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Visualization of Polyphenol Oxidase and Phenolics Distribution in Mesocarp of Fresh-cut Mango (cv. Kent) during Storage

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Sensory quality attributes are significant factors for fresh-cut fruits. The success of their commercialization is based on their safety, flavor, aroma, texture, and visual appearance. When fruits are processed (peeling, cutting, and slicing), enzymatic browning significantly increases due to the release and interaction between polyphenol oxidase (PPO) and phenolic compounds. In this study, enzymatic browning activity of mango (cv. Kent) mesocarp tissue was correlated with the incidence of browning during storage. PPO activity was tested by applying a catechol solution to different sections of the mesocarp, and color development was evaluated every minute during a 10-minute period. Total phenolic content was evaluated similarly using a Nitroso test which results in dark red color formation in the presence of phenolic compounds. PPO activity was found to be moderately uniform through the mesocarp tissue, but was more intense close to the endocarp. Phenolic compounds in the mesocarp tissue were present in higher concentrations near the endocarp and subtending the peel. Overall, the incidence of browning on the fruit cut surface was not considered to be a limiting visual quality factor for fresh-cut mango (cv. Kent) during storage.

The convenience and quality of fresh-cut fruit are factors in their increasing popularity in the food supply and so is the demand for various fresh-cut tropical and subtropical fruits. Mango (*Mangifera indica* L.), one of the most important tropical fruits in the world and currently ranked fifth in total world production among fruit crops (FAO, 2007), is considered to be a fruit with good potential for marketing as a fresh-cut product; however, little is known about mango physiology and shelf life when processed into fresh-cut slices or chunks.

The practice of fresh-cut processing causes wounding, increases metabolic activities, and decompartmentalizes enzymes and substrates on the cut surface. This may cause flesh browning, softening and decay, and off-flavor development (Gonzalez-Aguilar et al., 2004). These manipulations result in increased rates of respiration and ethylene production and may reduce the shelf life from 1 to 2 weeks for the intact fruit to only 1 to 3 d for the fresh-cut product even at optimal temperatures. Oxidative browning of fruits and vegetables is usually caused by the enzyme polyphenol oxidase (PPO) which, in the presence of O_2, converts phenolic compounds into dark-colored pigments. The quality degradation resulting from oxidative browning of fresh-cut mango due to the unpleasant color that develops is detrimental for the commercialization of this product.

Polyphenol oxidase (PPO) activity in mango displays a cultivar-specific logarithmic increase during postharvest ripening (Vásquez-Caicedo et al., 2007). Abou-Aziz et al. (1976) showed that the skin of 'Pairi' mango contained considerably more total phenolic compounds than the flesh and this content tended to decrease with duration of storage. To our knowledge, very little is known regarding the distribution of PPO activity and phenolic compounds within the mesocarp tissue of mango fruit, while abundant information is available regarding the PPO activity and phenolics profile in the peel and kernel, two by-products of mango processing (Ajila et al., 2007; Arogba, 2000).

In the present study, the correlation of visual browning during storage with the enzymatic browning activity of fresh-cut mango (cv. Kent) mesocarp tissue was analyzed. This study explores the application of visual assay methods to assess the browning potential of fresh-cut mango by locating the PPO activity and the phenolic compounds within the mesocarp tissue of mango fruit.

Materials and Methods

PLANT MATERIAL. 'Kent' mangoes used in this study were obtained from a wholesale market in Gainesville, FL. Fruit were held at room temperature (23 to 25 °C) until the desired ripeness stage was attained as determined by complete development of the characteristic yellow ground color of the peel along with flesh firmness that yielded to gentle hand pressure. For each visual assay of PPO activity and phenolic compounds, a mango fruit was sequentially sliced with a kitchen mandoline slicer (Zyliss® easy slice mandoline™, Model 11700.4, Zyliss USA, Irvine, CA) with a 1.5-mm-slice thickness attachment, following the longitudinal axis, from the peel to the endocarp or pit (Fig. 1). The outermost (peel) slice was discarded and the remaining slices were cut to obtain two series of rectangles. The rectangular pieces were then arranged side-by-side in order from the peel side to the pit side on a piece of white cardboard. One column was used as the control while the other received the chemical reagents. Photos of the slices

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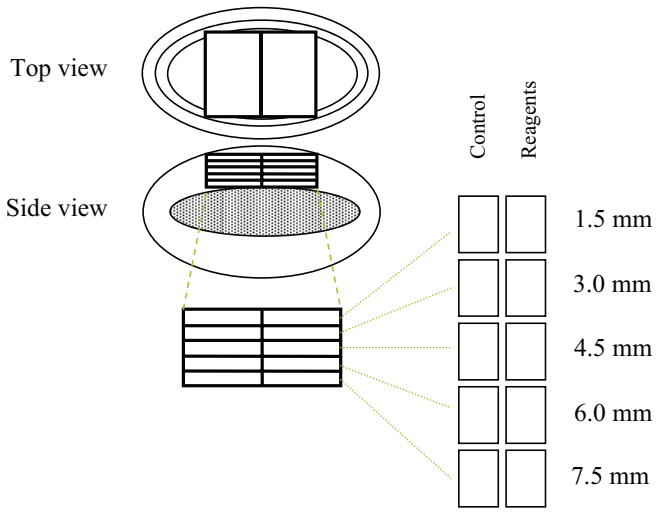

Fig 1. Sample preparation diagram.

Fig 2. Color development showing polyphenol oxidase activity in 'Kent' mango slices after 6 min of exposure to catechol solution.

were taken every minute for a period of 10 min with a Panasonic Lumix DMC-FZ20 digital camera to follow the color development subsequent to the application of reactant solutions.

Also, to have a view of the overall distribution of PPO and phenolics compounds in the mango flesh, two slices were taken from the middle and longitudinal section of one fruit half. The two slices were placed on a piece of white cardboard, the two facing sides showing (Figs. 2 and 3). One side was designated as the control while the other received the reactant solutions. Photos were also taken following the same procedure as previously described.

Polyphenol oxidase activity. PPO activity was determined following the visual assay method of Kader and Chordas (1984). The PPO activity test involves providing a substrate for PPO action and evaluating the extent of browning resulting from its oxidation and resulting formation of brown products. Cathecol was selected for use as the PPO substrate from among several phenolic compounds because of its relatively high susceptibility to PPO activity and solubility in cold water. Two to three drops of 0.1 M catechol reagent, enough to cover the whole surface, was applied to each slice. The catechol reagent was prepared by dissolving 0.55 g catechol in 50 mL of 0.1 M citric acid-phosphate buffer (one part 0.1 M citric acid solution and two parts 0.2 M disodium phosphate solution v/v; pH 6.2). Catechol reagent was freshly made on the day of its use.

Phenolic compounds. Phenolics concentration was determined following the visual assay method of Kader and Chordas (1984). In this test, sodium nitrite in the presence of acetic acid releases nitrous acid, which subsequently reacts with phenolic compounds, forming nitroso derivatives. Urea is used as a stabilizer. Nitroso derivatives, after addition of the base sodium hydroxide, are converted into sodium salts characterized by deep cherry-red color. The intensity of this color depends on the amount of phenolic compounds in the fruit tissues. To each slice of mango, one drop of each of the following reagents was applied in succession: 10% (w/v) sodium nitrite, 20% (w/v) urea [$CO(NH_2)_2$], and 10% (v/v) acetic acid. After 4 min, two drops of 8% (w/v) sodium hydroxide solution was applied to the slices.

Color assessment. Fruit surface color was measured with a handheld tristimulus reflectance colorimeter (Model CR-200b, Minolta Corp., Ramsey, NJ). Color was recorded using the CIE L*a*b* uniform color space (CIELab), where L* indicates lightness, a* indicates chromaticity on a green (–) to red (+) axis, and b* indicates chromaticity on a blue (–) to yellow (+) axis. Numerical values of a* and b* were converted into hue angle ($H = \tan^{-1} b^*/a^*$) (Francis, 1980).

Fig 3. Color development showing formation of nitroso derivatives of native phenolic compounds after application of 8% sodium hydroxide solution to 'Kent' mango slices previously treated with 10% (w/v) sodium nitrite, 20% (w/v) urea, and 10% (v/v) acetic acid.

Results and Discussion

Polyphenol oxidase activity. Development of brown color due to the action of PPO on the applied catechol during a period of 10 min varied as a function of the depth of the mesocarp tissue from the peel side to the pit (Fig. 4). Note that the control column illustrated in Fig. 1 is not shown in Fig. 4. This is because no color change was observed on the control slices during the 10-min period of the assay. For each mesocarp depth, the color changed over time from a yellow to a reddish color as expressed

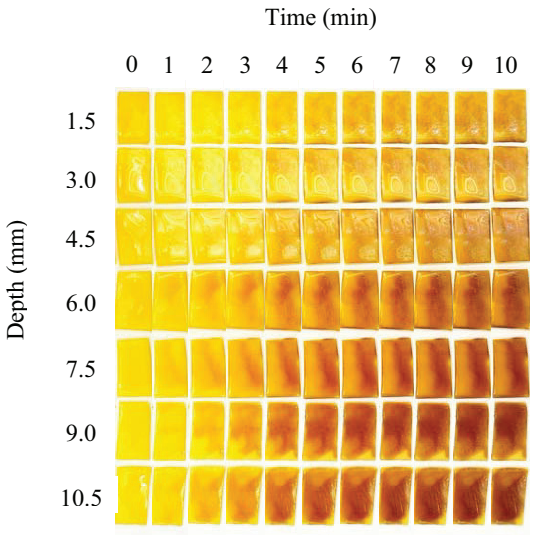

Fig 4. Color development during 10 min showing polyphenol oxidase activity at different depths of mesocarp tissue from the peel side to the pit of 'Kent' mango fruit.

by the decrease in hue angle (Fig. 5). The color change started to be obvious at 3 min after the application of the catechol solution. At this moment, the color intensified and it became possible to see that the reddish-brown color concentration varied with the depth of the mesocarp tissue: lighter near the peel side, at 1.5 mm under the peel, and becoming darker closer to the pit.

Since color development indicates the presence of PPO reacting with the catechol substrate; it is apparent that PPO is present in the mango mesocarp tissue, with higher enzyme concentrations occurring nearer the pit side. Two slices of mango mesocarp at times 0 (left slice) and at 6 (right slice) min after application of the catechol solution are presented in Fig. 2. The development of a brownish-red color throughout the tissue indicates the presence of PPO. The presence of darker coloration at the pit side of the slice is in agreement with the observation made with regard to Fig. 4.

Phenolic compounds. During the first 4 min after exposure to 10% (w/v) sodium nitrite, 20% (w/v) urea, and 10% (v/v) acetic acid, the slices did not change color (Fig. 6), which was confirmed by the constant a* and Hue angle values during that time period (Fig. 5). However, there were variations in color present within the depths of the tissue initially, with the slices from nearer

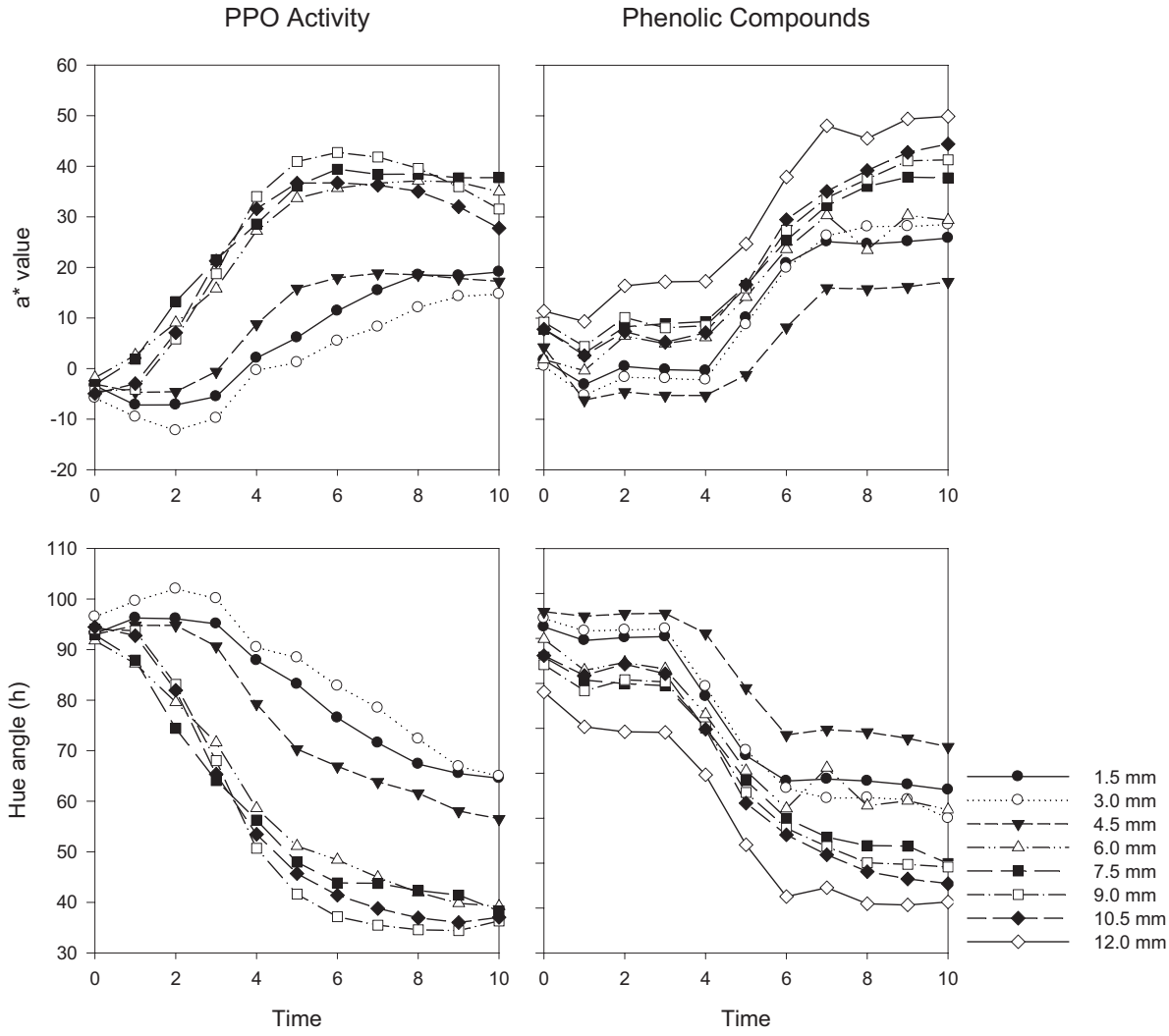

Fig 5. Changes in chromaticity values (a* and Hue angle) of 'Kent' mango slices as a function of the depth in the mesocarp tissue from the peel side (1.5 mm) to the pit side (10.5 mm and 12.0 mm for the PPO activity and the phenolic compound assays, respectively).

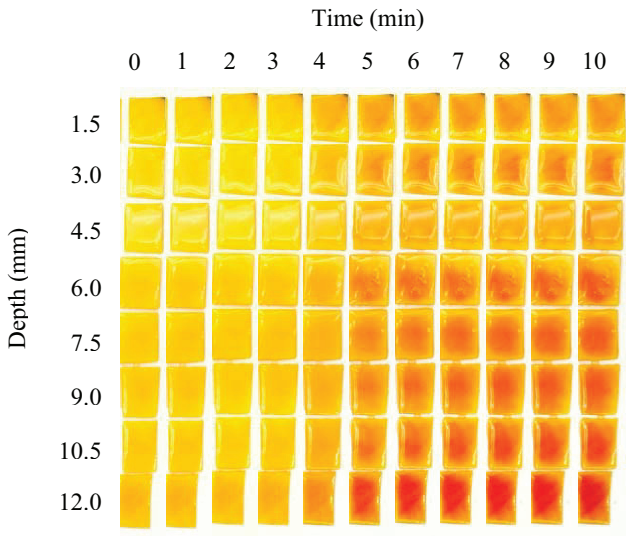

Fig 6. Color development during 10 min showing nitroso derivatives of native phenolic compounds at different depths of mesocarp tissue from the peel side to the pit of 'Kent' mango fruit.

the peel side being more yellow (lower a* value) compared to the reddish-orange color (higher a* value) of the slices nearer the pit side (Fig. 5). After 4 min, application of the 8% sodium hydroxide solution initiated the development of red color as the nitroso derivatives of the native phenolic compounds. Formation of nitroso derivatives of the native phenolic compounds varied with the depth of the mesocarp tissue from the peel side to the pit of 'Kent' mango (Fig. 6).

Color development on the slices after 6 min (Fig. 3), indicates the presence of phenolic compounds in a scattered pattern throughout the mesocarp tissue. Three main locations present an intense red color: one by the pit side and two located on the periphery of the peel. There were a few spots where very little color developed. Further investigation should be done to determine the histology and to quantify the amount of phenolics at those different locations.

Browning potential of fresh-cut mango. The assays demonstrated the presence of PPO and phenolics in the mango mesocarp, however, the fact that the controls did not discolor suggests that those levels in the mango mesocarp are low. In fact, browning of fresh-cut mango does not become noticeable until after 5 d of storage at 5 °C (S. Dea, unpublished results), which suggests that synthesis of PPO and phenolics increases during fresh-cut mango storage. Furthermore, since enzymatic browning appears at a slow rate during storage of fresh-cut mango, color change may not be the limiting factor by which the quality and shelf life of fresh-cut 'Kent' mango may be compromised. Nevertheless, the incidence of such browning development during fresh-cut mango storage would be expected to occur near the pit side of the mango slices or chunks, since higher PPO and phenolic concentrations have been demonstrated in this study to occur in that location.

Literature Cited

Abou-Aziz, A.B., F.K. Abdel-Wahab, and M.A. El-Ghandour. 1976. Effect of different storage temperatures on phenolic compounds in banana and mango fruits. Scientia Hort. 4:309–315.

Ajila. C.M., S.G. Bhat, and U.J.S. Prasada Rao. 2007. Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem. 10:1006–1011.

Arogba, S.S. 2000. Mango (*Mangifera indica*) kernel: Chromatographic analysis of the tannin, and stability study of the associated polyphenol oxidase activity. J. Food Composition Analysis 13:149–156.

FAO. 2007. FAOSTAT. Food and Agriculture Organization of the United Nations. FAO Stat. Div., Rome, Italy. <http://faostat.fao.org/> (accessed 18 May 2007).

Francis, F.J. 1980. Color quality evaluation of horticultural crops. HortScience 15:58–59.

González-Aguilar, G.A., S. Ruiz-Cruz, R. Cruz-Valenzuela, A. Rodríguez-Félix, and C.Y. Wang. 2004. Physiological and quality changes of fresh-cut pineapple treated with antibrowning agents. Lebensmittel-Wissenschaft und-Technologie 37:369–376.

Kader A.A. and A. Chordas. 1984. Evaluating the browning potential of peaches. California Agr. 38(3&4):14–15.

Vásquez-Caicedo, A.L., S. Schilling, R. Carle, and S. Neidhart. 2007. Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. Food Chem. 102:1172–1186.