Postharvest Biology and Technology 95 (2014) 1–6

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Investigating non-destructive quantification and characterization of pomegranate fruit internal structure using X-ray computed tomography

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ARTICLE INFO

Article history:
Received 17 January 2014
Accepted 25 March 2014

Keywords:
Fruit quality
Image analysis
Punica granatum L.
Air space
Fruit fractions
Non-destructive testing

ABSTRACT

In this study, X-ray computed tomography (CT) coupled with image analysis techniques was investigated for non-destructive characterization and quantification of internal structure of intact pomegranate fruit (cv. Shani-Yonay). X-ray tomograms of intact fruit were acquired using a V|Tome|X L240 commercial X-ray CT system based on X-ray radiation generated from a source voltage of 200 kV with the electron current set at 100 μA. Two-dimensional (2D) radioscopic images were acquired with a microfocus direct X-ray tube and used to reconstruct three-dimensional (3D) images to quantify volumes occupied by air space, albedo, and arils, using image processing software. The calculated volumes for these fruit fractions were 7.82 ± 1.09, 167.29 ± 16.54, and 182.11 ± 17.04 mL, estimated to contribute 2.22, 46.86 and 50.92% of total fruit volume, respectively. Destructive validation data were similar to non-destructive data, with volumes for albedo and arils of 166.08 ± 14.69 and 170.58 ± 14.25 mL, respectively, contributing 46.07 and 47.32% of total fruit volume. The remaining 6.61% of total fruit volume tested destructively could be due to the presence of air space and calyx. This work has demonstrated the capability of X-ray CT with image analysis as a useful non-destructive technique to study the quantity and distribution of edible and non-edible portions of pomegranate fruit.

1. Introduction

In the last decade, there has been a global expansion of interest in pomegranate fruit (*Punica granatum* L.) production and research. As a result, the global commercial production of pomegranate fruit has increased remarkably (Holland et al., 2009; Citrogold, 2011; Pomegranate Association of South Africa, 2012). The fruit has gained popularity due to increasing consumer awareness of its nutritional value in human diet and potential health benefits attributed to its consumption (Caleb et al., 2013; Fawole and Opara, 2013a). These benefits are linked to high antimicrobial effects and antioxidant contents, contributed by several groups of phytochemicals and polyphenols in the fruit (Kader, 2006; Opara et al., 2009). The fruit is also rich in organic acids, soluble solids, anthocyanins, vitamin C, fatty acids, and mineral elements (Fawole and Opara, 2013a; Caleb et al., 2013).

The increasing interest in pomegranate fruit has coincided with consumer demand for consistent supply of safe, nutritious and traceable food products. This need for quality assured fruit, has spurred the need for developing innovative non-destructive techniques for field and laboratory measurement as well as in-line sorting and grading, based on both external and internal quality attributes (Magwaza et al., 2012a,b, 2013a,b; Herremans et al., 2014). Such non-destructive methods would allow evaluation of fruit morphological structures and internal quality to ensure that all fruit meet minimum levels of acceptance in the market.

Physical properties of fruit, such as the volume of arils and juice content relative to inedible albedo fractions are important in the marketing of pomegranate fruit. Aril yield per fruit is a highly desirable property in the fresh fruit, food processing and beverage industries (Maskan, 2006; Al-Said et al., 2009). However, aril content (number of arils, mass and volume) has been previously reported to vary remarkably among cultivars grown in different parts of the world (Gil et al., 1996; Al-Said et al., 2009; Fawole and

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http://dx.doi.org/10.1016/j.postharvbio.2014.03.014
0925-5214/

Opara, 2013b). Gil et al. (1996) reported that for the 'Mollar' cultivar grown in Spain, arils constituted 57–66%, while aril content of 'Jabal 1', 'Jabal 2', Jabal 3' and a wild cultivar grown in Oman ranged from 50 to 67% (Al-Said et al., 2009). In six cultivars grown in Morocco, aril yield ranged between 53 and 61% (Martínez et al., 2012). High variability in aril yield between different cultivars and fruit of the same cultivar has prompted the need for the industry to develop techniques that can non-destructively visualize and quantify internal structures of the fruit.

A wide range of objective instruments and techniques for quality assessment of external and internal quality of fresh produce has been the subject of numerous reviews and research articles in the literature (Nicolaï et al., 2007; Lorente et al., 2012; Magwaza et al., 2012a, 2014a,b; Alfatni et al., 2013). Techniques such as magnetic resonance imaging (MRI, Lammertyn et al., 2003a; Defraeye et al., 2013), nuclear magnetic resonance (NMR, Zhang and McCarthy, 2013), visible to near infrared spectroscopy (Vis/NIRS, Magwaza et al., 2012b, 2014a), Vis/NIRS-based systems such as hyperspectral imaging (Haff et al., 2013) and optical coherence tomography (OCT, Magwaza et al., 2013a; Verboven et al., 2013) as well as X-ray computed tomography (CT, Kotwaliwale et al., 2014; Herremans et al., 2013) have been explored for non-destructive internal quality evaluation of different horticultural products. In conjunction with image analysis techniques, these imaging techniques have presented many potential avenues for non-destructive quality assessment of fresh fruit and vegetables.

X-ray CT has become one of the well-established research tools suitable for non-destructive analysis of internal morphological characteristics and detect internal defects of fruit and other horticultural products (Lammertyn et al., 2003a,b; Verboven et al., 2008; Herremans et al., 2013). Schatzki et al. (1997) applied X-ray CT to detect internal defects in apples. X-ray CT imaging has also been used to non-destructively characterize fruit physiological disorders such as translucency in pineapples (Haff et al., 2006), core breakdown in pears (Lammertyn et al., 2003a,b), watercore disorder of apples (Herremans et al., 2014). The main advantage of X-ray CT lies in its non-destructive characteristics and its large field of view, which allows scanning the entire sample without sample preparation (Léonard et al., 2008). Several studies comparing the performances of X-ray CT against other imaging techniques such MRI showed that X-ray is more convenient and less costly (Lammertyn et al., 2003a; Yacob et al., 2005; Herremans et al., 2014).

X-ray CT produces a stack of two-dimensional (2D) images. When coupled with different image analysis techniques, X-ray CT allows reconstruction of three-dimensional (3D) images from stacked series of image data allowing characterization of physical and physiological structures of biological materials. Although X-ray CT has recently gained significant attention and provided promising results for determining internal quality of other fresh produce, this technique has not been used on pomegranate fruit. The ability of X-ray CT to differentiate between structures with different density is envisioned to enable visualization, characterization and quantification of internal structures in pomegranate fruit.

The capability of X-ray CT technology together with associated image analysis could open a new avenue for using these techniques in pomegranate breeding programmes to assess cultivars with different proportions of arils to albedo. Considering that aril yield and postharvest storage performance are of particular interest to growers, breeders and postharvest technologists, using this non-destructive approach would allow internal quality evaluation and assessment of postharvest storability of the same fruit. The objectives of the study were (1) to investigate the feasibility of X-ray CT as a non-destructive technique for determining internal structures of pomegranate fruit, and (2) to demonstrate the capability of imaging analysis to characterize internal structures and quantify volumes of edible and non-edible portions.

2. Materials and methods

2.1. Pomegranate fruit supply

The study was conducted using 12 pomegranate fruit (*P. granatum* L. cv. Shani-Yonay) of uniform size (322.35 ± 17.52 g), purchased from a retail supermarket in Stellenbosch, Western Cape Province, South Africa. Based on information provided by the supplier (Freshmark (Pty.) Ltd., Brackenfell, South Africa), the fruit were imported from Israel. All fruit used for analysis of internal structure were free from external defects.

2.2. X-ray computed tomography scanning

X-ray CT images of intact fruit were acquired using a commercial X-ray computed tomography (CT) system (V|Tome|X L240, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorf, Germany) in the Central Analytical Facility at the University of Stellenbosch, South Africa. Various system settings were tested to optimize the scan quality. Optimal CT scans (tomograms) were obtained with an isotropic voxel size of 71.4 μm based on X-ray radiation generated from a source voltage of 200 kV and the electron current was set at 100 μA. Radioscopic images were acquired with a microfocus direct X-ray tube resulting in better signal to noise ratio and less reconstruction artefacts around the edges. The system was equipped with copper filter to cut out low energy X-rays. Pomegranate samples were mounted on a translation stage which was at a fixed physical distance of 250 mm from the X-ray source and 700 mm from the detector. Based on these system settings, the scan resolution was 71.4 μm to accommodate the size and volume of pomegranate samples.

A series of 2D X-ray images were obtained as the fruit was rotated 360 degrees, with 500 milliseconds of exposure time per image, without averaging or skipping of images, hence, recording 2500–3000 images in one rotation, depending on the size of sample and resolution. These image slices, covering the entire sample were acquired using a fully automated data acquisition system and saved onto a processing workstation, operated by system-supplied reconstruction software (Datos|x® 2.1, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorf, Germany). The total scan time for each sample was approximately 1 h.

2.3. Image processing

X-ray image slices were used to reconstruct 3D images for quantification purposes using volume graphics software (VG Studio Max 2.1. and 2.2, Volume Graphics GmbH, Heidelberg, Germany). The X-ray data was first smoothed to remove random noise using Guass (5 × 5) filtering method. Before computing volumes, beam-hardening correction was applied to the dataset to suppress beam hardening artefacts. Beam hardening correction was only applied at 7.5 units due to the use of a copper filter during acquisition. Copper filter suppressed low energy X-rays from the source, reducing beam hardening artefacts.

A representative slice from one of the 12 fruit was selected from the data set to obtain an average grey value for each of the fruit portions (albedo, arils and air spaces). Surface fitting of the data was performed using interactive thresholding of grey values and observing the fit line on a slice view. A typical graph showing grey values for different fruit parts is presented in Fig. 1. The average grey values were from 4,340 to 23,845 units for air spaces, from 23,846 to 44,660 units for albedo and from 44,661 to 56,200 for arils. After thresholding, a surface extraction procedure to non-destructively