
470 tissue zone. Therefore, the parietal composition of studied apricots varies significantly with  
471 clone compared to tissue zone, while there is no link with a precise tissue zone.

472 To highlight the impact of ripening stage on apricot texture, a reanalysis of the results with  
473 firmness and enzyme activities data for both maturity stages (M1 and M2) allowed better  
474 mapping of studied clones with a high variability (Fig. 1C and 1D). More than 78 % of the total  
475 variance was explained by the first both principal components (Fig. 2C and 2D). The negative  
476 correlation between firmness and enzymatic activities was shown on the factor map as a function  
477 of the tissue zones. This correlation is explained by the PC1 axis with 51.15% of the total  
478 variability on both sides of the PC origin. In addition, the three tissue zones are grouped for each  
479 measured parameter, indeed, they don't have a significant effect on apricot texture compared  
480 also to maturity stage. The distribution of individuals (Fig. 1D) distinguished the apricot clones  
481 according to a major impact of maturity stage. The factorial map (Fig. 1 D) indicated that  
482 ripening effect was related to the enzyme activities. M2 stage was characterized by a higher  
483 enzymatic activity compared to M1 stage and vice versa for apricots firmness.

484           Among studied clones, Adez LG2, Boum A2 and Agdez C2 were characterized a loss of  
485 firmness between M1 and M2, explained by high enzymatic activity observed for the two  
486 maturity stages. These results are supported with previous studies (Lazan et al., 2004; Arancibia  
487 & Motsenbocker, 2004; Lohani et al., 2004; Deng et al., 2005; Abaci et al., 2014; Ribas-Agustí  
488 et al., 2017) which reported the impact of ripening process in enzymatic activity increasing.  
489 However, Ab 5, Marouch 16, Mans 15 and Cg 2 (Fig. 3) were less influenced by the softening by  
490 looking at their firmness and the effect of the enzymatic activity both ripening stages. Hence the  
491 importance of these apricot clones which can represent a technological solution for the  
492 processing industry especially to canned halves, an important industry in Marrakesh region,  
493 whose apricot texture is the key element of the transformation.



**Fig. 2.** Principal component analysis of firmness, cell wall composition and pectinolytic enzyme changes in different tissue zones of ten apricot clones:  
 (A) and (B) Representation of all variables and segregation of 10 apricot clones according to tissue zones.  
 (C) and (D) Representation of firmness and pectinolytic enzyme variables and segregation of 10 apricot clones according to maturity stages.  
 Ped: Peduncle, Equ: Equatorial, Pis: Pistil  
 Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Cellulose: difference between total glucose measured after Saeman hydrolysis and readily hydrolysable glucose (No-cellulosic Glc) measured after 1M H<sub>2</sub>SO<sub>4</sub> hydrolysis. AUA: anhydrouronic acids, MeOH: methanol, PME: Pectinmethylesterase, BetaGal:  $\beta$ -Galactosidase, M1: commercial stage; M2: consumption stage.



509  
 510 **Fig. 3.** Cluster dendrogram realized on firmness and enzyme activity variables for ten apricot clones at  
 511 two maturity stages.

512

#### 513 4. Conclusion

514

515 Apricot texture was characterized with high heterogeneity between studied clones.  
 516 Apricots showed different texture profiles according to their cell wall composition and changes.  
 517 Indeed, the results could help to illustrate the fundamental of changes of apricot texture  
 518 according to tissue zone, genotype and ripening stage.

519 Apricot cell walls contained relatively high levels of neutral sugars such as glucose,  
 520 arabinose, xylose and galactose. Rhamnose, mannose and fucose were also found to be present in  
 521 small amounts. Also, studied apricots showed higher levels of uronic acid and of degree of  
 522 methylation. This composition depends especially to clone and to tissue zone. The measurement  
 523 of flesh firmness allowed a significant difference between the three tissue zones (peduncle,  
 524 equatorial and pistil). The ripening effect was observed in the evolution of firmness as a function  
 525 of enzymatic activity, in particular the increased PME activity with the maturity stage.

526 These associations allowed us to classify apricot clones according to the evolution of  
 527 softening. A group of clones constituted by Ab 5, Marouch 16, Mans 15 and Cg 2 were less  
 528 influenced by softening and have the advantage of a technological valorisation for processing  
 529 industry, especially for canned halves, thanks to the texture of fruit apricots of these clones  
 530 which is decisive in the production of apricot canned halves.

531

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537

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