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**Literature Cited**


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**EFFECT OF CELL WALL HYDROLYSIS ON BRIX IN CITRUS FRUIT**

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**cell wall digesting enzymes are required for total cell wall solubilization.**

Several varieties of citrus fruit continue to accumulate soluble solids (Brix) during storage (4, 6, 7, 11). In ‘Hamlin’ orange, for example, the increase in total soluble solids is accompanied by a parallel increase in sucrose and a concomitant decline in acid content (4). The presence of all the gluconeogenic enzymes in mature sweet oranges (5) suggests the possibility of de novo sugar synthesis from acid after the fruit is detached from the tree. In some instances, however, the postharvest increase in Brix gradually plateaus and declines, only to increase again during the latter stages of storage (4, 6, 7). At this time, the activities of the regulatory enzymes of the gluconeogenic pathway have declined and enzymes of sugar catabolism dominate. Thus, gluconeogenesis and de novo sugar synthesis do not seem to be involved in the second and final rise in Brix. Other varieties, such as ‘Robinson’ tangerine and ‘Palestine’ sweet lime do not show any direct correlation between the uninterrupted increase in Brix and changes in sugars after harvest (4). The above information suggests the involvement of a separate mechanism responsible for the increase in Brix during storage of some varieties and during the final stages of storage in others.

Maturation and senescence in fruit involves a series of physiological and biochemical events which include changes in membrane permeability, carbohydrate composition, and cell wall structure (2). In Citrus, degradation of cellulose, hemicellulose and pectin from cell walls of juice
vesicles within fruit segments may release soluble components which could have a direct effect on Brix. This study was initiated to determine the possible relationship between enzymatic cell wall digestion of citrus pulp and changes in Brix.

**Materials and Methods**

Orlando tangelo (*Citrus paradisi* Macf. x *Citrus reticulata* Blanco) finisher pulp was collected following extraction during January 1988 at the Citrus Research and Education Center, Lake Alfred, Florida. An FMC 291 B-100 extractor and model 35 finisher with 0.020 mesh screens and 46 psi pressure were used. The pulp was frozen at -10°C until use.

For experimental use, 100 g of pulp were thawed and washed twice with 250 ml of 0.5 M sucrose. The washed pulp (6 g) was added to the incubation media and immediately vacuum infiltrated. The incubation media (20 ml) contained 10% sucrose or sorbitol, 50 mM buffer (MES pH 5.6 for Cellulysin, hemicellulase, and combination treatments; Na-succinate pH 3.8 for pectinase; and Tris-HCl pH 7.5 for pectinesterase) and the appropriate enzyme or enzyme combination. The enzymes used and their final concentrations were Cellulysin (Cal-Biochem, cat no. 219466) at 5.5 units/ml, pectinase (Sigma, P5146) at 5.6 units/ml, hemicellulase (Sigma, H2125) at 0.13 units/ml, and pectinesterase (purified from 'Valencia' finisher pulp) at 1.25 units/ml. For commercial enzymes, units of activities are as defined by the manufacturer, and for pectinesterase, one unit of activity is described as 1 µeq ester hydrolyzed/min. After vacuum infiltration, the incubation media and pulp was agitated at 30°C in a gyratory shaker at 160 cycles/min.

Aliquots (1 ml) of the experimental solutions were taken upon addition of the pulp to the incubation media and after 0.5, 1, 2, 3, 4, and 5 hr. The samples were centrifuged at 13,000 x g for 2 min, and the supernatant collected and analyzed for Brix with an American Optical table top refractometer. The remaining supernatant was boiled for 5 min to inactivate cellulytic and pectolytic enzymes and frozen until further use. Each experiment was repeated at least three times.

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**Fig. 1.** The effect of cell wall digesting enzymes on Brix content of citrus finisher pulp. Treatments were (△) cellulase, (●) hemicellulase, (□) pectinesterase, (0) pectinase, and (*) all four enzymes combined. Each point is the mean of triplicate experiments. Bars indicate SD. Inset: time course of citrus pulp digestion in the presence of all four enzymes. Additional substrate (3 g) was added at the point indicated by the arrow.

The amount of various sugars solubilized by enzymatic treatment was determined by analysis of the inactivated supernatant. Glucose was analyzed by the glucose oxidase method (3), galacturonic acid as described by Blumenkranz and Asboe-Hansen (1), and reducing sugars as in Nelson (9).

Results

Incubation of 6 g citrus finisher pulp in a solution containing cellulase, hemicellulase, pectinase, and pectinesterase resulted in a 1.0 unit increase in Brix. Cellulase, hemicellulase, or pectinesterase treatment alone did not affect Brix, but incubation of the pulp with pectinase resulted in a 0.6 unit Brix increase (Fig. 1). No significant increases in Brix were measured after 3 hr in the pectinase and enzyme combination treatments where pulp digestion appeared complete. Enzymes remained active after 5 hr, as indicated by the rapid rise in Brix (Fig. 1, inset) following addition of fresh pulp to the combined enzyme treatment. Although cellulase, pectinase, and pectinesterase in combination increased Brix of the solution to levels similar to those of pectinase alone, addition of hemicellulase to the digestion mixture accelerated and increased the efficiency of digestion as indicated by the increase in Brix (Fig. 2).

Analysis of the incubation media during the 5 hr incubation period revealed a parallel increase in glucose, reducing sugars, and galacturonic acid when finisher pulp was digested in the presence of all four enzymes (Fig. 3). After 5 hr, pulp digestion yielded 929 mg reducing sugars. Glucose and galacturonic acid comprised 655 mg and 70 mg of the reducing sugar solubilized, respectively. Less than 15 mg of glucose and galacturonic acid were released as a result of cellulase, hemicellulase, or pectinesterase treatment alone. Only in the presence of pectinase did solubilization of sugars occur (data not shown). The effectiveness of the commercial pectinase preparation alone in increasing Brix may be explained by the presence of contaminating enzymes. When visibly examined, only treatments containing either pectinase alone or the enzyme combinations were digested.

The increase in Brix was not the result of microbial contamination, since inclusion of 15 μg/ml erythromycin did not affect the final Brix rise. Similarly, incubation of finisher pulp or boiled finisher pulp in incubation media in the absence of exogenous enzymes did not result in a rise in Brix. To determine the effect of osmoticum on pulp digestion, sorbitol was substituted for sucrose. Substitution of sorbitol (10%) as osmoticum did not affect the efficiency of digestion (Table 1).
Discussion

During postharvest storage, various citrus cultivars continue to increase in Brix while organic acids (citrate and malate) gradually decline. It has been suggested that the decline in juice acid content may be directly involved with energy production (11) and translocation to the peel (8). The physiological mechanisms involved in the postharvest increase in juice Brix, however, have not been thoroughly investigated.

Brix increases in harvested citrus fruit imply an increase in the sugar content of the juice. In a harvested fruit, the additional soluble sugar must originate from within the organ since translocation of photosynthates has already ceased. Conversion of organic acids to sugars through the gluconeogenic pathway is possible, but only during the first weeks after harvest (5). Other physiological events that release soluble sugars include starch and cell wall hydrolysis. Starch is not present in mature citrus fruit. Cell wall hydrolysis, however, is an inherent event in maturing and ripening fruits.

Our data demonstrate that the products of the enzymatic breakdown of citrus pulp can contribute to the postharvest increases in Brix observed in some citrus cultivars (Fig. 1-3). This conclusion is based on the observation that the Brix content of the incubation medium increases when citrus pulp was incubated in the presence of all cell wall hydrolytic enzymes. The presence of pectinase, however, was obligatory for the observed increases in Brix. The activities of other cell wall hydrolytic enzymes (cellulase, hemicellulase, and pectinesterase) alone did not have any effect on the Brix content of the experimental solution (Fig. 1, 2), but in combination with pectinase, their activities promoted an additional release of soluble solids.

The extent of the contribution of cell wall breakdown to the postharvest increases in Brix of citrus fruit cannot be assessed with the results presented in this communication since no data for in situ cell wall hydrolytic enzymes is

Table 1. The effect of various treatments on Brix content of citrus finisher pulp. Complete treatment denotes incubation media containing all four cell wall digesting enzymes. Data are expressed as mean Brix unit increases ± SD after 5 hr of digestion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brix increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Complete + erythromycin</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Pulp alone</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Boiled pulp</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Boiled complete</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Complete—hemicellulase</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Complete—hemicellulase + sorbitol</td>
<td>0.9 ± 0.0</td>
</tr>
</tbody>
</table>

available. Levels of pectinase reported in citrus fruit are very low (12); however, during prolonged storage, the total activity could be sufficient to promote changes in Brix. The levels of the remaining hydrolytic enzymes necessary for cell wall hydrolysis appear to be adequate to bring about complete cell wall solubilization (13). It should be noted that the hydrolytic products of citrus cell wall do not necessarily contribute significantly to quality improvement since they are organoleptically perceived differently (10).

In conclusion, cell wall breakdown can be a major contributing factor in the postharvest increase in Brix during prolonged storage of citrus fruits. The rate of cell wall breakdown is controlled by the activity of pectin degrading enzymes.

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MEASUREMENT OF COLOR CHANGES DUE TO BROWNING IN STORED GRAPEFRUIT JUICES

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Abstract. The Hunter color difference meter was used to determine color changes in canned and bottled grapefruit juices over a storage period of 21 weeks at temperatures ranging from 20° to 50°C. Grapefruit juices darkened to an undesirable brown color with increased storage temperature. Changes in Hunter L, a, and b values, hue, saturation index and total color due to browning were calculated and found to be significant. The rates for color changes between cans and bottles were compared, and correlations between color parameters and browning index (A420 nm) were described.

Citrus juices are susceptible to a number of deteriorative reactions during storage which result in the development of an objectionable appearance. These color changes are accompanied by losses in desirable flavor and nutrients, and by formation of brown pigments. Although this color defect is one of the main limiting factors in product quality and shelf-life reduction during storage of citrus juices, there has been limited research aimed at evaluating this color problem (9). In 1967, a citrus color difference meter was developed principally for the measurement of citrus juices (4); objective color studies related to variety, maturity and processing conditions became possible. Also, tristimulus color (15) values could be applied to study the storage behavior of orange juice and drinks, and a good relationship between CIE tristimulus values and browning of orange juice was reported (13).

The objective of the present work was to study the influence of storage conditions on color changes of canned and bottled grapefruit juices by using the Hunter color difference meter.

Materials and Methods

Grapefruit juice. Commercial single-strength grapefruit juices (cv. Marsh) packed in glass and cans were obtained from Citrus World, Inc., Lake Wales, FL. All products were produced from the same batch, and were packed in 6-oz tin-plated cans and 7-oz glass bottles. Samples were placed in storage lockers at 20°, 30°, 40°, and 50°C for 21 weeks. Control samples were stored at -10°C. Three samples were taken randomly from each storage locker for analysis at 3-wk intervals.

Sample preparation. A sample of 50 mL of juice in a 500 mL round bottom flask was freeze-dried overnight using a Thermovac Model FD-ULT-6 (Thermovac, Inc., Co.). The dried residue was resolved with 25 mL of methanol, filtered through filter paper (Whatman No. 42), and the volume adjusted to 50 mL with methanol. The methanol extract was placed in the freezer overnight, then filtered through 0.45 µm Magna nylon 66 membrane filter (Fisher Scientific Co.) before color measurement.

Color measurements. Hunter L, a and b color dimensions were measured in the transmittance mode using a Hunter Model D25D2 color difference meter. From these, values representing hue and saturation index were calculated (7). Total color difference between control and stored samples was expressed by the equation, ΔE = [(ΔL)² + (Δa)² + (Δb)²]¹/² (5). The browning index of the clarified juices was