Micromechanical Behaviour of Apple Tissue in Tensile and Compression Tests: Storage Conditions and Cultivar Effect

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Abstract.

The micromechanical behaviour of apple tissue was studied using a miniature tensile stage positioned underneath a microscope that allowed for simultaneous acquisition of force-displacement curves while the deformation of the individual cells was followed and recorded. Tensile and compression tests were performed on small samples of apple parenchyma of two different cultivars (Jonagored and Braeburn*)* and two storage conditions (*control* and *shelf-life*). Tests on the repeatability of the methods has provided satisfactory results and will allow the reduction of samples in further experiments. Under tensile loading, no differences for any of the mechanical parameters were found between cultivars, while a significant storage effect was observed for both cultivars. This opens the possibility of developing new sensors for quality assessment. Differences were found when studying the relationship of mechanical properties at the micro- and macro- level, which requires further investigation. The insights gained in this research will be useful when developing mathematical models based upon the mechanical behaviour of apple tissue.

1 Introduction

During harvest, transport, storage and packing, fruits are subjected to mechanical loading which may cause damage and loss in commercial value. The susceptibility to mechanical damage depends on the mechanical properties. Traditionally, in most of the techniques for the evaluation of fruit mechanical properties, it is assumed that the fruit behaves as a continuum material in which the mechanical properties essentially do not depend on the spatial scale. However, fruits consist of different tissues, which in their turn form a complex conglomerate of cells. The fleshy part of fruit is parenchyma tissue and is composed of three primary components: parenchyma cells, an adhesive middle lamella between adjacent cells and intercellular spaces; cell walls provide mechanical strength to the whole cell structure. Since macroscopic mechanical behaviour of fruits depends on several microscopic properties (cell size, internal turgor pressure, cell wall mechanical properties and thickness, etc) (Heredia et al., 1995; Konstankiewicz et al., 2001), a micromechanical approach is useful to understand the relative importance of these cellular and histological attributes on the overall mechanical behaviour of fruits and vegetables.

A significant amount of studies have been done on the micromechanics of potato tuber parenchyma tissue. Hiller and Jeronimidis (1996) assessed the fracture behaviour for two different varieties and predicted critical crack lengths (for different turgor states) by means of the compressive elasticity modulus and work of fracture. Konstankiewicz et al. (2001) concluded that structural parameters like cell area and cell perimeter exert a significant influence on mechanical parameters like strength and elasticity modulus. Hepworth and Bruce (2000) measured the deformation of individual cell within potato living tissue under uniaxial compression load. Cell deformation was successfully related

53to macroscopic tissue deformation, up to compressive strains of 20%. Onion epidermal 54tissue has been a subject of research as well. Mechanical properties and molecular 55dynamics in single cell walls were studied by Fourier-transform infrared spectroscopy 56under mechanically stressed conditions (Wilson et al., 2000). Recently, the variation in 57mechanical properties and structural parameters of onion epidermal peels originating 58from different layers has been investigated (Vanstreels et al., 2005).

59Little research has been carried out so far on the micromechanics of fruit tissue. Allende 60et al. (2004) studied the relationship between the histology of tomato skin and its rupture 61strength. Rojas et al (2002) proposed an empirical model to study the relative contribution 62of structural parameters to the rheology of kiwifruit by performing large deformation 63assays. Harker et al. (1997) investigated the cellular basis of textural diversity in different 64fruits (banana, avocado, melon) using tensile measurements of tissue strength and 65determined the mechanisms of tissue failure by low temperature scanning electron 66microscopy. Other studies on fruit focused on the influence of turgor on structural and 67mechanical properties using e.g. pear tissue (De Belie et al., 2000) or apple tissue (Oey et 68al., 2007).

69In the aforementioned experiments, only in very few cases the mechanical behaviour has 70been investigated simultaneously with visual observation of the actual deformations at the 71microstructural level. The objectives of our study were, therefore (i) to study the effect of 72external factors (e.g. acquisition zone) in the measurements, (ii) to compare the 73micromechanical behaviour of apple cultivars stored under different conditions, and (iii) 74to study the relationship of mechanical properties at the micro- (cellular) and macroscopic 75(tissular) level. For this purpose micromechanical experiments were carried out on two

different apple cultivars with known differences in structural parameters and differences in mechanical properties due to different storage conditions.

2 Materials and methods

2.1 Cultivars and sample preparation

The apple cultivars Jonagored and Braeburn were chosen since significant structural differences between them have been described (Schotsmans, 2003). Jonagored apples have less number of cells/mm^2, more intercellular space (%) and larger cells than Braeburn apples. Fruits were harvested in October 2004 at the experimental station PCFruit-PPS in Velm (Belgium). Apples were selected on the basis of uniformity, absence of damage or blemishes and size (average diameter between 85-90 mm for Jonagored, and 60-65 mm for Braeburn). For both cultivars, homogeneous batches of similar acoustic firmness index were selected. The acoustic firmness was measured using a commercial acoustic firmness tester (AFS, Aweta, Nootdorp, The Netherlands). The stiffness index ($S = f^2 m^{2/3}$) was calculated based on the resonant frequency (f) of the first peak frequency and the mass (m) of the fruit (Abbot et al., 1992), and was equal to 30.3 and 32.1 Hz^{2}kg$^{2/3}$ for Jonagored and Braeburn, respectively. After one month at 0.8ºC and 1ºC for Jonagored and Braeburn, respectively, and 65% RH for both cultivars, half of the apples were taken directly from the cool room for the experiment performance ("*control*"); the other half were stored for an additional period of 12 days under simulated shelf-life conditions (21ºC and 65% RH; "*shelf-life*") and had after storage an average stiffness index of 23.3 and 22.1 Hz^{2}kg$^{2/3}$ for Jonagored and Braeburn, respectively. The experiment thus consisted of four objects (2 cultivars × 2 storage conditions).

The Magness-Taylor firmness of apples before and after simulated shelf-life exposure was measured using a universal testing machine (LRX, Lloyd Instruments Ltd.,

100Hampshire, UK) equipped with a cylindrical plunger of 11 mm diameter. The probe was
101pressed into the fruit flesh at a penetration speed of 8 mm/s over a distance of 8 mm. The
102maximal force was used as firmness estimation and was equal to 75 N (*control*) and 62 N
103(*shelf-life*) for Jonagored and 85 N (*control*) and 64 N (*shelf-life*) for Braeburn.
104Apple flesh is mechanically very anisotropic. The cells in the inner part of the cortex are
105more or less oriented in radial columns of cylindrical cells stuck end to end (Khan and
106Vincent, 1990; Abbott and Lu, 1996), so we restricted our experiments to tangentially cut
107rectangular beam specimens where the load was applied parallel to cell columns
108distribution (Figure 1). Both a slicer and parallel razor blades to ensure constant
109dimensions and smooth surfaces were used for sample preparation.
110For every apple, six specimens for tensile tests and six for compression tests were taken
111from two subsequent slices of apple tissue: three samples were acquired from the first
112slice and second slice, respectively. Since strain rate has been proved to have an influence
113on dynamic failure properties (Bajema et al., 2000), a constant strain rate of 0.05 min^{-1} for
114both tensile and compression tests was selected for our study. The dimensions of the
115apple specimens were 11 mm × 5 mm × 2 mm for tensile tests and 3 mm × 11 mm × 5
116mm samples for compression tests. The deformation rates were 0.5 mm/min and 0.2 mm/
117min for tensile and compression tests, respectively, and were selected to keep a constant
118strain rate during the mechanical tests.

119*2.2 Determination of Isotonic Point*

120Since turgor pressure of cells in tissue affects their mechanical properties (Lin and Pitt,
1211986; De Belie, et al., 2000; Konstankiewicz and Zdunek, 2001), all the samples from
122every batch were equalised by soaking them overnight in its isotonic mannitol solution at

1234ºC. Thus, the possible differences in turgor among apples from the same batch were 124avoided.

125The average isotonic point of every object was determined by measuring relative volume 126and weight changes of cylindrical samples of apple tissue (12mm diameter by 5 mm 127height) after overnight soaking in one of several concentrations of mannitol (from 0 to 1280.8M). For every mannitol concentration, 10 apple cylinders coming from 10 different 129apples were used. In order to minimize cellular degradation of tissue strength during the 130experiment due to pH variation, and following the method described by Lin and Pitt, 1311986, the mannitol solutions were buffered with K_2HPO_4 (0.02 M) and KH_2PO_4 (0.02 M). 132Nine tissue cylinders were cut out the cortex of the green side of each apple and placed 133into each osmotic solution. Parenchyma samples represented approximately 1% of 134solution volume, ensuring a constant water activity value during soaking. Before and after 135the soaking, both weight and dimensions (diameter and height) of the samples were 136determined with an analytical balance (Sartorious, CP124S, Germany) and a digital 137calliper (Absolute Digimatic, Mitutoyo, UK, Ltd.), respectively. The apple-tissue 138cylinders were carefully blotted with tissue paper to remove excess of water before the 139measurements. Eventually, the isotonic point was calculated by interpolation to zero 140weight or volume change from the curve indicating the weight gain or loss of tissue as a 141function of the mannitol concentration.

142*2.3 Micromechanical Tests*

143The mechanical testing was carried out on a miniature tensile stage (Deben Microtest, 144Suffolk, U.K.) which, due to its reduced dimensions (12 cm × 8 cm), could be mounted 145underneath a stereomicroscope (SMZ1000, Nikon, Japan) equipped with a CCD camera 146(JVC, mod. TK-1360, colour ½¨ CCD). The whole setup allowed the acquisition of force-

displacement curves while the deformation of the individual cells was followed and recorded.

The experiments consisted of performing tensile and compression tests on tangential cut rectangular specimens of apple parenchyma as described above. In total 8 *control* and 8 *shelf-life* apples were analysed. For each apple 6 specimens for tensile and 6 specimens for compression tests were analysed. The day before the test was performed, the apples were removed from the cool room and cut, avoiding the outmost 5 mm of the fruit where the flesh is very heterogeneous. Cell turgor pressure was equalized by soaking the samples overnight in an isotonic mannitol solution which was previously determined per each apple set.

Additionally, a staining step was necessary to visualize and quantify cell deformations that occur during mechanical testing. Preliminary experiments (data not shown) indicated that a staining procedure for a small period of time (≤ 4 min.) did not affect either the mechanical nor structural properties of apple samples. The staining time was selected for being the shortest at which good images could be recorded. For both mechanical tests every sample was gently stained with methylene blue (7.5 mg / 100 mL mannitol isotonic solution) for 3 min. While for compression tests no specific adaptations were needed, for tensile tests the lateral edges of the apple beams had to be glued with cyanoacrylate adhesive to the two moving grips of the miniature tensile stage (Figure 2). After the glue was hardened and during the mechanical test humid air was blown over the samples to keep them from drying. Once the slope of the curve was stabilized, two loading and unloading cycles upon return to zero stress were carried out. Finally, samples were further elongated or compressed until rupture. Strain-stress curves were saved for further calculations.

2.4 Measurement of cell morphological parameters by image analysis

Cell morphological parameters were obtained from both the first image and the image where 80% of the maximal stress was reached in order to calculate cell strain (%) (Figure 3). The studied parameters were: perimeter, area (as cell projected area), aspect (Feret width-length ratio), roundness $\left(\text{perimeter}^2 / 4 \times \pi \times \text{area}\right)$, length and width. Length was estimated in two different ways: (i) as maximum Feret diameter (L_f) when studying morphological differences between cultivars and treatments; (ii) from the bounding box fitted for every cell(Length_box), where the length of the box pointed in the same direction as the mechanical load, when studying cell deformations under compression and tensile loading. Width was estimated as minimum Feret diameter (d_f). The average value of all cells per sample was considered. A list of symbols and abbreviations is given in Table 1.

The original images were digitized by means of a Matlab program (MATLAB version. 6.5, The MathWorks, Inc., Natick, Ma, USA) before determining cell structural parameters using the Image-Pro Plus 4.5 (Media Cybernetics, Silver Spring, USA) image analysis software. The same methodology was used in a previous study (Vanstreels, et al., 2005).

2.5 Data Analysis

Force (N) and deformation (mm) data were converted to stress (MPa) and strain (%) data. The strain was calculated as percentage of deformation after test performance. The stress values were obtained as force per unit of surface.

From the stress-strain curves a number of micromechanical parameters such as elasticity modulus in different parts of the stress-strain curve, as described below, stress at failure (ε_{max}) and strain at maximum stress (σ_{max}) were determined. The strain values when the

380% of the stress was reached ($\sigma_{80\%}$) and the 80% of the maximum stress ($\varepsilon_{80\%}$) were also calculated.

A linear mixed model was used to statistically analyze the data:

$$Y_{ijkmt} = \mu + C_i + T_j + CT_{ij} + A_{k(ij)} + S_{m(kij)} + R_{t(mkij)}$$

where Y_{ijkmt} is a mechanical property for the i^{th} cultivar (C), j^{th} treatment (T), k^{th} apple indicator (A) within 'cultivar' × 'treatment' combination, m^{th} apple slice (S) within apple and R is the random residual effect. 'Cultivar' and 'treatment' were considered as crossed fixed factors and 'apple' and 'slice' as random effects. Shapiro-Wilks tests applied over the residues of every variable (SAS, version 7, SAS Institute Inc., Cary, NC, USA) confirmed that the variables were normally distributed.

3 Results

3.1 Isotonic point and sampling consistency

The isotonic point of *control* Jonagored apples was -1.35 MPa while for *control* Braeburn apples it was -1.23 MPa. After simulated shelf-life storage there was an increase in soluble solids for both cultivars and the isotonic point was -1.56 and -1.48 MPa, respectively. The values of isotonic conditions for *control* Jonagored apples are similar to values reported by Quiong et al. (1989) for Empire apples.

No significant differences ($p<0.01$) among replicates and slices were found for micromechanical or structural parameters for any batch.

3.2 Micromechanical tests

Typical stress-strain curves of apple tissue subjected to tensile and compression tests are shown in Figure 4 and mean values of mechanical parameters are shown in Table 2 and Table 3. Additionally, a summary of the most relevant results is given in Table 4.

Compression Tests

Compression stress-strain curves for apple samples were sigmoid (Figure 5a, b). The elasticity modulus was denoted by E_{C1} for the first part of the curve and by E_{C2} for higher strains, corresponding to 80% stress, approximately (Figure 4). The cyclic part of all tests revealed that, after unloading to zero stress some plastic deformation remained and this plastic deformation was more pronounced ($p \leq 0.05$) for *shelf-life* apples (6.84 %) than for *control* apples (4.63 %).

The average value of both elasticity moduli was different; E_{C1} was about five times higher than E_{C2}. Moreover, as expected, for E_{C1} and E_{C2} a significant effect of storage conditions ($p \leq 0.001$) was found: *control* apples were stiffer than *shelf-life* apples. However, no significant differences between cultivars were observed.

For maximum strain, statistically significant differences were found between cultivars; Jonagored specimens were more compressible (ε_{max} = 28 %) than Braeburn samples (24%), while no storage effect could be observed.

The maximum stress of *control* Braeburn (σ_{max} =0.56 MPa) apples was larger than that of *control* Jonagored samples (σ_{max} =0.36 MPa) for α = 0.05 (data not shown). After shelf life storage, the differences were not statistically significant (0.34 vs. 0.15 MPa). When considering the whole apple, however, *control* Braeburn apples were harder (MT firmness of 85) than *control* Jonagored apples (MT firmness of 75).

Tensile Tests

An almost linear relationship was found between stress and strain values for *control* samples while S-shaped curves were found for *shelf-life* (Figure 5c,d).

As in the case of compression loading, the plastic deformation of *shelf-life* specimens subjected to tensile tests was more pronounced ($p \leq 0.05$) than for *control* samples (2.73 % vs. 1.47 %).

For most *control* samples, an abrupt decrease in stress when the tissue fails was observed while for *shelf-life* samples the peak reached at maximum stress became broader. Also, for some Braeburn *shelf-life* samples a certain tissue extension before the specimen was completely broken was observed ('*Shelf-life*_2' in Figure 5c).

When comparing cultivars, no significant differences were found for average values of strain at maximum stress; however, *control* Jonagored specimens were more extensible than *control* Braeburn samples (11.36 vs. 7.23 %); after shelf life storage, these differences disappeared.

When micromechanical parameters were calculated from stress-train curves we found statistically significant differences ($p \leq 0.001$) between storage conditions for E_T (elasticity modulus for tensile test); *control* apples had considerably higher E_T (5.02 MPa) than *shelf-life* apples (2.10 MPa) (Figure 5). A similar decrease of the elasticity modulus for lower turgor pressures was also detected in Granny Smith (Tu et al., 1996) and Jonagored apples (Oey et al., 2007).

Stiffness of Braeburn apples was more affected by shelf life storage conditions during the interval studied than stiffness of Jonagored apples, corresponding to a more pronounced decrease of the elasticity modulus for the former. Similar to $\boldsymbol{\varepsilon}_{max}$, no differences between cultivars were found in terms of stiffness after shelf-life storage. It seems that since the middle lamella is mainly affected during ripening processes, the original mechanical differences found for *control* apple tissue subjected to tensile tests, disappeared after 12 days storage at 20ºC.

3.3 Cell morphological parameters

The average cell projected area of Braeburn samples taken from 'zone A' was larger ($32.6 \cdot 10^3$ μm^2) than the average cell projected area of Jonagored specimen ($29.1 \cdot 10^3$ μm^2) (Table 5). For L_f, d_f and perimeter the same trend was found. However, no significant differences were found between cultivars for samples taken from 'zone B.'

According to cell shape related parameters (i.e., aspect and roundness), no statistically significant differences ($\alpha = 0.05$) between cultivars were found. Therefore, in Table 6 the values were pooled over cultivar. In this same table, an effect of sampling zone is observed between *control* and *shelf-life* specimens but no differences were found between storage conditions when samples were taken from zone B. The cells from samples extracted from zone A that were stored under shelf life conditions were more round (1.12 and 1.30 for roundness and aspect, respectively) than cells from *control* apples (1.16 and 1.38).

Apples subjected to tensile tests had lower cell deformations (about 6%) than those under compression loading (about 10%) (Table 7).

4 Discussion

4.1 Sampling consistency

Since there were no significant differences among replicates and slices for micromechanical or structural parameters, we can assume that sample acquisition and manipulation were carried out in a repeatable way and had no influence on the observations. Therefore, in future experiments the number of samples taken from the same apple can be reduced such that more apples can be included in the study to better assess biological variation.

4.2 Micromechanical tests

Compression tests

The sigmoid shape found for specimen subjected to compression loading may be explained by the microstructure of the tissue. For small strains the elasticity modulus was relatively small, this may be due to cell reorganization and compression of the intercellular space in response to compression loading. From the recorded images it was observed that while initially cells suffered almost no deformation, from a certain point cell deformation became more and more clearly. Moreover, since the boundary cells of the apple specimen were cut during sample extraction, some fluid would be expelled at the beginning of the test. This was also observed by Drazeta et al. (2004). For larger strains, the elasticity modulus increased, probably reflecting cell pressurization.

It is known that tissue failure under compression loading generally occurs due to cell wall rupture because of extreme stresses during test performance (De Belie et al., 2000; Diehl et al., 1980) and that after shelf life storage, degradation processes of the cell wall start. The major changes are related to pectins which are present in both the amorphous matrix where the cellulose microfibrils of the primary cell wall are embedded, and the middle lamella (Kunzek et al., 1999). For samples subjected to compression tests, the former factor will have a foremost influence on tissue failure. Therefore, since tissue rupture mainly depends on the resistance that the cell wall exerts to compression loading, it seems logical that *control* specimen had higher values of maximum stress than *shelf-life* apples. This corresponded with the mechanical parameters obtained from the stress-strain curves. Higher strain values for Jonagored apples might be explained on the basis of the structural characteristics of apple tissue. Since Jonagored apples have more intercellular spaces than Braeburn apples, the former would be more susceptible to compression than Braeburn.

For *shelf-life* apples, ripening and senescence processes which include water loss, degradation of pectin substances, etc. might reduce the differences in the mechanical properties between cultivars that have been found between *control* apples.

Tensile tests

Stress-strain curves obtained from apple tissue subjected to tensile loading were compared with those obtained from other vegetative tissues like *Aristolochia brasiliensis* (Köhler, 2000) or onion epidermis (Wilson et al., 2000; Vanstreels et al., 2004). The clear and typical biphasic behaviour observed in the latter was not observed in this research. Such behaviour was explained by these authors in terms of cell wall composition where fibrils of cellulose are embedded in flexible pectin. Two processes were assumed to be involved: reorientation of microfibrils and slip of microfibrils past each other due to yielding of the pectin matrix. In apple parenchyma, the intercellular spaces represent an important part of the total volume. Also, apple parenchyma tissue consists of many cell layers in which different cells undergo different stresses, which would lead to different shape curves.

As we found in apples, smooth peaks at failure in force-displacement curves were also observed for fruits such as muskmelon and banana (Harker et al., 1997b), and pears (De Belie et al.,2000). Pectin, which acts as a cementing substance in the middle lamella, starts its solubilisation reducing the adhesion between cells during ripening. Tissue failure for *shelf-life* apples would start where the middle lamella was weaker and from that point on the apple parenchyma specimen would finally break as a sequential failure of cells groups.

Unlike compression tests, tissue failure under tensile loading involves cell wall tearing and/or cell-to-cell debounding. These different ways of rupture would be related to fruit

aging. Thus, cells of *control* apples would break due to tearing of the cell wall while failure of ripened apple tissue would occur due to a breakdown of the inter-lamellar region (Harker et al., 1997a; Tu et al., 2000). Therefore, since middle lamella is considered a weaker material, less applied force is needed to break *shelf-life* samples than *control* samples under tensile loading.

Cell morphological parameters

Contrary to Schotsmans (2003) we found that Braeburn cells were larger than Jonagored cells, what may be attributed to different maturity levels of both apple cultivars in this experiment. On the other hand, the values of the aspect ratio of cells of *control* apples were in agreement with values found for Braeburn apples by Drazeta et al. (2004) and for other cultivars (Khan and Vincent, 1990).

From the morphological results, it appears that both sampling zones are different. In principle, cells should be similar for specimen extracted from both zones, however, the fact that the samples were not exactly taken from the very same part of the apple might explained these differences. Despite the sample acquisition procedure was designed such that similar specimens could be obtained (equatorial and tangentially orientated beams) for tensile and compression test performance, it seems that beams extracted from zone B were closer to the bottom of the fruit, resulting in smaller cells.

Related to cell deformations, apple specimens (tissue level) were deformed positively or negatively as they were elongated or compressed. Through the recorded images it was observed that most of the cells subjected to compression loading undergo negative deformations and cells subjected to tensile tests undergo positive deformations. Some cells, however, underwent positive deformations under compression loading and negative

deformations when the tissue was subjected to tensile tests. This is probably caused by the lack of information about the deformations in the third dimension (cell depth).

Relationship between mechanical properties at micro- (cellular) and macro-(tissular) level

Differences were found when studying the relationship of mechanical properties at the micro- and macroscopic level. Hence, although *control* Braeburn apples were harder (had higher MT firmness index) than *control* Jonagored apples, they showed lower σ_{max} than Jonagored. This should be further investigated and interpreted in terms of the structural parameters of apple parenchyma.

On the other hand, samples subjected to compression tests reached significant higher values of ε_80% than samples under tensile loading (21.47 % vs.7.52 %) (Table 3). However, no significant differences of cell deformation values between the two tests were found at the cellular level.

It has to be considered that, when studying deformations at tissue level, deformation of air spaces represents an important portion of total volume. Moreover, due to the experimental design of the compression tests, the specimen was not completely confined (the upper and lower surfaces were not) and at the beginning of the tests there were a certain displacement of the grips; they may have slightly distort the measurements. These facts might explain the high strain values obtained for compression tests when comparing to tensile tests and also that the differences found at tissular level were not found at cellular level.

5 Conclusions

The experiments have shown that sample acquisition and manipulation was conducted consistently and had no influence on the measurements. This can reduce the amount of

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samples taken from each apple and will allow focusing on the study of biological variation in further experiments.

Under tensile loading, we found no differences between the two cultivars employed. However, they had been selected because they had significant structural differences according to number of cells/mm^2, intercellular space (%) and cell size. Nevertheless, a significant effect of storage conditions was found through this micromechanical approach. This fact may lead to the development of sensors capable of to infer the senesce stage of apples, which can be a useful means to assess apple quality.

When comparing tests at different scales different trends have been found. For instance, the apparently harder apples (with higher MT index, which is a macro test) have shown lower σ_{max} during compression tests. Moreover, when comparing cellular deformations (microscale) against observed strain values of tissues (macroscale), major differences between tensile and compression tests were found in the former than in the later. This may have been caused by the influence of the distribution and quantity of intercellular spaces and also because of the lack of 3-dimensinal information. These facts highlight the importance of the scale in the results and open the way for further research on the comprehension of textural properties of apple fruit.

Eventually, the insights gained from this research will prove valuable when developing mathematical models based upon the histological properties of apple tissues to predict strain and failure of fruit tissue as a consequence of static loading and impact.

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476**Tables**

477Table 1. List of abbreviations and symbols

478

Symbol or abbreviation	Description
Mechanical parameters	
E_{C1}	elasticity modulus of samples under compression tests obtained from the first part of stress-strain curve
E_{C2}	elasticity modulus of samples under compression tests corresponding to 80% stress
E_T	elasticity modulus of samples under tensile tests
ε_{max}	strain at maximum stress
σ_{max}	stress at failure
Morphological parameters	
Length_box	length of the imaginary surrounding box of a cell whose major axis is in the same direction as the applied mechanical load. This parameter is used for the cell deformation study.
L_f	maximum Feret diameter
d_f	minimum Feret diameter

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483Table 2. Mean values ± standard error of micromechanical parameters for apple tissue subjected to compression loading. For cultivar, the
484values are pooled over storage treatment while when comparing storage effect the data are pooled over cultivar. Values of p smaller than
4850.05 indicate a significant difference at the 95% confidence level.

486

	Cultivar			Storage conditions		
	Braeburn	Jonagored	p-value	*Control*	*Shelf-life*	p-value
σ_{max} (Mpa)	0.25 ± 0.04	0.40 ± 0.03	0.0121	0.47± 0.04	0.21± 0.03	0.0018
ε_{max} (%)	23.76 ± 1.32	28.26 ± 1.23	0.0299	28.13± 1.32	24.49± 1.23	0.2242
E_{C1} (MPa)	0.35 ± 0.04	0.42 ± 0.04	0.2556	0.54± 0.04	0.26± 0.04	0.0013
E_{C2} (MPa)	1.72 ± 0.14	2.01 ± 0.13	0.1019	2.35± 0.14	1.48± 0.13	0.0027
Plastic def. (%)	6.57 ± 0.38	4.90 ± 0.40	0.2214	4.63± 0.38	6.84± 0.41	0.0082

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489Table 3. Mean values ± standard error of micromechanical parameters for apple tissue under tensile loading. For cultivar and storage
490conditions data are pooled over storage conditions and cultivar, respectively. P-values are also given.

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	Cultivar			Storage conditions		
	Braeburn	Jonagored	p-value	*Control*	*Shelf-life*	p-value
σ_{max} (Mpa)	0.22 ± 0.02	0.24 ± 0.02	0.1909	0.34 ± 0.02	0.11 ± 0.02	< 0.0001
ε_{max} (%)	7.83 ± 0.72	10.21 ± 0.66	0.0782	9.22 ± 0.72	8.93 ± 0.66	0.7398
E_T (MPa)	3.91 ± 0.17	3.19 ± 0.16	0.1491	5.02 ± 0.17	2.10 ± 0.16	<0.0001
Plastic def. (%)	1.69 ± 0.12	2.52 ± 0.11	0.0595	1.47 ± 0.12	2.73 ± 0.11	0.0164

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Table 4. This table summaries the results obtained under compression and tensile tests for the main mechanical parameters under study. The > symbol is used to indicate that a certain parameter (on the left column) reaches a higher value for the experimental factor shown in that column.

Parameters	**Test type**	**Between cultivars**	**Between storage conditions**
Differences found for compression tests			
Curve form	C	No difference (Sigmoid)	No difference (Sigmoid)
σ_{max}	C	Jonagored > Braeburn	Control > Shelf-life
ε_{max}	C	Jonagored > Braeburn	No difference
E_2	C	No difference	Control > Shelf-life
Plastic deformation	C	No difference	Shelf-life > Control
MT	C	Braeburn > Jonagored	N.D.*
Differences found for tensile tests			
Curve form	T	No difference	Linear for control Sigmoid for shelf-life
σ_{max}	T	No difference	Control > Shelf-life
ε_{max}	T	No difference	No difference
E_2	T	No difference	Control > Shelf-life
Plastic deformation	T	No difference	Shelf-life > Control

*not determined

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501Table 5. Mean values ± standard error of initial cell size related parameters for apple tissue. For cultivar the values are pooled over storage
502treatment while when comparing storage effect the data are pooled over cultivar. Values of p smaller than 0.05 indicate a significant
503difference at the 95% confidence level.

		Cultivar			Storage conditions		
	Zone	Braeburn	Jonagored	p-value	*Control*	*Shelf-life*	p-value
Area (μm^2)	A	32597 ± 536	29095 ± 497	0.0190	31771 ± 536	29788 ± 497	0.1207
	B	24730 ± 568	23920 ± 526	0.3431	21896 ± 656	26471 ± 622	0.0035
L_f (μm)	A	237.52 ± 1.75	223.90 ± 1.59	0.0153	239.00 ± 1.75	222.62 ± 1.59	0.0042
	B	206.53 ± 1.66	199.90 ± 1.53	0.2612	193.00 ± 1.91	212.01 ± 1.81	0.0119
d_f (μm)	A	180.0 ± 1.18	171.86 ± 1.09	0.0402	177.21 ± 1.18	174.29 ± 1.09	0.3886
	B	154.75 ± 1.50	156.09 ± 1.51	0.9938	148.43 ± 1.88	161.78 ± 1.78	0.0057
Perimeter (μm)	A	663.12 ± 5.57	628.14 ± 5.16	0.0252	661.27 ± 5.57	629.73 ± 5.16	0.0298
	B	573.60 ± 5.99	563.04 ± 5.55	0.3600	540.91 ± 6.92	592.46 ± 6.57	0.0068

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506Table 6. Mean values ± standard error of initial cell shape related parameters of *control* 507and *shelf-life* apple tissue. Values were pooled over cultivar. p-values <0.05 indicate a 508significant difference at the 95% confidence level.

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		Storage conditions		
	Zone*	*Control*	*Shelf-life*	p-value
Aspect	A	1.38 ± 0.02	1.30 ± 0.02	0.0005
	B	1.32 ± 0.01	1.33 ± 0.01	0.6845
Roundness	A	1.16 ± 0.01	1.12 ± 0.01	0.0003
	B	1.14 ± 0.01	1.11 ± 0.01	0.0890

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512Table 7. Mean values of cell deformation (%) (judging from length_box deformations) 513and tissue deformation (judging from $\varepsilon_{80\%}$), for apple parenchyma under tensile and 514compression loading. ± Standard deviation is given next to each value.

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Test	Compression	Tensile
Macroscale	21.47 ± 3.55	7.57 ± 1.65
Microscale	9.63 ± 5.58	6.46 ± 1.57

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Figure captions

Figure 1. **a)** Diagram showing how tangential beam specimens were extracted from an apple. The grey bars represent the slices where the samples were extracted from. Specimens for compression test were obtained from 'Zone A' while specimens for tensile tests were obtained from 'Zone B'. Dimensions of the specimens subjected to compression (**b**) and tensile (**c**) tests are also shown. The arrows represent the direction of the applied load. In **b** and **c**, the mark over the beams indicates the place from where cell deformations were observed and recorded.

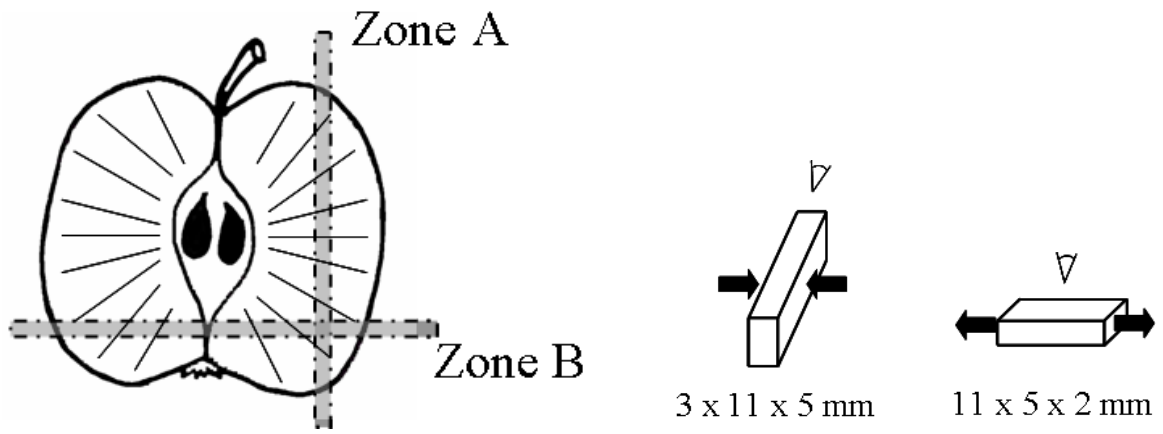
Figure 2. Miniature tensile stage (left) with the grips used for compression tests. Close up of how the apple specimens for tensile tests were glued to the grips of the bench (right).

Figure 3. Original images of Jonagored apple tissue as seen through the stereomicroscope. (Left) Image obtained at the beginning of the compression test. (Right) Image obtained at 80% stress of the compression test. The same cells are denoted with **a**, **b** and **c** so differences in their dimensions can be appreciated.

Figure 4. Representative stress-strain curves of *control* apple tissue subjected to compression (a) and tension (b) loading. The bold lines on the curves represent the region from where the elasticity modulus and the associated images were extracted. $E_{C1:}$ elasticity modulus of samples under compression loading obtained at the beginning of the test; $E_{C2:}$ elasticity modulus of samples under compression tests, corresponding to 80% stress; E_T: Elasticity modulus of samples under tensile tests; σ_{max} : maximum stress; ε_{max}: strain at maximum stress.

Figure 5. Typical stress-strain curves for Braeburn and Jonagored apple tissue under different storage conditions (*control* and *shelf-life*) subjected to tensile and compression tests.

542 Figures



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544**Figure 1. a) Diagram showing how tangential beam specimens were extracted from**
545**an apple. The grey bars represent the slices where the samples were extracted from.**
546**Specimens for compression test were obtained from 'Zone A' while specimens for**
547**tensile tests were obtained from 'Zone B'. Dimensions of the specimens subjected to**
548**compression (b) and tensile (c) tests are also shown. The arrows represent the**
549**direction of the applied load. In b and c, the mark over the beams indicates the place**
550**from where cell deformations were observed and recorded.**

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553Figure 2. Miniature tensile stage (left) with the grips used for compression tests. Close up

554of how the apple specimens for tensile tests were glued to the grips of the bench (right).

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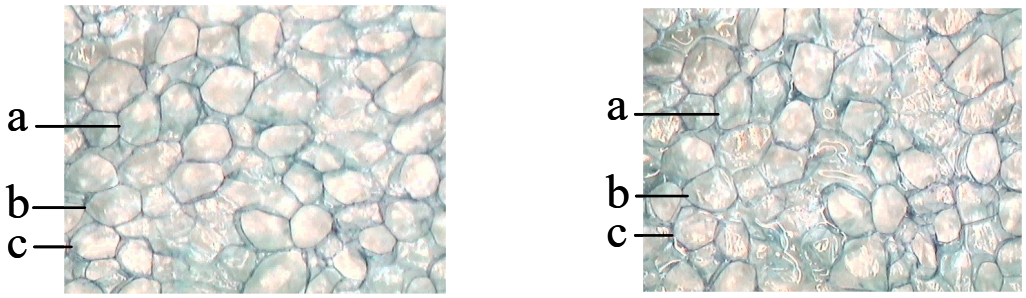

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558Figure 3. Original images of Jonagored apple tissue as seen through the stereomicroscope.
559(Left) Image obtained at the beginning of the compression test. (Right) Image obtained at
56080% stress of the compression test. The same cells are denoted with a, b and c so
561differences in their dimensions can be appreciated.

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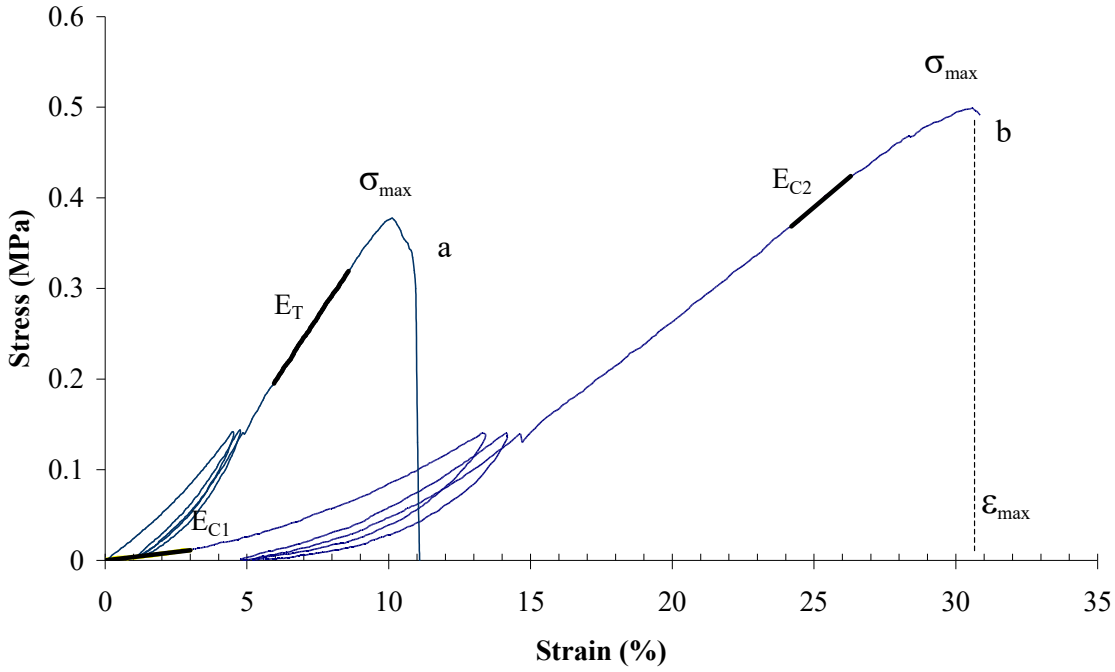

563

564**Figure 4. Representative stress-strain curves of control apple tissue subjected to**
565**compression (a) and tension (b) loading. The bold lines on the curves represent the**
566**region from where the elasticity modulus and the associated images were extracted.**
567**EC1: elasticity modulus of samples under compression loading obtained at the**
568**beginning of the test; EC2: elasticity modulus of samples under compression tests,**
569**corresponding to 80% stress; ET: Elasticity modulus of samples under tensile tests;**
570**? max : maximum stress; ⌐ max: strain at maximum stress.**

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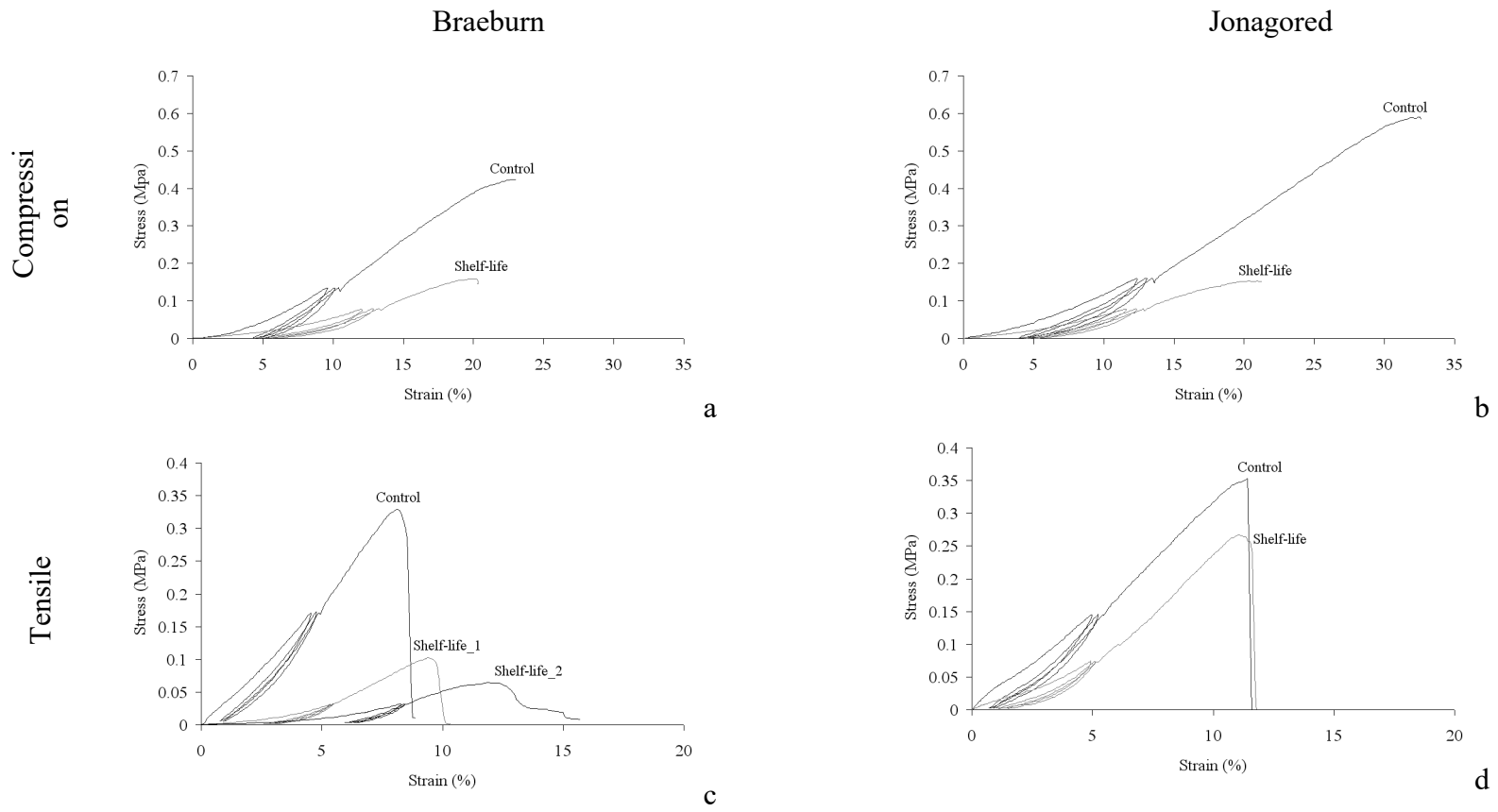

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575Figure 5. Typical stress-strain curves for Braeburn and Jonagored apple tissue under different storage conditions (control and shelf-life)

576subjected to tensile and compression tests.

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