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

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Keywords: Apple, cuticle, cell wall, stiffness.

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 \textit{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 \textit{et al}.., 1991; Schreiber and Riederer, 1996; Baur \textit{et al}., 1996; Blanke and Holthe, 1997) and mass losses (Morice and Shorland, 1973). Jenks \textit{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 peel 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, Fr (in N): strength or rupture force, E* (in N.mm⁻¹): stiffness or young modulus, and Ff (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:

\[
CQ \cdot \text{or} \cdot CWQ(\%) = \frac{\sum [\text{black \cdot pixels}]}{\sum [\text{black \cdot pixels} + \text{white \cdot pixels}]} \times 100
\]
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 account: 00day, 14th day (CR and SL) and 28th day (CR).The three rheological measurements were correlated to cell wall quantity with R² 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 (R² = 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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Literature Cited


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.

<table>
<thead>
<tr>
<th>Apple cultivar</th>
<th>00 day Cooled room</th>
<th>14th day Cooled room</th>
<th>28th day Cooled room</th>
<th>14th day Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu )</td>
<td>( \mu )</td>
<td>( \mu )</td>
<td>( \mu )</td>
</tr>
<tr>
<td></td>
<td>55.96 ± 0.44</td>
<td>63.33 ± 1.05</td>
<td>58.99 ± 1.64</td>
<td>55.69 ± 1.23</td>
</tr>
<tr>
<td>( n )</td>
<td>34</td>
<td>48</td>
<td>46</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>55.69 ± 1.23</td>
<td>60.51 ± 2.10</td>
<td>52.12 ± 1.32</td>
<td>52.12 ± 1.32</td>
</tr>
<tr>
<td>( n )</td>
<td>42</td>
<td>22</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>54.22 ± 1.50</td>
<td>63.31 ± 1.38</td>
<td>54.53 ± 1.53</td>
<td>54.53 ± 1.53</td>
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<tr>
<td>( n )</td>
<td>35</td>
<td>36</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>49.47 ± 2.28</td>
<td>56.80 ± 1.56</td>
<td>57.55 ± 1.77</td>
<td>57.55 ± 1.77</td>
</tr>
<tr>
<td>( n )</td>
<td>20</td>
<td>39</td>
<td>38</td>
<td>39</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Rheological measurements</th>
<th>Apple cultivars</th>
<th>[El + Ga + Sm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_p )</td>
<td>0.05</td>
<td>0.77</td>
</tr>
<tr>
<td>( E^* )</td>
<td>0.94</td>
<td>0.69</td>
</tr>
<tr>
<td>( F_f )</td>
<td>0.87</td>
<td>0.56</td>
</tr>
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</table>

Analyse de la quantité de la paroi de cellules et d'épaisseur de cuticule des fruits de pomme et relations avec certaines de leurs propriétés rhéologiques

Mots-clés : pomme, cuticule, paroi de cellules, rigidité

Résumé

L’épaisseur des cuticules de pomme et la quantité de cellules dans la région épidermique, exprimées en pourcentages de pixels, étaient mesurées sur trois cultivars : Gala (Ga), Elstar (El) et Smoothee (Sm). La zone de cuticules (CQ) est plus épaisse pour Sm à 32% ; El et Ga ont une CQ similaire à respectivement 22% et 19%. La CQ pourrait être liée à les pertes de masse durant le stockage. Sm montre la CQ la plus épaisse et une perte de masse moins importante comparée à El et Ga. La quantité de la paroi de cellules (CWQ) permet de classifier les trois cultivars comme suit : Ga > El > Sm avec respectivement 63%, 59% et 56% (pourcentage de pixels). La CWQ était mesurée également sur des fruits stockés pour 14 et 28 jours en chambre froide (CR) et 14 jours avant la vente (SL). Les relations entre la CQW et les propriétés rhéologiques des pommes, mesurée par une pénétramètre, étaient calculées. La CWQ était plus corrélé avec le résistance \( F_p \) pour Ga (\( R^2=0.77 \)), avec la rigidité \( E^* \) et fermeté de chair \( F_f \) pour El (\( R^2=0.94 \) and \( R^2=0.87 \) respectivement), mais avec aucun paramètre pour Sm.