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Morphometric analysis and tissue structural continuity evaluation of senescence progression in fresh cut papaya (*Carica papaya* L.)

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ABSTRACT

Senescence prompted after cutting and environmental exposure was evaluated in fresh cut papaya in two ripening stages and two tissue locations by confocal laser scanning microscopy and digital image analysis to stablishing tissue structural stability. Self-fluorescence images from two emission channels were analysed through multifractal parameters, lacunarity, and skeleton attributes. Skeletons features reflected tissue integrity by the number and length of branches, and amount of junctures as key elements in the microarchitecture of the cellular supportive structure, depending on pectin variation. Tissue stability could be described through the integrity of continuity lines given by the connected cell walls and middle lamella. The patterns of structural continuity lines showed properties of a multifractal set. Ripened and exposed tissues addressed lower singularity. Digital Image analysis allowed to determining stability status associated to tissue integrity and structural continuity by establishing singularities in heterogeneous tissue netting when describing senescence progression of fresh cut papaya.

1. Introduction

Papaya (*Carica papaya* L.) is a fruit that originated in the Caribbean coast of Mesoamerica and spread around the world. It is an important crop given its high agricultural yield, sensory attributes, functional properties (Jiménez et al., 2014), and nutritional value, particularly as a good source of carotenes (Schweiggert et al., 2014). The metabolic phenomena that occurs during fruit development and ripening causes sensorial and compositional changes, which intensify after minimal processing in cut fruits ready-to-eat due to abiotic stress (Lara et al., 2014). Abiotic stress tolerance challenges shelf life (Ma et al., 2017). Natural senescence and abiotic stress-induced senescence are associated with structural degradation and cell death (Brummell et al., 2004; Gepstein and Glick, 2013). Cellular structure determines mechanical, optical, chemical, microbiological as well as shelf life stability. Therefore, cells from different type of tissue or different microscale arrangement report diverse physical responses when facing the same adverse factors (Li and Thomas, 2014). Fruit and vegetable living cells are enclosed in a primary cell wall, which consists of a three-dimensional (3D) multicomponent hydrated matrix of cellulose microfibrils, pectin, hemicellulose and structural proteins, surrounded by the middle lamella formed mainly by pectin responsible for cell-to-cell adhesion (Cosgrove, 2016; Goulao and Oliveira, 2008). Cell wall and middle lamella integrity maintain tissue morphology and functionality, depending on ripening and processing (Phothiset and Charoenrein, 2014). Therefore, disruption of cell walls and middle lamella leads to softening, senescence progression and other changes in macro- and micro-structural levels (Cáez-Ramirez et al., 2015). Structural integrity evaluation has been applied to follow processing changes in onions to identify parenchymal cell integrity changes after the application of high pressure (Gonzalez et al., 2010); in strawberry authors studied softening due to pectin depolymerisation by atomic force microscopy (Posé et al., 2015); and in apple, to determine tissue continuity affected by pressure impregnation (S. Wang et al., 2015). Complex fruit microstructures were also assessed using 2D and 3D images. For example, morphometric parameters and linear discriminant analysis described structures in apple mesocarp tissue from Confocal Laser Scanning Microscopy (CLSM) images (Pieczywek and Zdunek, 2012). Other studies on apple, pome, and mango applied 3D X-ray

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microtomography and synchrotron radiation tomography evaluating fruit structures (Cantre et al., 2014; Mebatsion et al., 2009; Mendoza et al., 2007, 2010).

Digital image analysis (DIA) involves the construction of explicit, meaningful, and quantitative descriptions of objects from their images. It is used to describing the material structure by means of the relationships between structures, properties, processes, functions, and other aspects. Processing Several algorithms have been proposed to evaluate image features by describing certain degrees of self-similarity. These include texture image analysis by fractal dimension (F_D), lacunarity, and multifractal attributes (Florindo et al., 2015). F_D is an indicator of the irregularity of the evaluated object or part of it while lacunarity (Λ) can explain image heterogeneity based on the distribution of gaps in the image of an object (Li et al., 2009). Fractal descriptors have been applied to address changes in the mottled skins of bananas (Quevedo et al., 2008) and avocados (Arzate-Vázquez et al., 2011), chilli peppers tissues (Sánchez-Segura et al., 2015), red bayberries (Lu et al., 2011), and others. Multifractal analysis considers the decomposition of imaged objects based on self-similar measures inside the entangled fractal sets or sub-regions, which are characterised by their singularity. It has been useful to classify fruits as in melon skin texture to differentiate varieties (Li et al., 2012) and in apple pore distributions (Mendoza et al., 2010).

Papaya has shown large variations in quality associated to structural modifications in a short period of time (Cáez-Ramirez et al., 2017). Several studies in papaya, apple and strawberry recognize pectin key role in structural modifications (Posé et al., 2015). In this sense, quantifying microstructural changes in a short period of time, under typical consumption conditions, could offer alternative tools to follow up senescence by key compounds variations, such as pectin, common component for fruits and vegetables. The novelty of the present work consists in the application of DIA, specially fractal and multifractal descriptors to explain microstructural changes leading to senescence, and to identify self-similar structures associated to the inherent complexity of a living tissue. The objective of this work was to evaluate senescence advance in fresh-cut papaya at two ripening stages and two locations from papaya core, as a result of abiotic stress prompted after cutting and environmental exposure, by confocal laser scanning microscopy and DIA morphometric parameters.

2. Materials and methods

2.1. Vegetal material and sample preparation

Papaya variety Maradol, in two ripening stages, 65 and 85% (10°–12° Brix, range commonly accepted for processing and consume as ready-to-eat fresh fruit (Cáez-Ramirez et al., 2017) were obtained from a local market in Mexico City, Mexico. Slices of 10 ± 2 mm thick from the middle longitudinal axis of the fruit were obtained. Once cutting was performed, 8-mm-wide rectangular block sections were then cut and divided according to their tissue locations in two parts, as shown in Fig. 1. The external tissue corresponded to the portion near the exocarp or peel, while the internal tissue corresponded to the portion near the core. Tissue location was included as an experimental variable considering the potential microscale arrangement modification, as found for sensitivity variation to bruising by mechanical damage along parenchymal apple tissue (Li and Thomas, 2014). In order to evaluate edible tissue structural changes, peel, seeds and their supporting tissues were removed prior analysis.

Tissue samples, unwrapped and without immersion in sugar solutions, were evaluated immediately after cutting as initial condition and after 4 h of environmental exposure under controlled temperature, light and air composition, simulating typical conditions of a salad bar: 16 ± 2 °C, 60 ± 4% RH; Illumination was carried out by using dichroic halogen lamps, 36°, 3000K, 950 lm, and considering oxygen concentration as a factor that could affect respiration rate and accelerate stress-induced senescence (Ma et al., 2017). Air composition at atmospheric conditions was, 20.8% of oxygen and 0.03% of CO_2 (Martins and de Resende, 2013) was kept by interchanging ambient air by using a cooling automated conditioned air system. The maximum period of environmental exposure time was established according to the US Food and Drug Administration (FDA) recommendations for ready-to-eat food, such as fresh-cut fruit for salad bars (FDA, 2009).

2.2. Image acquisition

A confocal laser scanning microscope, CLSM 710 (Carl Zeiss, Jena, Germany) was used to capture tissue images in optical and fluorescence modes (Espinosa-Velázquez et al., 2016). The laser wavelength excitation applied were 405, 481, 651, and 724 nm. Sample fluorescence emission were compared with standard of the expected compounds: β-carotene, low methoxyl pectin (LMP), and cellulose (Sigma–Aldrich, St. Louise, MO, USA). RGB images (8 bits) were saved with a resolution of 1025×1025 pixels in Tagged Image File format (TIFF) (Corona et al., 2015).

Compounds distributions changes were analysed (Phothiset and Charoenrein, 2014) considering the wavelength of self-fluorescence with the maximum intensity of 255 arbitrary intensity units (AIU) from standard expected compounds in two separate channels. Green channel showed self-fluorescence response between 422 and 522 nm, related to cell wall components: low methoxyl pectin (LMP), with a maximum at 461 nm and cellulose with a maximum at 500 nm; Red channel showed responses between 522 and 622 nm, related to β-carotene distribution changes with a maximum at 598 nm, all of them were in accordance to the literature expected fluorescence peaks values (D'Andrea et al., 2014; Egea et al., 2011).

2.3. Digital image analysis methodology

DIA was conducted by performing pre-processing, segmentation, feature extraction, and classification, as reported in a previous study with plant tissues (Fig. 1) (Perea-Flores et al., 2011). RGB images of 1025×1025 px were analysed by means of the ImageJ v.1.49 software (Rasband, 1997-2015) with consideration of the reference scale. Then, the images from each channel emission were separately evaluated by their grayscale intensity. The image segmentation applied the Li algorithm to extracting the relevant information from the image background (Sankur, 2004). Binary images were then applied to evaluate Λ and multifractal parameters, and to obtain the continuity line in the skeletonised images of the tissues.

The skeletonisation evaluation has the advantage of reducing image objects to a centre line, while preserving their morphological data, such as for evaluating porosity in apples (Mendoza et al., 2007), and to describing the growth of *Rhizopus oligosporus* (Camacho-Diaz et al., 2010), among others. The process of skeletonisation was performed by using the ImageJ Skeleton analyser plugin v.2.0.4 (Fig. 2). Accordingly, the number of branches, total junctions (pixels with more than two neighbours), and three-and-four junctions in the skeleton branches were evaluated. In addition, net length of each branch (NLB), length of the longest path (Maximum distance of the continuous backbone in which branches are bonded) and the relative length of the branch (RLB, relationship between length of the longest path and number of branches) were assessed. All of the above components were considered as descriptors of structural continuity (Arganda-Carreras et al., 2010).