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# Methods to analyze physico-chemical changes during mango ripening: A multivariate approach

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#### ABSTRACT

Canonical discriminant analysis (CDA) was used to identify the best method to discriminate between maturity and ripening stages, assessed in terms of dry matter content, firmness, color (peel and flesh), total soluble solids content attributes, before and during 'Keitt' mango ripening at 20 °C. Dry matter content was determined by hot-air oven and microwave oven methods. Fruit firmness was determined nondestructively by hand squeezing, with a durometer, using acoustic resonance and low-mass elastic impact methods (AWETA), as well as destructively by the penetrometer. Peel and flesh color were expressed as  $L^*$ ,  $a^*$ ,  $b^*$ ,  $h^0$  and  $C^*$  values. Total soluble solids content was analyzed from filtered juice from whole fruit tissue and from unfiltered juice squeezed out by hand. Canonical discriminant analysis indicated that the durometer and the penetrometer were better methods to assess firmness than hand firmness, acoustic resonance or impact methods. The best color attributes to follow changes during early stage of 'Keitt' mango ripening were  $a^*$  and  $b^*$  values of the flesh, whereas  $b^*$  value of the peel was considered better during later stages of ripening. Results of total soluble solids content in filtered juice from whole fruit tissue were less variable compared to unfiltered juice squeezed out by hand. Dry matter content was better assessed by drying the sample in a microwave oven than in a hot-air oven. A combined CDA including the best methods to assess each ripening attribute, as well as titratable acidity, showed that the best tools to assess changes in fruit during ripening were the penetrometer, followed by flesh  $a^*$  value and total soluble solids content (from filtered juice from whole fruit).

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#### 1. Introduction

Mango (*Mangifera indicia* L.) is a tropical fruit generally harvested at the mature green stage when shipped to distant markets to minimize over-ripening and losses in quality during postharvest handling and transportation. The stage of ripeness and eating quality of mango fruit is judged by a variety of attributes evaluated by various methods at specific steps in the supply chain. The main physico-chemical attributes related to ripening quality of mango fruit include firmness, flesh color (sometimes peel color), total soluble solids content, titratable acidity, and aroma volatiles (Lalel et al., 2003; Li et al., 2009; Yashoda et al., 2006). Accurate determination of fruit ripening stage is important for fresh cut products,

and to provide a consistent supply of good quality fruit for retail marketing (Saranwong et al., 2004). Recent studies have focused on developing non-destructive techniques to evaluate the internal quality and stage of ripeness of mangoes (Delwiche et al., 2008; Jha et al., 2005).

Firmness has been considered a reliable indicator of mango maturity at harvest and ripeness during commercial mango handling, and an important tool for growers, importers, retailers and consumers. Fruit firmness changes from very hard at harvest to very soft at the fully ripe stage. Penetrometers (hand held and automated) have been the most common destructive method used to measure the firmness of mango fruit (Al-Haq and Sugiyama, 2004a; Mitcham and McDonald, 1992). The penetrometer measures the force (Newtons) required to plunge a metal probe of known diameter to a certain depth in the fruit flesh. Polderdijk et al. (2000) reported that penetrometer firmness gave less reliable results than hand firmness (degree of force required by palm of hand or fingers to compress fruit cheeks by 1–2 mm), especially during the later stages of fruit ripening. There has been interest

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to develop a non-destructive firmness method for mango fruit. A firmness test, where fruit was compressed on the most convex part (the cheek), using a texture analyzer was not able to discriminate small changes in firmness as occur during mango maturation (Sirisomboon et al., 2008). Recently, a handheld impact firmness tester was designed to measure fruit firmness while the fruit remain attached to the tree (Slaughter et al., 2009). Previous studies have shown that a non-destructive acoustic transmission technique and ultrasonic wave attenuation can be used to measure flesh firmness in mango (Al-Hag and Sugiyama, 2004b; Mizrach et al., 1999; Valente and Ferrandis, 2003). The use of a sound velocity technique has been suggested to measure the stage of ripeness of mango, although it does not assess the same characteristics as a penetrometer (Subedi and Walsh, 2009). Based on a principle similar to the acoustic method, the sound velocity method uses a 'hand gun' with a plunger that moves along the barrel and lightly taps the fruit surface to produce vibrations, which are detected by 2 unidirectional microphones.

Most of the previous studies focused on analyzing firmness changes using only one technique at a time and a very little information is available comparing different methods to determine firmness changes during mango ripening. Even though acoustic and ultrasonic wave attenuation methods are fast and nondestructive, commercial use of these techniques has been very limited because of the high installation cost of automated systems and variability in results depending on fruit type. A reliable non-destructive method to determine ripening stage during packing and distribution will help to improve the quality of mangoes delivered to retailers and consumers.

The amount of soluble sugars and acidity are important components of the flavor of ripe mangoes, along with aroma volatiles. Mature green 'Keitt' mangoes accumulate approximately 7% starch which is converted to soluble sugars during fruit ripening (Simao et al., 2008). Recent studies have indicated that non-destructive near-infrared (NIR) technology can be used to segregate mangoes based on total soluble solids content (Delwiche et al., 2008; Saranwong et al., 2003). However, refractometers are commonly used to quantify total soluble solids content under commercial conditions. Commercial mango growers generally squeeze juice from a half mango (cheek) onto the reflecting mirror of a refractometer, while quality laboratories squeeze a wedge of tissue or the entire half mango and filter the juice through cheesecloth before reading with a refractometer. The difference between the 2 methods is unknown. Total soluble solids content has been strongly correlated with sweetness, and sucrose was the predominant sugar responsible for sweetness in ripe 'Keitt' mangoes (Silva et al., 2008). Both citric and malic acids contribute to titratable acidity which decreases during ripening of 'Keitt' mangoes (Medlicott and Thompson, 1985).

Fruit flesh color is an important indicator of maturity and ripeness. All mango cultivars develop orange and yellow pigments in the flesh with maturity and ripening, but changes in skin color are not always correlated with maturity, ripeness or internal eating quality. During ripening, peel color may change from green to yellow or deep orange, depending on the cultivar, or may remain green. 'Tommy Atkins' mango peel developed more red and yellow pigments than 'Keitt' mango (Mitcham and McDonald, 1992).

The final eating quality of mango fruit depends largely on harvest maturity. Discrimination of mature and immature mango fruit at harvest is extremely important because an immature mango fruit never attains its full eating quality potential. The initial development of internal flesh color is a good indicator of fruit maturity. Dry matter content has also been shown to be a good indicator of harvest maturity of mango (Saranwong et al., 2004) and is correlated to the final total soluble solids content achieved in the ripe fruit. However, the hot-air oven method used to quantify dry matter is very slow (~2 d) and inefficient and has not been adopted by the mango industry. Recent research has shown the potential use of a NIR technique to assess dry matter as a harvest maturity index for mango (Subedi et al., 2007). However, this technology is new and requires further improvements to make it reliable and affordable for mango growers. Previously, a simple microwave technology was developed to determine dry matter content in avocado (Lee et al., 1983), corn (Beewar et al., 1977), kiwifruit (Ragozza and Colelli, 1990), and olives (Micelbart and James, 2003). As reported in other crops, use of microwave technology to determine dry matter content can be a quick and simple method to judge harvest maturity in mango fruit, although a non-destructive technique would be advantageous for fruit sorting after harvest.

ANOVA is commonly used to analyze the significance of differences between groups (such as ripening stages or treatments) for each parameter measured. However, ANOVA does not show how treatments or groups compare when all attributes are considered together, or how those attributes may be inter-related. This is relevant, for example, when the main objective is to identify the best methods and/or ripening attribute(s) to track changes in fruit ripening. Multivariate analysis techniques, such as canonical discriminant analysis (CDA), can be used to identify the best method to assess each physico-chemical attribute during fruit ripening, as well as the main attribute(s) to discriminate postharvest ripening changes. Canonical discriminant analysis provides standardized canonical coefficients (SCC), which are used to rank attributes in order of their contribution to the separation of groups and to characterize the canonical discriminant functions (CDFs), and canonical correlation (r) between CDFs and the original attributes. While SCC provide information about the attributes contributing jointly (multivariate contribution), r shows the importance of each attribute independent of the others (univariate contribution) to the separation of groups (such as ripening stages) (Cruz-Castillo et al., 1994). The use of parallel discriminant ratio coefficient (DRC), a product of SCC and r, has been suggested to assess the relative importance of attributes in a CDF, with attributes having large and positive DRC's having more power in discriminating groups (Thomas, 1992; Thomas and Zumbo, 1996).

The objective of this study was to use CDA to determine the most reliable methods to analyze the major physico-chemical changes during ripening of 'Keitt' mangoes, as well as to investigate the best physico-chemical indices to monitor changes in mango ripening. In addition, the use of the microwave oven was compared to hot air oven drying as a quick and reliable method for quantifying mango dry matter content, an indicator of fruit maturity and eating quality potential.

#### 2. Materials and methods

Mature green 'Keitt' mango fruit were procured from a commercial packinghouse in Corona, CA. Fruit were harvested one day before packing, and transported on the same day, in an air conditioned vehicle, to the Postharvest Pilot Plant at the University of California, Davis. Fruit with poor quality, misshapened, sunburned or immature based on cheek fullness and shoulder shape were discarded. After sorting, boxed fruit were allowed to ripen at 20 °C and 85-90% relative humidity. Twenty fruit were analyzed on day zero (immediately after sorting) and every other day until fully ripe (14d). All quality measurements and methods to measure quality (firmness, color, total soluble solids, dry matter, and titratable acidity) were made on the same fruit for the same period of evaluation, starting with the non-destructive measures. Each evaluation period during ripening was considered as a treatment, and each treatment had 20 single fruit replications.

#### 2.1. Firmness

To determine the most reliable and applicable method to measure firmness changes during ripening, fruit were assessed: (1) by hand squeezing, (2) with a Durometer (model Digital DD-3, Rex Gauge Company Inc., Buffalo Grove, IL), (3) with an acoustic, (4) impact firmness sensor (AFS) unit (AWETA, Nootdorp, The Netherlands), and (5) with a penetrometer (Fruit Texture Analyzer, GÜSS Manufacturing Ltd., South Africa).

Mango fruit assessed by hand were gently squeezed at the cheeks and a firmness score was given according to a 5-1 hedonic scale where 5 = very firm, 4 = sprung, the flesh deforms 1-2 mm with firm finger pressure, 3 = near ripe, 2-3 mm deformation achieved with slight finger pressure, 2 = ripe or eating soft, fruit deforms with moderate whole-hand pressure, and 1 = over-ripe, whole fruit deforms with slight whole-hand pressure.

The durometer equipped with a type 'E' tip (hemispherical in shape with 2.5 mm radius, 8.5 N spring force at full displacement, ASTM D2240) and fitted on an operating stand (model OS-2H, Rex Gauge Company, Inc., Buffalo Grove, IL) was used to measure firmness. The operating stand featured a load weight and a dampener which lowered the durometer at the same rate for each measurement, eliminating operator error and resulting in superior repeatability when measuring viscoelastic materials like mango. Results were expressed as the mean value of 2 separate readings taken at the center of the cheek on each side of the fruit. The fruit deformation by the probe was quite small (<2.5 mm) and usually did not cause noticeable damage to the peel or flesh.

Two additional non-destructive measures of firmness, a sonic stiffness (Abbott and Massie, 1998) index by acoustic resonant frequency and a low-mass elastic impact response (Chen et al., 1996) were also conducted. Mango fruit placed on the measurement cup receives an impact from a round-ended tapper. The elastic impact response is measured in addition to main resonant frequency, and fruit weights are recorded, and these variables are used to calculate the 2 'firmness indices' (Polderdijk et al., 2000). The average of two measurements, one on each cheek, were made for each mango fruit.

For penetrometer measurement using the Fruit Texture Analyzer, the flesh from both cheeks was cut from each fruit. Inner flesh tissue from one cheek was measured at 4 locations, 2 at the proximal ends and 2 in the center, using an 8 mm Magness–Tayler type probe (Abbott, 1998). The firmness (*N*) was determined as the mean of these 4 measurements from one cheek.

#### 2.2. Color

Peel color was measured at the equator on opposite cheeks of the fruit. Flesh color was measured in the center of one cut cheek, with two measurements per fruit. Color measurements were made using the Minolta CR-200 colorimeter (Minolta, Ramsey, NJ) and were expressed as lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), chroma [color saturation, ( $a^{*2} + b^{*2}$ ) 0.5], and hue angle [arctangent ( $b^*/a^*$ ) × 360/(2 × 3.14)].

#### 2.3. Total soluble solids content and titratable acidity

Collection of juice for determination of total soluble solids content was made by two different methods; one commonly used by the mango industry and one used by research personnel. In the first method, one cheek was cut off the fruit and a few drops of juice were squeezed by hand directly onto the reflecting mirror of an automatic refractometer (AR6 Series/Reichert Analytical Instruments, Depew, NY). In the second method, the second cheek from the same mango fruit was peeled, chopped into small pieces and juiced through 4 layers of cheese cloth using a manual juicer. Total soluble solids content was measured by placing a few drops of filtered juice onto the refractometer. A portion of the filtered juice was used for determination of titratable acidity. Juice was titrated against  $0.1 \text{ mol } \text{L}^{-1}$  NaOH to pH 8.2 using an automated titrator (TIM 850 titration manager connected to a SAC80 sample changer, Radiometer Analytical SAS, Lyon, France). Only one standard method was used to determine changes in titratable acidity and results were expressed as malic acid equivalent in the juice.

#### 2.4. Dry matter

To quantify dry matter content of flesh tissue, thin slices  $(\sim 1 \text{ mm})$  were removed from the equatorial region of each fruit. Approximately 5 g of tissue was placed onto filter paper in a Petri dish. The sample was dried in a microwave oven (1.45 kW) and was weighed after every 5 min until reaching a constant weight. Our initial assessment indicated that a low power setting resulted in burning of the tissue samples. Total time to dry the tissue to a constant weight was approximately 35 min, depending on the power of the microwave oven. Another sample weighing approximately 5 g was placed in a pre-weighed aluminum dish and left to dry in a hot air oven  $(60 \,^\circ\text{C})$  until a constant weight was reached (2 d). Dry matter content was calculated as the percentage of fresh weight. The results for dry matter measured using these two methods were subjected to regression analysis and compared by CDA.

#### 2.5. Statistical analysis

Statistical analysis of the data was carried out using SAS software (SAS Institute, 2002). The PROC GLM procedure was used for analysis of variance (ANOVA) and multiple comparison tests (Bonferroni; P < 0.05). The best fit between dry matter content analyzed with the microwave and hot-air oven was achieved using PROC REG procedure. Canonical discriminant analysis was performed to identify the best methods to assess dry matter content and changes in fruit firmness, total soluble solids, and peel and flesh color during fruit ripening using the PROC CANDISC procedure. The power of each method to discriminate between ripening stages was investigated by calculating the DRC. In addition, the best methods to assess each ripening attribute as well as titratable acidity were collectively submitted to CDA to categorize them for power (DRC value) in discriminating fruit ripening stages. A plot of individual scores was used to visualize how the first 2 canonical discriminant functions  $(CDF_1 \text{ and } CDF_2)$  accounted for separation between ripening stages. Also, a biplot of canonical correlations (r) between the variables in the model and  $CDF_1$  and  $CDF_2$  was constructed. These values of "r" represent factor loadings of the variables on each CDF.

#### 3. Results and discussion

#### 3.1. Firmness

All firmness methods detected a decrease in firmness during the period of fruit ripening (Fig. 1). None of the methods showed any significant firmness changes during the first 2 d at 20 °C. The penetrometer showed substantial loss of firmness between 2 d and 8 d after harvest, with slow changes thereafter (Fig. 1C). This pattern is consistent with the change in firmness after harvest in mango observed by Jarimopas and Kitthawee (2007). The durometer readings showed a more gradual loss of firmness until day ten, followed by a rapid loss of firmness (Fig. 1D). This pattern was similar to that obtained with the Aweta impact and hand firmness methods. The Aweta impact firmness method and hand squeezing method were not able to differentiate firmness changes during the first



**Fig. 1.** Postharvest changes in firmness of 'Keitt' mango fruit measured during ripening at  $20 \degree C$  by (A) Aweta acoustic impulse, (B) Aweta impact response, (C) Penetrometer (Güss Fruit Texture analyzer), (D) Durometer, and (E) hand. Each value represents the mean of 20 fruits analyzed at each ripening stage. For each method, firmness values during ripening followed by the same letter were not significantly different (Bonferroni; P < 0.05).

6 and 4 d of ripening, respectively. These results were not unexpected since the methods rely on a measureable change in the elastic deformation of the tissue. Unlike, the penetrometer, where energy applied during measurement is only limited by the model of instrument used, nondestructive firmness measurements, by design, only impart a small amount of energy to the fruit during measurement in order to prevent damage. When the fruit are hard, the low level of energy delivered to the fruit by nondestructive methods often results in very small levels of deformation, reducing the sensitivity of the method. The durometer provided better resolution for low fruit firmness and would be more useful than the penetrometer in tracking firmness changes during later stages of ripening. Similar results have been shown with pear fruit assessed for changes in firmness with the durometer and penetrometer (Slaughter and Thompson, 2007). However, the penetrometer provided better resolution at high fruit firmnesses and would be more useful than the durometer in tracking firmness changes of less ripe fruit or fruit maturity at harvest. Overall, the penetrometer firmness decreased from 61 N at harvest to 5.3 N on the fourteenth day of ripening.

The CDA for firmness assessment methods showed that the first canonical discriminant function  $(CDF_1)$  explained 70.6% of the total variation, with a canonical correlation between firmness attributes and days after harvest of 95.1%. Therefore, most of total discrimination between changes in firmness with fruit ripening

was accounted for by  $CDF_1$ . The Wilks' Lambda multivariate statistic test indicated a highly significant (P < 0.0001) difference in fruit firmness between ripening stages for  $CDF_1$ .

The  $\text{CDF}_1$  for firmness showed high DRC values for durometer and penetrometer measurements of firmness (Table 1) indicating that these methods provided better discrimination of changes in firmness during ripening, while maintaining low variability within the group of fruit at the same stage of ripening. As previously observed, the penetrometer is better suited for monitoring early ripening changes and the durometer is better suited for later ripening stages. Acoustic and hand firmness were the least reliable methods to follow the changes in firmness during mango fruit ripening (Table 1).

Al-Haq and Sugiyama (2004b) found that the asymmetrical shape and presence of a hard stone resulted in differences in transmission velocities measured by a portable acoustic firmness tester at different positions on a mango fruit. The higher acoustic firmness values on the less fleshy side of the fruit than on the more fleshy side (i.e., fruit cheeks) were attributed to the reduced distance from the seed. Sensitivity to fruit orientation and shape has been reported as a limitation of the acoustic method (Shmulevich et al., 2003), which has been confirmed here by comparing acoustic firmness with other firmness measurements on same mango fruit. Further, flesh temperature and moisture status of the fruit may also affect the acoustic response (Slaughter and Thompson, 2007).

#### Table 1

Parallel discriminant ratio coefficients (DRC) for canonical discriminant function 1 ( $CDF_1$ ) of firmness, skin color, flesh color, total soluble solids (TSS) content, and dry matter content, measured by different methods or parameters, in 'Keitt' mangoes during ripening at 20 °C for 14 d.

Attribute	CDF1
Firmness	
By hand	0.059
Durometer	0.640
Acoustic response by AWETA	0.023
Impact force by AWETA	-0.248
Penetrometer	0.525
Color	
Flesh L*	-0.024
Flesh a*	1.068
Flesh b*	1.227
Flesh C*	-1.411
Flesh h <sup>0</sup>	-0.096
Peel L*	-0.014
Peel a*	-0.143
Peel b*	2.878
Peel C*	-2.351
Peel h <sup>0</sup>	-0.134
Dry matter	
Microwave oven	1.467
Hot-air oven	-0.467
TSS content	
Filtered juice	1.099
Hand squeezed unfiltered juice	-0.099

#### 3.2. Color

Color changes in skin and flesh tissue are presented as  $L^*$ ,  $a^*$ ,  $b^*$ ,  $h^0$  and  $C^*$  values (Fig. 2). Peel color showed no significant changes until 8–10 d after harvest while flesh color changed from whitish green to bright yellow/orange. As has been shown before, 'Keitt' mango fruit accumulated significant soluble solids from starch degradation during ripening without an obvious change in peel color (Mitcham and McDonald, 1992). There was a gradual increase in peel  $a^*$  value (Fig. 2a) beginning on day eight, and higher  $b^*$  value on days twelve and fourteenth (Fig. 2b), whereas  $L^*$  value of the peel showed no change (Fig. 2c). Previously, Medlicott et al. (1986) reported that mature green 'Tommy Atkins' mangoes showed no degradation in peel green color until after 9 d at 22 °C.

Mesocarp tissue color changed throughout ripening, exhibiting a progressive decrease in  $L^*$  value (Fig. 2c). Flesh  $a^*$  value increased consistently during 14 d of ripening compared to  $b^*$  value that showed significant changes only for the first 4 d of ripening (Fig. 2a and b). These results indicate that 'Keitt' mango flesh may develop full yellow color during early stages of ripening, but will continue to accumulate red pigments (carotenoids), until the full ripe stage, as indicated by its deep orange colored flesh (Medlicott et al., 1986). The continuous increase in  $a^*$  value in the flesh paralleled the increase in  $C^*$  value (Fig. 2d); however, the decrease in  $h^0$ value during fruit ripening was very slow (Fig. 2c).

The CDA for color assessment parameters showed a canonical correlation of 93.3% for the CDF<sub>1</sub>. The Wilks' Lambda multivariate statistic test indicated a highly significant (P < 0.0001) difference in fruit color between ripening stages for CDF<sub>1</sub>, which explained 80.7% of the total variation of skin and peel color with ripening.

The CDF<sub>1</sub> showed high DRC values for  $b^*$  value of the peel, followed by  $a^*$  value of the flesh, and then by  $b^*$  value of the flesh (Table 1). The other color attributes had DRC values that were very low ( $L^*$  value of the flesh) or negative ( $C^*$  and  $h^0$  of the flesh and  $L^*$ ,  $C^*$ ,  $h^0$  and  $a^*$  values of the peel) and were considered to be less important to discriminate between ripening stages. The high DRC value for  $b^*$  value of the peel might be the result of a rapid and substantial change in peel color between days ten and twelve, from green to yellowish green (Fig. 2d). A significant change in  $b^*$  value

of the peel after 10 d at 20 °C indicated that the  $b^*$  value of the peel shows a high power only to discriminate fruits in advanced ripening stage, as a result of peel yellowing, but not at early stages. Therefore, only  $a^*$  and  $b^*$  values of the flesh should be considered for a better discrimination of changes in color during the entire fruit ripening period for 'Keitt' mango. The gradual and substantial change in color of the flesh to a deep orange colored flesh along the entire period of fruit ripening is consistent with the high power of flesh  $a^*$  value and, to a lesser extent, the flesh  $b^*$  value, to discriminate between ripening stages.

#### 3.3. Dry matter

The presence of high dry matter content ( $\sim$ 22%) at harvest indicates that the fruit harvested for this study were physiologically mature (Saranwong et al., 2004). A high correlation coefficient (r=0.92) was observed between dry matter content analyzed with the microwave and hot-air oven methods (Fig. 3). The DRC value for CDF<sub>1</sub> for dry matter content measured using the microwave oven was higher than that of the hot-air oven method indicating that the former method provided less variable results than the latter. Our results suggest that the microwave oven could be used as a simple and rapid method to measure percent dry matter. Dry matter content in mango has been shown to be a reliable indicator of harvest maturity and eating quality during ripening (Saranwong et al., 2004) and can be used as an indicator of harvest maturity, as commonly used in the avocado industry (Lee et al., 1983). In the future, a dry matter content standard will need to be established for each mango cultivar and correlated to final ripening quality of the fruit.

#### 3.4. Total soluble solids and titratable acidity

In agreement with previous research, total soluble solids content and titratable acidity followed the expected trends during the ripening period: the total soluble solids content in the fruit increased from 9.1% to 17.3%, while the titratable acidity decreased by a factor of 3, from 0.6% to 0.2% (Fig. 4). There was a consistent increase in total soluble solids content during ripening, but a significant decrease in titratable acidity was only observed after 6d of ripening at 20°C. Citric acid and malic acid are the main organic acids in 'Keitt' mango, and decreases in titratable acidity with ripeness might be due to their utilization as substrates for respiration (Medlicott and Thompson, 1985). The mean values of total soluble solids content obtained through 2 different methods (filtered juiced from whole fruit and unfiltered juice squeezed out by hand) were similar, but total soluble solids content in juice from the hand squeezed method was always higher on average, even for fully ripe fruit (Fig. 4). This may be due to naturally higher total soluble solids in the center of the fruit, as ripening starts in the middle portion of the fruit, near the seed. However, the DRC value in CDA identified that filtered juice separated the total soluble solids content at different stages of ripening better than the hand squeezing method (Table 1). The use of filtered juice showed less variability within samples analyzed at the same stage of ripening and provided better separation between mean values at different ripening stages (particularly from 0 d to 8 d at 20 °C) (Fig. 4). Considering the time constraints in the fruit industry, the hand squeezing method to measure total soluble solids content can be used with adequate sample numbers to decrease variability and with recognition that the readings might be slightly higher than for whole fruit.

#### 3.5. CDA of all ripening attributes

After identifying the best methods to assess each ripening attribute described in Table 1 (firmness, peel color, flesh color, and total soluble solids content), a CDA was performed with all of the



**Fig. 2.** Changes in mango flesh and peel color during ripening at 20 °C. (A) CIE *a*<sup>\*</sup> value (increasing values indicating change from green to red color). (B) CIE *b*<sup>\*</sup> value (increasing values indicating change from blue to yellow color). (C) CIE *L*<sup>\*</sup> value (represent the lightness, 0 for black and 100 for white). (D) CIE *C*<sup>\*</sup> value (represent chroma, 0 for gray and 100 for vivid color). (E)  $h^0$  value (represents hue angle, 180 for green, 90 for yellow and 0 for red). Each value represents the mean of four replications, with five fruits per replication and two measurements per fruit. Standard error (±SE) bars are smaller than symbols. Values followed by the same letter within each tissue type are not significantly different (Bonferroni; *P* < 0.05).

best methods or parameters to assess each maturity and ripening attribute, to identify the best attributes to discriminate between ripening stages of 'Keitt' mangoes. The CDA showed a canonical correlation of 95.7% for the CDF<sub>1</sub>, which explained 72% of total



**Fig. 3.** Correlation between mango fruit dry matter (DM) content determined with microwave oven and hot-air oven.

variation. The second function ( $CDF_2$ ) explained only 24.2% of total variation, indicating that the maximum possible variation of fruit ripening was explained by the  $CDF_1$ . The Wilks' Lambda multivariate statistic test indicated a highly significant (P < 0.0001) difference between ripening stages for  $CDF_1$ .

Canonical scores revealed clear discrimination between ripening stages on CDF<sub>1</sub>, as shown by the clustering of data for each separate ripening day along the CDF<sub>1</sub> axis in Fig. 5. Along this axis (which explained most of total variation), firmness atributes (measured with penetrometer and durometer) and TA had positive rvalues, while SSC of filtered juice, color  $a^*$  of the flesh and color  $b^*$ of flesh and peel had negative "r" values (Fig. 6). Therefore, high values of firmness and TA (attributes with positive "r" values along CDF<sub>1</sub> in Fig. 6) points towards less ripen fruit (with positive canonical scores in Fig. 5), while high SSC and deep orange colored flesh and peel (attributes with negative "r" values along CDF<sub>1</sub> in Fig. 6) points towards more ripe fruit (with negative canonical scores in Fig. 5).

The  $\text{CDF}_1$  showed high DRC values for firmness measured with the penetrometer, followed by flesh  $a^*$  value and total soluble solids content measured on filtered juice (Table 2). Therefore, these results show that, in a multivariate sense, the destructive measurement of flesh firmness is still the best attribute to assess ripening of 'Keitt' mangoes. However, given the advantages of the non-



**Fig. 4.** Changes in total soluble solids content and titratable acidity during ripening of mango fruit. Each value represents the mean ( $\pm$ SE) of four replications, with five fruits per replication. Values followed by the same letter within each ripening attribute are not significantly different (Bonferroni; *P* < 0.05).



**Fig. 5.** Canonical scores of the first two canonical discriminant functions (CDFs) during ripening at 20 °C of 'Keitt' mango fruit, calculated with methods or parameters described in Table 2.



**Fig. 6.** Biplot of canonical correlation (r) between the variables and the first two canonical discriminant functions (CDFs) during ripening at 20 °C of 'Keitt' mango fruit, calculated with methods or parameters described in Table 2. The "r" value represents the variable loading on each CDF.

#### Table 2

Parallel discriminant ratio coefficients (DRC) for canonical discriminant function 1 (CDF<sub>1</sub>) of all the best methods or parameters to assess each ripening attribute described in Table 1 (firmness, skin color, flesh color, and total soluble solids content) plus titratable acidity measured in 'Keitt' mangoes during ripening at  $20^{\circ}$ C for 14d.

Variable	CDF1
Firmness with durometer	0.127
Firmness with penetrometer	0.406
Total soluble solids of filtered juice	0.263
Titratable Acidity	0.046
Peel color b*	-0.006
Flesh color a*	0.227
Flesh color b*	-0.063

destructive nature of the durometer, it would still be of value for the industry and researchers. By incorporating the other ripening attributes in the CDA, the durometer's power to discriminate ripening stages by tracking loss of firmness was reduced. The  $b^*$  value of the peel and flesh showed a suppressive behavior (negative DRC value) and titratable acidity also exhibited poor power (low DRC values) to discriminate between ripening stages.

#### 4. Conclusions

Canonical discriminant analysis showed that firmness with the penetrometer is the best tool to assess changes during mango ripening followed by total soluble solids content and flesh  $a^*$  value. The durometer was the most accurate non-destructive firmness method tested for mangoes. The microwave can be used as a quick method to analyze dry matter content to assess mango fruit maturity.

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