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Textural, biochemical and micro-structural changes in mesocarp tissue of imported avocado from Peru during ripening

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Abstract

Avocado (*Persea americana* Mill.) fruit is a valuable product and notorious for the difficulties encountered in determining quality. Typically the degree of ripeness of many climacteric fruits, such as avocado, is measured by assessing flesh firmness. The aim of the presented work was to elucidate the temporal and spatial changes in texture, biochemistry and micro-structure in different avocado tissues from the same fruit. Fruit were first treated with ethylene and then ripened at 12°C. Samples were taken four times over 10 days storage. Maximum load and viscoelasticity of horizontally-cut slices from fruit (n = 24) imported from Peru were measured during ripening. These texture parameters were measured using an Instron 5542 universal testing machine fitted with a 500N or 5N load cell. Non-structural carbohydrates (NSCs; *viz.* sucrose, mannoheptulose, perseitol) and fatty acid methyl esters from the same samples were identified and quantified using standard HPLC coupled to evaporative light scattering detection and gas chromatography coupled to flame ionisation detection, respectively. Samples taken from adjacent mesocarp tissue slices were examined: Each specimen included the sclerenchymatic exocarp, fleshy mesocarp and endocarp including the coat of the seed. An analysis of variance was performed to elucidate the change of parameters during fruit ripening. NSC content and textural properties changed during ripening and showed spatial heterogeneity within individual fruit. Micro-structural changes were evident during the latter stages of ripening and coincided with fruit softening and degradation in mannoheptulose. These findings might be used to enhance quality monitoring of imported avocado fruit.

INTRODUCTION

Avocado (*Persea americana* Mill.) fruit are increasingly valued by consumers for not only their unique flavour and texture, but also for their reported health benefits (Ashton et al., 2006; Ding et al., 2007; Henrotin, 2008). Avocado mesocarp tissue has inherently high concentrations of unsaturated fatty acids (FAs), and seven carbon (C7) carbohydrates. Research has shown that diets rich in avocado fruit pulp may contribute to lowering cholesterol levels (Ledesma et al., 1996).

Commercially, the quality of avocado fruit is rated according to size, estimated oil content (dry matter), absence of defects and firmness (OECD, 2004). The degree of ripeness of many climacteric fruits like avocado can be correlated with fruit firmness. Oil content is used as an indicator of fruit maturity, and thus commonly defines the optimum harvest period. Lipids accumulate during avocado fruit development and constitute ca. 70% of dry matter at maturity.

The fact that fruit often do not soften uniformly due to physiological gradients within the fruit is generally accepted (Schroeder, 1985). Past research has looked at the changes in dry matter and pigments in various spatial locations within avocado fruit during postharvest ripening (Schroeder, 1985; Ashton et al., 2006), but has not considered

the distribution of carbohydrates and FAs. There is little published information on the interdependence and spatial distribution of quality-related compounds and textural parameters in avocado mesocarp tissue during ripening. Therefore, in this research the FA composition, non-structural carbohydrate (NSC) content and texture of avocado fruit were determined in a number of predefined localities within individual fruit during ripening and analyzed. For textural analysis a stress-relaxation test was applied, which is suitable for measuring viscoelastic properties of tissues (Sakurai and Nevins, 1992). The test procedure was optimized to minimize tissue damage to allow biochemical analyses on the same avocado mesocarp tissue.

Past structural examination of avocado tissue concentrated on e.g. skin structure (Schroeder, 1950), idioblasts (Platt and Thomson, 1992), disorders (Kaiser, 1993) and texture evaluation in general (Harker et al., 1997). A temporal examination of the ultrastructure of mesocarp cell walls during fruit ripening was previously presented in conjunction with respiration and ethylene-production measurements (Platt-Aloia et al., 1980; Platt-Aloia and Thomson, 1981).

The present study aimed at linking spatio-temporal textural and physiological changes in ripening avocado fruit and compare them to micro-structural changes to possibly assist quality monitoring of imported avocado fruit.

MATERIALS AND METHODS

Methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate, sucrose, D-glucose, D-fructose and D-mannoheptulose standards were purchased from Sigma (Dorset, UK). Perseitol (D-glycero-D-galacto-heptitol) was obtained from Industrial Research Ltd. (IRL - Fine Chemicals, New Zealand). All other chemicals used were of analytical grade and purchased from Fisher Scientific Chemicals (Leics., UK).

All imported avocado 'Hass' fruit (size code 16; OECD, 2004) were supplied by M. W. Mack Ltd. (Kent, UK; 24/06/2007). The experiment was conducted on unripe fruit originating from Peru (Camposol S.A.). Produce was held in the laboratory overnight at 6°C, after which fruit were placed into two hermetically sealed polypropylene boxes (ca. 32 cm x 14.5 cm x 28 cm). Only 6 fruit to be measured the same day (day 0 = "before") were held aside. Controlled ripening was initiated using exogenous ethylene (100.3 $\mu L\ L^{-1}$; British Oxygen Company (BOC) Gases Ltd., Surrey, UK; Terry et al., 2007) and avocado fruit kept overnight at ambient temperature. The ethylene concentrations in boxes were monitored and were found to decline from 96.7 $\mu L\ L^{-1}$ to 61.9 – 41.8 $\mu L\ L^{-1}$. After 24 h the boxes containing the avocado fruit were opened and transferred to 12°C for subsequent ripening. Fruit subsamples (n = 6) were selected during 10 days of storage (day 3 = "early", day 7 = "later", day 10 = "store end": n = 3 randomly from each box).

Each fruit was cut manually using a sharp knife. Initially, each avocado fruit was cut vertically with the stem facing the operator. The right half of the avocado fruit was discarded. The left half was placed with the flat surface downwards and a 1 cm-thick slice cut horizontally from the stem end; this piece was discarded. Thereafter, ten slices were sequentially cut starting from the apical end (near stem) towards the basal end of the fruit. Alternate slices were selected resulting in five ca. 1 cm-thick slices for textural measurement, which took place immediately after cutting. The seed (if appropriate) and skin were removed prior to texture tests.

Textural evaluation

All texture tests were performed on an uni-axial testing machine (5542, Instron, MA) equipped with calibrated 500 N or 5 N load cell, depending on the maximum loads recorded on the test day. Textural tests for each slice from top, middle and bottom of the fruit were done vertically such that the planar surface of the slice was in contact with the

specimen stage. Three replicate tests were carried out on each tissue slice. The machine was programmed (Bluehill 2, version 2.11, Instron) such that the probe indented the sample to a depth of 0.6 mm with the cross head speed set at 10 mm min^{-1}, and then held at this position for 1 min (Sakurai and Nevins, 1992). A cylindrical probe of 6 mm diameter was used. At least 5 g of the measured tissue were snap-frozen in liquid nitrogen immediately after testing and stored at -40°C to preserve them prior to biochemical analysis.

The apparent elasticity modulus was calculated. The minimum relaxation time, which relates to viscosity of the sample, was calculated using Matlab (7.0.4.365 R14 Service Pack 2, The MathWorks Inc., MA) according to Landahl et al. (submitted).

Frozen tissue samples of top, middle and bottom slices (n = 72) were freeze-dried in a Christ freeze dryer with cooling trap ALPHA (100 400, Osterode, Germany) for 7 days at 0.05 hPa. Dry mass (DM) was recorded and samples returned to -40°C until further processing. Lyophilized mesocarp tissue was ground, weighed (to 1 g) and a hexane extraction performed as described by Meyer and Terry (2008) to obtain oil and powder residue from the same sample.

Fatty acid composition

Fatty acid methyl esters (FAMEs) were produced according to International Olive Oil Council (IOOC, 2001) modified by Meyer and Terry (2008). Briefly, 0.2 ml of methanolic KOH (2 M) was added to 0.1 g avocado oil extract in 2 mL hexane. The mixture was shaken vigorously for 30 s and left to stratify until the upper layer became clear. The hexane layer containing the methyl esters was decanted and kept for no more than 12h at 5°C until needed. This solution was diluted 1:100 (v/v) with hexane immediately before injection into an Agilent 6890N GC (Agilent Technologies, Cheshire, UK) equipped with a G1540N flame ionization detector (FID) and a 7683B autosampler. The FA composition was calculated as percentage of total of the five detected FAMEs, after comparison of sample peak areas and peak areas of a mixed standard of known composition.

NSC identification and quantification

NSCs were extracted and quantified as described previously (Davis et al., 2007) with some modification (Meyer and Terry, 2008). In summary, NSC content in the avocado extracts was determined using a high performance liquid chromatography (HPLC) system comprising a P580 pump, and GINA 50 autosampler (Dionex, CA). Twenty µL of diluted avocado extract (1:10; v/v), was injected onto a Rezex RCM-Monosaccharide Ca^{+} (8%) column, 300 x 7.8 mm size (00H-0130-K0, Phenomenex, CA) with a Carbo-Ca^{2+} guard column, 4 x 3 mm size (AJ0-4493, Phenomenex). The mobile phase was set at a flow rate of 0.6 mL min^{-1}. Column temperature was held at 75°C using a column oven (STH 585, Dionex). Eluted NSCs were detected by an evaporative light scattering detector (ELSD 2420, Waters, MA) connected to the HPLC system. Mixed standards of known composition and concentration were used and presence of each NSC quantified by comparing sample peak areas to the standards. The minimum quantification limit for the NSCs was 20 mg g^{-1} powder residue. Results below quantification limit were set at zero.

Light Microscopy

All samples were stored in formol acetic acid (FAA), a botanical fixative, for at least ten days. (FAA = 63 v/v ethanol, 27 v/v water, 5 v/v formalin (36 % formaldehyde), 5 v/v glacial acetic acid). The avocado pieces were taken from a radial slice immediately below the middle slice. Each specimen included the sclerenchymatic exocarp, fleshy mesocarp and endocarp including the coat of the seed.

Samples were examined after embedding in paraffin wax. The fixed samples were placed into individually numbered histology cassettes and placed into an automated tissue

processor (Leica microsystems, Wetzlar, Germany). This processor dehydrates the samples through a series of alcohols, from ethanol (70 %) to absolute ethanol and then into xylene, until finally the samples become impregnated with molten wax. The avocado chunks were then oriented into metal moulds, filled with molten wax and allowed to solidify on a cool-plate. These blocks were then trimmed of excess wax, cooled on a chiller plate and sectioned on a rotary microtome (2035, Leica microsystems) at 5 µm, using disposable steel blades. The wax sections were floated on a bath of distilled water at 43°C, mounted onto glass slides and dried horizontally on a hot plate at approximately 37°C. These were then stored in a 50°C incubator until they were stained.

The slides were de-waxed in xylene, then re-hydrated using a series of alcohols and then stained with periodic acid and Schiff's reagent and counter-stained using light green (PAS-LG). The sections were then protected by DPX mounting medium (Fluka Chemie AG, Neu-Ulm, Germany) and a cover slip applied. The slides were then examined by bright field microscopy, using a DMRB microscope (Leica microsystems). Observations were recorded using a digital camera (KY-F75U, Victor Company of Japan, Ltd. (JVC), Japan) with KY-LINK software (JVC).

Statistical analysis

All statistical analyses were carried out using Genstat for Windows (vers. 10, VSN International Ltd., Herts., UK). Data were subjected to analysis of variance (ANOVA). Least significant difference values (LSD $P = 0.05$) were calculated for mean separation. Storage-duration and the vertical location of the tissue slice within the fruit were the treatment factors in the ANOVA. The data were grouped by fruit (blocks n = 24).

RESULTS AND DISCUSSION

The ultimate aim of this study was to describe the spatio-temporal ripening process of avocado fruit with respect to not only textural properties, but also nutritional parameters by means of biochemical examination of the same samples. This approach provided a greater insight into the relationship between both physical and biochemical changes within different regions of the fruit mesocarp during ripening. In the present study, spatial variability was observed for all measured parameters in mesocarp tissue.

Fatty acid composition

In general, the composition of FAMEs was not affected by storage. In order of dominance, oleic acid, palmitic acid, linoleic acid, palmitoleic acid and linolenic acid were detected. Some substantial variation in the spatial distribution of FAMEs was observed in avocado fruit. Palmitic, palmitoleic and linoleic acid were highest in the basal region (Table 1). Also linolenic acid was more abundant towards the basal region in the fruit, but oleic acid content declined towards the bottom end of the avocado fruit (Tables 1 & 2).

Avocado is classed as a lipid-based fruit due to its high content of oil. In most avocado varieties maturity is based on lipid metabolism and rapid oil accumulation can be found at the onset of maturity (Bower and Cutting, 1988). The FA composition of avocado oil has been shown before to remain relatively unchanged during postharvest ripening (Meyer and Terry, 2008). The FAME composition reported herein was rather unusual in that others had found a higher oleic acid content as well as a smaller palmitic and palmitoleic acid content (Meyer and Terry, 2008; Landahl et al., submitted). This could be related to seasonal variability or the growing conditions of the fruit.

NSC identification and quantification

Sucrose content was highest at early storage and was generally higher towards the basal slice, perseitol distribution was similar as it was highest at the beginning of storage and lowest in the middle of the fruit (Tables 1 & 3). In contrast, mannoheptulose content

showed highest values in the mesocarp tissue near the stem and decreased during storage (Fig. 1).

Sucrose, mannoheptulose and perseitol were the most abundant free NSCs in avocado mesocarp tissue as previously reported (Liu et al., 1999; Cowan, 2004; Bertling and Bower, 2005; Meyer and Terry, 2008). Sucrose is not considered as important for judging postharvest quality (Bertling and Bower, 2005). In metabolic processes of avocado fruit the C7 carbohydrates are most important (Liu et al., 1999; Liu et al., 2002; Cowan, 2004). The decrease found in the mannoheptulose and the decrease in perseitol during storage are in agreement with that reported elsewhere (Shaw et al., 1980; Liu et al., 1999; Bertling and Bower, 2005; Meyer and Terry, 2008).

Distribution of sucrose and perseitol concentrations within the fruit were generally higher towards the base of the fruit. Considerable heterogeneity of mannoheptulose concentration was found in the fruit tissue from stem-end to base with the largest concentration in the apical region. The higher mannoheptulose levels measured at the stem-end of the avocado fruit may indicate an accumulation of the C7 sugar at the entry point into the fruit.

Textural evaluation

Significant changes in elasticity and minimum relaxation time of avocado mesocarp tissue were found during storage and spatial location of tissue within the fruit (Table 2). Apparent elasticity declined concomitantly during storage whilst minimum relaxation time did not follow an exponential decline. The highest elasticity values were recorded in the stem-end region of the fruit. At the beginning of storage the difference in apparent elasticity between the stem-end region and the middle / basal region was greatest, yet values converged during ripening. In contrast, differences in viscosity between slices remained, but were small.

As expected, textural parameters decreased and thus, were appropriate to describe effect of ripening. Before and during early storage significant spatial variation was found in textural values. Thus, the stress-relaxation parameters were well suited to describe avocado fruit ripening, if care is taken to measure similar locations in the fruit especially during early storage. Fruit softening is based on the biochemical change in constituents within the fruit tissue (Bower and Cutting, 1988; Sakurai and Nevins, 1997). The softening also seemed to be represented in the change in the tissue micro-structure, where a development from tightly attached cells towards detached cells with swollen cell walls was visible.

Light Microscopy

In agreement with Platt-Aloia et al. (1980), the tissue of unripe avocados consisted of homogenous angular cells and very small intercellular spaces, which indicates good adhesion and fully turgid cells. In addition, the idioblasts were not prominent. After 10 days ripening the cells were round and appeared plasmolysed. Idioblasts were prominent and approximately the same size as the mesocarp cells, so it can be assumed that adhesion was low and oil could be liberated easily upon tissue maceration. The idioblast cells could be easily identified in ripe avocado tissue and appeared to be the same irregular shape as reported by Platt and Thomson (1992).

At day 0 all tissues showed tightly packed cells of different organisation according to the position from seed to fruit skin. After 10 days a clear and definable gap was visible between exocarp and mesocarp, where cells had separated. This corresponds to the observation that skin can be easily peeled off from the mesocarp tissue of ripe avocado and may be related to breakdown of the middle lamella (Platt-Aloia et al., 1980).

Platt-Aloia et al. (1980) found that in postclimacteric very soft fruit the cell walls itself degraded. Cell wall hydrolysis seemed to occur due to an increase in polugalacturonase and cellulase during ripening, which appeared to be synthesized by

exocytosis indicated by vesiculation of the rough endoplasmatic reticulum and its apparent fusion with the plasma membrane (Platt-Aloia and Thomson, 1981). In the present study, tissue breakdown was visible in the endocarp close to the seed. It is unclear if this was due to mechanical damage during removal of the seed.

CONCLUSIONS

By examining the spatial and temporal change in biochemistry, micro-structure and texture during ripening it was evident that avocado fruit do not ripen uniformly such that quality analysis needs to take position of test into account.

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Tables

Table 1 Mean values at main effect of position within 24 Peruvian avocado fruit.

slice	top	middle	bottom	LSD
sucrose/DM	8.58	6.77	10.13	2.623
perseitol/DM	8.40	6.46	12.66	1.951
palmitic acid	26.63	27.61	28.89	0.546
palmitoleic a.	12.85	12.65	13.20	0.264
oleic acid	45.94	42.38	39.92	0.875
linoleic acid	13.60	16.00	16.62	1.069

DM: dry mass. Sucrose and perseitol [mg g^{-1} DM]. Palmitic, palmitoleic, oleic, linoleic acid [% total FAMEs].

Table 2 Mean values at storage time and individual location within 24 Peruvian avocados.

storage × slice		top	middle	bottom	LSD (P = 0.05)	
apparent	before	1546	1027	1049	218.1	storage × slice
elasticity	early	1298	971	981	188.9	at the same
	later	495	262	340		level of storage
	store end	152	125	138		
min.	before	34.68	33.35	32.60	3.032	storage × slice
relaxation	early	32.17	30.85	32.12	1.413	at the same
time	later	19.09	18.83	18.44		level of storage
	store end	15.60	16.85	16.66		
linolenic	before	0.732	1.095	1.353	0.4149	storage × slice
acid	early	0.700	1.274	1.483	0.3147	at the same
	later	1.328	1.471	1.309		level of storage
	store end	1.167	1.609	1.352		

Apparent elasticity [N mm^{-2}], min. relaxation time [s], linolenic acid [% total FAMEs].

Table 3 Mean values at main effect of storage measured on 24 Peruvian avocado fruit.

storage	before	early	later	store end	LSD
sucrose/DM	5.25	13.57	5.72	9.42	5.403
perseitol/DM	12.9	13.18	4.14	6.48	3.781

DM: dry mass. Sucrose and perseitol [mg g^{-1} DM].

Figure

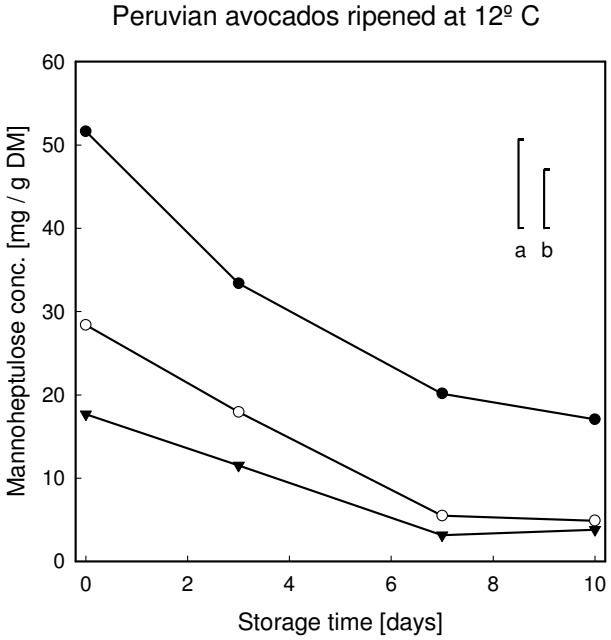

Fig. 1 Mean values of the mannoheptulose concentration for 24 avocado fruit 'Hass'. ● Top slice, ○ middle slice, ▼ bottom slice (each point n = 6). LSD (P = 0.05) a) for storage × slice, b) for slice at the same level of storage.