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ORIGINAL PAPER

The effect of Ca^{2+} and cellular structure on apple firmness and acoustic emission

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Abstract This study presents the influence of calcium lactate treatment (0–6 %) and cellular composition on two mechanical attributes: firmness and total acoustic emission (AE) events registered in the puncture test of apple tissue. The experiment was performed on five apple cultivars stored for nine months in a normal or controlled atmosphere. The microstructure was characterized quantitatively on control samples by confocal scanning laser microscope followed by image analysis. The mean area and perimeter of detected objects, which were either cells or spaces, and estimated cell wall fraction were used for the characterization of the tissue microstructure. Treatment with increased Ca^{2+} concentration caused a significant increase in firmness and total AE events. The increase was more pronounced in the case of total AE events which better reflected an increase in brittleness of apple tissue than firmness parameter. The effect was particularly positive in the case of very soft apples (stored in a normal atmosphere) due to greater extent of de-esterification of homogalacturonan. Analysis of the results, together with a review of the literature, suggests that calcium importantly changes the cracking mode of tissue, from intercellular debonding to cell wall rupturing. Firmness and the total AE events significantly relate negatively to the object's size and positively to estimated cell wall fraction. A rough accordance of the number of split cells open across cell walls by puncture probe and the total AE events registered in the test, which was saturated in high Ca^{2+} concentrations, was noticed.

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Introduction

Firmness, measured in a very simple puncture test, is popular parameter used for fruit classification and is often compared with sensory attributes as an instrumental-method of texture evaluation [1, 2]. Acoustic emission (AE) is the phenomena of sound generation during rapid mechanical breakdown of a material and, as a method of material characterization, is also currently investigated for instrumental texture evaluation of parenchymatous tissue [3–7].

The mechanical properties of parenchyma tissue are governed mainly by turgor, cell size, shape and packing, cell wall thickness and strength, and the extent of cell-to-cell adhesion [8]. Higher turgor causes increased tissue rigidity and increased brittleness of tissue [9]. Tissue, if is composed of smaller cells, has a larger content of cell walls, a lower relative amount of cytoplasm and vacuole, a greater area of cell-to-cell contact, a lower amount of intercellular spaces [8], and such tissue reveals higher rigidity and strength for compression [10]. One of the roles of cell walls is to create, together with middle lamella, a mechanical skeleton of tissue. The mechanical properties of cell walls depend on polysaccharide composition [11], assemblage and their interlinking [12]. Cell-to-cell adhesion plays a very significant role in the biomechanics of soft plant tissues [13], and although is often interrelated with other structural properties of tissue, it is considered also as important factor for the firmness and sensory texture of fruit [8]. Crispy fruit tends to rupture through cells splitting open across the primary cell walls [2]. However,

cell separation may be a typical failure mode for soft fruit when ripe [14].

In the commonly accepted model of primary cell walls, hemicelluloses adhere to cellulose, cross-linking them to form a three-dimensional framework which is imbedded in a matrix of pectic polysaccharides [15]. Large changes occur in both pectins and matrix glycans during fruit ripening [16]. Among matrix glycans, glucomannans and xylans, which are weakly bound to cellulose, show little depolymerization compared with those tightly bound to cellulose (xyloglucan). During ripening, pectins become increasingly depolymerized and soluble [17]. Depolymerization increases cell wall porosity, which initially may be low and may limit access of cell wall hydrolases to glycan substrates. During fruit ripening, pectins become increasingly de-esterified. Pectin de-esterification leads to loss of integrity of cell walls, cell-to-cell adhesion, increase in intercellular spaces and a change of tissue structure.

De-esterified negatively charged homogalacturonan (HG) molecules can be bridged by calcium ions, which adds rigidity to the cell wall [8, 18] and preserves the intercellular debonding in the middle lamella [13]. The capability of creating calcium bridges depends on the degree of pectin methylation [19]. Low-methylated pectins in the presence of Ca^{2+} make gel at low temperatures and a low concentration of calcium. High-methylated pectins require elevated temperatures for gel composition, but in this case cross-binding occurs through hydrogen bridges between methyl groups. The treatment of fresh fruit with calcium has a significant influence on its firmness; for example, it delays unfavorable textural changes, improves tissue structure, decreases susceptibility to disease during storage, lengthens shelf life and maintains the firmness and quality of fruit [20–24].

Recently, the AE method, which uses a sensor in contact with the sample, has been shown as a useful method for testing plant tissue cracking [5, 9] and for instrumental evaluation of apple texture [6, 7, 25]. For the instrumental prediction of the sensory attributes of apples, like crispness, crunchiness, hardness or overall texture, the use of a summary number of AE events [7] or AE counts [6] in puncture tests gave satisfactory results. The prediction could be improved when both the sum of AE counts and fruit firmness were included in a principal component regression (PCR) model [25]. This improvement has resulted from the fact that the AE detector, combined with the force detector in the method, records the two most important textural features sensed by humans during eating: bone-conducted vibrations and force necessary to bite a piece of food [26, 27].

The previous studies proposed hypothesize that AE signal is generated mainly by ruptured cell walls due to elastic properties and the capability of generating elastic waves when ruptured [5, 7]. The amplitude of AE signal is proportional to stress at the moment of energy release [28]; therefore, one can expect that the tensile strength of cell walls affects acoustic measurement due to the greater amount of energy released in the process. On the other hand, acoustic measurement in a standardized mechanical test should be affected by the number of ruptured cell walls, that is, microstructural characteristics like cell and pore size or cell wall fraction. However, these hypotheses are not confirmed yet by obtaining direct relationships with structural properties of tissue. Since calcium ions could add some rigidity to cell walls, this study is aimed at verification of the hypothesis that properties of cell walls modified by calcium treatment affect acoustic measurement. The second aim of this study is to check out the relation of cellular structure characteristics with AE measurement. Verification of both hypotheses is important for the future application of the AE method and for the interpretation of the texture of apples in terms of structural properties.

Materials and methods

Apples [*Malus domestica* (Borkh.)] of five cultivars stored for nine months in a normal atmosphere (NA, 1 °C, RH ~80 %) or controlled atmosphere (CA, 1 °C and 2 % CO_2 and 2 % O_2) were used for the experiment. Two cultivars 'Topaz' and 'Szampion' were stored in both atmospheres (NA and CA), whereas 'Gloster', 'Idared' and 'Ligol' were stored only under CA conditions. The fruit were purchased from the orchard of the Research Institute of Pomology and Floriculture in Skierniewice, Poland. Apples were conditioned at room temperature for one day before testing. Thirty apples of similar size from each cultivar/storage variant were selected for experiment. The apples were divided into six groups for certain treatments (one for control and five for calcium treatments). From each apple, two 13-mm diameter and 10-mm height cylindrical samples were taken from the fruit equator without consideration of the skin color, from the depth of about 5–7 mm under the skin. The cylinder orientation and sampling scheme are shown in Fig. 1. Then, 12 samples were put to one of the treatments: calcium lactate solutions of 0 (water), 1, 2, 4 and 6 % (w/v). Additionally, control samples were left untreated for comparison. Calcium lactate was chosen as a texture agent because it has little influence on the taste. Firstly samples were subjected to under pressure for 30 min. at room temperature in order to remove air from intercellular spaces and to increase Ca^{2+} penetration, and then, the samples were kept in plastic bags at 2 °C for 24 h to reach the equal distribution of calcium ions inside the tissue. Directly before the tests, samples were conditioned for 1 h at room temperature.

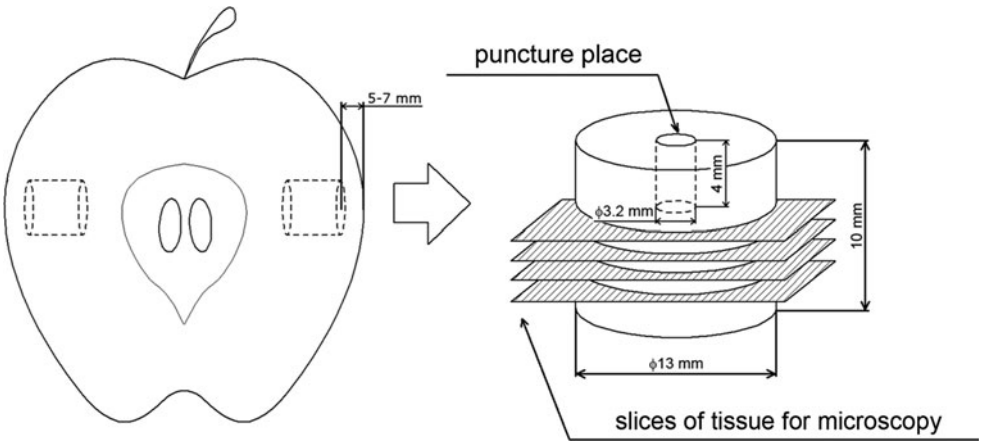

Fig. 1 Sampling scheme in the experiment

Firmness test with acoustic emission

The device used for mechanical testing was composed of two integrated systems: for puncturing and for AE recording during puncturing. The firmness test was performed with a Lloyd LRX universal testing machine (Lloyd Instruments Ltd., Hampshire, UK) and the Nexygen software provided with the apparatus. A 10 N load cell with an accuracy of ±0.5 % was used. The trigger force for starting the force-deformation curve was 0.1 N. Due to the small sample size, the puncture test was modified in comparison with those usually performed on apples; however, the methodology of firmness determination was not changed: Firmness was determined as the maximum force in the test. A probe of 3.2 mm diameter was pushed 4 mm into the sample with a speed of 20 mm/min. The probe diameter was chosen to puncture a much smaller area than the sample surface; similarly, the depth of penetration was limited by the sample height (Fig. 1). A smaller puncturing volume has the additional advantage of allowing as close as possible relation of mechanical measurements with microstructure characteristics.

For AE measurement, exactly the same system as described by Zdunek et al. [7] was used. The system consisted of an AE head with a sensor and amplifiers with filtering and signal processing. A signal from the 4381V (Bruel & Kjear, Narum, Denmark) sensor, with maximum sensitivity in the audible range 1–16 kHz, was amplified by a low noise amplifier (EA System S.C., Warsaw, Poland). The amplifier additionally filters the signal to pass the 1–20-kHz band. After filtering, the signal is converted into a digital signal by the A/D board Adlink PCI 9112 (Adlink Technology Inc., Taipei, Taiwan). The sampling rate was 44 kHz. The second channel of the A/D board was used for recording an analogue signal of force delivered from the Lloyd LRX machine in order to integrate the AE signal with the force-displacement curve. The AE device records WAV files with the 44 kHz; however, for further analysis, the signal is also converted off-line into a few AE descriptors. From available AE descriptors, the AE event has been chosen in this study since this descriptor previously showed the best correlation with crispness and crunchiness of apples compared to AE mean amplitude or the product of AE event and mean AE amplitude [7]. The AE event is the section of the AE time signal where oscillations with amplitudes higher than a preset threshold called the AE discrimination level are measurable [5]. The threshold level has been found experimentally by running the Lloyd LRX machine with the speed and other settings (including amplification) as in the proper experiment, but without the test material. The desired level was the minimum voltage value of the signal from the 4381V sensor at which no AE event was detected in this test. The AE discrimination level and other settings were the same for the entire experiment. Despite the AE events being counted in the device in periods of 0.1 s during the entire puncturing, it has been shown that the total number of AE events counted in the test is a very simple and satisfactory predictor of the sensory texture attributes of apples [7].

Microstructure evaluation and image analysis

Samples for microscopy observation were taken as shown in Fig. 1. Five randomly chosen samples from the each control group were examined in this way. After puncturing, samples were glued to the holder of the Vibratome Leica VT 1000S (Leica Microsystems GmbH, Germany) which cut them into four slices, each of 250 μm thickness. The procedure for staining and microscopy observation was based on the protocol developed by Zdunek and Umeda [10]. In this study, slices were stained in a water solution of acridine orange for 10 s. Then, they were mounted on microscope slides without cover glass. To stick the slice to the slide and to make the slice surface as flat as it possible, the water that remained on the slice was carefully absorbed by tissue paper. This ensured that within the whole field of

observation, the slice surface did not extend out from the focal plane. Only small adjustments were necessary while the slice was positioned under the microscope to improve brightness and contrast.

A confocal scanning laser microscope (CSLM), Fluoview FV300 (Olympus Europa Holding GmbH, Hamburg, Germany) with He–Ne Green laser (1 mW, 543 nm), was used. An objective of 10× was chosen as the most suitable for revealing the cellular structure, having about 100–150 cells per image. One image from each slice from the place corresponding to central point of puncturing was recorded. Images had 512 × 512 pixel resolution which corresponded to the field of observation 1.4 × 1.4 mm. In total, 20 images were collected for the each cultivar/storage variant.

In the confocal images, the cellular structure was revealed as brighter cell walls surrounding darker interiors (Fig. 2). The interiors could be both cells or intercellular spaces which the later would consists of about 20 % of apple tissue volume [29]. Two-dimensional images from CSLM were subjected to image analysis procedures in order to measure the properties of cellular structure. To perform this, an image analysis protocol used previously by Zdunek and Umeda [10, 30] was applied. This protocol has been created for confocal images and allows segmentation images into objects very closely representative of cells or pores (red line in Fig. 2). The procedure includes skeletonization of bright lines in the original image; thus, the object's edges were detected in the middle of visible walls. Due to various imperfections of the images, the procedure also allows checking and eventual manual deleting of incorrectly segmented objects. In this study, human inspection and manual correction have been made to remove from the analysis objects that are very badly represented. Moreover, objects attached to the image border were removed from the analysis (Fig. 2 shows a reconstruction after removing the boundary object) since they do not represent a structure correctly. After correction, the procedure measured a set of geometrical parameters of the each object detected. In this study only area and perimeter, expressed in pixel count, were used. Cell walls were represented as one pixel line so cell wall thickness was neglected because of the large uncertainty in its estimation from the images. Additionally, a cell wall fraction was estimated as the ratio of the total length of perimeters of all objects and the summary area of objects.

Statistical analysis

Statistical analysis was performed using Statistica 9.0 (StatSoft, Inc., Tulsa, OK, USA). The mean values and standard deviation (SD) were determined from 12 replicates at each treatment. The calcium treatment effect was investigated with one-way ANOVA followed by the post hoc Tukey's HSD test. To reveal the correlations in detail, Pearson's correlation coefficients were calculated with Statistica and significance was estimated at $p < 0.05$.

Results

Calcium effect on firmness

Table 1 presents mean firmness of apple samples treated with different calcium concentrations for 24 h. The one-way ANOVA showed that a significant effect ($p < 0.05$)

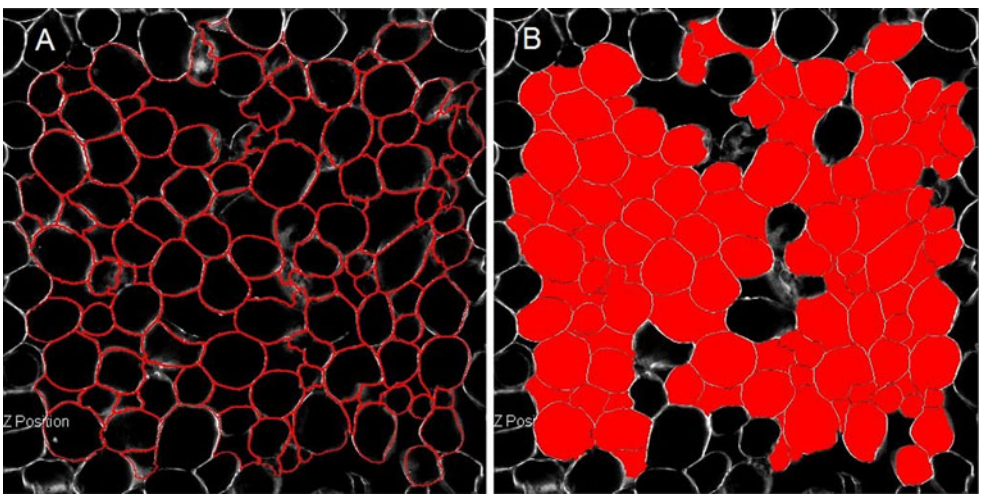

Fig. 2 Confocal images of apple with segmentation. **a** Example of apple cellular structure and the result of image segmentation (*red line*), **b** the same image with objects (inversed representation of objects) left after manual correction of badly reconstructed objects and automatic removal of boundary objects. Only the remaining objects, as shown in **b**, were used for calculation of mean area, perimeter and cell wall fraction

Table 1 Firmness of apple samples after Ca^{2+} treatment for 24 h

Ca^{2+} treatment	Firmness (*N*)						
	Topaz		Szampion		Ligol	Gloster	Idared
	NA	CA	NA	CA	CA	CA	CA
Control	3.63^{a}	5.48^{a}	2.36^{a}	3.49^{a}	5.09^{a}	5.57^{a}	6.43^{a}
0 %	2.87^{a}	5.53^{a}	3.05^{b}	3.28^{a}	4.63^{a}	4.37^{a}	5.33^{ab}
1 %	3.56^{a}	5.37^{a}	3.87^{b}	3.15^{a}	5.80^{a}	6.52^{a}	6.92^{ab}
2 %	4.75^{b}	6.56^{a}	4.06^{bc}	3.73^{ab}	5.07^{a}	5.51^{a}	6.74^{ab}
4 %	6.49^{c}	5.80^{a}	4.93^{c}	4.03^{ab}	4.70^{a}	6.34^{a}	6.32^{ab}
6 %	6.61^{c}	6.44^{a}	5.97^{c}	4.44^{b}	5.80^{a}	6.02^{a}	7.02^{b}
F value	42.1	1.1	23.7	3.7	2.8	1.2	2.5
p	0.000	0.391	0.000	0.006	0.025	0.310	0.040

The same superscript letters mean no significant differences in post hoc HSD Tukey's test at $p = 0.05$

NA storage at normal atmosphere, *CA* storage at controlled atmosphere

existed for 'Topaz' and 'Szampion' stored in NA, and 'Szampion', 'Ligol' and 'Idared' stored in CA. No significant calcium effects were observed in the case of 'Topaz' and 'Gloster' stored in CA (F value = 1.1, $p = 0.4$ and F value = 1.2, $p = 0.3$, respectively). However, considering the F value of the ANOVA test, one can notice the very pronounced effect of calcium treatment obtained for the two cultivars stored in NA: 'Topaz' and 'Szampion' (F value = 42 and 24, respectively). In the case of these two cultivar/storage variants, continued increase in firmness was observed from pure water (0 %) to concentration 4 %. Treatment in 6 % of calcium did not cause further significant increase in firmness; however, the mean value was slightly higher. Maximum firmness for samples stored in NA was about 3 N, so almost twice compared to the control and 0 % samples. Also, in the case of samples stored in CA, increase in firmness with calcium concentration was observed, but post hoc analysis showed that there are no significant differences among consecutive treatments or an important difference only existing between the control or 0 % samples and the highest concentrations (6 %). In the case of CA storage, the firmness increase was not higher than 1 N (~20 %). It should be noticed that the control samples stored in CA had clearly higher firmness than samples stored in NA.

Calcium effect on acoustic emission

Figures 3 and 4 present changes of total AE events versus Ca^{2+} concentrations for studied variants. Figure 3 compares results obtained for cultivars stored under different conditions (NA and CA): 'Topaz' and 'Szampion'. Graphs show that the effects are similar for the two storage methods, but their extent is different. The F value for both cultivars stored in NA conditions is much larger (>100) than for fruits stored in CA (<20), despite in both cases the effects with significance ($p < 0.05$). The highest difference between two storage methods was observed in the case of control samples and samples treated with low Ca^{2+} concentrations. 'Topaz' and 'Szampion' stored in NA had very low initial numbers of AE events (<10,000 for 'Topaz' and ~1,500 for 'Szampion'), whereas the same cultivars stored in CA started from more than 30,000 events in the case of 'Topaz' and more than 10,000 events in the case of 'Szampion'. Moreover, calcium treatment up to 1–4 % (depending on the case) significantly increased the number of total AE events, and further calcium impregnation did not cause any effect on this parameter. Interestingly, in Fig. 3 and also in Fig. 4. The maximum number of AE events in puncture tests did not reach more than ~40,000, and it must be stressed that this limitation was not caused by the device used for AE detection. For some cultivar/storage variants, particularly for these stored in CA, the value 40,000 of AE events was constant from control samples through all treatments. Post hoc analysis presented in Figs. 3 and 4 confirms that samples from apples stored in CA condition show a weak but significant effect, observed mainly at low calcium concentrations. In the particular case of 'Idared' stored in CA, post hoc analysis actually showed no significant difference. The positive effect, if any, on total AE counts is much more pronounced than in the case of firmness. For apples stored in a normal atmosphere, the increase is at least fourfold, while for apples stored in a controlled atmosphere, the increase is approximately double initial values. This is also reflected in the higher F values of ANOVA.

Microstructure versus acoustic emission and firmness

Table 2 presents a mean area and a mean perimeter of objects detected from microstructure images, and an estimated cell wall fraction for the studied cultivar/storage

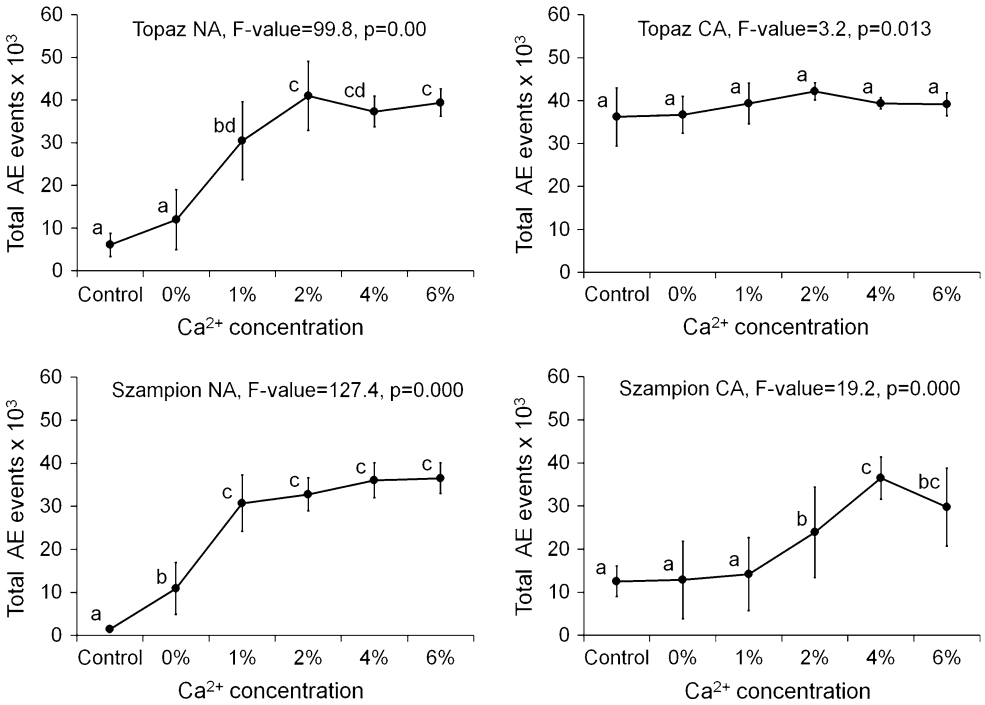

Fig. 3 Calcium treatment effect on total AE events for 'Topaz' and 'Szampion' cultivars stored in a normal atmosphere (NA) or a controlled atmosphere (CA). The *same letters* mean no significant differences ($p > 0.05$)

variants. Post hoc analysis showed that results could be divided into three groups according to the structure characteristics. Cultivars 'Idared', 'Ligol' and 'Topaz' stored in CA had the smallest objects detected. For these cases cell wall fraction was the highest and consisted of more than 14 %. On the opposite side, 'Szampion' samples, both stored in NA and CA, had the largest objects; thereby, their cell wall fraction was the smallest (about 12 %). 'Gloster' CA samples and 'Topaz' NA samples were built of average size objects (14,000 and 12,900 μm^2, representatively), resulting in cell wall fraction a little bit more than 12 %.

Table 3 presents Pearson's correlation coefficients between mechanical parameters and microstructural characteristics. For calculation, the mean values from each cultivar/storage variants were used. The correlations are significant ($p < 0.05$) for the relation between object area and both mechanical parameters, and between cell wall fraction and both mechanical parameters. Figure 5 presents plots of total AE counts and firmness versus mean object area, whereas Fig. 6 presents the same mechanical variables versus cell wall fraction. It is clear that larger objects (smaller cell wall fraction) in the tissue corresponded with a smaller number of total AE events recorded in the puncture test. Similarly, it also corresponded to smaller firmness. In other words, a denser structure, in terms of cell wall, caused an increase of total AE events and higher tissue firmness. This graph shows that difference of about 5,000 μm^2 between two extreme tissue structures resulted in difference of about 35,000 of total AE events and 4 N of firmness.

Discussion

Apple parenchyma tissue is composed mainly of primary cell walls. Primary cell walls are extensible and somewhat elastic [8]. The middle lamella acts as the glue holding neighboring cells together and as such is the primary determinant of cell-to-cell adhesion. The middle lamella is composed almost entirely of HG belonging to pectins which are structurally complex and heterogeneous. The presence of pectins has been proven to be important for cell wall plasticity [11]. The HG is abundant also in the primary cell wall providing some plasticity and having impact upon cell expansion, cell development and defense mechanisms [31]. Stretches of HG, negatively charged due to removal of methylester groups by pectin methylesterase (PME), can associate by calcium cross-linking which promotes the formation of supermolecular pectic gels [32]. The association is important in controlling porosity and the mechanical properties of cell walls and contributes to the maintenance of intercellular adhesion [15]. When pectins and glycans in cell walls and in the middle lamella are degraded, the adhesion is smaller and pore fraction increases. The decrease in cell-to-cell adhesion is considered as the main factor influencing firmness, and in consequence, the intercellular debonding mode of tissue failure is promoted. On the other hand, in a fresh and firm tissue, adhesion between cells is high and the cracking mode through cell walls is preferential [14]. The above simplified view on the biomechanics of fruit tissue helps with interpretation of the data obtained in this experiment.

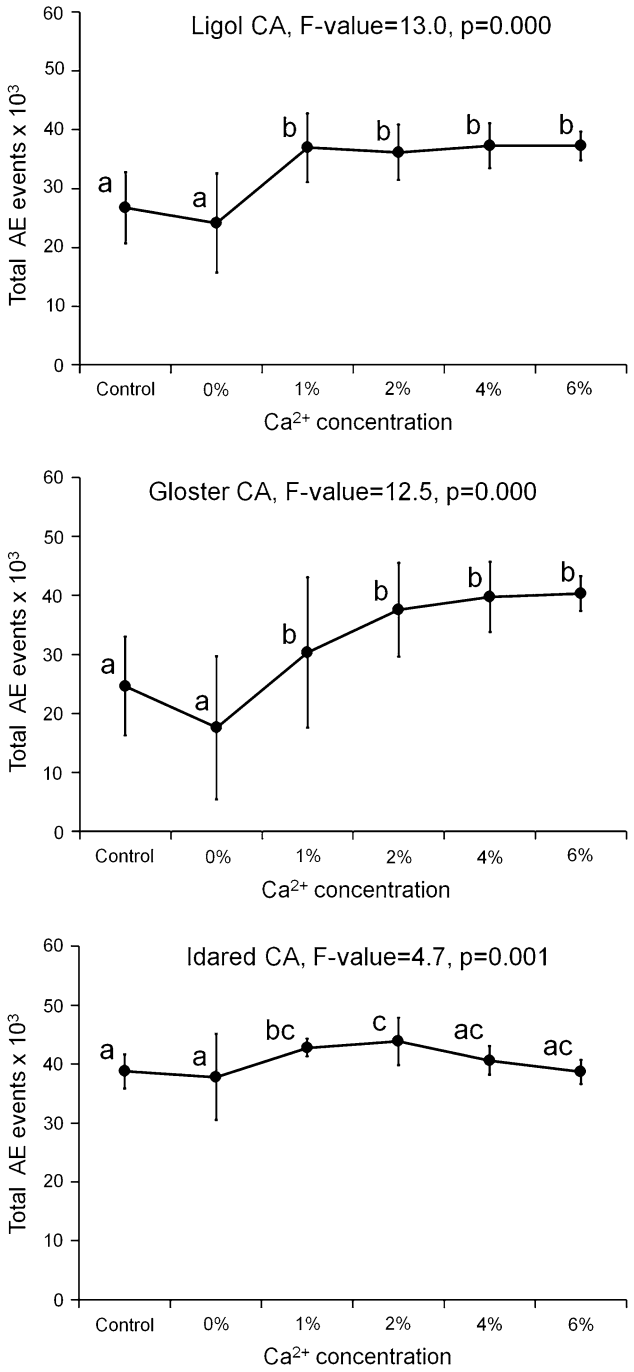

Fig. 4 Calcium treatment effect on total AE events for 'Ligol', 'Gloster' and 'Idared' cultivars stored in a controlled atmosphere (CA). The *same letters* mean no significant differences ($p > 0.05$)

In the experiment, besides firmness increase (Table 1), total AE events also increased with calcium lactate concentration (Figs. 3, 4). In general, AE events are generated one by one during puncturing as a result of succeeding rupturing of elements creating a mechanical skeleton of tissue. AE is generated in a process where some part of energy could be released in the form of acoustic waves. Usually it must be a sudden process. In the tissue structure, rupturing of elastic cell walls could be the source of acoustic signal. Middle lamella, due to its plastic properties, probably does not contribute significantly to sound generated during mechanical damage. This is why mealy fruits are usually not juicy (no release of intracellular soap) and do not emit a lot of sound. When the cell wall suddenly ruptures, due to turgid cell compression beyond the cell wall strength, an acoustic signal is generated and the amplitude of this signal relates to the magnitude of tension force within cell walls required for the rupturing. The tension force required for rupturing would be higher when cell walls are strengthened by calcium ions; thus, the positive effect of calcium concentration on total AE events was observed in this experiment. Moreover, an increase in cell-to-cell adhesion, as a result of forming gel by HG in the presence of Ca^{2+}, causes an increase in the probability of cracking through cell walls instead of intercellular propagation. These two effects contribute together to increase the number of total AE events with Ca^{2+} concentration.

The calcium impregnation effect was more pronounced in the case of total AE events than in the case of firmness. The increase in total AE events suggests that material from ductile-like becomes more brittle. The increase in brittleness means that it breaks at lower deformation accompanied with snapping sound. In such material plastic deformation is limited and energy stored in tissue is smaller: The energy is released during breaking. Firmness parameter from the puncture test has important limitation in analysis of brittleness because it reflects only the maximum value of the force-deformation curve and does not reflects directly a puncture history before and after reaching the maximum force. It reflects only that tissue is strengthened generally. Therefore, when only force-deformation curve is available, analysis of jaggedness of the whole puncture curve seems to be more appropriate to evaluate crispness and other sound-related texture attributes, which has been already applied for other food materials in compression tests [33, 34]. AE events are collected during the whole puncturing, and the total value represents a total energy released mainly as elastic waves resulted by rupturing of the tissue. Therefore, AE is very suitable method for the investigation of material brittleness and eventually for instrumental evaluation of sound-related texture attributes [3–7, 9, 33, 34].

For both firmness and total AE events, the observed effects were more pronounced for samples stored in a normal atmosphere (NA) than in a controlled atmosphere (CA). The CA storage inhibits pectin degradation; thus, one can expect that HG de-esterification level by PME to be smaller compared to typical NA. It was reflected for the control groups which showed higher firmness and total AE events after CA storage. Therefore, due to lower amounts

Table 2 Structure characteristics of apple tissue samples from the control group

Cultivar	Storage	Number of objects detected	Area (μm^2)		Perimeter (μm)		Cell wall fraction (%)
			Mean	SD	Mean	SD	
Topaz	NA	1,619	12,920[a]	7,609	602[a]	184	12.61
	CA	2,198	10,699[b]	6,810	560[b]	180	14.17
Szampion	NA	1,061	15,295[c]	8,937	662[cd]	212	11.70
	CA	535	15,252[c]	8,388	679[c]	204	12.04
Gloster	CA	1,574	14,078[d]	9,014	647[d]	213	12.44
Idared	CA	1,920	10,106[b]	6,019	560[b]	172	14.99
Ligol	CA	1,783	10,704[b]	6,346	591[a]	189	14.95

Mean area and mean perimeter were calculated from objects detected using the image analysis procedure from 20 images of the each cultivar/storage variant

The same superscript letters mean no significant difference at $p < 0.05$

SD standard deviation

Table 3 Pearson's correlation coefficient R and significance level p for relation of structural parameters of apple tissue (mean area, mean perimeter and cell wall fraction) with mechanical attributes (total AE events and firmness)

Structural parameter	Total AE events		Firmness (*N*)	
	R	*p*	*R*	*p*
Mean area	−0.81	0.026	−0.78	0.040
Mean perimeter	−0.73	0.061	−0.70	0.081
Cell wall fraction	0.79	0.035	0.88	0.009

The statistics were obtained for mean values in groups

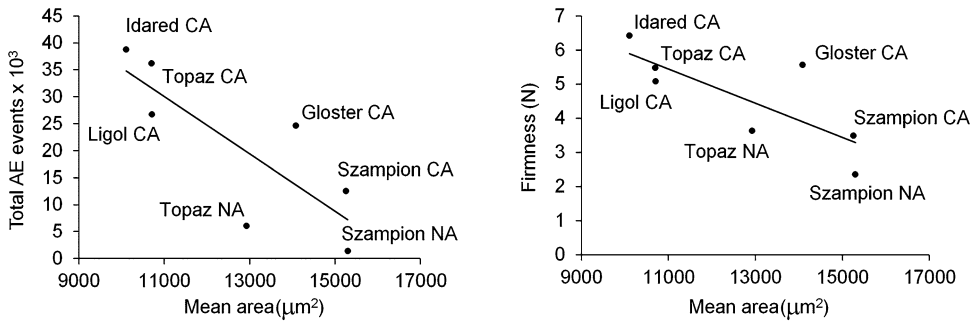

Fig. 5 Relation of mean area with total AE events and with firmness for studied cultivar/storage variants

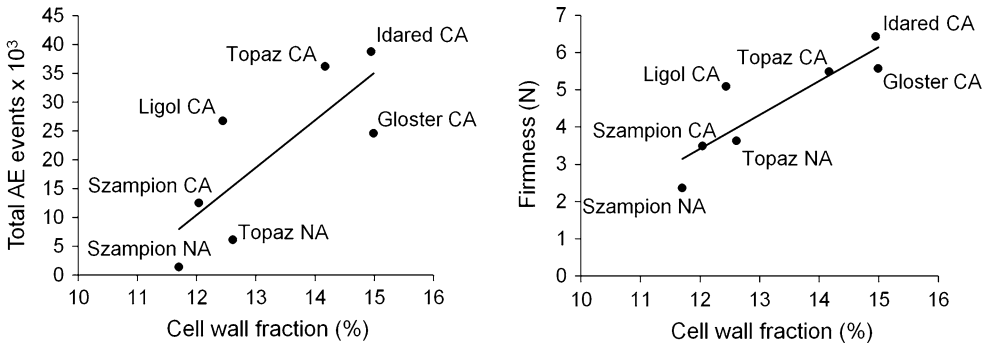

Fig. 6 Relation of cell wall fraction with total AE events and with firmness for studied cultivar/storage variants

of free carboxylic acid groups in the CA samples, calcium treatment of these samples had a less pronounced effect compared to samples stored in NA.

In this experiment, the calcium effect on firmness and AE has been studied in the puncture test with a probe of 3.2 mm. Considering the maximum depth of penetration (4 mm) at

least a tissue volume of 32 mm^3 has been destroyed. The calcium effect on total AE events was very pronounced at low concentration and then was saturated at about 40,000 events almost in each of the cultivar/storage variants. This means that there would be a finite number of cells damaged under the puncture probe. This maximum value of AE events was reached for samples impregnated by calcium of at least 2–6 % concentration; thus one can posit that cell walls and middle lamella have already been strengthened and tissue failure occurs mainly through cell wall rupture. Taking into account that a mean object radius (calculated from Fig. 5) is about 60 μm, the estimated number of objects in the volume taken by the puncture probe is about 35,000. Moreover, when tissues with the smallest objects are considered ("Idared" CA in Fig. 5), mean object radius was ~56 μm which corresponds to 42,000 objects in 32 mm^3 taken by the puncture. This is very close to the maximum number of total AE events recorded in this experiment. A similar estimation was obtained in previous study, when a mean value of apple cell size taken from the literature was used for calculation, showing compatibility of results with the present experiment [7]. These results together suggest that indeed a single AE event would be the result of a single cell rupture. However, it must be emphasized that in the present research the "object" detected from the image could be either cell or intercellular space. No discrimination has been made in this research due to many doubts about an unambiguous decision as to which class the object should be classified. It must also be considered that the AE signal could be damped on the way to the sensor or other sources would introduce events too. Thus, the above assignment of an AE event to a single cell rupture must still be treated as a rough estimation.

This experiment also showed that the samples with smaller sized objects detected and higher cell wall fraction had higher firmness and numbers of total AE events (Fig. 6). This supports the observation that total AE events relate to the number of ruptured cells. Considering firmness, the negative relation with object size and the positive relation with cell wall fraction are compatible with previous studies reviewed by Toivonen and Brummell [8] and with the study of failure properties versus cell size for potatoes and carrots [10]. Significant linear relationships have been obtained when different cultivars were included in the construction of the relationships (Figs. 5, 6). It suggests that apple apparent structure, considered as either object size (both cells and intercellular spaces) or cell wall fraction, is dominant for mechanical properties, independent of cultivar or storage history.

Conclusions

This study presents the influence of calcium treatment and cellular composition on two mechanical attributes: firmness and total AE events registered in the puncture test of apple tissue. The results confirmed that treatment with an increased concentration of calcium ions caused a significant increase in firmness. However, a much more pronounced positive effect of Ca^{2+} treatment was found in total AE events which better reflects the increase of brittleness of apple tissue after impregnation. This suggests that calcium impregnation improves post-harvest and post-storage firmness and also gives an advantage in sound-related properties. However, the effect is particularly positive in the case of very soft apples due to greater extent of de-esterification of HG. Interpretation of results, together with a review of the literature, suggests that calcium importantly changes the cracking mode of tissue, from intercellular debonding to cell wall rupturing, the latter event producing sound. Total AE events relates to the strength of cell walls due to the higher amplitude of the signal generated and is determined by the cellular composition. The rough accordance of the number of split cells open across cell walls by puncture probe and total number of AE events registered in the test, which was saturated in high Ca^{2+} concentrations, confirms that the AE method could be used for the evaluation of the cracking-related properties of food, such as crispness, juiciness and hardness.

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