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Spatial and Temporal Analysis of Textural and Biochemical Changes of Imported Avocado cv. Hass during Fruit Ripening

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The ripeness degree of climacteric fruits, such as avocado (*Persea americana* Mill.), can be correlated with rheological properties. However, there remains little information on not only the postharvest changes in texture of avocado fruit from different origins but also the spatial variation within fruit. In addition, the relationship between changes in texture and composition of fatty acids and major nonstructural carbohydrates (NSCs) of fruit tissue during ripening is unknown. The texture of different horizontally cut slices from individual fruits within a consignment was measured during ripening using a previously unreported technique. The composition of fatty acids and NSCs in fruit mesocarp tissue was determined. The composition of fatty acids and oil and dry matter contents varied significantly according to origin. Significant changes in texture, mannoheptulose and perseitol contents, and linoleic acid percentage were found in avocado fruit flesh during ripening. Spatial variation within fruit was detected in both textural and biochemical characteristics.

KEYWORDS: Fatty acids; *Persea americana*; postharvest; sugars

INTRODUCTION

Avocado (*Persea americana* Mill.) fruits are increasingly valued by consumers for not only their unique flavor and texture but also their reported health benefits (*1*−*3*). Avocado mesocarp tissue has inherently high concentrations of unsaturated fatty acids and seven-carbon (C7) carbohydrates. Research has shown that diets rich in avocado fruit flesh may contribute to lowering cholesterol levels (*4*). D-Mannoheptulose (*5*) and its reduced form polyol, perseitol (*6*), have been reported to have anticancer activity.

Commercially, the quality of avocado fruit is rated according to size, estimated oil content (dry matter), absence of defects, and firmness (*7*). 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 composition of fatty acids of avocado oil has been shown to remain relatively unchanged during postharvest ripening (*8*).

The fact that fruit often do not soften uniformly due to physiological gradients within the fruit is generally accepted (*9*). Past research has looked at the changes in dry matter and pigments in various spatial locations within avocado fruit during postharvest ripening (*1*, *9*), but has not considered the distribution of carbohydrates and fatty acids. There is no 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 composition of fatty acids, major nonstructural carbohydrates (NSCs), and texture of avocado fruit tissue were determined in a number of predefined localities within individual fruits during ripening and analyzed using chemometrics. For textural analysis a stress−relaxation test was applied, which is suitable for measuring viscoelastic properties of tissues (*10*). The test procedure was optimized to minimize tissue damage to allow biochemical analyses on the same avocado mesocarp tissue. Imported cv. Hass fruits were sourced from three different major avocado-producing countries (viz. Spain, Peru, and Chile) as it is recognized that imported avocado quality is, in part, reliant on preharvest conditions and thus origin.

The present study aimed at enhancing the spatiotemporal understanding of the ripening process in avocado fruit and thus may ultimately assist quality selection by aiding prediction of ripeness.

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MATERIALS AND METHODS

Reagents. Methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate, sucrose, D-glucose, D-fructose, and D-mannoheptulose standards were purchased from Sigma (Dorset, U.K.). 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., U.K.).

Plant Material. All imported avocado cv. Hass fruit [$n = 60$, size code 16 (*7*)] were supplied by Mack Multiples Division (M. W. Mack Ltd., Kent, U.K.). Three experiments were conducted on fruit shipped to the United Kingdom originating from Malaga, Spain; La Libertad, Peru; and Quillota Province, Chile. Fruit from Spain (late season 2007) was grown at approximately 36° 43′ N and 4° 25′ W. Fruits were transported and cooled within 3 days and then brought to the United Kingdom by road in ca. 3 days. Peruvian (early season 2007) and Chilean (mid-season 2007) fruits were grown at approximately 8° 25′ S and 78° 46′ W and 32° 50′ S and

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71° 05′ W, respectively. Transit time to the United Kingdom for fruit from South America ranged between 3 and 4 weeks.

Fruits were unripe on arrival as confirmed by initial color and firmness measurements. The exact physiological age of the imported avocados was not specified at arrival. Fruits were not treated with 1-methylcyclopropene. Produce was held in the laboratory overnight at 5−6 °C, after which fruits were placed into hermetically sealed polypropylene boxes (approximately 32 cm×14.5 cm×28 cm). Fruit to be measured the same day (day 0) were removed. Controlled ripening was initiated using exogenous ethylene (100 μL L^{-1}; BOC Gases Ltd., Surrey, U.K.), and avocado fruits were then kept overnight at ambient temperature (*11*). The ethylene concentrations in boxes were monitored and were found to decline from 100 to ∼40−75 μL L^{-1}. After 24 h, the boxes containing the avocado fruit were opened and transferred to 12 °C for subsequent slow ripening. Fruit subsamples (Spanish, $n = 3$; Peruvian and Chilean, $n = 6$) were randomly selected four times during 11 days of storage and objective color changes recorded (*11*).

Sample Preparation. Each fruit was cut manually using a sharp knife. Initially, each avocado fruit was cut vertically, with the stem facing the operator (**Figure 1**). The right half of the avocado fruit was discarded. The left half was placed with the flat planar surface downward and a 1 cm thick slice cut horizontally from the stem end; this piece was discarded. Thereafter, 10 slices were sequentially cut starting from the apex (near stem) toward the basal end of the fruit. Alternate slices were selected, resulting in 5 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 ($n = 300$).

Textural Evaluation. All texture tests were performed on a uniaxial testing machine (model 5542, Instron, Norwood, MA) equipped with a calibrated 500 or 5 N load cell, depending on the maximum loads recorded on the test day. Textural tests for each slice ($n = 300$) were done vertically such that the planar surface of the slice was in contact with the specimen stage. Three replicate measurements were carried out on each mesocarp slice as indicated in **Figure 1**. The machine was programmed (Bluehill 2, version 2.11, Instron) such that the right circular cylinder probe of 6 mm diameter indented the sample to a depth of 0.6 mm with a cross head speed of 10 mm min^{-1} and then was held at this position for 1 min (*10*). A number of cells were damaged due to this test, amounting to a volume of 3 times ca. 17 mm^3, which is a relatively small volume compared to the whole sample kept for biochemical analysis of at least 1.5 cm^3 (maximum 3% damaged). At least 5 g of the measured mesocarp tissue of slices 1, 3, and 5 (**Figure 1**) was cut and immediately snap-frozen in liquid nitrogen after testing and stored at −40 °C to preserve them prior to biochemical analysis ($n = 180$).

Force (N), deformation (mm), and time (s) were recorded as raw data throughout each test using the Bluehill software (at force changes of 20 or 2 mN depending on the load cell used, or at a maximum rate of 10 Hz). The apparent elasticity modulus (E_{ap}) was calculated as

$$E_{ap} = (l_0/A_0) \times \tan \phi$$

where l_0 is the actual thickness of the sample slice at the beginning of the test. l_0 or the distance between the specimen stage and the tip of the probe was recorded at the beginning of each test when a force of 0.4 N was reached. The slice thickness was included in the statistical analysis to evaluate if a possible deviation from 1 cm caused by hand cutting influenced other variables. A_0 is the cross-sectional area of the probe: 28.27 mm^2. tan ϕ is the highest slope of the first portion of the stress−relaxation curve (**Figure 2**, "Stress") calculated by least-squares fit on six regions of the curve.

The minimum relaxation time was calculated using Matlab software (7.0.4.365 R14 Service Pack 2, The MathWorks Inc., Natick, MA). The exponential law of relaxation of the Maxwell model was applied to find the relaxation time (T_{rel}) of the second part of the stress−relaxation curves recorded for every avocado slice (**Figure 2**, "Relaxation"). Vectors of varying relaxation times were obtained from these calculations.

$$T_{rel} = -t/\ln(\tau_t/\tau_{t=0})$$

where t is the time during the test. τ_t is the recorded load during the test. $\tau_{t=0}$ is the maximum load at an indentation of 0.6 mm. The minimum relaxation time values were calculated.

The mean value of the three replicates was calculated for each slice after it was confirmed by ANOVA that the position of each replicate did not have a significant effect on the variables.

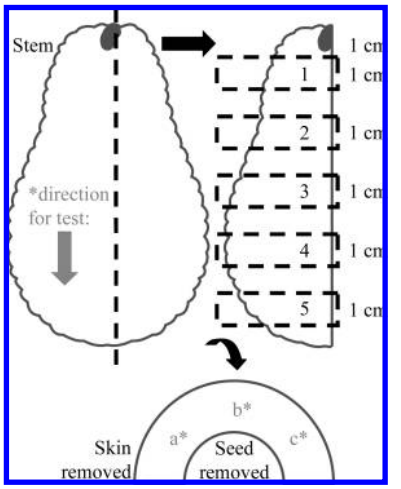

Figure 1. Illustration of sample preparation of five avocado tissue slices for texture measurement. Dotted line = cut; a, b, c = position for replicates (e.g., slice 3 or 4).

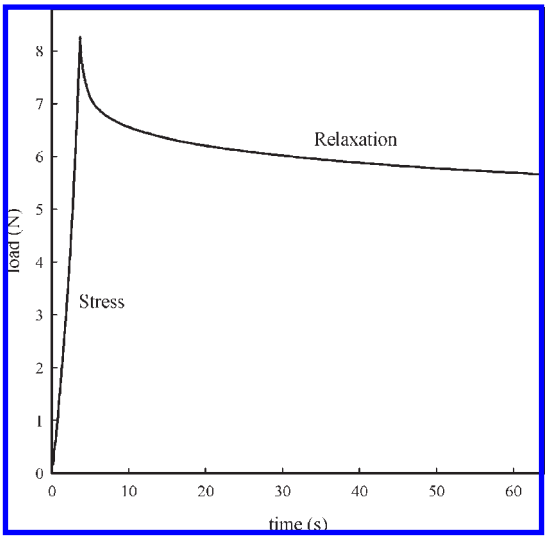

Figure 2. Stress−relaxation curve of avocado tissue measured at 10 mm min^{-1} to a depth of 0.6 mm with a cylindrical probe of 6 mm diameter.

Fatty Acids and NSCs Extraction. Frozen mesocarp tissue samples of slices 1, 3, and 5 ($n = 180$) 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 were returned to −40 °C until further processing. Lyophilized mesocarp tissue was ground and weighed (to 1 g), and a hexane extraction was performed as described by Meyer and Terry (*8*) to obtain oil and powder residue from the same sample. Briefly, the samples were homogenized with hexane and filtered through a Fisherbrand QL 100 filter paper (Fisher Scientific, Leics., U.K.). The powder residue was recovered from the filter paper and the solvent evaporated from the lipid-containing filtrate. The recovered oil and powder were weighed and returned to −40 °C for subsequent fatty acids and NSCs analysis, respectively.

Composition of Fatty Acids. Fatty acid methyl esters (FAMEs) were produced according to the International Olive Oil Council method (*12*) as modified by Meyer and Terry (*8*): 0.2 mL of methanolic potassium hydroxide (2 M) was added to 0.1 g of avocado oil extract in 2 mL of 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 12 h 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, U.K.) equipped with a G1540N flame ionization detector (FID) and a 7683B autosampler.

The identification and quantification of selected compounds was performed on a CP-Sil 88 fused silica capillary column (30 m×0.25 mm i.d., 0.2 μm film thickness; Varian, Harbor City, CA). Column temperature was programmed at 55 °C for 3 min and then raised to 175 at a rate of 13 °C min^{-1} followed by an isothermal period of 1 min and increased again to a final temperature of 220 at 8 °C min^{-1} (*8*). The carrier gas was He at a constant flow rate of 1.6 mL min^{-1}. The injector and detector temperatures were set at 220 and 250 °C, respectively. The composition of fatty acids was calculated as percentage of total of the five detected FAMEs, after comparison of peak areas of samples and peak areas of mixed standards of known composition.

NSCs Identification and Quantification. Starch content of the fruit was not analyzed. Major NSCs of the fruit flesh were extracted as described previously (*13*) with some modifications (*8*): In summary, the recovered residue powder (150 mg) was mixed with 3 mL of 62.5% (v/v) aqueous methanol, placed in a water bath with shaker at 55 °C for 15 min, with the samples shaken vigorously by hand every 5 min to prevent layering, and then left to cool. Then the samples were filtered through a syringe-driven filter unit (0.2 μm, Millipore Corp., Bedford, MA), and the clear extract was analyzed.

NSCs were quantified as described previously (*8*): Briefly, NSC content in the avocado extracts was determined using a high-performance liquid chromatography (HPLC) system comprising a P580 pump and a GINA 50 autosampler (Dionex, Sunnyvale, CA). Twenty microliters of diluted avocado extract (1:10; v/v), was injected onto a Rezex RCM-Monosaccharide Ca^{+} (8%) column, 300 × 7.8 mm size (00H-0130-K0, Phenomenex, Torrance, CA) with a Carbo-Ca^{2+} guard column, 4 × 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, Milford, MA) connected to the HPLC system. Mixed standards of known composition and concentration were used, and the presence of each NSC was quantified by comparing sample peak areas to the standards. The minimum quantification limit was 20 mg of NSC per gram of powder residue (ca. 0.2% NSC per gram of fresh mass). Results below the quantification limit were set at zero, which causes an unavoidable underestimation of the mean value of NSC content. For clarity, the number of values below quantification are reported for particular regions within the avocado fruits.

Chemometric Analysis. All statistical analyses were carried out using Genstat for Windows vers. 10 (VSN International Ltd., Herts., U.K.). Data were subjected to analysis of variance (ANOVA). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation. Significant differences were compared with appropriate LSD according to examined groups (number of repetitions: minimum, minimum−maximum, maximum). Different letters per variable indicate significantly different groups. Model adequacy checks were performed by examining plots of the residuals. Some variables were transformed to interpret data with normally distributed residuals. Graphs of nontransformed data (**Figure 3**) are presented with standard errors of the means. Origin, storage duration, and the vertical location of the tissue slice within the fruit were the treatment factors in the ANOVA. Different test days were combined by storage duration and termed "before", day 0; "early", day 3 or 4; "later", day 6 or 7; and "store end", day 10 or 11. The data were grouped by fruit (blocks $n = 60$).

Multivariate analysis was performed using principal component analysis (PCA). All sample properties were compared by analyzing slices 1, 3, and 5. A full cross-validation was done; data were centered and variables were weighted by dividing by the standard deviation. Total explained variance per principal component and loadings plots and score plots showing treatment factors were used for interpretation. To investigate in more detail if the variation of results of slices from different locations within the fruit could be significantly different at one time during storage or one origin, respectively, subgroup PCAs were examined (two examples are represented: Peru, "early").

RESULTS

Complex interactions between temporal and spatial changes in texture and quality-related compounds were found for avocado fruit from different origins (**Table 1**).

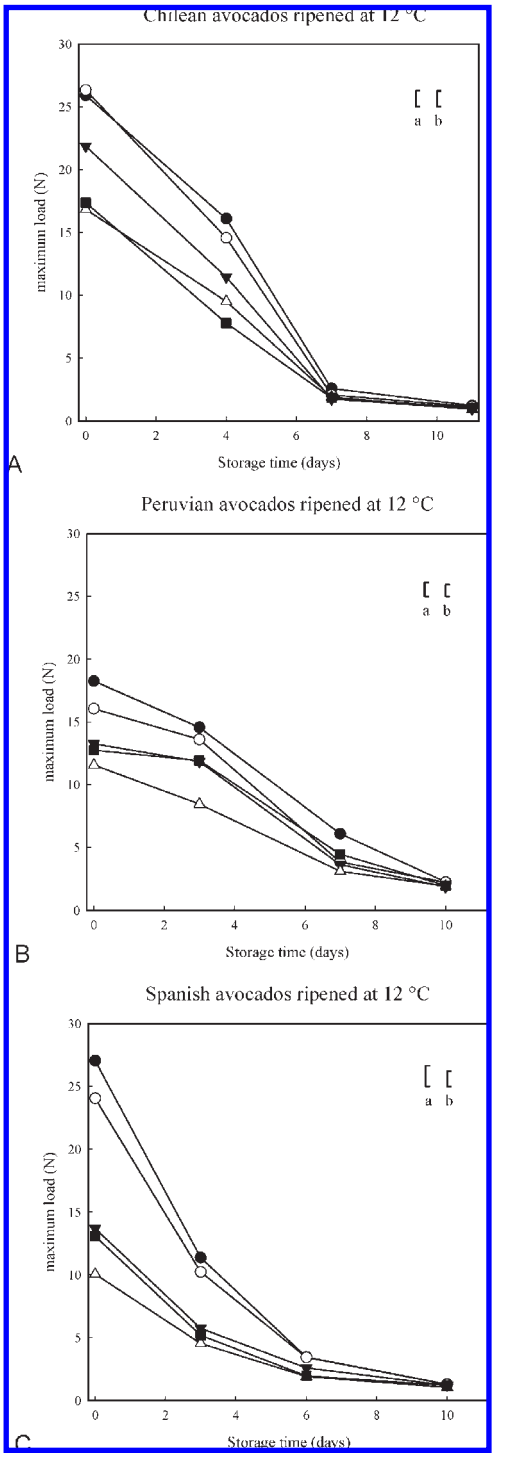

Figure 3. Development of the maximum load of mesocarp tissue of avocado fruit (**A**, **B**, $n = 24$; **C**, $n = 12$) during storage at 12 °C measured by means of a stress−relaxation test: ●, slice 1, closest to the stem of the fruit; ○, slice 2, apical region within fruit; ▼, slice 3, middle of the fruit; △, slice 4, both near the seed; ■, slice 5, base of the fruit. The bars indicate standard errors of the means: (a) for levels of storage time; (b) for levels of slice.

Quality Parameters. Significant differences in dry matter content, oil content (**Table 1**), and objective color were observed between avocado fruits from different origins. Objective color of avocado fruit predictably changed during storage. Hue angle ($h°$) development from green ($h° \sim 125$) to reddish/black was more extreme after 10 days of storage for Chilean-derived fruit ($h° \sim 37$) as compared to fruit from Peru ($h° \sim 102$). In contrast, the

Table 1. Probabilities of an *F* Test in ANOVA of Quality Parameters Measured on Avocados from Different Origins during Ripening

source of variation[a]	origin	storage	origin × storage	slice	origin × slice	storage × slice	origin × storage × slice
% DM/FM	<0.001	0.338	0.666	<0.001	<0.001	0.023	0.004
% oil/DM	<0.001	<0.001	0.029	0.004	0.263	0.045	0.027
height	0.259	0.426	0.124	<0.001	0.350	0.467	0.237
$\log_{10}$ max load	<0.001	<0.001	<0.001	<0.001	0.005	0.003	0.174
$\log_{10} E_{ap}$	<0.001	<0.001	<0.001	<0.001	0.031	<0.001	0.081
min T_{rel}	<0.001	<0.001	0.003	0.002	0.180	<0.001	<0.001
sucrose/DM	0.733	0.015	<0.001	<0.001	<0.001	0.083	0.616
mannoheptulose/DM	<0.001	<0.001	0.006	<0.001	<0.001	0.060	0.385
perseitol/DM	0.002	<0.001	0.094	<0.001	0.002	0.601	0.302
power2 palmitate	<0.001	0.712	0.450	0.029	<0.001	0.065	0.022
power2 palmitoleate	<0.001	0.466	0.550	0.244	<0.001	0.435	0.344
oleate	<0.001	0.749	0.257	<0.001	<0.001	0.094	0.162
linoleate	0.009	0.005	0.338	<0.001	<0.001	0.089	0.426
linolenate	<0.001	0.536	0.063	<0.001	<0.001	0.759	0.868

[a] $\log_{10}$, values were transformed with logarithm for normality; power2, values were transformed by square for normality; FM, fresh mass; DM, dry mass; E_{ap}, apparent elasticity modulus; min T_{rel}, minimum relaxation time.

lightness of Peruvian fruit at the beginning of storage was lower than that of Chilean fruit, after which values of both fruit decreased to similar values during ripening (data not shown). Fruit could be distinguished from one another on the basis of both dry matter and oil content (**Table 2**). Late season Spanish fruit had the highest dry matter and oil contents compared to mid-season Chilean and these, in turn, showed higher values than early season Peruvian. Chilean and Peruvian avocados tended to have less dry matter content in the middle of the fruit close to the seed (**Table 2**). In Spanish fruit there was significantly less dry matter content in the middle and basal regions of the fruit than the apex, toward the latter end of storage. For all origins, oil content within the fruit was typically less toward the basal end of the fruit (**Table 2**). Only in Chilean fruit was significantly higher oil content found at the base compared to the stem region after short storage ("early", **Table 2**). In Chilean and Peruvian fruits the oil content tended to increase during storage, but no significant trend was observed in Spanish fruit (**Table 2**).

Textural Parameters. Significant changes in maximum load, elasticity, and minimum relaxation time of avocado mesocarp tissue were found during storage (**Tables 2**−**4**). Moreover, textural measurements also differed according to origin and spatial location of tissue within the fruit (**Tables 2** and **5**). Maximum load (**Figure 3**) and elasticity declined concomitantly during storage, while minimum relaxation time did not follow an exponential decline. Chilean fruit tissue had first the highest and then the lowest maximum loads at the beginning and end of storage, respectively (**Figure 3A**). Peruvian fruit tissue maintained comparatively greater resistance to load throughout storage despite having the lowest initial maximum load (**Figure 3B**). Maximum loads of Spanish avocado tissues were lowest after 3 days of storage at 12 °C compared to the fruit from other origins (**Figure 3C** and **Table 3**).

Regardless of origin, the highest maximum load and elasticity values were recorded in the stem-end region of the fruit (**Tables 4** and **5**). At the beginning of storage the difference in maximum load between the stem-end and middle and basal regions was greatest, yet values converged during ripening (**Figure 3**). This decline was more pronounced for elasticity. In contrast, minimum relaxation time differed less between slices, yet decreased more rapidly during storage, and at late storage minimum relaxation time already reached a minimum. For Spanish fruit minimum relaxation time was significantly less than for avocados from the two other origins, especially in the apical region after short storage ("early") and in the middle and basal regions after longer storage ("later", **Table 2**). In contrast, Peruvian fruit had a significantly higher minimum relaxation time value in the middle and basal regions after 3 days of storage (**Table 2**).

Biochemical Parameters. *Fatty Acids*. The composition of fatty acids in fruit was governed by origin (**Table 1**). In order of dominance, oleic acid (ca. 63%; monounsaturated), palmitic acid (ca. 19%; saturated), linoleic acid (ca. 11%; biunsaturated), palmitoleic acid (ca. 6%; monounsaturated), and linolenic acid (ca. 1.0%; triunsaturated) were detected in Spanish and Chilean avocado fruits. However, the composition of fatty acids found in Peruvian fruit was significantly different (ca. 43% oleic acid, ca. 28% palmitic acid, ca. 15% linoleic acid, ca. 13% palmitoleic acid, and ca. 1.2% linolenic acid).

Some substantial variation in the spatial distribution of fatty acids was observed in avocado fruit (**Tables 2** and **5**). For Spanish and Chilean fruits the palmitic acid and palmitoleic acid ratios were lowest at the base of the fruit, whereas in the Peruvian fruit palmitic acid was lowest in the apical region (**Tables 2** and **5**). The distribution of oleic acid content in Peruvian avocados declined toward the basal end. In contrast, oleic acid tended to be equally distributed across the vertical profile of fruit mesocarp tissue derived from either Spain or Chile. Linolenic acid was always found to be highest in the basal region of fruit from all origins.

NSCs. Origin had no main effect on the amount of sucrose in fruit (**Table 1**). The sucrose content of fruits from different origins showed no common trend during storage (**Table 3**). Sucrose concentration was generally higher toward the basal slice, contrasting with that observed for mannoheptulose, which showed highest values in the mesocarp tissue near the stem (**Table 5**) and generally decreased during storage (**Table 3**). The findings are underpinned with the counts of values below quantification limit per 60 fruit measured (sucrose, apex $n = 20$, base $n = 12$; mannoheptulose, apex $n = 9$, base $n = 31$). More mannoheptulose was present in fruit from Chile than from Peru, and more mannoheptulose was present in Peruvian fruit than in Spanish (**Table 5**), which in fact showed quantifiable levels of mannoheptulose only in the slice of the stem region.

No significant course of development was observed for perseitol concentration of fruits from different origins (**Table 1**); however, a decreasing trend was found (data not shown). The distribution of perseitol was more random than for the sugars (**Table 5**); this said, a lower perseitol content was observed in the middle of the fruit nearest the seed (values below limit, apex, $n = 8$; middle, $n = 12$; base, $n = 5$).

Multivariate Analysis. The thickness of the mesocarp tissue slice ("height") had a negligible influence on the texture measurements (**Figure 4**).

Table 2. Mean Values of Variables Measured on Avocados from Various Origins at Different Levels of Storage at Several Locations within the Fruit, Which Were Found to Have Significance in the *F* Test as Seen in **Table 1**

origin × storage × slice[a]			1	2	3	4	5
% DM/FM	Chile	before	29.25 b,c		29.23 b,c		29.49 b,c
		early	26.71 c,d		26.46 c,d		27.06 c,d
		later	27.54 c,d		25.95 c,d,e		26.49 c,d
		store end	28.84 b,c		27.58 c,d		29.24 b,c
	Peru	before	24.97 d		22.76 d,e		25.42 c,d
		early	25.82 c,d		23.27 d,e		24.43 d,e
		later	24.67 d		23.50 d,e		23.55 d,e
		store end	25.79 c,d		24.13 d,e		24.25 d,e
	Spain	before	39.06 a		36.73 a,b		35.13 a,b
		early	37.71 a		33.52 a,b		35.25 a,b
		later	37.74 a		33.14 a,b		29.55 b,c
		store end	35.51 a		32.36 b		31.04 b,c
% oil/DM	Chile	before	57.97 a,b		60.00 a,b		58.48 a,b
		early	51.91 c,d		54.78 b,c		56.31 b
		later	65.13 a		61.61 a,b		60.76 a,b
		store end	66.64 a		66.74 a		64.02 a,b
	Peru	before	47.98 c,d		45.00 d		42.84 d,e
		early	50.62 c		50.66 c		47.48 c,d
		later	53.20 b,c		51.38 b,c		51.99 b,c
		store end	57.77 a,b		55.70 b,c		53.70 b,c
	Spain	before	63.08 a,b		67.24 a		69.87 a
		early	64.57 a		66.95 a		61.55 a,b
		later	66.93 a		63.02 a,b		62.88 a,b
		store end	71.28 a		69.29 a		60.44 a,b
min T_{rel}	Chile	before	34.39 a,b	32.68 a,b,c	34.29 a,b	36.01 a	36.32 a
		early	29.97 b,c	29.43 b,c	26.65 d	25.48 d	24.54 d,e
		later	17.56 e,f	16.68 e,f	17.10 e,f	17.69 e,f	17.49 e,f
		store end	16.34 e,f	16.51 e,f	17.19 e,f	17.21 e,f	16.66 e,f
	Peru	before	34.68 a	33.31 a,b	33.35 a,b	34.19 a	32.60 b
		early	32.17 a,b	30.93 b,c	30.85 b,c	30.40 b,c	32.12 a,b
		later	19.09 e,f	18.51 e,f	18.83 e,f	18.19 e,f	18.44 e,f
		store end	15.60 f,g	16.10 f	16.85 e,f	16.84 e,f	16.66 e,f
	Spain	before	32.39 a,b	31.68 a,b	32.95 a,b	36.82 a	37.44 a
		early	24.64 d	23.24 d	22.55 d,e	24.06 d	16.75 e,f
		later	13.61 f,g	13.34 f,g	11.92 g,h	12.18 g	11.51 g,h
		store end	15.31 f,g	14.36 f,g	14.71 f,g	15.02 f,g	14.66 f,g

origin × storage × slice[a]			1	re-transformed	3	re-transformed	5	re-transformed
power2 palmitate	Chile	before	441.2 d	21.00	399.4 d	19.98	339.6 d,e	18.43
		early	303.4 d,e	17.42	454.8 d	21.33	385.0 d,e	19.62
		later	317.7 d,e	17.82	337.9 d,e	18.38	290.6 d,e	17.05
		store end	424.6 d	20.61	382.5 d	19.56	371.1 d	19.26
	Peru	before	716.9 a,b,c	26.77	798.4 a,b	28.26	819.2 a	28.62
		early	684.1 b,c	26.16	740.0 a,b	27.20	804.0 a	28.35
		later	726.0 a,b	26.94	758.9 a,b	27.55	898.4 a	29.97
		store end	718.4 a,b	26.80	761.2 a,b	27.59	827.4 a	28.76
	Spain	before	408.0 d	20.20	396.8 d	19.92	310.0 d,e	17.61
		early	395.3 d	19.88	330.6 d,e	18.18	265.1 d,e	16.28
		later	388.2 d	19.70	394.0 d	19.85	299.4 d,e	17.30
		store end	340.3 d	18.45	330.9 d,e	18.19	278.3 d,e	16.68

[a] power2, values were transformed by square for normality; FM, fresh mass; DM, dry mass; % DM/FM (g g^{-1}); % oil/DM (g g^{-1} DM); min T_{rel}, minimum relaxation time (s); palmitate (%).

Origin. Visual inspection of the score plot (**Figure 5**) revealed that the first principal component (PC1) explained mostly variation in origins, whereas PC2 explained the effect of storage time (explained variance: PC1 = 34%, PC2 = 25%). The loadings plot revealed (**Figure 4**) that both oil and dry matter contents, and oil content and oleic acid percentage were related. Linoleic acid was inversely correlated to oleic acid, and oleic acid was inversely correlated with palmitic acid and palmitoleic acid, respectively. Palmitic acid and palmitoleic acid were strongly correlated.

In PC1 palmitic acid, palmitoleic acid, oleic acid, linoleic acid, dry matter, and oil content were most important (**Figure 4**). These 6 variables were subjected to further PCA, by which an excellent separation was found between Peruvian fruit and Chilean/Spanish-derived fruit. In addition, a moderate separation between Chilean and Spanish fruits was observed by means of PC2 (explained variance: PC1 = 73%, PC2 = 13%; plot not shown).

Storage Duration. Mannoheptulose, perseitol, linoleic acid, and texture parameters were the most important variables in PC2 (**Figure 4**). All texture parameters were correlated. Using only the important variables in a further PCA, it was shown that 67% of the variability of the results could be explained in the new PC1. The samples "before" storage were plotted completely separately from those at "later" and at the end of storage (plot not shown).

Table 3. Mean Values of Variables Measured on Avocados from Various Origins at Different Levels of Storage, Which Were Found to Have Significance in the *F* Test as Seen in **Table 1**

origin × storage[a]		before	re-transformed	early	re-transformed	later	re-transformed	store end	re-transformed
$\log_{10}$ max load	Chile	1.31 a	20.49	1.05 b	11.24	0.29 e	1.97	0.01 f	1.02
	Peru	1.13 a,b	13.64	1.06 b	11.50	0.59 d	3.86	0.28 e	1.93
	Spain	1.20 a	16.01	0.80 c	6.30	0.41 e	2.57	0.08 f	1.19
$\log_{10} E_{ap}$	Chile	3.22 a	1664.56	2.95 a,b	901.36	2.12 e	131.19	1.81 f	64.97
	Peru	3.06 a,b	1158.24	3.00 a,b	999.54	2.46 d	286.75	2.12 e	131.10
	Spain	3.09 a	1217.59	2.68 c	481.84	2.31 d	205.35	1.88 f	75.11
sucrose/DM	Chile	7.83 b		10.05 a,b		13.80 a		5.99 b	
	Peru	5.25 b		13.57 a		5.72 b		9.42 a,b	
	Spain	2.93 b,c		1.23 b,c		14.49 a		18.63 a	
mannoheptulose/DM	Chile	28.45 a,b		41.15 a		26.23 a,b		9.53 c	
	Peru	32.56 a		20.95 b		9.60 c		8.58 c	
	Spain	2.24 c		2.99 c		0 c		1.14 c	

[a] $\log_{10}$, values were transformed with logarithm for normality; DM, dry mass; max load (N); E_{ap}, apparent elasticity modulus (N cm^{-2}); sucrose and mannoheptulose (mg g^{-1} DM).

Table 4. Mean Values of Variables Measured on Avocados at Different Levels of Storage at Several Locations within the Fruit, Which Were Found to Have Significance in the *F* Test as Seen in **Table 1**

storage × slice[a]		1	re-transformed	2	re-transformed	3	re-transformed	4	re-transformed	5	re-transformed
$\log_{10}$	before	1.34 a	21.96	1.32 a	21.01	1.20 b	15.90	1.09 b,c	12.43	1.14 b	13.72
max load	early	1.13 b	13.64	1.11 b,c	12.76	0.99 d	9.82	0.87 e	7.49	0.92 e	8.22
	later	0.57 f	3.70	0.46 g	2.88	0.39 g	2.46	0.35 g,h	2.23	0.40 g	2.54
	store end	0.19 i	1.53	0.17 i	1.48	0.10 i,k	1.26	0.08 i,k	1.22	0.12 i	1.33
$\log_{10} E_{ap}$	before	3.24 a	1739.80	3.21 a	1608.42	3.10 b	1258.64	3.04 b,c	1092.19	3.07 b	1176.25
	early	3.06 b	1140.51	3.02 b	1042.56	2.90 d	797.99	2.78 e	608.84	2.83 e	676.86
	later	2.44 f	278.10	2.31 g	202.96	2.24 g	174.42	2.20 g,h	159.88	2.27 g	184.59
	store end	2.00 i	99.95	1.97 i	94.23	1.91 i,k	81.94	1.90 i,k	79.25	1.95 i	89.08

[a] $\log_{10}$, values were transformed with logarithm for normality; max load (N); E_{ap}, apparent elasticity modulus (N cm^{-2}).

Table 5. Mean Values of Variables Measured on Avocados from Various Origins at Different Locations within the Fruit, Which Were Found to Have Significance in the *F* Test as Seen in **Table 1**

origin × slice[a]		1	re-transformed	2	re-transformed	3	re-transformed	4	re-transformed	5	re-transformed
$\log_{10}$ max load	Chile	0.77 b	5.83	0.73 b,c	5.37	0.65 d	4.42	0.60 d	3.98	0.59 d,e	3.90
	Peru	0.87 a	7.37	0.81 b	6.42	0.74 b,c	5.46	0.67 b,c,d	4.62	0.76 b	5.70
	Spain	0.77 b	5.93	0.75 b	5.58	0.59 d,e	3.88	0.47 e,f	2.97	0.53 d,e	3.41
$\log_{10} E_{ap}$	Chile	2.63 c	429.54	2.57 c,d	372.05	2.50 c,d,e	314.92	2.47 e	292.89	2.46 e	291.68
	Peru	2.77 a	594.16	2.71 b	511.09	2.61 c	407.38	2.56 c,d	363.33	2.65 c	442.18
	Spain	2.61 c	410.39	2.57 c	375.40	2.48 d,e	301.79	2.35 f	225.48	2.43 e	266.20
sucrose/DM	Chile	6.38 b				11.65 a				10.23 a	
	Peru	8.58 a,b				6.77 b				10.13 a	
	Spain	5.91 b				8.44 a,b				13.61 a	
mannoheptulose/DM	Chile	37.74 a				27.10 b				14.18 c	
	Peru	30.55 b				14.18 c				9.05 c	
	Spain	4.78 c,d				0 c,d				0 c,d	
perseitol/DM	Chile	13.34 a				12.44 a				12.01 a	
	Peru	8.40 b				6.46 b,c				12.66 a	
	Spain	9.99 a,b				8.76 a,b				12.56 a	
power2 palmitoleate	Chile	36.86 c	6.07			38.76 c	6.23			33.52 d	5.79
	Peru	166.32 b	12.90			161.10 a,b	12.69			175.96 a	13.26
	Spain	43.75 c	6.61			34.98 c,d	5.91			24.56 c,d,e	4.96
oleate	Chile	63.89 a				63.25 a				64.20 a	
	Peru	45.94 c				42.38 d				39.92 e	
	Spain	62.86 a				61.64 a,b				63.39 a	
linoleate	Chile	10.03 c				10.00 c				10.49 c	
	Peru	13.60 b				16.00 a				16.62 a	
	Spain	10.68 c				12.45 b				13.52 b	
linolenate	Chile	1.10 a,b				0.96 a,b				1.27 a	
	Peru	0.98 a,b				1.36 a				1.37 a	
	Spain	0.36 c				1.02 a,b				1.25 a	

[a] $\log_{10}$, values were transformed with logarithm for normality; max load (N); E_{ap}, apparent elasticity modulus (N cm^{-2}); power2, values were transformed by square for normality; DM, dry mass; sucrose, mannoheptulose, and perseitol (mg g^{-1} DM); palmitoleate, oleate, linoleate, and linolenate (%).

Spatial Location within Fruit. The PCA calculated with all variables did not show a separation between results of different avocado slices. However, PCAs calculated for subgroups of avocado data revealed differences in spatial distribution of parameters. For example 1 (Peru), dry matter content and oleic acid and palmitic acid percentages were used in a new PCA of results of Peruvian avocados alone to evaluate the variation of slices during storage (n = 72). The results of slice 1 could be grouped (explained variance: PC1 = 60%, PC2 = 29%). For example 2 ("early"), another PCA was performed with texture parameters of short-stored avocados of fruit from different origins (n = 45). The results of slice 1 could be grouped (explained variance: PC1 = 93%, PC2 = 6%, plots not shown).

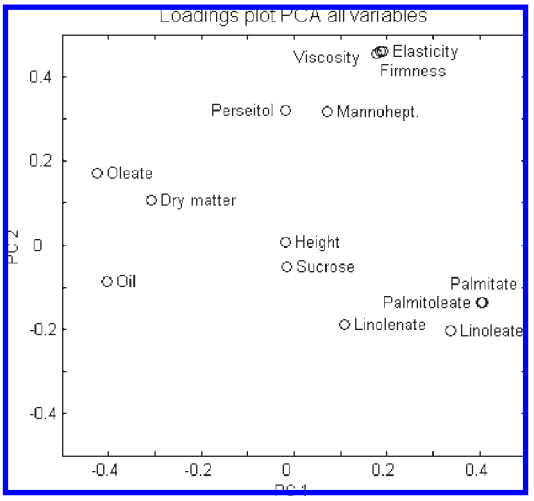

Figure 4. Loadings plot of a PCA with all variables measured on avocado fruit from different origins during 12 °C storage.

DISCUSSION

Avocado cv. Hass fruit were sourced from three different major exporting locations in the state as they arrive commercially in the United Kingdom. It was expected that growing conditions at the different sources (climate, season, soil, agronomic practices) and transit time would influence avocado quality. The ultimate aim of this study was to describe the spatiotemporal ripening process of avocado cv. Hass 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. The most differentiating parameters to evaluate origin, storage time, and location within the fruit were dry matter content (after freeze-drying), oil content, minimum relaxation time, and palmitic acid percentage.

In the present study, spatial variability in mesocarp tissue was observed for nearly all measured parameters and was differently expressed according to origin. Often, textural and biochemical variables showed gradients between the apical region and the basal region; only dry matter content (and, to a lesser extent, perseitol) tended to be less toward the middle of the fruit.

Quality Parameters. As expected, the fruit color changed from green to an almost black red.

The values of dry matter (after freeze-drying) and oil content were not as closely related as expected, because in industry dry matter content (after oven-drying) is taken as an indicator of oil content and maturity (*7*). Other studies advise caution when using the dry matter and oil contents as a maturity index (*14*). Dry

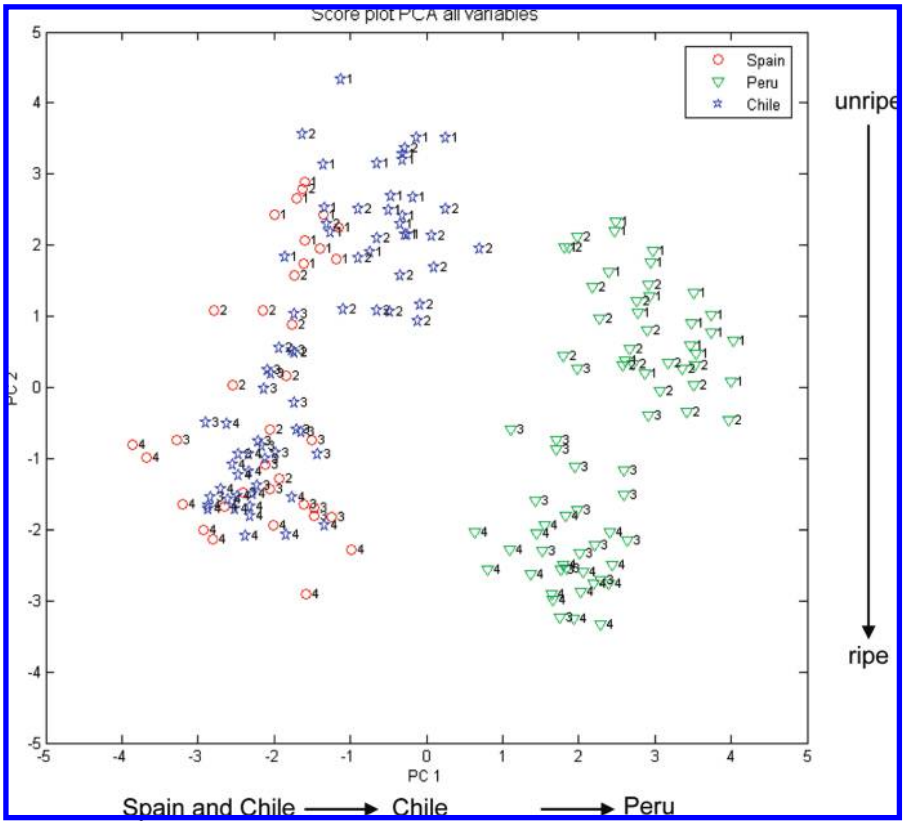

Figure 5. Score plot of a PCA with all measured variables (**Figure 4**) of avocado fruit from different origins during ripening (n = 179, of 60 fruit with 3 slices). Origin: red circles, Spain; blue stars, Chile; green triangles, Peru. Storage time: 1, "before"; 2, "early"; 3, "later"; 4, "store end".

matter content varied more according to spatial distribution within the fruit, whereas oil content tended to increase during storage. Very little research has been conducted to investigate the spatial differences of quality parameters in avocado fruit during ripening (*1*, *9*). Schroeder (*9*) measured dry matter content (after heat-drying) at different locations within avocado cv. Hass fruit and from that deduced that oil content in tissue near the rounded base of the fruit was higher than for other regions. The ripeness of the fruit was not specified. The results above show that dry matter content (after freeze-drying) tended to be lower in the middle and basal regions of the fruits; the same was found for oil content in ripe fruit. It was observed that late season fruit had a higher dry matter and oil content than mid-season followed by early season avocados. More detailed research is planned to examine this phenomenon in detail with fruit from the same orchard.

Textural Parameters. As expected, values of textural parameters decreased and, thus, were appropriate to describe the effect of ripening. The maximum load is the measured parameter most comparable to the firmness evaluation used by industry. Typically, the firmness of avocado fruit is measured in commercial environments to assess the degree of ripeness and thus the suitability of a consignment for sale and consumption. Firmness is defined as the maximum force that a sample can support before fracture; during textural evaluation firmness can be defined as the force that is necessary to achieve a previously defined deformation (*15*). The measured maximum load was not measured at fracture, but at a small deformation. Therefore, a strong correlation to the apparent elasticity modulus was observed.

Textural parameters were the most important variables able to predict the effect of different storage times. Before and during early storage, significant spatial variation was found in textural values. Thus, the stress−relaxation parameters are well suited to describe avocado fruit ripening, if care is taken to measure similar locations in the fruit, especially during early storage.

The mechanical measurement of fruit softening is based on the biochemical change in constituents within the fruit tissue. The minimum relaxation time is thought to reflect auxin-induced changes within cell walls, but detailed examination of the stress−relaxation parameters led to the belief that avocado softening differs from that induced by auxin in stems (*16*). This research confirmed findings that minimum relaxation time decreases during avocado ripening, which suggests a decrease in cell wall viscosity according to the findings of Sakurai and Nevins (*16*). Bower and Cutting (*17*) suggested that early stages of softening in avocado fruit appear to be due to increased cellulase activity, which leads to disorganization in the cell walls and allows greater access to polygalacturans in the wall matrix, so that polygalacturonase is believed to be responsible for final fruit softening. Molecular weight downshift of xyloglucan within the soluble hemicellulose fraction reflects consequences of depolymerization in the cell wall by degradation of cross-links between cellulose microfibrils (*16*). The elastic properties of cell walls are likely to be dependent on this microfibril network, and a rapid decrease in mechanical stress may have been due to endotype hydrolytic enzymatic cleavage of xyloglucan molecules (*16*).

Biochemical Parameters. *Fatty Acids*. Avocado is classed as a lipid-based product 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 (*17*). The composition of fatty acids reported herein is generally in the range of that reported by others (*8*, *18*−*21*), but significant differences were observed between avocados from Peru and the other two origins. The different oil contents and compositions of fatty acids found in fruits from different origins were expected to be related to the growing conditions.

NSCs. Glucose and Fructose values were not considered, since most were below quantification limit. Sucrose, mannoheptulose, and perseitol were the most abundant free NSCs in avocado mesocarp tissue as previously reported (*8*, *22*−*24*). No discernible pattern was observed for the development of sucrose concentration of fruit from three different origins during slow ripening, which could confirm the suggestion that sucrose is not suitable for judging postharvest quality (*22*). In metabolic processes of avocado fruit the C7 carbohydrates are most important (*23*−*25*), and perseitol and mannoheptulose were needed in the PCA to predict ripeness stage. The decrease found in the mannoheptulose content in Peruvian and Chilean fruits during storage is in agreement with that reported elsewhere (*8*, *22*, *24*, *26*). In an experiment, Liu et al. (*25*) detected a threshold value of maximally 20 mg g^{-1} C7 sugar per fruit sample before ripening started. The present study was conducted under different conditions: namely, with fruit from other origins, different ripening regimens, and different seasons and of varied biological age. Here, mannoheptulose concentrations higher than 20 mg g^{-1} of DM were found at the beginning of ripening for avocados from Peru and Chile. Ripening occurred as measured by means of decreasing mannoheptulose content and softening (**Table 3**). Spanish avocados showed the lowest overall mannoheptulose content. In those late season fruit, low sugar content was expected (*24*).

Distribution of sucrose concentrations within the fruit was generally higher toward the base of the fruit, and perseitol concentrations were lower in the middle region. Cowan (*23*) reported that perseitol content increased in the seed following exogenous perseitol application to the detached fruit via the pedicel. It remains to be examined if the seed influences the concentration of carbohydrates in the surrounding tissue. Considerable heterogeneity of mannoheptulose concentration was found in the fruit tissue from stem end to base, with the largest concentration in the apical region. It was reported that mannoheptulose is metabolized in the leaves and that the C7 sugar is translocated via phloem during fruit growth (*24*, *25*). A possible explanation for the finding of firmer mesocarp tissue in the apical region is the suggested role of the C7 sugar as a ripening inhibiting factor (*23*−*25*). Another possible explanation could be an involvement of the seed in ethylene evolution and responsiveness to ethylene during ripening (*27*).

The measured delay in softening of the tissue near the pedicel in comparison to other tissue regions provides physical and biochemical evidence for suggested functions of ripening factors in avocado fruit via a combination of tests of texture, NSC content, and composition of fatty acids in different locations within the fruit.

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