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Microstructural characterisation of commercial kiwifruit cultivars using X-ray micro computed tomography

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ABSTRACT

The skin is the physical barrier between the fruit and the environment in which it develops. Environmental conditions during fruit development have a large influence on fruit quality, both at the time of harvest and during subsequent storage. It is hypothesised that some features of the skin and sub-epidermal tissues could provide information about the past growing conditions to which the fruit was exposed and therefore be of predictive value for storage quality. In this study, five commercial kiwifruit cultivars ('Hayward', 'Hort16A', 'G3', 'G9' and 'G14') were studied, and 'Hayward' fruit were manipulated during growth with different cultural practices. After harvest at horticultural maturity, X-ray micro computed tomography (μCT) was used to investigate features of the skin and the immediate parenchyma tissue. Despite orchard management practices (crop load and girdling) being observed to effect macro fruit quality parameters (mass, firmness, SSC, and DM), differences in microstructure (e.g. porosity) caused by these practices were not observed. However, porosity and pore size were found to be highly variable between cultivars. The thickness of dense sub-epidermal tissue could be readily measured and the 3-D distribution of raphide bundles was visible as high density particles distributed within the parenchyma. Overall, μCT was found to be a powerful technique to explore fruit epidermal and sub-epidermal structures in three dimensions at a micro level. However, the length of time required for data capture and analysis and the large number of samples required to overcome natural variation within horticultural products need to be considered. Future work may define the impact of differences in porosity or sub-epidermal anatomy on kiwifruit physiology (e.g. firmness change or sensitivity to low oxygen storage atmospheres). With this information, μCT could be used as a screening tool during plant breeding, or to determine the response to agronomic treatments, without conducting lengthy storage trials.

1. Introduction

Kiwifruit quality and storability are known to be affected by environmental factors during growth and development. Sunlight exposure during development can enhance pigment development, stimulate fruit maturation, enhance skin colouration and result in firmer fruit with less incidence of rot in storage (Tombesi et al., 1993) and better fruit storability (Antognozzi et al., 1995). This sunlight exposure is directly influenced by canopy density (Snelgar et al., 1998). Similarly, application of trunk girdling has been applied to result in both increased fruit mass and dry matter, depending on timing, while fruit crop load is known to affect final fruit size (Patterson and Currie, 2011). Further studies in manipulating appropriate growth conditions may also provide an insight into alleviating low temperature breakdown (LTB) in kiwifruit during storage. It is hypothesised that membrane deterioration plays a critical role during ripening and LTB development (Marangoni et al., 1996). Membrane deterioration affects porosity either through filling of pores with cellular fluid as a result of cellular breakdown (Kuroki et al., 2004), or creation of larger air spaces as a result of tissue collapse (Herremans et al., 2013a). This membrane deterioration leading to changes in porosity is also suggested to play a critical role in chilling injury in mangoes. Chilling injury damages cell walls, causing cell wall disassembly and cell leakage (Han et al., 2006). Thus, cellular fluid may fill the intercellular gas-filled spaces, completely separating the pores (Narain et al., 1998).

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The decrease in pore size and pore connectivity in cucumbers during ripening also suggests leakage of intracellular substance into the gas-filled intercellular space (Kuroki et al., 2004).

The importance of a pore network and void volume fraction for gas exchange in apples and pears has been extensively studied (Herremans et al., 2013a,b; Ho et al., 2013a,b; Verboven et al., 2008). For example, some apple cultivars (e.g., 'Braeburn' and 'Cripps Pink') develop internal browning disorders in storage (Herremans et al., 2013a; James and Jobling, 2009), while others (e.g. 'Granny Smith') tend to develop skin related injuries (Fan et al., 1999). Contemporary research suggests that these differences in storability and susceptibility to disorders are associated with skin and flesh properties that influence gas diffusion within the fruit tissue (Ho et al., 2009, 2010, 2011) including differences in porous microstructure (Ho et al., 2013b), with those differences strongly affecting optimal storage conditions (Ho et al., 2013c). Whether such relationships, of flesh properties to optimal storage conditions, exist for kiwifruit is currently unknown.

Techniques to visualise and quantify microstructure have been used to answer valid research questions and to provide a better understanding on the relation of microstructure to quality characteristics during storage. Unlike light and electron microscopy, non-destructive techniques provide a more accurate depiction of the microstructure since cells and tissues remain intact and undamaged and no tedious sample preparation is required (Veraverbeke et al., 2001; Musse et al., 2010). One such non-destructive technique is X-ray micro computed tomography (μCT), a three-dimensional visualisation technique that creates an image based on X-ray attenuation within the sample. The difference in X-ray attenuation of different materials creates contrast to differentiate low density and high density materials (Karathanasis and Hajek, 1996). In comparison with other non-destructive techniques, μCT has excellent spatial resolution that resolves intercellular space down to the submicron range, and depth of penetration, allowing subsurface features to be studied. This technique can help to characterise and understand foods by measuring cell size and shape, void space and spatial distribution (Lim and Barigou, 2004). The use of μCT has allowed visualisation of the fruit void network architecture, showing a volume fraction of 5.1% and 23% for 'Conference' pear and 'Jonagold' apple cortex tissue, respectively (Verboven et al., 2008; Mendoza et al., 2007). This technique was also successfully used in 3-D analysis of raphides in rose peduncles and *Lotus miyakojimae* seeds (Matsushima et al., 2012; Yamauchi et al., 2013).

The main objective of this study was to gain insights into the potential differences in 3-D microstructural properties of commercial kiwifruit genotypes. μCT was used as a tool to explore the fruit peel and tissue from five cultivars. Additionally, treatments to deliberately manipulate 'Hayward' kiwifruit during growth via altering crop load and application of girdling were applied as a preliminary study to investigate if any of the crop manipulation techniques would influence cellular arrangement and density within the fruit. Insights from this study were expected to reveal relationships between the potential for storage capability of cultivars to changes in cellular arrangement and density as affected by environmental factors during growth and development.

2. Materials and methods

2.1. Plant material and treatment manipulation

Currently there are five commercial cultivars exported from New Zealand: green cultivars 'Hayward' (*A. deliciosa*, Zespri™ Green) and 'G14' (*A. deliciosa* × *chinensis*, Zespri™ Sweet Green), and yellow fleshed cultivars (all *A. chinensis*) 'Hort16A' (Zespri™ Gold), 'G3' (Zespri™ Sun Gold) and 'G9' (Zespri™ Charm). Single tray samples of 'G14', 'Hort16A', 'G3', and 'G9' were obtained from commercial orchards in New Zealand and delivered via airfreight to KU Leuven, Belgium, in June 2013. Five fruit from each cultivar were scanned for X-ray μCT. At the time of the measurements, fruit could be considered as eating ripe.

In a growing condition manipulation experiment conducted in the 2013 harvest season, a mature 'Hayward' kiwifruit orchard trained on a pergola system and located in Te Puna, Bay of Plenty, New Zealand was used. This experiment consisted of a 2 × 2 matrix of plant manipulation treatments, fruit crop load (36 or 43 t/ha) and use (or not) of trunk girdling (Patterson and Currie, 2011). Crop thinning occurred on 4–5 Jan 2013 and trunk girdling occurred on 10 Dec 2012 and 2 Feb 2013. A single sample of 15 fruit was harvested from each of the 4 treatments on 7 May 2013 and delivered by airfreight to KU Leuven. Five fruit from each treatment were measured in June at which time the fruit could be considered eating ripe. Commercial harvest of this experiment occurred on 15 May 2013 at which time 90 fruit samples were collected and subsequently analysed for at harvest quality (mass, soluble solids content, firmness and % dry matter).

2.2. Micro X-ray CT imaging

Kiwifruit tissue samples measuring 5 mm × 5 mm × 10 mm including the skin were excised from the equatorial region and wrapped in parafilm to prevent dehydration prior to subsequent CT scanning. A Skyscan 1172 high resolution μCT scanner (Bruker, Kontich, Belgium) was used for acquiring projection images with a source power of 10 W at 60 kV and 167 μA. Each projection image was averaged from three frames with each frame taken with an exposure time of 295 ms. The sample was rotated on the stage at an increment of 0.4° until a rotation angle of 204.8° (180° + fan beam angle) was completed generating 512 shadow projections with a pixel size of 4.87 μm. Cross section slices were generated from the shadow projections using the Feldkamp reconstruction algorithm (Feldkamp et al., 1984) implemented in Nrecon 1.6.5.8 software (http://www.skyscan.be/next/downloads.htm). Reconstruction parameters for beam hardening, ring artefact reduction and smoothing were set to 35, 8 and 2, respectively. The dynamic range or linear attenuation range was limited to 0–0.0854 to generate an 8-bit bitmap grayscale cross section slice.

2.3. Image processing

Undamaged and intact μCT tomographs measuring 2 mm × 2 mm × 2 mm, left after virtual cropping, were utilised for 3-D image processing and analysis (Fig. 1A). Each image was segmented with a manual threshold (Fig. 1B) to obtain the pores and cell assembly, followed by individual pore labelling (Fig. 1C) and 3-D image rendering of the pore network (Figs. 1D and 2B). A spatial graph representation of the skeleton using TEASAR algorithm (Sato et al., 2000) was made to show the essential geometry and local thickness of the pore network (Fig. 2C). Microstructural parameters (Table 1) as described by Herremans et al. (2013a) were analysed. Raphide bundles were revealed as a substantial number of high density oblate spheroid particles in the epidermal and sub-epidermal regions. These particles were segmented with a manual threshold and labelled prior to surface rendering and subsequent 3-D analysis. Image processing and 3-D analysis were performed using CTAn 1.12 (Bruker microCT, Kontich, Belgium) and Avizo 7.1 (VSG, Bordeaux, France). Results of the quality measurements and microstructural analysis were subjected to analysis of variance using PROC GLM procedure in SAS 9.3 (SAS Institute, Cary, NC, USA).