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Postharvest Biology and Technology

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

Assessment of bruise volumes in apples using X-ray computed tomography

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ARTICLE INFO

Article history: Received 7 October 2016 Received in revised form 26 January 2017 Accepted 26 January 2017 Available online xxx

Keywords: X-ray computed tomography Bruise Postharvest Image processing Fruit Non-destructive quality inspection

1. Introduction

X-ray computed tomography (CT) is a visualization technique that can render three-dimensional images of the internal structure of agricultural commodities. X-ray CT detects internal structures based on differences in X-ray attenuation resulting from 3D variation in the composition of the material. This makes X-ray CT an excellent technique to visualize the porous structure of products (Cantre et al., 2014; Herremans et al., 2014a, 2014b; Magwaza and Opara, 2014), as X-ray scattering in biological products is limited, while the absorption by air is negligible compared to that by water-rich tissues. As a result, X-ray techniques have been explored to detect several kinds of internal defects in agricultural products. These defects are often detectable with X-ray imaging because they change the tissue density locally or the distribution of the water content (Donis-González et al., 2014; Herremans et al., 2013, 2014a, 2014b; Kotwaliwale et al., 2014; Lammertyn et al., 2003). Bruise damage in apples is one of these defects that has been found to be detectable by using linescanning X-ray equipment (Diener et al., 1970; Schatzki et al., 1997). Besides radiographs of bruised apples from latter research, low quality X-ray CT images of apples showing bruises have been

http://dx.doi.org/10.1016/j.postharvbio.2017.01.013 0925-5214/© 2017 Elsevier B.V. All rights reserved.

ABSTRACT

A non-destructive methodology was developed to automatically detect and quantify bruise volumes in the equatorial region of apples, using X-ray CT images. Grey level threshold values were calculated to segment bruises in 'Jonagold', 'Joly Red' and 'Kanzi' apple fruit using the multi-level Otsu's threshold method. Comparisons were made between the CT-based bruise volume estimates and bruise volume estimates that were based on destructive measurements in combination with simple geometric assumptions. Visualisation of the bruises in both 2 and 3D showed that bruises, resulting from a pendulum impact with a spherical impactor, can be highly irregularly shaped which implies that the bruise volume estimations based on simple geometric assumptions cannot deliver accurate results.

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published in a study not focused on bruise damage (Tollner et al., 1992).

Apple bruises are caused by failure of cells when the internal pressure exceeds a critical value (Baritelle and Hyde, 2001) and can be identified by the flattening, softening or discoloration of the tissue (Pang et al., 1994). Both softening and browning of the tissue are the result of enzymatic reactions taking place after the enzymes are brought into contact with polyphenols due to rupturing of membranes and cell walls (Li and Thomas, 2014).

Until now, most research into the damage susceptibility of fruit made use of destructive measurements to quantify bruise volume (V_B) (Chen and Yazdani, 1991; Lu and Wang, 2007; Pang et al., 1992; Zarifneshat et al., 2010). Similar to a method based on magnetic resonance (MR) images (Lin et al., 2003), a method using X-ray CT images allows for the investigation of the temporal changes of bruises in fruit, since the measurements are non-destructive. Quantifying bruise damage based on X-ray CT images may solve the difficulty to measure the V_B in complex cases including overlapping multiple impacts. A 3D non-destructive CT analysis may also be applied to other agricultural products like tomatoes and strawberries, whose bruises are difficult to quantify by manual measurements, because there is no clear discoloration as in apples (Van Zeebroeck, 2005).

Bollen et al. (1999) propose several formulae for computing V_B , which all assume that a bruise can be approximated as either an ellipsoid or a section of an ellipsoid or sphere made by a flat or

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convexly shaped cutting plane. A 3D visualization technique may be used to assess the appropriateness of such formulae.

Bollen et al. (1999) and Lin et al. (2003) compared several destructive V_B estimation methods with V_B estimates derived from RGB and MR images, respectively. The RGB images were made from several parallel slices through the bruises. The brown area in each slice was summed up to calculate the total bruise volume. According to Bollen et al. (1999), the enclosed volume formula with elliptical bruise shape assumption was the most appropriate one for estimating $V_{\rm B}$ only when the bruise widths could be measured across both perpendicular directions and in case both small and large bruises were investigated. In the work of Lin et al. (2003), MR images were made of intact bruised apples. Bruises were automatically segmented from these MR images in a predefined window containing the bruise. The reconstructed bruise volumes were compared to two conventional destructive V_B estimation methods, namely the full-depth method and ellipsoid method with circular bruise shape assumption. They concluded that both these methods yield reasonable bruise volume estimates. However, bruise volume estimates using MR were considered more accurate

In this work, we develop a method to quantify bruise volume (V_B) from X-ray computed tomography (CT) measurements of bruised apples and we compare the presented X-ray CT-based V_B estimation method to destructive V_B estimation methods. Besides elaborating a new non-destructive technique for bruise size quantification, the high quality X-ray CT images produced in this work permit the observation of bruise shape and features such as cracks, and can help understand spatial changes in water content and tissue density in (and near) the damaged tissue. This is the first study that investigates these characteristics of apple impact bruises of commercially realistic size in 3D.

2. Materials and methods

2.1. Apple samples

The experiment was carried out on apples (*Malus* × *domestica* Borkh.) of three different cultivars, namely 'Jonagold', 'Joly Red' and 'Kanzi'. These three cultivars are commercial cultivars in Belgium and were chosen for their availability and good storability, and to evaluate bruises in different cultivars. Fifteen apples per cultivar were picked at the end of September 2014 and stored under controlled atmosphere conditions (VCBT, Heverlee, Belgium) for approximately 15 weeks. 'Jonagold' and 'Joly Red' were stored at $1 \,^{\circ}$ C, $1\% \,O_2$ and $3\% \,CO_2$, while 'Kanzi' was stored at $4 \,^{\circ}$ C, $2\% \,O_2$ and $0.7\% \,CO_2$ since these are the optimal storage conditions for long term storage of these cultivars (VCBT, 2014). One day before bruising the apples were taken out of storage, where after they were left at room temperature during the remainder of the entire experiment.

2.2. Pendulum measurements

Apple fruit were damaged in a controlled way by releasing the rod of a pendulum device (Fig. 1) onto three different places on the equator of the apple that was mounted to the anvil by a rubber band. Five different release angles were used resulting in a wide range of impact energies and consequently bruise volumes. The pendulum device has a spherical aluminum impactor (radius of curvature: 15 mm) as previously described by Diels et al. (2016). The encoder (RON 275-TTLx5, Heidenhain, Traunreut, Germany) and force sensor mounted on the device allow for measuring the deformation and force during impact, respectively. Impact energy was calculated as the area under the loading curve.



Fig. 1. Schematic representation of the pendulum device.

2.3. X-ray computed tomography

2.3.1. Set-up

Five days after impacting the 'Jonagold' and 'Joly Red' apples and four days after impacting the 'Kanzi' apples, each apple was scanned with a Microfocus Computer Tomography Tomohawk system (AEA Technology, Harwell, UK). This long time interval between bruising and imaging was chosen to let the bruises stabilise and to allow for plenty moisture removal from the bruises which could enhance the difference in X-ray absorption between bruised and healthy tissue. The X-ray source was operated at a voltage of 80 kV and a current of 439 mA, and the shadow image was acquired by a CCD camera (Adimec MX12P). The apple was placed in a styrofoam box on a rotation stage that rotated over 187.8° in steps of 0.3°. The total scanning time for one apple was approximately 30 min.

2.3.2. Image processing

NRecon (1.6.9.18, Bruker microCT, Kontich, Belgium) was used to reconstruct cross-sectional images from the X-ray transmission images using the filtered back projection method. The reconstruction resulted in 3D stacks of about 900 8-bit grevscale images (image plane perpendicular to the stem-calyx axis, pixel intensity values between 0 and 255) covering the entire apple. During reconstruction, the data were downsampled with a factor of two to reduce the computational load during further image processing in Matlab (R2014a, The MathWorks Inc., Nattick, MA). This downsampling procedure led to an isotropic voxel size of $210.79^3 \,\mu m^3$. Further steps to process the CT data are graphically represented in Fig. 2 on a 2D cross-sectional image of a stack. First, a global image threshold was calculated per stack using Otsu's method (Otsu, 1979). This threshold was used to separate the apple from the background (Fig. 2a). Holes in the image stacks were filled and small objects originating from the surrounding styrofoam were removed using an image morphological opening (Fig. 2b). In order to automatically select a region of interest (ROI) (see Fig. 2c) that doesn't includes the core, but does include all the bruises made on the equator of the apple, the centroid location of the segmented apple was used as the centre of the circle (radius 17 mm) defining a cylinder that was excluded from the ROI. Thereafter, the multilevel Otsu's method (Otsu, 1979) was used to calculate several thresholds per apple that converted the grevscale image stacks into image stacks with several discrete levels (Fig. 2d), whereby the discrete level corresponding to regions with intensities that were

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