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An ASABE Meeting Presentation

Paper Number: 084563

Spatio-temporal postharvest changes in texture and fatty acid profiles in avocado fruit from different origins

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Written for presentation at the
2008 ASABE Annual International Meeting
Sponsored by ASABE
Rhode Island Convention Center
Providence, Rhode Island
June 29 – July 2, 2008

Abstract. *The degree of ripeness of many climacteric fruits, such as avocado, can be correlated with flesh firmness and other rheological properties. However, there remains a paucity of information on not only the postharvest changes in texture of avocado fruit from different origins, but also on the spatial variation in texture within individual fruit. In addition, the relationship between changes in texture and lipid profile of fruit tissue during postharvest ripening is unknown.*

The aim of the present study was to assess and discriminate between avocado cv. Hass fruit from three different origins (viz. Spain, Peru and Chile) on the basis of temporal and spatial changes in both texture and fatty acid profiles of fruit flesh. Texture of different horizontally-cut slices from individual fruit within a consignment was measured during ripening using a previously unreported technique. Maximum load, elasticity and viscosity of fruit tissue was measured using an Instron 5542 universal testing machine fitted with either a 500 N or 5 N load cell. The same fruit slice was immediately snap-frozen in liquid nitrogen and freeze-dried prior to subsequent extraction, identification and determination of fatty acid methyl esters (FAME) profiles using gas chromatography coupled to flame ionization detection (GC-FID). The results were used to differentiate

avocado fruit into definable groups using partial least squares discriminant analysis. Significant differences in maximum load, elasticity, viscosity were found in avocado fruit flesh during ripening, and between origins and to a lesser extent between different locations within the fruit. Lipid profiles showed dissimilar composition according to origin and changed slightly from apex to base. The textural changes and lipid profile in avocado fruit is therefore related to origin and the spatial variation within individual fruit.

Keywords. *Persea americana,* stress-relaxation, lipids, ripeness

Introduction

Commercially, the quality of avocado fruit (*Persea americana* Mill.) is rated according to size, estimated oil content (dry matter), absence of defects and firmness (OECD 2004). Oil content is used as an indicator of fruit maturity, and thus commonly defines the optimum harvest period. Avocado mesocarp tissue has inherently high concentrations of unsaturated fatty acids. Lipids accumulate during avocado fruit development and constitute approx. 70% of dry matter at maturity. The fatty acid profile of avocado oil has been shown to remain relatively unchanged during postharvest ripening (Eaks 1990, De La Plaza et al. 2003, Ozdemir and Topuz 2004). The degree of ripeness of many other climacteric fruits like avocado can be correlated with fruit firmness.

Past research has looked at the changes in dry matter and pigments in various locations within avocado fruit during postharvest ripening (Schroeder 1985, Ofelia et al. 2006), but has not considered the distribution of fatty acids. There is no published information on the interdependence and spatial distribution of textural parameters and lipids in avocado mesocarp tissue. Therefore, in this research the fatty acid profile and texture of avocado fruit were determined in a number of predefined localities within individual fruit during ripening and the effect of origin, storage time and location within the fruit investigated. For textural analysis a stress-relaxation test was applied, which is suitable for measuring visco-elastic properties of tissues. The test procedure was optimized to minimize tissue damage to allow biochemical analyses on the same avocado mesocarp tissue. Imported *cv*. Hass fruit were sourced from three different avocado producing countries (*viz*. Spain, Peru, Chile) as it is recognized that imported avocado quality can be reliant on pre-harvest conditions and thus origin.

The present study aimed at enhancing spatio-temporal understanding of the ripening process in avocado fruit and thus ultimately assists optimization of quality assessment.

Materials and Methods

Reagents

Methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate standards were purchased from Sigma (Dorset, UK). All other chemicals used were of analytical grade and purchased from Fisher Scientific Chemicals (Leics., UK).

Plant material

All imported avocado *cv*. Hass fruit (size code 16 fruit; OECD 2004) were supplied by M. W. Mack Ltd. (Kent, UK). Three experiments were conducted on preclimacteric fruit originating from Malaga, Spain (late season 2007), La Libertad, Peru (early season 2007), and Quillota Province, Chile (mid season 2007). The physiological age of the imported avocados was different at arrival due to the respective distance from origin to the UK. Produce was held in the laboratory overnight at 5-6°C, after which fruit were placed into hermetically sealed polypropylene boxes (approx. 32 cm x 14.5 cm x 28 cm). Controlled ripening was initiated using exogenous ethylene (100 µL L^{-1}; BOC Gases Ltd., Surrey, UK; Terry et al. 2007) and avocado fruit were kept overnight at ambient temperature. The ethylene concentrations in boxes were monitored and found to decline from 100 µL L^{-1} to approx. 40 – 75 µL L^{-1} (data not shown). After 24h the boxes containing the avocado fruit were opened and transferred to 12°C for subsequent ripening (Terry et al. 2007). Fruit sub-samples (Spanish n = 3, Peruvian and Chilean n = 6) were

randomly selected four times during 11 days of storage and objective color changes recorded (Terry et al. 2007).

Sample preparation

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 planar 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 (rounded) of the fruit. Alternate slices were selected resulting in five approx. 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 Instron (model 5542, MA) uniaxial testing machine equipped with a calibrated 500 N or 5 N load cell, depending on the maximum (max.) loads recorded on the test day. Textural tests for each slice were done vertically such that the planar surface of the slice was in contact with the sample stage. Three replicate measurements were carried out on each mesocarp 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 this position for 1 min (Sakurai and Nevins, 1992). A right-circular-cylinder probe of 6 mm diameter was used to cover a large number of cells (approx. 7500), which were damaged during the test. However, the low indentation depth ensured minimal destruction of the tissue sample. At least 5 g fresh mass (FM) of three measured mesocarp tissue samples (apex, middle, base) were snap-frozen in liquid nitrogen immediately after testing and stored at -40°C to preserve these for lipid 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 mN or 2 mN depending on the load cell used, or at a maximum rate of 10 s^{-1}). The apparent elastic modulus was calculated.

The viscosity data, *viz*. the minimum (min.) relaxation time, was calculated later using Matlab software (7.0.4.365 R14 Service Pack 2, The MathWorks Inc., 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 (Eq. 1). Vectors of varying relaxation time values were obtained from these calculations. The min. relaxation time values were chosen as an indicator for viscosity.

Equation 1:

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

t is the time during the test. τ_t is the recorded load during the test. $\tau_{t=0}$ is the max. load at an indentation of 0.6 mm.

Lipid extractions

The above mentioned frozen mesocarp tissue samples (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 assessed and samples returned to -40°C until further processing. Lyophilized mesocarp tissue was ground, weighed (to 1 g) and a cold hexane extraction performed as

described by Meyer and Terry (2008) to obtain oil from the same sample. Briefly, the samples were homogenized with hexane and filtered through a Fisherbrand QL 100 filter paper (Fisher Scientific, Leics., UK). The recovered oil was weighed and returned to -40°C for subsequent fatty acid analysis.

Fatty acid methyl esters (FAMEs) were produced according to Meyer and Terry (2008): 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 identification and quantification of selected compounds was performed on a CP-Sil 88 fused silica capillary column (30 m x 0.25 mm i.d., 0.2 µm film thickness; Varian, CA). Column temperature was programmed at 55°C for 3 min, and then raised to 175°C at 13°C min^{-1} intervals followed by an isothermal period of 1 min and increased again to a final temperature of 220°C at 8°C min^{-1} (Meyer and Terry 2008). 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 fatty acid profile was calculated as percentage of total detected FAMEs, after comparison of peak areas of samples and peak areas of mix of standards of known composition.

Statistical analysis

All statistical analyses were carried out using a trial version of The Unscrambler (9.7, Camo Software Inc., NJ). Multivariate analysis was performed using partial least squares discriminant analysis (PLS-DA). Data were centered and variables were weighed by dividing with the standard deviation. Total explained variance, loading plots and score plots were used for interpretation. A full cross validation was done to perform a Martens' uncertainty test to identify the important variables in the prediction of origin, storage duration and the vertical location of the tissue slice within the fruit (y-variables or "treatment"). Different test days were combined by storage duration and termed: "before" = day 0, "early" = day 3 or 4, "later" = day 6 or 7, "store end" = day 10 or 11. The x-variables included in the PLS-DA were the measured parameters: dry matter content [%DM FM^{-1}], oil content [g g^{-1} DM], max. load [N] and apparent elastic modulus [N cm^{-2}] (both transformed by calculating log10), min. relaxation time [s], palmitate [%] and palmitoleate [%] (both transformed by calculating power of 2), oleate [%], linoleate [%], linolenate [%]. Solely avocado tissue samples which were tested for texture and lipids were included in the analysis (apex, middle, base).

Results and Discussion

In avocado, an evaluation of the stress-relaxation properties of the tissue showed that a high elasticity could not be found in conjunction with a low viscosity (Sakurai and Nevins 1997). These findings were confirmed with the obtained results (Fig. 1).

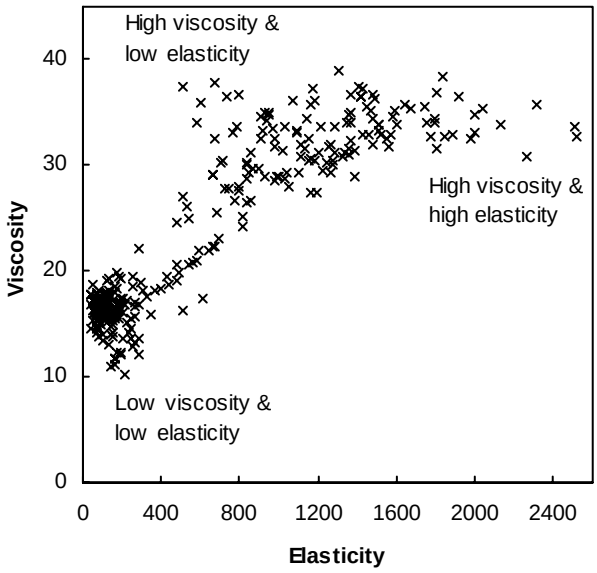

Figure 1. Min. relaxation time [s] plotted versus apparent elastic modulus [N cm^{-2}]. Results of 180 avocado mesocarp tissue slices of 60 fruit.

Origins

Avocado is classed as a lipid-based product due to its high content of oil. Bower and Cutting (1988) stress that an acceptable taste of avocado fruit is achieved after a certain level of maturity is reached on the tree. The ripening process leads to changes in color, taste and texture of the avocado. In most avocado varieties the maturity is based on lipid metabolism and rapid oil accumulation can be found at the onset of maturity (except for varieties originating in Florida, Bower and Cutting, 1988). The FAME profiles reported herein are generally in the range of that reported by others (Eaks 1990, Requejo-Tapia et al. 1999, De La Plaza et al. 2003, Ozdemir and Topuz 2004, Vekiari et al. 2004), but significant differences were observed between avocados from Peru and the other two origins. The different oil content and FAME profiles found in fruit from different origins could be related to seasonal variability or to length of transit time.

The avocado samples from Peru could be segregated from the Chilean and Spanish avocado fruit samples, which in turn overlapped slightly (Fig. 2). Almost all parameters (except linolenate) were important in the model to explain different origins as the loading plot of the PLS-DA indicates (Fig. 3). The textural parameters were closely related. Palmitate, palmitoleate and linoleate percentages seemed to be linked. No clear relationship between textural parameters and lipids was found. Only the oil content seemed to be negatively related to texture, which could be due to the different water contents observed between fruit from the three origins (data not shown).

Figure 2. Score plot of PLS-DA to predict the three origins of 60 avocado fruit (slices n = 180).

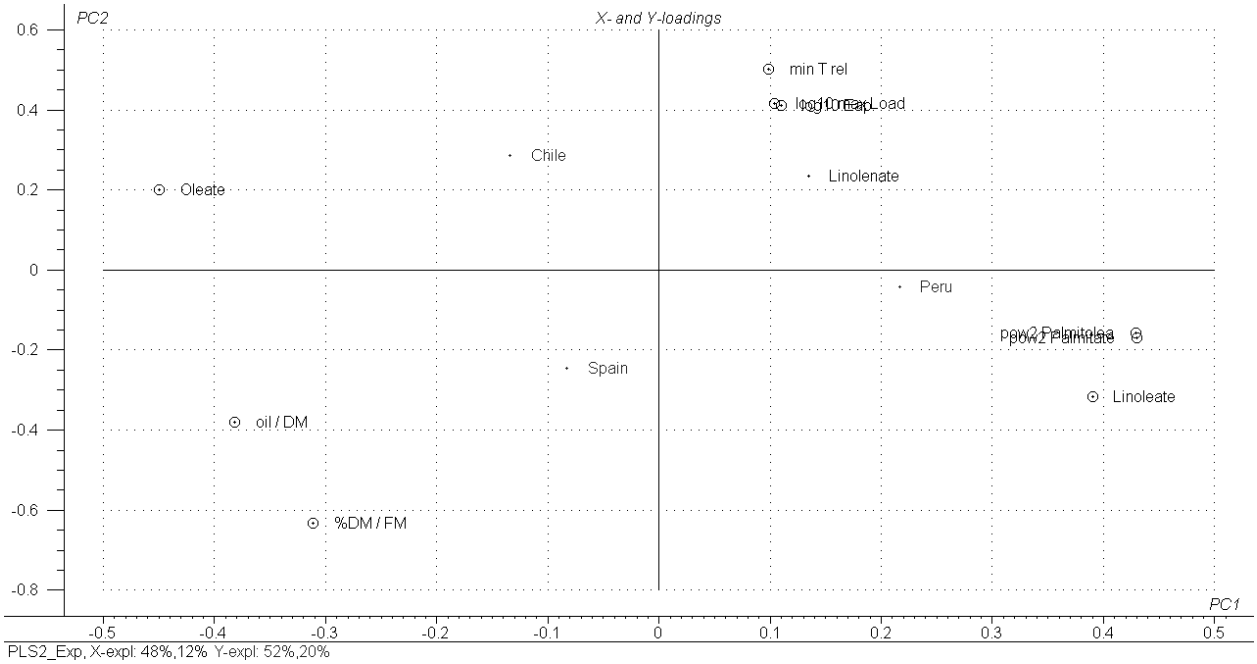

Figure 3. Loadings plot of PLS-DA to predict the three origins of 60 avocado fruit (slices n = 180). Circled variables were important for the model.

Storage time

The avocado fruit measured at the beginning and the end of the experiments could be separated using PLS-DA. Textural parameters were the most important variables required to predict effect of different storage time (Fig. 4 and 5). This confirms that the measurement of texture is appropriate to interpret fruit quality. Interestingly, the viscosity was not closely related

to elasticity and max. load. Sakurai and Nevins (1997) found decreases in min. relaxation time and suggested a decrease in cell wall viscosity during avocado ripening. The decrease in viscosity of the cell wall matrix has been shown to be due to depolymerization of cell wall polysaccharides (Sakurai and Nevins 1992), therefore it is suggested that max. load and elasticity are related to another mode of structural change. Bower & Cutting (1988) suggested that early stages of softening in avocado fruit appear to be due to cellulase activity, which leads to disorganization in the cell walls. Polygalacturonase is believed to be responsible for the final fruit softening during ripening, which causes a loss of density in the tissue by weakening of the middle lamella. It is known that fruit firmness partly depends on cell adhesion and tissue density, this seems to be the case for max. load and elasticity as well.

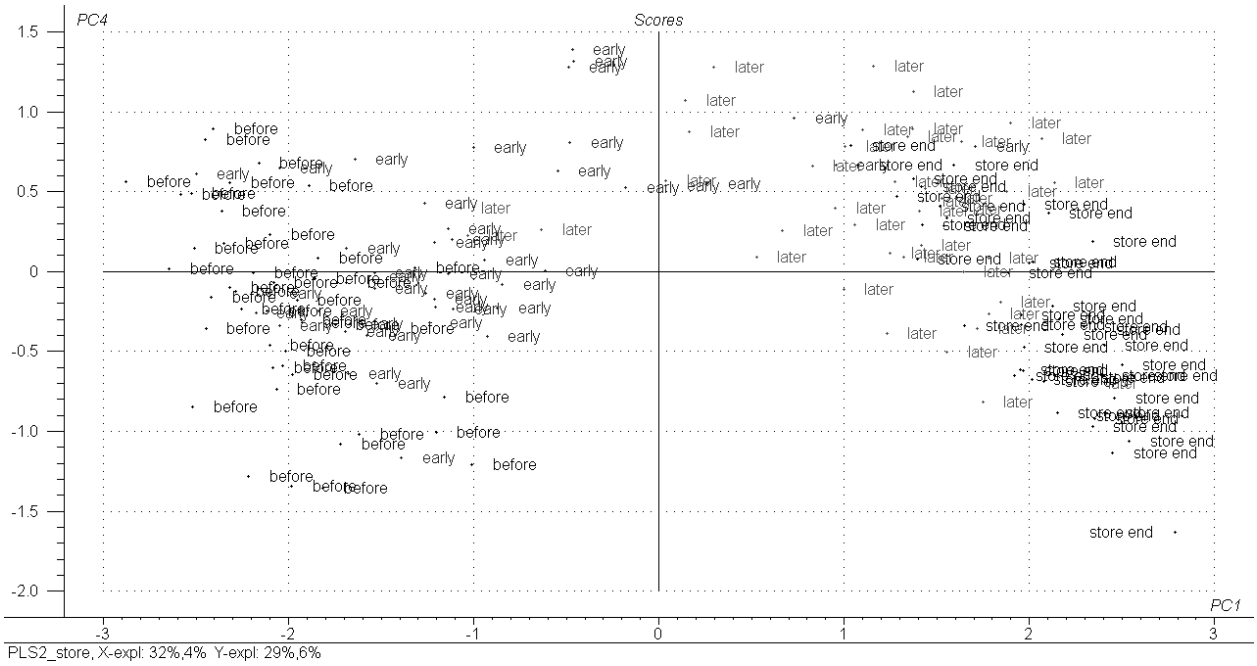

Figure 4. Score plot of PLS-DA to predict the four storage times of 60 avocado fruit (slices n = 180).

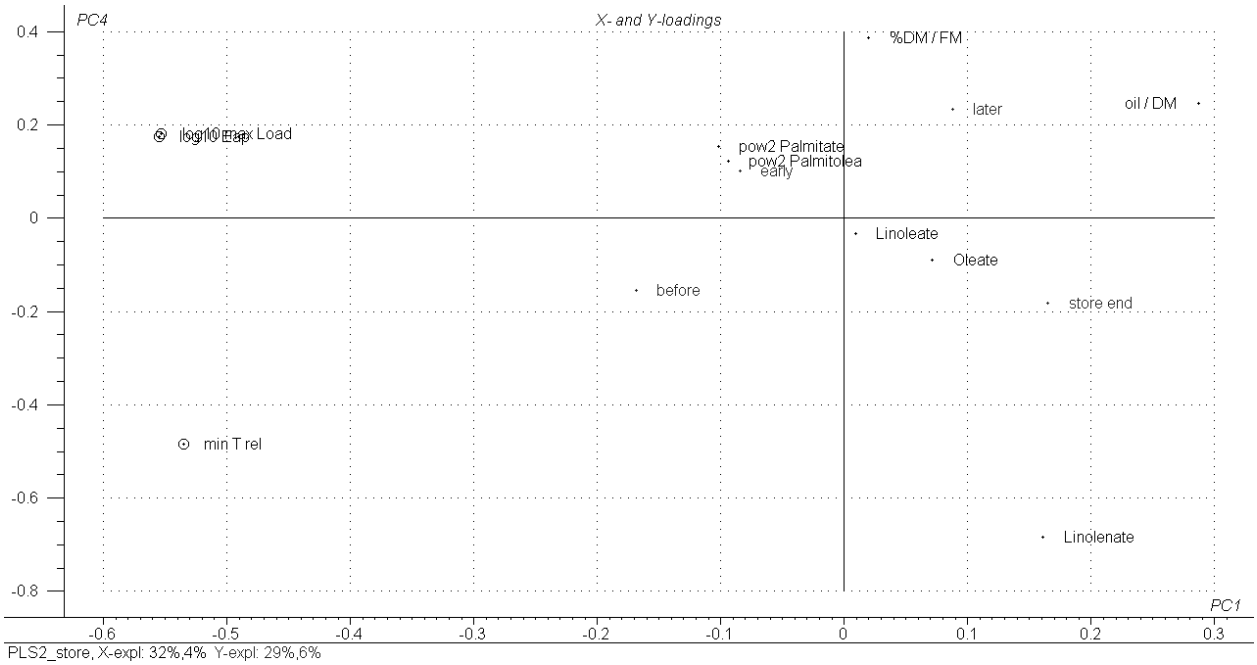

Figure 5. Loadings plot of PLS-DA to predict the four storage times of 60 avocado fruit (slices n = 180). Circled variables were important for the model.

Vertical location within fruit

The classification of tissue from different vertical locations within the avocado fruit was not clear when all origins and storage times were included in the PLS-DA since the explained variance of location within the fruit amounted only to 7% for the first two latent variables (Fig. 6). That said, the apical slice could be distinguished from the middle and basal tissue slices, when a single origin and storage time were selected (data not shown). The most important variables were the texture parameters, which were closely related. In addition, palmitate, palmitoleate and linoleate were weighted closely in the loadings plot. Schroeder (1985) measured dry matter content at different locations within avocado *cv*. Hass fruit and deducted that oil content in tissue near the rounded base of the fruit was higher than for other regions. This finding was not clearly shown in the experiments presented herein.

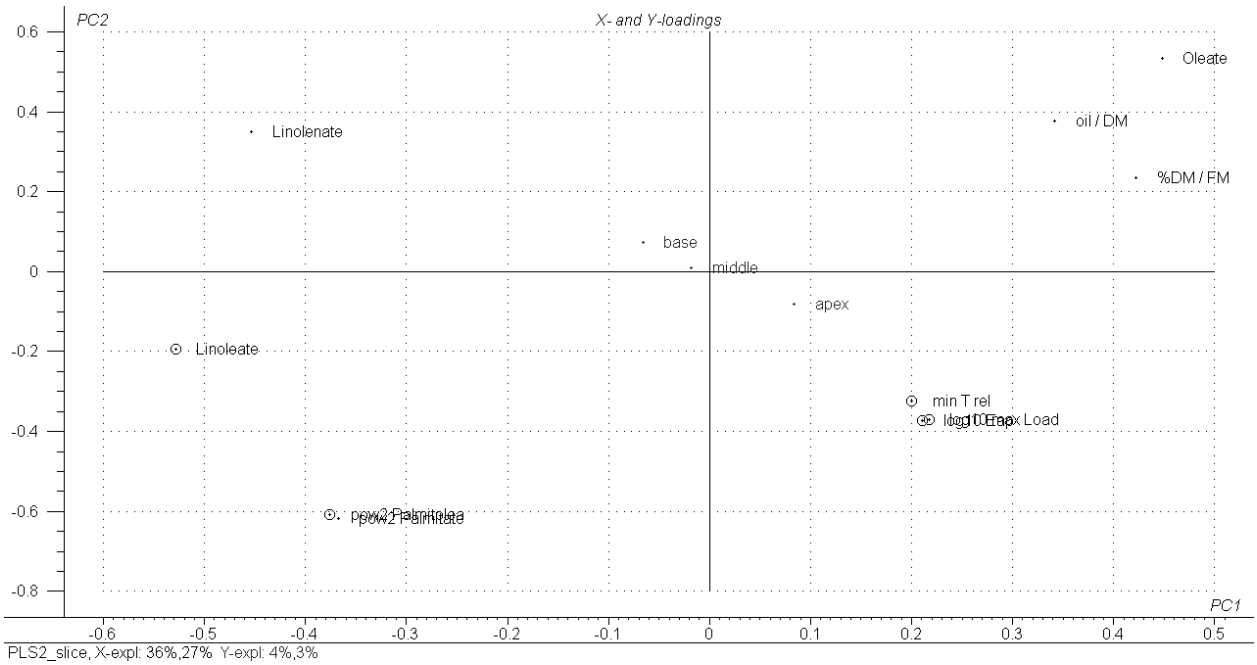

Figure 6. Loadings plot of PLS-DA to predict the three vertical locations within each of 60 avocado fruit (slices n = 180). Circled variables were important for the model.

Conclusions

Spatial distribution of fatty acids and texture within avocado fruit from the same consignment showed that sampling location is critical when interpreting fruit status and ultimately quality. The current study has demonstrated that stress-relaxation measurements are well suited to monitor origin and ripeness of avocado fruit. A mathematical correlation between lipids and textural parameters was not found, but these parameters are equally well suited to provide the basis to predict the location within individual avocado fruit, and fruit origin.

Acknowledgements

This work forms part of a larger FoodLink project (AFM 235) and is financially supported by the UK Government (Department for Environment, Food and Rural Affairs, Defra) and UK industry representatives (Lambda Photometrics Ltd., M. W. Mack Ltd., Marks and Spencer Plc, Unilever R&D). We thank Dr. Conrad Bessant, Cranfield University, for advice on statistics. The technical support of Balwinder Cook and Rosemary Burns, Cranfield University, is gratefully acknowledged.

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