Analysis of Cell Wall Quantity and Cuticle Thickness of Apple Fruits and Relations with some of their Rheological Properties

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Abstract

Thickness of apple cuticle and cell wall quantity in epidermis region, expressed in percent of image pixels, were measured for three apple cultivars: *Gala* (*Ga*), Elstar (*El*) and Smoothee (*Sm*). Cuticle zone (CQ) was thicker for *Sm* with 32%, *El* and *Ga* had similar CQ with 22% and 19%, respectively. CQ could be linked to limited mass losses during storage: *Sm* showed the most important CQ and less mass losses compared to *Ga* and *El*.

Cell wall quantity (CWQ) allowed classifying the three studded cultivars as following: *Ga* > *El* > *Sm* with 63%, 59% and 56%, respectively (percent of image pixels). CWQ was also measured on fruits stored 14 and 28 days in cooled room (CR) and 14 days in shelflife (SL). Relations between CWQ and rheological properties of apples, measured by penetrometry, were calculated. CWQ showed highest correlation with strength (F_p) for *Ga* (R^2=0.77), stiffness (E*) and Flesh firmness (F_f) for *El* with correlation coefficient values of R^2=0.94 and R^2=0.87, respectively. Sm didn't show any correlation.

INTRODUCTION

Rheological properties of fruits are important for assessing the quality of texture. They are frequently estimated by an unique measurement: the firmness value (Harker *et al*. 1997). This value is estimated by penetrometric tests which record the maximal force needed to penetrate the apple (with or without skin) until a determined depth (Hoehn, 2003; Johnston, 2001).

For apples, these rheological properties are supposed to depend on different characteristics of inner fruit structure as cell morphology, cell-to-cell adhesion and cell wall composition (Zhu and Melrose, 2003). They also depend on water status of fruits and thus could be influenced by the cuticle thickness and composition. As the first protective barrier, cuticle allows to limit the surface permeability and consequently water losses (Araus *et al.*, 1991; Schreiber and Riederer, 1996; Baur *et al*., 1996; Blanke and Holthe, 1997) and mass losses (Morice and Shorland, 1973). Jenks *et al.* (1994) showed that cuticle degradations mainly occurred during storage and depended on cultivars and storage conditions (Rinallo and Mori, 1996).

The aim of this work was first to measure the cultivar differences according to their cuticle thickness (CQ) and cell wall quantity (CWQ) in epidermis region, and then to study the relations between CQ and mass losses during storage and the relations between CWQ and some rheological measurements extracted from penetrometric tests.

MATERIALS AND METHODS

Fruits

Three apple cultivars: *Gala* (*Ga*), *Elstar* (*El*) and *Smoothee* (*Sm*) were analyzed. In order to maximize the variability of their rheological properties, the fruits were stored in cooled room (***CR***, 02°C and 95% of relative humidity) or in shelf life (***SL***, laboratory temperature and relative humidity). Fruits were analysed before storage (00 day), after 14 and 28 days of CR storage and after 14 days of SL storage. A total of 180 fruits were used (15 fruits/sample). Fruits were produced in the orchards of the National Institute of Agronomic Research (INRA) of Angers in north west of France.

Penetrometric test

A penetrometric test was performed on whole unpealed apples with a 4mm diameter probe fitted on a texture analyzer (TA-XT2). The probe was moved from the surface of the fruit through the peal and flesh to a final depth of 15mm at a constant speed of 3.3mm.sec^{-1}. The force, measured in Newton, was expressed as a distance function. Three parameters were extracted from the curve, F_p (in N): strength or rupture force, E* (in N.mm^{-1}): stiffness or young modulus, and F_f (in N): flesh firmness (Duprat *et al*., 2000; Mehinagic *et al*., 2003).

Mass losses

Mass losses were measured on each apple by the difference between initial mass (00 day) and mass after 120 days of CR storage.

Histology and image analysis

5 fruits per storage duration (00, 14 and 28days) and storage condition (CR and SL) were selected for the histological study. Histological slides were performed from apple pieces (l=5mm, L=10mm, H=5mm) sampled on the epidermis region of the fruits and then included in methylacrylate resin.5 slides (minimum) to 10 slides (maximum) were obtained from each sample. They were coloured with a black Sudan B colorant (fig.1A) or a PAS (periodic acid + Schiff) colorant to underline apple cuticle or cell wall, respectively. Then, images of these slides were shot under microscope and recorded in a computer. All image analyses were performed with the Scion image software.

Quantification of cuticle thickness and cell walls in epidermis region

Slices coloured with the black Sudan B (fig.1A) or PAS were transformed on grey levels images (fig.1B), and then binarized and rotated to obtain a horizontal position of cuticle (fig.1C). The analysed region was delimited in a dimensioned rectangle (300 x 200 pixels) located on cuticle for *CQ* (fig.1D) or just below cuticle for *CWQ*. Results (*CQ* and *CWQ*) were expressed in percent and calculated according to equation:

$$CQ \cdot or \cdot CWQ(\%) = \frac{\sum [black \cdot pixels]}{\sum [black \cdot pixels + white \cdot pixels]} \times 100$$

RESULTS

Cuticle Quantification and mass loss

Figure 2 shows a typical coloration of cuticle of each apple cultivar where cuticle of *Sm* appears clearly thicker than cuticle of *Ga* and *El*. This observation was confirmed by the quantitative approach. *CQ* was significantly higher for *Sm* than for the two other cultivars with 32%, 22% and 19% for *Sm*, *El* and *Ga*, respectively (figure 3). The difference between *El* and *Ga* cultivar was also significant but not important enough to be pointed out.

Masse loss (ML), measured at 120 days of storage in CR for each apple cultivar, was significantly less important for *Sm* (7,79%) than for *Ga* (9,47%) and *El* (11,40%).

Cell wall quantity and penetrometry

CWQ was significantly higher for *Ga* than for the two other cultivars whatever the duration of storage in CR (table 1). Differences measured between *El* and *Sm* were not significant. For *El* and *Ga*, *CWQ* showed a drastic decrease after 14 days of SL storage, which was not observed for *Sm*.

The initial value and the evolution during storage of the three rheological measurements extracted from the penetrometric tests ($F_{p:}$ rupture force needed to fail the epidermis), E*:stiffness and $F_{f:}$ flesh firmness) were compared for the three cultivars (figure 4). At 00 day, the initial values of F_p, E* and F_f were higher for *Ga* than for *Sm* and *El*. F_p didn't show any noticeable variation with durations and conditions of storage. E* and F_f showed a continuous decrease from time 0 until 28th or 14th day of storage for CR or SL condition, respectively (figure 4). Yet, differences between CR and SL condition were quite smaller for *Sm* than for the two other cultivars.

CWQ and each rheological measurement at 00 day allowed to classify the three cultivars in a same order: *El* < *Sm* < *Ga*. The relations between the mean of each rheological parameter at one day and the mean of cell wall quantity at the same day were independently studied for each cultivar and inside a common data set pooling the three cultivars (table 2). In all the cases, the four storage conditions were taken into acount: 00day, 14th day (CR and SL) and 28th day (CR).The three rheological measurements were correlated to cell wall quantity with R^2 values of 0.63, 0.60 and 0.58 for F_p (figure 5), E* and F_f, respectively. The same correlations calculated for each cultivar showed great differences between them. Thus, only *Ga* showed a correct correlation between F_p and *CWQ* ($R^2 = 0.77$). The correlations of E* and F_f to *CWQ* were particularly strong for *El* and correct for *Ga*. *Sm* didn't showed correct correlation.

CONCLUSION

This study showed that it was possible to classify the apple cultivars according the cuticle thickness and the cell wall quantity in epidermis region.

Apple cuticle is generally known to be a protection against water losses for plants. We noticed that a thicker cuticle and a less important mass loss were measured for *Sm* cultivar. Thus, cuticle thickness of *Sm* could partially explain the better water retention. Nevertheless, the difference of mass loss between *El* and *Ga* can not be related with cuticle thickness. Jenks *et al.* (1994) have shown that biochemical composition of cuticle could limit mass losses during storage. According our preliminary results we supposed that cuticle thickness could be an other relevant parameter to avoid or limit water losses in apples.

Besides, linear relations were shown between some rheological properties and cell wall quantity. These correlations were cultivar dependent: *Sm* didn't show any link between *CWQ* and penetrometric measurements. In order to complete the knowledge on relationships between inner structures and rheological properties of whole apple fruit, others properties must be studied in further works like structural characteristics and organization of parenchyma cells. Measurements of biochemical composition of cuticle and cell walls could be complementary to cell structure analysis.

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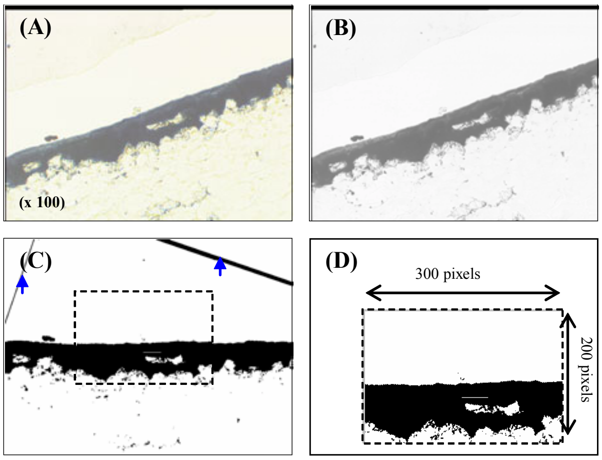

Figure 1. Image treatment for cuticle measurements. A: coloured image, B: grey level image, C: image rotation, D: analyzed region (rectangle of '300x200' pixels).

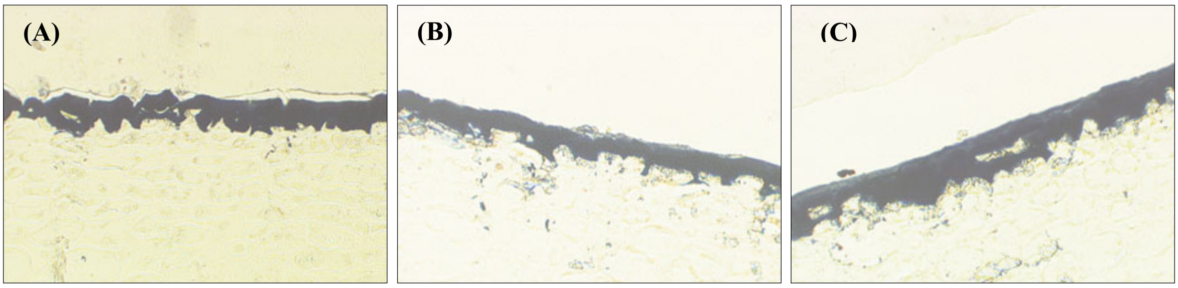

Figure 2. Coloration of apple cuticle. A: Elstar, B: Gala, C: Smoothee. (x100).

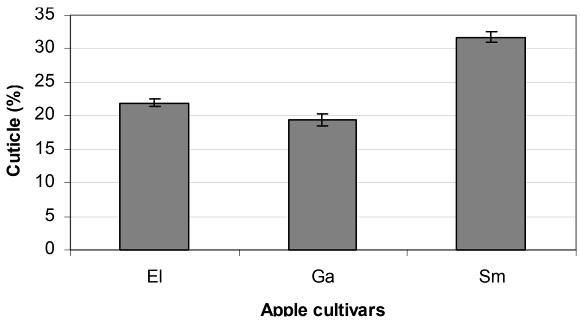

Figure 3. Cuticle quantity according to the cultivar *El*: Elstar, *Ga*: Gala, *Sm*: Smoothee.

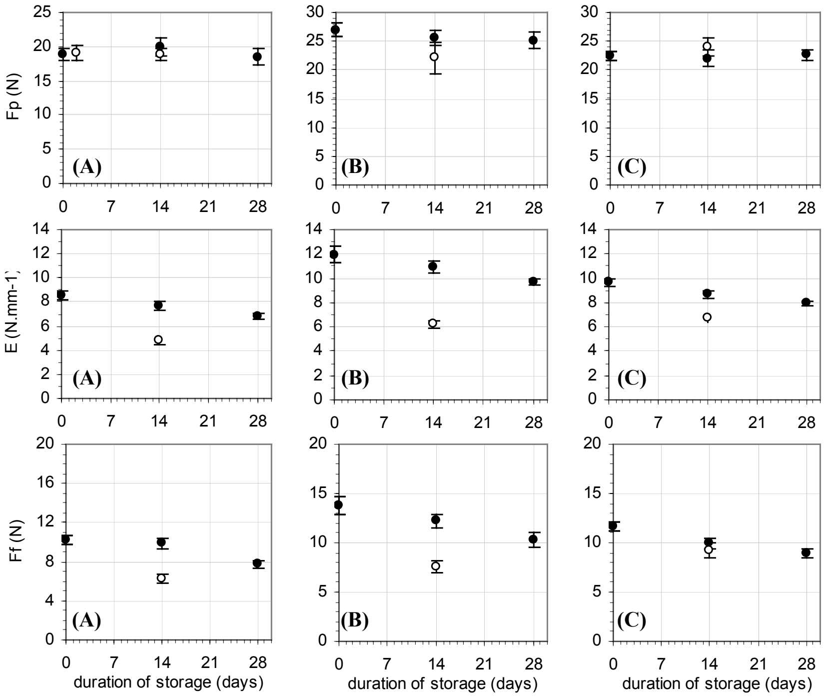

Figure 4. Measurement of F_p, E* and F_f parameters during storage. ●: CR condition, ○: SL condition. ***A***: Elstar, ***B***: *Gala*, ***C***: Smoothee.

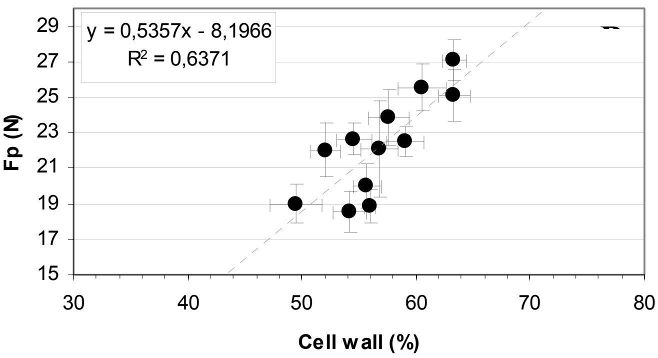

Figure 5. Relation between F_p and cell wall quantity. Each point represents the mean of values and the confidence intervals for one cultivar and one storage condition..

Table 1. Mean values and confidence intervals (threshold = 0.05) of cell wall quantity. Mean values are expressed in percent. *n* = number of analyzed images.

			Apple cultivar		
			Elstar	Gala	Smoothee
00 day	*Cooled room*	μ	55,96 ± 0,44	63,33 ± 1,05	58,99 ± 1,64
		n	34	48	46
14th day	*Cooled room*	μ	55,69 ± 1,23	60,51 ± 2,10	52,12 ± 1,32
		n	42	22	30
28th day	*Cooled room*	μ	54,22 ± 1,50	63,31 ± 1,38	54,53 ± 1,53
		n	35	36	32
14th day	*Shelflife*	μ	49,47 ± 2,28	56,80 ± 1,56	57,55 ± 1,77
		n	20	39	38

Table 2. Correlation coefficients between means of rheological measurements and means of cell wall quantity calculated for each of the four storage conditions.

Rheological measurements	Apple cultivars			
	Elstar	Gala	Smoothee	[*El* + *Ga* + *Sm*]
F_p	0.05	0.77	0.30	0.63
E*	0.94	0.69	0.00	0.60
F_f	0.87	0.56	0.24	0.58