Table 2. Changes in standard curve correlation coefficient (r) through time.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-0.997149</td>
</tr>
<tr>
<td>5</td>
<td>-0.996521</td>
</tr>
<tr>
<td>10</td>
<td>-0.994151</td>
</tr>
<tr>
<td>15</td>
<td>-0.990756</td>
</tr>
<tr>
<td>20</td>
<td>-0.988862</td>
</tr>
<tr>
<td>25</td>
<td>-0.984617</td>
</tr>
<tr>
<td>30</td>
<td>-0.980215</td>
</tr>
</tbody>
</table>

rimetric estimates were in close agreement, giving a value of 288.6 ± 11.21 units • ml⁻¹ (n = 16). Colorimetric assay of 0.006, 0.009, 0.012 and 0.015 units of OPPME indicated that the microplate assay was accurate between 0.009 and 0.015 units of enzyme (Table 3). Higher concentrations could be assayed but were limited by the rapidity of the change in optical density. The activity estimate obtained by assaying 0.006 units was only one-half of the predicted value (0.0029 units).

The commercial non-pasteurized orange juice contained 0.0133 ± 0.0005 μ equivalents acid released • min⁻¹ • μl⁻¹ (n = 18) while the hand-expressed juice contained only 0.001 ± 0.00003 μ equivalents acid released • min⁻¹ • μl⁻¹ (n = 60). These values are equal to 13.3 units • ml⁻¹ and 1.0 unit • ml⁻¹ respectively.

**Conclusions**

The colorimetric assay for PME activity introduced by Hagerman and Austin (1986) has been adapted successfully to a kinetic microplate reader. Results presented here demonstrate that the method is accurate and applicable to a commercial PME as well as a commercial, non-pasteurized and a hand-expressed orange juice. The sensitivity of the assay is slightly greater than originally reported by Hagerman and Austin (1986). As little as 0.009 units of PME could be measured accurately. The assay requires very small volumes of substrate and enzyme. The microplate format allows for extensive replication of individual samples, making statistical treatment possible. Finally, adaptation of the colorimetric PME assay to a kinetic microplate reader saves a tremendous amount of time. After becoming familiar with the procedure, 24 samples could be assayed, with each sample replicated three times, in less than one hour. Additionally, computer software is available to operate the microplate reader, collect and manipulate data and present final estimates of enzyme activity.

**Literature Cited**


**Abstract.** Carambola fruit were harvested at four stages of ripeness: dark green (DG), light green, color break (CB) and ripe. Additional ripe fruit were stored at 21°C until overripe (OR). The ratio of CIE color a/b increased during ripening inversely to the decrease in fruit firmness. Respiration and ethylene production of carambola fruit of different ripeness stages suggested a possible climacteric pattern. However, daily monitoring of individual fruit respiration and ethylene production provided inconclusive evidence as to the climacteric/nonclimacteric nature of carambola fruit. The cell walls of DG carambola fruit were comprised mainly of cellulose (60%) and hemicellulose (27%), with pectin polymers accounting for only 13%. There was an increase in the proportion of less tightly bound chelator-soluble pectin and a decrease in

**Table 3. Activity estimates from assaying known amounts of OPPME; \( r = 0.99986 \) for 0.009, 0.012 and 0.015 units.**

<table>
<thead>
<tr>
<th>Added Activity (units)</th>
<th>Estimated Activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.006</td>
<td>0.00294 ± 0.00022 (n = 16)</td>
</tr>
<tr>
<td>0.009</td>
<td>0.00898 ± 0.00057 (n = 32)</td>
</tr>
<tr>
<td>0.012</td>
<td>0.01205 ± 0.00056 (n = 32)</td>
</tr>
<tr>
<td>0.015</td>
<td>0.01495 ± 0.00042 (n = 16)</td>
</tr>
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</table>

**Acknowledgements:** The authors would like to thank Craig Campbell of J.R. Brooks & Son for hand-selecting and donating the fruit used in this study. We also acknowledge the excellent technical assistance of Ms. Heather Tucker.

covalently-bound pectin during ripening. The amount of total cell wall uronic acid decreased at the OR stage. The amount of hemicellulose decreased beginning at the CB stage and the proportion of cellulose increased throughout the ripening process.

Although carambolas have been commercially grown in Asia for many years, the industry in South Florida is relatively new. While production has increased rapidly in recent years, little is known about the physiology of carambola ripening. Although changes in color, soluble sugars and acids have been documented during fruit ripening (Campbell et al., 1989; Vines and Grierson, 1966), there is little or no information available on the textural changes that occur during ripening of carambola fruit. The softening process is of great commercial importance because the postharvest shelf life of the fruit is limited by increased softness which leads to an increase in susceptibility to mechanical damage and disease. Softening of fleshy fruits is mainly due to a modification of cell wall structure. Cell wall structure and modification thereof have not been investigated in the carambola fruit.

Also, uncertainty exists about the classification of carambola fruit as climacteric or nonclimacteric. Vines and Grierson (1966) observed a respiratory climacteric in 'Golden Star' carambola and classified carambola as climacteric. However, Oslund and Davenport (1983) found no evidence that 'Golden Star' carambola was climacteric. Similarly, Lam and Wan (1983) found no evidence for a climacteric pattern in carambola (cv. B10), and exposure to Ethrel, a treatment which releases ethylene gas, failed to induce ripening (Lam and Wan, 1983). It was recently reported that treatment with 100 ppm ethylene can promote carambola ripening (Sargent and Brecht, 1990). This type of response to ethylene treatment is normally associated with climacteric fruit. It has been suggested that carambola is a climacteric fruit and that the climacteric occurs very late in the ripening process.

The objective of this investigation was to increase our knowledge of the physiology of carambola ripening by exploring changes in fruit texture and cell wall composition during ripening, and additionally, to search for evidence of a climacteric pattern in carambola.

### Materials and Methods

**Fruit material.** Carambola (Averrhoa carambola L., cv. Arkin) fruit were harvested in Homestead, Fla. at four different stages of development as determined subjectively by color development: 1) dark green (DG), no yellow color; 2) light green (LG), green with pale yellow between ribs; 3) color break (CB), mostly yellow with some green remaining, orange color between ribs; and 4) ripe (R), yellow to light orange. Fruit were transported to Orlando, Gently dipped in 0.015% sodium hypochlorite (30C, pH 7.7), rinsed with deionized water (dH2O) and dried. Fruit were stored at 21C, 90% RH overnight. Eight to 12 fruit of good condition representing DG, LG, CB, and R stages were selected for respiration, ethylene production, color, firmness, and cell wall analysis. Additional R fruit were stored (1 to 3 weeks) until overripe (OR, dark orange color), at which time they were also sampled.

**Respiration and ethylene production.** Eight single fruit or pairs of small fruit at each stage of ripeness were sealed in 930 ml jars at 21C. After 15 min, 1 ml gas samples were analyzed for CO2 using a Hewlett Packard 5880 gas chromatograph equipped with a Porapak Q column and a thermal conductivity detector (TCD). Nitrogen served as the carrier gas at a flow of 30 ml·min⁻¹. Only five measurements of CO2 production were obtained from OR fruit as fruit showing any sign of decay were not used. The same fruit were then sealed in 930 ml jars at 21C with 30 g of soda lime (sodium hydroxide with calcium oxide or hydroxide) to absorb CO2. After 22 to 24 hr, 1 ml gas samples were analyzed for ethylene using a Hewlett Packard 5880 gas chromatograph equipped with an alumina column and a flame ionization detector (FID). Nitrogen served as the carrier gas at a flow rate of 30 ml·min⁻¹.

An additional 20 fruit of various stages of ripeness from LG to R were monitored daily for CO2 production as described above. A separate group of 15 fruit were monitored every second day over a 20-day period for ethylene production as described above.

**Color.** After gas measurements, color measurements (CIE L*a*b) were taken on the same 8 to 12 fruit at each ripeness stage. A Minolta CR200 Colorimeter fitted with an aperture plate (11 mm diameter) was used to measure external color. The mean of three measurements per fruit, on the side of three wings, were averaged for fruit of each ripeness stage.

**Firmness.** Carambola fruit were supported by a triangular platform with the flat side of one wing perpendicular to a round, convex probe (8 mm diameter) attached to an Instron Universal Testing Instrument. The Instron was fitted with a 100 kg load cell and operated at a crosshead speed of 25 mm·min⁻¹. Resistance to compression (3 mm) was measured on three wings per fruit.

**Cell wall extraction.** Fruit were peeled, and seeds and endocarp removed. The mesocarp from two to three fruit per stage were combined, frozen in liquid nitrogen, and stored at −80C for subsequent cell wall extraction. Later, tissue was thawed, homogenized 3 min with a Polytron in 3 volumes of 80% ethanol, treated in a cell disruption bomb to break remaining whole cells and rinsed through Miracloth with 2 volumes 20 mM Hepes-NaOH (pH 6.9). The residue was stirred in 2 volumes phenol:acetic acid:dH2O (2:1:1, v/v/v) for 20 min, and successively rinsed in 3 volumes chloroform:methanol (1:1, v/v) and 3 volumes acetone. Cell walls were dried in a vacuum oven at 40C over phosphorous pentoxide.

**Cell wall uronide determination.** Cell walls (10 mg) were incubated on ice in 2 ml concentrated sulfuric acid on a gyratory shaker. Two 500 μl aliquots of dH2O (4C) were added slowly and the solution incubated on a shaker until dissolved (6 hr). Uronic acid concentration was estimated by the carbazole method (Dische, 1947).

**Cell wall fractionation.** Cell walls (150 mg) were fractionated into chelator-soluble pectin (CSP), covalently-bound pectin (CBP), a hemicellulosic fraction (HF), and a cellulose fraction (CF) as described previously (Mitcham et al., 1989), except that the HF was extracted using a single incubation in 8 N KOH for 3 hr. Carbohydrate concentration of cell wall fractions was determined by the phenol-sulfuric acid method (Dubois et al., 1956). Uronic acid concentration was determined using the carbazole method (Dische, 1947).

**Cellulose determination.** Cellulose content of crude cell walls was estimated according to the procedure of Updegraff (1969). Cell walls (10 mg) were incubated in teflon-cap-
ped tubes with 1 ml 2 N trifluoroacetic acid (TFA) for 1 hr at 121°C to hydrolyze noncellulosic neutral sugars. The TFA solution was removed and the residue rinsed twice with dH₂O and incubated at 30°C for 1 hr with 1 ml 78% sulfuric acid. The volume was brought to 25 ml with dH₂O and incubated at 121°C for 1 hr. Cellulose content was estimated using the Anthrone method for total hexose (Spiro, 1966) with glucose as the standard.

Results and Discussion

Color and firmness. CIE color “a” and “b” values both increased during ripening of carambola fruit (data not shown); however, the ratio of a/b provided the most linear relationship to ripeness stages which had been selected based on subjective color analysis (Fig. 1). Resistance to compression (firmness) decreased linearly as ripening progressed (Fig. 1). The decrease in firmness was inverse to the increase in a/b color indicating that determination of ripeness stage based on fruit color is very accurate.

Respiration and ethylene production. The rate of CO₂ production of fruit picked at different ripeness stages (Fig. 2) was 30 ml·kg⁻¹·h⁻¹ at the DG and LG stages, decreased to 20 ml·kg⁻¹·h⁻¹ at the CB stage, then increased to the original rate by the OR stage. The pattern of CO₂ production indicated a possible preclimacteric minimum at the CB stage with a peak in respiration at the OR stage. However, there is no evidence for a decrease in CO₂ production after the peak. Ethylene production from the same fruit began to increase at the CB stage and increased sharply at the OR stage (Fig. 2). The results for ethylene production were similar to those of Oslund and Davenport (1983).

To search for additional evidence of a climacteric pattern in carambola fruit, CO₂ and ethylene production was monitored over time (Fig. 3) in fruit harvested at different stages of ripeness. No consistent pattern in respiration was observed in fruit harvested at the CB stage (Fig. 3A) or any other ripeness stage (data not shown), and we did not observe a peak in CO₂ production like that reported by Vines and Grierson (1966). As fruit became OR, decay often interfered with respiration measurements. The presence of decay was associated with an increase in respiration, which may have been due to a wound response or to respiration of the decay organisms.

Ethylene production, however, followed a climacteric pattern with a sharp peak in production at approximately the R stage (Fig. 3B). All fifteen fruit monitored underwent a peak in ethylene production, but the timing and magnitude varied with the ripeness stage at harvest. Two CB fruit underwent an immediate peak in ethylene production while the ethylene peak of R fruit occurred several days later and was lower in magnitude (Fig. 3B). It is possible that R fruit underwent peak ethylene production prior to harvest. Two LG fruit underwent a peak in ethylene pro-
duction at the CB to R stage. Most fruit exhibited signs of
decay 2 to 4 days after the peak in ethylene production.
The decay organisms did not appear to contribute to
ethylene production since the production rate continued
to decrease after decay began. However, ethylene production
by the carambola fruit may have been a pathogen-induced,
wound response that occurred before decay became
visibly noticeable. Alternatively, normal production of
ethylene by the fruit may have stimulated decay develop-
ment.

Our data regarding the climacteric nature of carambola
are inconclusive. However, as explained by Biale (1960),
placing a fruit in the nonclimacteric category calls for a
more tentative decision than listing one as climacteric be-
cause positive evidence can be experimentally obtained
much more readily than convincing negative proof.

Cell wall composition. The carbohydrate portion of DG
carambola cell walls was comprised largely of cellulose
(60%) and hemicellulose (27%) polymers with pectin polym-
ers comprising only 13%. In unripe fruit, a greater percent-
age of the pectin was covalently-bound pectin (CBP) which
is more tightly bound within the cell wall (Fig. 4A). The
proportion of loosely associated chelator-soluble pectin
(CSP), which can be solubilized from the cell wall with cal-
cium chelators, was lower than CBP in the unripe fruit (Fig.
4A). However, in OR fruit, the increase in CSP and decrease
in CBP resulted in equal amounts of the two types of pectin
polymers. In addition to a shift in the type of pectin polym-
er present during ripening, there was also a slight decrease
in cell wall uronides, the major component of pectin, after
the R stage (Fig. 4B). The modification of pectin polymers
from CBP to CSP began after the LG stage along with the
decrease in fruit firmness; however, there was no net loss
of uronic acid from the cell wall until after the R stage.

The changes in carambola pectin polymers during ri-
pening are similar to those found in strawberry fruit. The
amount of cell wall uronide decreases late in ripening (Knee
et al., 1977), and a shift to CSP also occurs during ripening
of strawberry fruit (Woodward, 1972). Woodward pro-
posed that a change in the cell wall polysaccharides takes
place in such a way that the uronide polymers become
rearranged to allow more plasticity in the walls of the fruit.
Neal (1965) suggested that chelation of calcium ions, which
are thought to form crosslinks between carboxyl groups of
polyuronide chains, may lead to softening of the tissue.

In addition to the pectin changes, there was also a de-
crease in the amount of hemicellulosic material which began
at the CB stage (Fig. 5A). The amount of cellulosic material
increased linearly throughout ripening (Fig. 5B). The in-
crease in the proportion of cellulose may be due to a loss
of other cell wall polymers.

It is interesting that the amount of all four types of cell
wall polymers (mg/150 mg cell wall) increased from the DG
stage to the CB stage (Figs. 4 & 5). These data indicate a
decrease in another cell wall component, perhaps protein.

Conclusions

The development of carambola fruit CIE a*/b* color
was inverse to the decrease in fruit firmness indicating color
is an accurate means by which to determine fruit ripeness.
Carambola cell walls were comprised mainly of cellulose
and hemicellulose with only 13% pectin; however, the proportion of less tightly bound chelator-soluble pectin increased with ripening.

**Literature Cited**


**AN EXPERT SYSTEM FOR HYDROCOOLERS**

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Additional index words. cantaloupe, celery, cucumber, peaches, sweet corn, cooling.

Abstract. This paper describes an expert system that has been developed to give the specifications required to cool produce with a hydrocooler. Provided with a user friendly interface, this expert system can perform a wide range of tasks such as: hydrocooler design, precooling process optimization, quality control, energy conservation and investigation of new applications. In order to execute these tasks, the expert system needs information provided by the user such as: kind of product, loading capacity, entering and exit temperature of the product, water temperature and residence time. Depending on the information given, the expert system will use default values for the missing information to compute the residence time, product temperature, refrigeration capacity, water flow rate and the maximum shelf life of the product if stored at the exit temperature. Assumptions made in this expert system are: cooling efficiency of 50%, 3°F rise in temperature of the water going through the hydrocooler, exit product temperature determined by the 7/8 cooling time unless the chilling injury threshold temperature of the product is higher. Further improvement will make the software more versatile and applicable to a wider variety of commodities.

The temperature of fresh fruits and vegetables is the greatest determinant of the rate of deterioration by decay and senescence, and consequently, of the potential market life (Kader et al., 1985). Produce temperature control is critical from the moment of harvest when the process of deterioration begins; delay in cooling the product can cause loss of quality.

Several methods for rapid removal of heat from produce are in commercial use (Ryall and Lipton, 1979). The choice of a particular method depends mainly on the rate of cooling desired, product surface to volume ratio, product susceptibility to water damage, availability of equipment, the value and the perishable nature of the product. It is very important to select the optimal precooling system to satisfy the commodity and the market structure needs. A knowledge-based system for selecting precooling methods was developed by Morey et al. (1988) to help producers faced with a decision of selecting a precooling method. This computer program (Morey et al., 1988) gives the users the opportunity to specify the crop and information about their operation, and uses this information to select possible cooling methods, which can be room cooling, forced-air cooling, hydrocooling, vacuum cooling or package icing. However, the above system does not provide any pertinent information on the possible cooling methods selected.

Once a precooling method is selected, the service of an expert to characterize the system is required. With their knowledge, experts are able to determine the specification needed to design and operate a precooling unit. This kind of expertise can be costly and may not be readily available. The same problem can be observed for owners of precooling units who want to change operating conditions of their units or to use them for other crops. Only an expert can provide this kind of information. In many cases, a joint effort of a team of experts in postharvest physiology and engineering would be useful. These teams cannot always be available at the time the information is needed. For these reasons, computer programs that simulate human experts in this area are needed. By storing the knowledge of many experts in its memory bank a computer program ("expert system") can provide answers to questions from users. Com-