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The relation of apple texture with cell wall nanostructure studied using an atomic force microscope

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ABSTRACT

In this study, the relation of the nanostructure of cell walls with their texture was investigated for six different apple cultivars. Cell wall material (CWM) and cellulose microfibrils were imaged by atomic force microscope (AFM). The mean diameter of cellulose microfibrils for each cultivar was estimated based on the AFM height topographs obtained using the tapping mode of dried specimens. Additionally, crystallinity of cellulose microfibrils and pectin content was determined. Texture of apple cultivars was evaluated by sensory and instrumental analysis. Differences in cellulose diameter as determined from the AFM height topographs of the nanostructure of cell walls of the apple cultivars are found to relate to the degree of crystallinity and pectin content. Cultivars with thicker cellulose microfibrils also revealed crisper, harder and juicier texture, and greater acoustic emission. The data suggest that microfibril thickness affects the mechanical strength of cell walls which has consequences for sensory and instrumental texture.

1. Introduction

Many macroscopic properties and sensory qualities of plant foods relate to the micro- and nanostructure (Aguilera, 2005; Brummell & Harpster, 2001). Cell walls, beside turgor pressure, are the main architecture responsible for the texture of fruit and vegetable parenchyma tissue. The texture, in particular sensory attributes such as crispness, hardness and juiciness, determine consumer acceptance and usefulness in numerous technological processes. Moreover, cell walls are a valuable component of one's daily diet as dietary fibre since they contribute to the correct functioning of the alimentary canal and thereby reduce the tendency toward obesity (Jarvis, 2011).

The cell walls of apple parenchyma have an advantageous chemical composition from the dietary and mechanical point of view. Apple cell walls are composed mostly of polysaccharides; they can include 40% pectin, 25% hemicelluloses (the dominant hemicellulose in apple parenchyma is xyloglucan) and 20% cellulose (Brummell & Harpster, 2001):

1. Cellulose is composed of (1-4)-β-linked D-glucose chains, assembled together by hydrogen bonding into long crystalline microfibrils. Cellulose microfibrils involve a highly crystalline core surrounded by less crystalline regions and interrupted by amorphous forms of cellulose.
2. Hemicellulose occurs in cell walls in various forms, such as xyloglucan, glucomannan and glucuronoarabinoxylan, with xyloglucan being the most abundant. Xyloglucan coats cellulose microfibrils by hydrogen bonding and spans adjacent microfibrils, thereby linking them together. Because xyloglucan forms long chains (hundreds of micrometres), this polymer plays a significant role in cell wall mechanics. Others hemicelluloses, less abundant in the primary cell walls of dicotyledonous plants, also cross-link microfibrils by hydrogen bonding, although more weakly than xyloglucan.
3. Pectins are a complex polysaccharide rich in galacturonic acid which occur in the primary cell walls in the three distinct forms of homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. The most abundant pectin in apple and pear parenchyma is homogalacturonan, which usually is initially highly methyl-esterified. Pectins, normally largely hydrated, fill the space between the cellulose/xyloglucan network. They also form a network, linked together by ester bonds between pectin molecules and by ionic calcium bridges between de-methyl-esterified homogalacturonans (Cybulska, Zdunek, & Konstankiewicz, 2011).

The macromolecules are organized in a complex network supporting numerous functionalities and with architecture that have a significant influence on the whole parenchyma system, including

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mechanical properties. However, it must be emphasized that the exact role of each polysaccharide and the interactions between them are still under discussion. According to one of the recent cell wall models, pectins form an independent network, in parallel to that of the cross-linking glycans, which works as a plasticizer and water binding agent (Cybulska, Vanstreels, et al., 2010). The role of pectin and cross-linking glycans is to bind to cellulose microfibrils in the cell wall. In an alternate cell wall model, pectins also interact with cellulose, however, they compete with glycans (Żykwińska, Thibault, & Ralet, 2008). When xyloglucan concentration is high, pectin absorption onto cellulose is lower, thus the cellulose–pectin interactions are weaker than interactions between cellulose and xyloglucan, whereas at low xyloglucan abundance, the main function of pectins is to bind the gap between microfibrils.

Analysis of the cell wall structure requires microscopes that allow observation at the nanometre scale due to the dimensions of the polysaccharides macromolecules. The atomic force microscope (AFM) has been shown to be a very useful tool for observing the molecular structure of a single macromolecule and cell wall assemblies (Cybulska, Konstankiewicz, Zdunek, & Skrzypiec, 2010; Cybulska, Vanstreels, et al., 2010; Morris et al., 1997) with minimal sample preparation (Kirby, Gunning, Waldron, Morris, & Ng, 1996). AFM provides much valuable information which was not always obtainable by analytical methods (Round, Rigby, MacDougall, & Morris, 2010; Zhang et al., 2012). Davies and Harris (2003) applied AFM to measure cellulose microfibrils in partially hydrated sampled of model dicotyledon *Arabidopsis thaliana* and monocotyledon – onion. Water content in cells significantly influences the diameter of cellulose microfibril which was documented in the experiment with different water levels of hydrations (Thimm, Burritt, Ducker, & Melton, 2000). Changes of arrangement of the cellulose microfibrils of growing cucumber cells were investigated by means of AFM (Marga, Grandbois, Cosgrove, & Baskin, 2005). Based on AFM observation, a model of a 36-chain elementary cellulose fibril and its biosynthesis of the maize parenchyma cell wall was proposed (Ding & Himmel, 2006). AFM was also used for studying the action of cellulases enzyme on cellulose which showed an increase in the proportion of crystalline regions during hydrolysis (Liu, Fu, Zhu, Li, & Zhan, 2009). Differences in the nanostructure of commercial citrus pectin, orange albedo pectin and lime albedo pectin were described quantitatively with the use of AFM imaging (Fishman, Cooke, & Coffin, 2006). Degradation of water-soluble pectin was detected using AFM on the basis of pectin aggregates separation (Yang, An, Feng, Li, & Lai, 2005). Pectin–protein complexes, found in extractions from unripe tomato, can play the role of emulsifying factors (Kirby, MacDougall, & Morris, 2008). AFM allowed observation of changes of pectin chains after fruit storage and Ca^{2+} treatment (Liu, Chen, et al., 2009). It shown that immersion of the strawberry fruit in a Ca^{2+} solution delays undesired physicochemical changes, as well as degradation of pectins, by strengthening the ionic cross-linkages among pectin molecules (Chen et al., 2011). Hydrolysis of pectin was analysed on the basis of the backbone length, branching and branch length distributions for individual polymers (Round et al., 2010).

So far, AFM has been used mainly for comparison of nanostructures among different species. Very rarely are differences between cultivars within the same commodity analysed from the point of view of nanostructure of cell walls. Recently, Zhang et al. (2010) showed differences in nanostructure of pectins between soft and crispy peach cultivars which was also related to differing firmness of these cultivars, whereas Chen et al. (2009) found differences between the nanostructure of hemicelluose macromolecules of crisp and soft Chinese cherry cultivars. At present, a degradation of pectins in the cell wall and middle lamella is considered as the main factor determining the dynamics of fruits softening. However, the properties of cellulose microfibres, like diameter or degree of crystallinity, or their spatial assembly, have not been investigated with relation to the macromechanical properties of fruit tissue. This is particularly important in the case of apples due to the wide range of available cultivars which differ in terms of sensory texture. The crystallinity degree of cellulose has not been considered yet as an important factor influencing fruit texture, however, the presence of crystal and amorphous parts of microfibrils is important with regard to the interaction with other biopolymers and water binding capacity. Therefore, in this study, firstly six apple varieties at approximately the same stage of ripening were investigated to verify whether significant differences exist in terms of their cell wall nanostructure properties: cellulose microfibrils' diameter, cellulose crystallinity and pectin content. Then, the nanostructure properties were compared with sensory texture attributes and instrumental parameters often used for texture evaluation.

2. Materials and methods

2.1. Materials

Six apple [*Malus x domestica* (Borkh.)] cultivars were used in the experiment: 'Cortland', 'Honeycrisp', 'Ligol', 'Mutsu', 'Rubin' and 'Jonagold'. Fruits were purchased from the orchard of the Research Institute of Pomology and Floriculture in Skierniewice, Poland. The apples have been harvested at the optimum maturity time for each cultivar based on ethylene test from seeds ovary and starch iodine test (Konopacka & Płocharski, 2004). Before experiment the apples were stored at 2 °C in a normal atmosphere for two months after harvest.

2.2. Cell wall material (CWM) and cellulose isolation

Apple cell wall material (CWM) was isolated using the modified phenol buffer method proposed by Renard (2005). Frozen apple slices were homogenized in a cold buffer, simulating the ionic conditions in apple juice (1.2 mM $CaCl_2$, 2.0 mM $MgCl_2$, 0.5 g L^{-1} KCl, 60 mg L^{-1} ascorbic acid, 4 g L^{-1} apple acid, 1 g L^{-1} sodium disulphite supplemented to pH 3.5 with 5 M NaOH) with Triton 100 (2 g L^{-1}) and 1-octanol (4 mL). The suspension was then filtered under reduced pressure and washed in a 60% water solution of acetone. The resultant paste was blended with phenol at a volumetric ratio of 1:4 and left for 1 h at room temperature. Next, the blend was dissolved in the buffer and filtered. The material was washed successively in 70% and 96% ethanol, and finally in acetone.

Cellulose from apple tissue was obtained during sequential extraction of cell walls in order to remove pectin and hemicelluloses according to the method proposed by Redgwell, Melton, and Brasch (1988) with some modifications. Cell walls were stirred in deionized water for 6 h at 20 °C and then in 0.1 M cyclohexane-trans-1.2-diamine tetra-acetate (CDTA) (pH 6.5) at 25 °C for 2 h, since 6 h after the previous step. Then the residue was diluted in 0.05 M sodium carbonate (Na_2CO_3) with addition of 20 mM sodium borohydride ($NaBH_4$) and stirred for approx. 20 h at 1 °C, filtered, and again stirred for 2 h at 20 °C. After filtration, depectinated cell walls were stirred sequentially in 0.5 M, 1 M and 4 M of potassium hydroxide (KOH) with an addition of 20 mM $NaBH_4$ each for 2 h at 20 °C every time. Finally all cellulose from the apple tissue was rinsed several times in deionized water and ethanol.

2.3. Pectin content determination

Supernatants after CDTA and Na_2CO_3 treatment were used for pectin determination. Galacturonic acid content was