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Changes in cell wall stiffness and microstructure in ultrasonically treated apple

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ABSTRACT

Ultrasounds (US) enhances mass transfer in fruit, however, evidence of changes triggered at cell wall level are still very scarce. Apple tissue was subjected to ultrasound for 7.5, 15 or 30 min in order to evaluate the effect on the cell wall. The distribution of galacturonic acid (GalA) in water (WSP), calcium chelator (CSP) and sodium carbonate (DASP) soluble pectin fractions extracted from cell walls was measured. Cell wall stiffness was evaluated using an atomic force microscope. The cellular structure was studied using a confocal microscope. Area, perimeter, roughness and circularity of objects in the images were analyzed. Cell wall stiffness gradually decreased from 8.97 MPa to 4.24 MPa after 30 min. Low content of GalA in WSP fraction for the control samples showed a relatively low level of pectin solubilization in the apples used. Most GalA was distributed in DASP. A significant increase in GalA content in WSP as a function of time of US treatment was observed. Similarly, US increased the GalA content in CSP and DASP fractions, but a significant effect only occurred after the longest treatment time (30 min). The content of GalA in the insoluble fraction finally increased after 30 min. This showed that ultrasounds increased the overall pectin solubilization in cell walls. The exposure to ultrasound caused a significant decrease in the average area and the mean perimeter of the objects due to an increase in the area contributed by small objects (<6000 μm^2). This has confirmed that ultrasounds creates intercellular spaces in fruit tissue.

1. Introduction

Power ultrasound (US) refers to sound waves beyond audible frequency within the range of 20 kHz to 1 MHz, which are able to interact with both solid and liquid media. Depending on the wave frequency and the level of energy applied, ultrasound can produce chemical, mechanical or physical changes in processes or products (Leong et al., 2011). When power ultrasound is passed through subjected media, sequential compressions and expansions are generated, which in turn leads to acoustic cavitation effects, seen in the form of gas bubbles in the liquid medium that can explosively collapse and generate localized pressure as well as temperature increases (Simal et al., 1998; Wan et al., 1992).

Having the ability to interact with a wide range of media, ultrasound is becoming a regular feature in many aspects of agro-food technology (Awad et al., 2012; Cárcel et al., 2012; Pingret et al., 2013). Ultrasound pre-treatment offers new possibilities for biomatrix modification, this has been thoroughly investigated due to its usefulness as a tool for enhancing biomaterial drying efficiency (García-Pérez et al., 2007; Mothibe et al., 2011). An intensification of the phenomena of mass exchange in fruit and vegetable tissue, due to the power (Garcıa-Perez et al., 2006) or duration (Fernandes et al., 2008) of US treatment, is attributed to changes in tissue microstructure, especially the creation of microscopic channels that facilitate moisture removal.

Mass transfer in fruit tissue structure is affected by several morphological features, such as; the intercellular network of spaces, cell wall properties, and plasmalemma permeability (Aregawi et al., 2013; Fanta et al., 2014; Ho et al., 2006, 2008, 2009, 2010, 2011). The duration of US treatment can affect the microstructure of tissue by inducing the formation of microscopic channels in the fruit structure (Fernandes et al., 2008, 2009) or disrupting the cellular structure (Schössler et al., 2011, 2012). There is also evidence that US facilitates the extraction process of a variety of food components (e.g., herbal, oil, protein, polysaccharides) as

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Abbreviations

AFM	atomic force microscope
AIR	alcohol insoluble residue
CSP	calcium chelator (CDTA) soluble pectins
CWM	cell wall material
DASP	diluted alkali (sodium carbonate) soluble pectins
GalA	galacturonic acid
US	ultrasound
WSP	water soluble pectins

well as bioactive ingredients (e.g. antioxidants) from plant and animal resources (Awad et al., 2012; Pingret et al., 2013; Vilkhu et al., 2008). However the effect of US on the structure and properties of plant cell walls, that are considered to be the key determinants of fruit texture, is still not well known.

The cell wall structure is considered to be a continuum of matrix polysaccharides and glycoproteins. Three main classes of polysaccharides have been distinguished in primary cell walls: cellulose, hemicelluloses and pectins which amount to approximately 15–40%, 20–30% and 30–50% of the primary cell wall, respectively (Cosgrove and Jarvis, 2012; Fischer and Bennett, 1991; Jarvis, 2011). Pectins and hemicelluloses are considered to be matrix polysaccharides where cellulose microfibrils are embedded. The mechanisms of cell wall pectins, hemicelluloses and cellulose assembly and their function in cell wall mechanics are not fully established yet. Recently discovered covalent linkages between hemicelluloses and pectins and with wall glycoproteins provide new evidence demonstrating the complexity of the cell wall assembly and that the cell wall should be considered as a continuum of matrix polysaccharides and glycoproteins interacting with cellulose (Park and Cosgrove, 2015; Tan et al., 2013). Pectins play the role of a hydrophilic filler, which determine cell wall porosity (Fleischer et al., 1999; Rondeau-Mouro et al., 2008) with consequences for cell wall diffusivity as well as for cell wall water uptake and cell wall swelling. Pectins are also the main component of the middle lamella which binds adjacent cells (Knox, 1992; Taiz and Zeiger, 2002) therefore they are considered to be the main determinant of the mechanical properties of the cell wall and plant tissue.

In this study we hypothesize that US causes a loosening of the cell wall assembly and a decrease in the stiffness of the cell wall. This factor, along with the creation of microscopic channels, could be another reason for the changes in the macroscopic properties of tissue due to US. Therefore this work focused on the effect of time of ultrasound treatment on cell wall stiffness and on the tissue microstructure. We have chosen apple as a model fruit material. Cell wall properties were characterized by the distribution of galacturonic acid (GalA) in different pectin fractions and by the stiffness evaluated by an atomic force microscope (AFM). The latter method was recently used to characterize fruit cell walls (Zdunek et al., 2016). On the microstructure level the tissue was characterized by the geometrical attributes of objects detected by computer image analysis of confocal images in order to confirm the creation of microscopic channels formed due to ultrasound.

2. Materials and methods

2.1. Sample preparation

Apples from the 'Idared' cultivar (*Malus domestica Borkh*) were purchased at a local store. Two types of samples were used: pieces of cell wall material for stiffness evaluation and cylinders of tissue for microstructure characterization.

Cell wall material (CWM) was isolated from parenchyma tissue as alcohol insoluble residue (AIR), (Renard, 2005). Twenty grams of apple pulp, from the same part of the apple that was sampled for microstructure characterization, was boiled with 70 ml of 70% ethanol for 20 min. Then the sample was chilled before being filtered using a nylon filter and mixed with 30 ml of 70% ethanol. After filtration, if a phenol-sulphuric acid assay for the presence of sugars produced a negative result, the sample was washed twice with 10 ml of 96% ethanol and 50 ml of 99.5% acetone and dried at 40 °C. Twenty two and a half milliliters of CWM water suspension (concentration of 1 mg/1 ml) was prepared. Initially ultrasound was applied to the suspension and then 12 randomly selected CWM pieces were used to characterize cell wall stiffness according to the procedure published in Zdunek et al. (2016).

For microstructure characterization, the same apples were used. Four cylindrical samples were cut from the equatorial region of the apple (~from a depth of 10 mm below the apple peel) (Fig. 1). The dimensions of the cylinders were 15 × 6.5 mm and they had an average weight of 0.32 g. This sampling location was chosen due to the relatively homogenous and isotropic structure of the tissue in that place. For ultrasound treatment and microscopic observations, each cylinder was glued to a metal plate and placed at the bottom of a laboratory beaker. The beaker was always filled with 150 ml of deionized water. First, ultrasound was applied and then slices were cut from the samples and characterized using a confocal scanning laser microscope. This part of experiment was repeated three times.

2.2. Ultrasound treatment

Both types of samples received US treatment in deionized water. A Vibra Cell VCX 130 (SONICS & MATERIALS Inc.) processor with a net power output equal to 130 Watts and a frequency of 20 kHz was used. The processor was equipped with a 6 mm diameter probe with the maximum oscillation amplitude equal to 114 μm. Ultrasound was applied for different lengths of time, either 7.5, 15 or 30 min. The treatment time was chosen based on a literature review (Cao et al., 2010; Fernandes et al., 2008, 2009). Control samples were not treated with ultrasound (0 min). For each exposure time the amplitude of the acoustic pressure wave was set to 20% of the maximum oscillation amplitude of probe. This was equal to a power output of 2.5 W. The specific energy applied to samples was 10 kWh/kg for the cylinders and 100 kWh/kg for CWMs. The temperature increase after 30 min was 4 °C in the case of cylindrical samples and 24 °C in the case of CWM samples. In the case of the cylindrical samples, the probe of the ultrasonic processor was placed in the beaker directly above the sample, at a distance of 10 mm (Fig. 1). In the case of the CWM suspension the probe was placed ~2.5 cm below the surface of the suspension. Further analyses were performed for cooled samples to room temperature (~22 °C).

2.3. Image acquisition for microstructure characterization

For microstructure characterization, the samples, that were still attached to the metal plate were transferred to a Vibratome LEICA VT 1000S (Leica Microsystems, Germany) and were cut into 200 μm thick slices. Slices were cut from the region about 1–1.5 mm below the top of the cylindrical sample. The slices obtained were then stained using acridine orange (for about 15 s) according to previously developed protocol (Zdunek and Umeda, 2005, 2006). Next, the samples were washed with deionized water and placed on microscope slides for immediate observation.