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Prediction of the nanomechanical properties of apple tissue during its ripening process from its firmness, color and microstructural parameters

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ABSTRACT

Nanomechanical properties of fruit determine macroscopic firmness. Young's modulus (YM) of apple tissue obtained by atomic force microscopy (AFM) at cellular level and its correlation with other macroscopic physical parameters are used to evaluate ripening when apples were stored at 25 °C during 40 days. The YM of the tissue decreased from 0.96 ± 0.42 MPa (first day) to 0.11 ± 0.06 MPa (last day). The best linear correlation of YM was obtained with firmness (F) which decreased from 21.15 ± 0.79 N to 12.74 ± 0.34 N. Also various physical parameters obtained as peel color difference (ΔE) from 57.80 ± 0.97 to 66.51 ± 0.71, environmental electron microscopy (ESEM) such as entropy: Ent (from 8.67 ± 0.12 to 9.60 ± 0.17) and fractal dimension: FD (from 2.67 ± 0.05 to 2.75 ± 0.03) changed as well. Significant correlations ($P < 0.05$) were found between YM, F, ΔE, Ent and FD using Pearson analysis. Predictive models to evaluate YM from F, ΔE, Ent, FD were obtained by multiple linear regression, $R^2 > 0.95$ was found.

Industrial relevance: The study of the nanomechanical properties of fruit cells by AFM may provide insight into internally fruit properties and how changes in these properties over time influence the quality of the fruit. The determination of cell/tissue mechanics could be used to follow changes in the nanomechanical properties that occur during the processing and storage of climacteric fruits such as apples. The study of the nanomechanical properties of plant cells as well as their correlation with other useful food properties such as firmness, peel color and image texture could lead to a better understanding of the ripening process. Mathematical models to predict cell mechanical properties at the nanometric level through food quality parameters could be an innovation in food engineering and be a novel tool in evaluating the quality of apples. Benefits of the methods herein could be extended to address current issues, such as extending the storage life of other climacteric fruits and predicting nanostructural modifications to the cells when they are physically modified or chemically treated.

1. Introduction

Apples (*Malus domestica*) are climacteric fruits with enormous demand on the world market. During recent years, the apple has become an ever more important export in many producing countries. The main consumers are the USA and Europe owing to the large consumer demand for both fresh and processed apple products. In 2013 the FAO reported that China was ranked first for production (tons of apples produced), while Mexico was ranked 15th among top apple producing countries. Mexico produces more than 364,000 metric tons (MT) of Golden Delicious apples (FAOSTAT, 2013). However, in Mexico roughly 43% of the apple crop is lost due to changes in quality features such as firmness, defects, external damage, microbial contamination, and color changes among others (Flores, 2013). Most apple losses are attributed to dry weather, improper management of the postharvest fruit and scarce technology for fruit classification. For this reason, more studies related to understanding the ripening process are required.

Ripening process of apples takes place at room temperature within 32 days after harvest (Cen et al., 2013). During this period fruit softening increases, while in some cultivars as Golden Delicious, its peel color changes from green to yellow (Rutkowski et al., 2008). Apples firmness is a key attribute for consumers, many of whom expect a high turgidity and an adequate firmness for purchase. Texture softening also plays an

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important role in consumer acceptance, since it can indicate the shelf life, freshness, durability and quality of the fruit. The texture of fruits and their susceptibility to damage are determined by their mechanical properties such as the firmness and stiffness. Essentially, in the climacteric fruit, the firmness loss is due to depolymerization of cell wall polysaccharides by a variety of hydrolytic enzymes (Dintwa et al., 2011). Several studies on the biochemical and physicochemical changes that occur during the ripening of climacteric fruits have already been conducted (Wang et al., 2013; Costa et al., 2012; Billy et al., 2008; Yashoda et al., 2007; Ferrer et al., 2005). These have focused mainly on understanding the ripening process for diverse fruits by using different indicators such as total soluble solids, titratable acidity, firmness, pigment content and color parameters. The main intention of these studies has been to predict the ripening stages of the climacteric fruits and find correlations between these stages and its physical, chemical and biochemical properties (Vélez-Rivera et al., 2013; Jha et al., 2010). During ripening, the softening of the cell wall as well as the fruit firmness breakdown, are intrinsically related with the changes occurring in the polymeric structure of the cells. Also, it has been demonstrated that apple fruit maturation can be followed by changes to the skin color using non-destructive methods and that this in turn can be associated with changes in the cellular microstructure. Thus, quality loss in the fruits depends strongly on the color changes and the structural network that confers turgidity and support to plant tissues (Rutkowski et al., 2008; Blanke and Notton, 1992).

Scanning electron microscopy (SEM) has been used to evaluate cellular microstructure in plant tissue. The study of biologic materials using SEM in high vacuum mode is common but limited due to the required sample preparation which involves numerous time consuming steps, pieces of equipment and reagents. Usually, sample preparation methodology for observations in SEM involves fixation, alcohol dehydration processes, critical point dehydration and coating with gold. This procedure invariably causes structural modifications to the biological samples thus providing unreal images of the structure (Stokes, 2003). This could be a problem when kinetic studies are performed using an SEM in conventional mode. A variant of the SEM is the environmental scanning electron microscopy (ESEM). This operation mode provides distinct advantages in the evaluation of the microstructure of fresh biological materials in comparison to conventional mode SEM. ESEM greatly facilitates the observation of biological materials with minimal damage and almost without sample preparation. The benefits that ESEM provides can be used to evaluate numerically the complex structure of the biological materials under study. In the same way, texture image analysis has been suggested in order to measure quantitatively the microstructural changes of foods from images (Chanona et al., 2003; Santacruz-Vázquez et al., 2010). Texture features such as entropy and fractal dimension of the digital images can be determined using texture image analysis. These parameters have been used in different studies, for instance, Kerdpiboom et al. (2006) followed the microstructural changes in carrot tissue during the drying process. Arzate-Vázquez et al. (2011) used texture image analysis to analyze images of chitosan and alginate films obtained from different microscopy techniques.

Atomic force microscopy has become an important tool to study intrinsic cellular nanomechanical properties. Used to directly manipulate and examine whole and subcellular reactions, AFM allows mechanical characterization of the cells properties as well as its polymeric components (Haase and Pelling, 2015; Cybulska et al., 2013). The determination of the nanomechanical properties of cells is also a new approach for understanding the mechanisms of physiological softening and deterioration of the quality of fruits during postharvest storage (Zdunek et al., 2016) because cells and cell walls are the main tissue constituents responsible for macroscopic firmness. However, few studies have evaluated the changes occurring to the structure at subcellular and nanometric levels as well as their correlation with macroscopic indicators such as firmness and peel color. Zdunek et al. (2014) have shown that pear fruit with thicker and more branched pectins were also firmer. It has been evidenced as well that during on tree maturation and postharvest storage of pears substantial changes in cell wall stiffness occurred (Zdunek et al., 2016).

The main issue in evaluation of mechanical properties with AFM is sample handling at cellular and subcellular level. One approach is to use cell wall pieces therefore the stiffness evaluation is not affected by turgor that is present in the intact cells. On the other hand studying of mechanical properties of intact cells is challenging due to keeping sample alive and necessary deconvolution of turgor and the cell wall Young's modulus from the force curve if the cell wall modulus is necessary to be evaluated (Zdunek and Kurenda, 2013). It may be solved by applying low indentations that limits the effect of turgor (Radotić et al., 2012). Another approach is to use AFM to probing cells mechanical properties in fresh-cut tissue. This approach has been recently used to study both tissue properties and intact separated cells from apple tissue, and a decent agreement between these two systems has been demonstrated (Cárdenas-Pérez et al., 2016).

Cárdenas-Pérez et al. (2016) demonstrated also the complexity of the surface of apple cells from atomic force microscopy images. This approach could be useful to evaluate kinetically the microstructural changes that occur during fruits maturation. According to various studies (Wang et al., 2013; Cybulska et al., 2013; Costa et al., 2012; Billy et al., 2008; Ferrer et al., 2005), the ripening kinetic is associated with several structure and compositional changes that occur and which clearly determine whole fruit firmness. They discuss that the separation and degradation of water-soluble pectin substances, erosion of the middle lamella due to carbohydrate-degrading enzymes, lead to fruit flesh softening. Billy et al. (2008) demonstrated that pectin substances are the most abundant class of macromolecules within the cell-wall matrix of fruits and are the main components of the middle lamella. They have a role in the adhesion between cells and in regulation of intercellular adhesion. Therefore, enzymatic pectin degradation cause important changes in the structural arrangement of mesocarp cells which are strongly related to macroscopic changes such as firmness loss and peel color variations. However, there are few studies related with the nanomechanical behavior occurring during the kinetics of ripening which are important as a mechanical-structural overview. The study of the relationships between external parameters through the microstructure and the cell mechanics at a nanometric level could provide valuable information to help preserve the quality of climacteric fruits. The present work was conducted to evaluate the cell mechanics of apple tissue (*Malus domestica* v. Golden Delicious) at a cellular scale using atomic force microscopy (AFM) and how it correlates with firmness, peel color and the global structural arrangement of tissue throughout the ripening process. This approach could be useful in food engineering to evaluate and predict the nanomechanical properties of apple cells and a more in-depth knowledge of structure–functionality relationships in apple fruit.

2. Materials and methods

2.1. Samples

The apples (*Malus domestica* cv. Golden Delicious) used for this study were harvested at their green stage during mid-October 2015. The apples were kindly provided by Agropecuaria La Norteñita (Cuauhtémoc City, Chihuahua, Mexico). The fruits were sorted by means of sensorial inspection and with an analytical balance (accuracy 0.1 mg, Explorer OHAUS, USA), in order to remove blemished and irregular fruit and obtain samples with similar shape, size, color and weight (156–160 g). Next, the apples were placed in separate stores at room temperature 25 °C and a relative humidity of 75% (Hotpack 435314, USA). They were stored for 40 days and all experimental measurements were performed every 8 days.