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Cell walls as determinant of apple texture quality

Evolution of hemicelluloses with fruit development and ripening

Marc Lahaye, Mandy Malick, Bernard Quemener
UR1268 Biopolymères Interactions Assemblages
INRA
44316 Nantes, France
marc.lahaye@nantes.inra.fr

François Laurens
UMR1259 Génétique et Horticulture
INRA
49071 Beaucouzé, France

Abstract—**Apple fruit texture is a key quality trait orienting consumer choice and agro-industrial processes. Among the several factors at different scales involved in texture, cell walls and their constitutive hemicelluloses are determinant in controlling the mechanical properties of the cells and tissues. In this report, the dynamic of cell wall composition and hemicelluloses fine structure during fruit development and ripening was studied from different apple genotypes collected over 2 years. Typical loss of cell wall galactose was observed along apple expansion and ripening. The combination of cell wall enzymatic hydrolysis and MALDI-TOF mass spectrometry revealed important hemicelluloses modifications along the fruit construction and maturation affecting both xyloglucan and the manor amount of glucomannans. Similar modifications were observed for individuals collected over the two years. The fine-tuning of hemicelluloses structure during apple development and ripening appears as an addition mean by which the fruit controls its mechanical properties and resulting texture.**

Keywords-component; Apple, texture, cell wall, hemicelluloses, MALDI-TOF mass spectrometry, glucanase

I. INTRODUCTION

Fruit texture is a major quality trait orienting consumer choice, impacting post-harvest itineraries and diseases resistance [1,2]. Its control is particularly strategic for the promotion of fruit consumption for their health benefits. Fleshy fruit texture depends on different factors at different scales: histology, cell turgor pressure and cell wall structure and organization [3-5]. Cell walls are central to these factors as they shape cells, assure tissue cohesion by the cell-cell adhesion at their interfaces and allow cells to withstand turgor pressure [6]. For these reasons, regarding fruit softening, many studies focused on cell walls composition and metabolism as well as on the different enzymatic and non-enzymatic players involved in wall disassembly [5, 7]. Among fleshy fruits, apple represents the 2nd world production [8]. Like other fruits, its cell walls are composed of three macromolecular networks: cellulose, hemicelluloses, pectins together with structural proteins, enzymes, small metabolites (sugars, organic acids…), ions and water [6]. Its texture elaboration starts with fruit development [9] and particularly at the ripening stage and during storage after harvest [10-13]. These changes have been qualified at the cell wall levels with the decrease in pectin associated neutral sugars. In particular galactose and arabinose are metabolized during fruit ripening [14-16]. Little is known about the fate of hemicelluloses during apple development and texture elaboration although their wall interactions and molecular weight are likely affected on ripening [17, 18]. These were identified as composed of xyloglucans, glucomannans and xylans [19-20]. In the light of the important roles of xyloglucans on fleshy fruit texture [5, 7], the fine structure of these polysaccharides was characterized and defined as being build on majority of **XXXG**, **XXFG**, **XLFG** with lower amount of **GFG**, **XXG** and **XLXG** [21-24] taking the oligosaccharide nomenclature from [25] (Table 1). Since early fleshy fruit development can impact on texture of the fully mature fruit [9], we more specifically followed the evolution of hemicelluloses structure in apples. Recent developments in mass spectrometry made it possible to rapidly detect minute modifications in cell wall structural features in relation with plant mutations or development stages by analysis of cell walls enzymatic hydrolyzate [26-28]. In this report we applied this strategy coupled to multivariate analyses to follow the fine structural evolution of apple hemicelluloses during fruit development until harvest.

TABLE I. NOMENCLATURE USED TO REFER TO HEMICELLULOSES STRUCTURES (ADAPTED FROM [25])

Hemicelluloses	Chemical structure	Code
Xyloglucan		
	-β-D-Glc*p*-(1→4)	**G**
	-β-D-Glc*p*-(1→4)- α-D-Xyl*p*-(1→6)↑	**X**
	-β-D-Glc*p*-(1→4)- β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)↑	**L**
	-β-D-Glc*p*-(1– α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)↑	**F**
Glucomannan		
	–β-D-Glc*p*-(1→4)-	**Hex**
	–β-D-Man*p*-(1→4)-	**Hex**
Substituting group		
	CH_3-CO-O-	**a**

Elementary building block of xyloglucan structures: **XLFG** is made of the linkage of **X**, **L**, **F** and **G** elements; Number after code refers to the number of residues in the oligosaccharide. The number following the code **a** refers to the number of acetyl groups.

II. Materials and Methods

A. Fruits

Fourteen and 17 genotypes were studied in 2006 and 2007, respectively. All the 2006 samples and 15 in the 2007 samples were progenies from crosses between parents with extreme scald sensitivity (X681, X683 from the INRA, GenHort, Angers, France). Apples were collected in July and at commercial maturity in 2006 and in June, July, August and at commercial maturity in 2007.

B. Cell wall material preparation

The freeze-dried flesh from 1 to 7 different fruits per genotypes and collect date was milled to a fine powder (Fast-Prep 24, MP Biomedicals). Cell walls were prepared as alcohol insoluble material (AIM) from these powders by sequential extraction with 70% and in 96% ethanol (85°C, 100 bars; ASE 200, Dionex, Sunnyvale USA).

C. Chemical analyses

All analyses were carried out on dry AIM.

Sugars composition in the AIM was identified following sulfuric acid hydrolyzis according to [29]. A 30 min prehydrolysis in 13 M at 25° C was followed by 2 h hydrolysis at 100° C in 1M acid containing allose as internal standard. Neutral sugars were identified and quantified by HPAEC using retention times and weight response factors determined from a standards. Sugars were chromatographed through a CarboPac PA1 column at 25°C eluted by 75% water and 25% NaOH (500 mM) at 1 ml/min. Elution was monitored by pulsed amperometry (Electrochemical Detector 6D50, Dionex).

Uronic acids were quantified by colorimetry using meta-hydroxydiphenyl [30].

Cell wall hemicelluloses enzymatic degradations were realized sequentially using endo β-(1,4)-D-mannanase (*Aspergillus niger*) and endo β-(1,4)-D-glucanase (*Trichoderma longibrachiatum*; Megazyme, Bray, Ireland). Dry AIM (5 mg) was dispersed in 1 ml deionized water containing mannanase (30 µl, 300 U/ml). Incubation under agitation was done at 40° C for 15 h. Hydrolyzate recovery was achieved by centrifugation of the suspension (12 000 g, 20° C, 30 min) and the supernatant solution was boiled for 10 min. The pellet was washed three times with 900 µl water prior to addition of 1 ml water containing glucanase (37 µl, 540 U/ml). This second hydrolysis was carried out and processed as above.

For MALDI-TOF MS analysis, enzymatic hydrolyzates in quadruplets were mixed (10 µl) with matrix (10 µl; 6ATT-DHB matrix) [31] and analyzed on a M@ldi-TOF LR MS (Waters) calibrated by oligosaccharides of known masses.

D. Statistical analyses

All data treatment and statistical analysis were performed on R [32]. Principal component analysis was performed using the FactoMineR package [33].

III. Results and Discussion

Cell wall composition of developing apples: The sugar composition of cell walls according to the collect period is given in Table II. Principal component analysis on the composition data (Fig. 1) clearly points to the effect of development status on starch contamination in AIM and on cell wall galactose and mannose content. The first component depicted the starch content decrease for fruit reaching commercial maturity. The second component reflected the decrease in galactose and that of the minor amount of mannose as fruit development progressed. An analysis of variance on these data showed no significant effect of year of collect and of genotype on the sugar content while all sugars content were significantly affected by the fruit development ($p < 0.05$). Galactose content decrease was typical of what was previously reported for pectic structure evolution on fruit development [14-16].

TABLE II. Mean sugar composition in apple AIM according to collect time (%dry weight; ± standard deviation)

Collect	Cell wall sugars								Starch	n[b]
	Fuc[a]	Rha	Ara	Gal	Glc	Xyl	Man	UA		
June	0.5 ± 0.1	0.5 ± 0.1	5.1 ± 0.9	11.3 ± 2.0	21.2 ± 4.1	5.2 ± 7.2	1.3 ± 0.3	12.3 ± 1.4	25.1 ± 6.7	14
July	0.5 ± 0.1	0.5 ± 0.1	4.6 ± 0.8	9.1 ± 1.5	25.7 ± 5.7	2.2 ± 0.3	1.0 ± 0.3	11.1 ± 1.5	31.4 ± 6.2	26
August	0.5 ± 0.1	0.5 ± 0.1	4.7 ± 0.8	8.3 ± 1.8	19.9 ± 4.7	2.3 ± 0.5	0.7 ± 0.2	11.3 ± 1.5	36.3 ± 6.4	14
Harvest	0.8 ± 0.2	0.8 ± 0.1	7.6 ± 1.0	7.6 ± 2.5	25.1 ± 6.3	5.3 ± 3.3	0.8 ± 0.3	17.5 ± 3.0	14.5 ± 8.9	39

a Fuc, Rha, Ara, Gal, Glc, Xyl, Man, UA refer to fucose, rhamnose, arabinose, galactose, glucose, xylose, mannose and uronic acids; b n refers to the number of samples

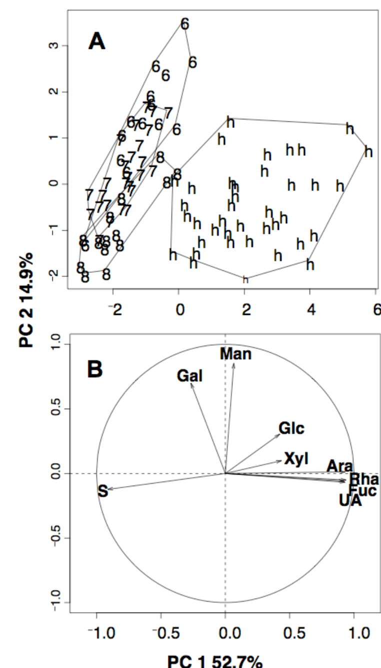

Figure 1. Principal component analysis of sugar composition in apple AIM. Maps of the first 2 components for A: individuals according to collect period june (6), july (7), august (8), commercial maturity (h) and B: variables with Gal, Man, Glc, Xyl, Ara, Rha, Fuc, UA, S referring to galactose, mannose, glucose, xylose, arabinose, rhamnose, fucose, uronic acids and starch.

Evolution of hemicelluloses structure during apple development: Cell walls of apples at different developmental stages were hydrolyzed sequentially by mannanase and glucanase. Mannanase yielded trace amounts of low molecular weight oligosaccharides observed by HPAEC (data not shown) with mass below the detection limit of MALDI-TOF MS. Glucanase hydrolyzates yielded MALDI-TOF MS spectra, which mean profile reflected major xyloglucan structures that were in agreement with literature data [21-24]. However the spectra showed that the **XXFG** and **XLFG** structures were mainly present as acetylated derivatives (Fig. 2), which were not previously shown due to artefactual deacetyl-esterification introduced in the analyses of the literature. Minor structures were identified on their mass basis as hexose containing structures (**Hex**) more or less acetyl-esterified (**Hexa**). On the basis of this substitution, part of these oligosaccharides was attributed to glucomannans [34]. The **Hex** oligomers cannot be ascribed to either hemicelluloses or galactan pectic structures as the amylase-free commercial glucanase is known contain trace of β-1,4-D-galactanase activity. Although MALDI-TOF ions intensities are not quantitative on an absolute basis, on the relative basis used in this study, peaks intensity variations reflected modifications in structure content in the hydrolyzate [26,27].

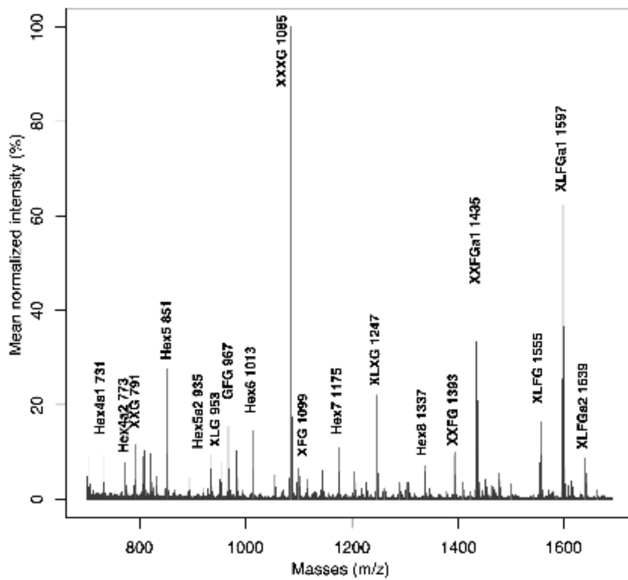

Figure 2. Mean MALDI-TOF MS spectrum of the entire collection of apple collected in 2006 and 2007 at different periods of development. See Table 1 for oligosaccharides nomenclature.

Principal component analysis of the series of spectra showed an overall good reproducibility in the developmental evolution of hemicelluloses structures over the two years (Fig. 3A). The first principal component (71.8% of total variance) indicated that **Hex** and **Hexa** oligomers proportions decreased with fruit ripening (Fig. 3B) which is in keeping with the lowering of galactose and mannose content measured in the cell wall. Distinct groupings were observed for **Hex** and **Hexa** oligomers. Within these, strong correlations were observed, which indicated that they originated very likely from the same polysaccharide families. Along this first component reflecting a "ripening" axis, oppositions in xyloglucan structures were also observed, notably between **XXGa1** and **XXG** as well as between **XLFG** and **XLXG**. These oppositions most likely resulted from acetyl esterases and various osidases dynamics. Xyloglucan structures were also differently distributed according to the second principal component axis (12.1% of total variance), which more likely revealed the effect of fruit expansion from June to August.

These results clearly showed that hemicelluloses fine structure are markedly modulated during fruit development and ripening. Different cell wall enzymes and proteins reshuffling the hemicelluloses-cellulose network play key roles in fruit development and softening [5, 7]. The fine structure modulation of these hemicelluloses either during the biosynthesis step or later in muro during ripening adds on to the means by which the fruit controls its mechanical properties and resulting texture. The methodological approach presented provides a convenient mean for evaluating key chemical characteristics for fruit quality.

IV. Acknowledgments

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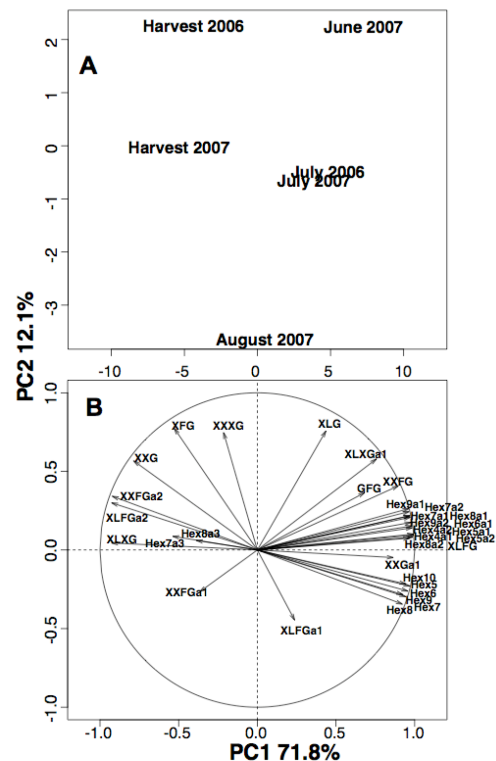

Figure 3. Principal component analysis of the MALDI-TOF MS spectra from cell wall glucanase hydrolyzate of the apple collected in 2006 and 2007 at different period during fruit development. A) Individual map: dates represent the position of the mean spectra, B) Variable map (see Table 1 for oligosaccharides nomenclature)

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