

# Induction of chilling injury in jicama (*Pachyrhizus erosus*) roots: changes in texture, color and phenolics

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## Abstract

Storage of jicama roots at 10 °C resulted in characteristic chill-induced changes in pulp color (decreased  $L^*$  values and increased chroma) and texture (increased distance to rupture) after 7–14 days. Discoloration or browning of the pulp occurred first in exterior pulp tissue and then progressed to the interior tissue. Changes in texture during storage were similar in exterior and interior pulp tissues. Chill-induced color changes generally occurred before changes in texture. After 10 °C storage, transfer to 20 °C enhanced chill-induced changes in color, texture, and concentrations of phenolics. Roots stored at 13 °C for 6 weeks began to exhibit changes in pulp color but not in texture. Roots stored at 20 °C for 6 weeks had no color or textural changes although they lost about 40% of their fresh weight. Chill-induced browning was associated with increased concentrations of soluble phenolic compounds and increased phenylalanine ammonia lyase activity. Phenolic compounds in chilled jicama root had UV spectra similar to those of catechins, though (+)-catechin and (−)-epicatechin were not present based on HPLC. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Soluble phenolics; PAL; Dry weight; Weight loss;  $L^*$  color value; Chroma; Transfer period; Gradients in pulp

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## 1. Introduction

Jicama (*Pachyrhizus erosus* (L.) Urban) is a tropical legume root that is chilling sensitive when stored at 10 °C or below (Bergsma and Brecht, 1992; Cantwell et al., 1992; Mercado-Silva and

Cantwell, 1998; Paull and Chen, 1988). Typical symptoms of chilling injury are increased external decay, internal color changes, and loss of crisp texture. The whiteness of the pulp is an important quality characteristic (Cantwell et al., 1992), and browning discoloration can occur because of chilling injury and senescence. A crisp texture is also an important attribute for good quality jicama (Mudahar and Jen, 1991), and storage at chilling temperatures results in loss of crispness (Mercado-Silva and Cantwell, 1998). Chilling, however, does not affect rupture or penetration force,

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but mainly results in increased distance to rupture point, indicative of sponginess and loss of crispness (Jackman and Stanley, 1995).

In some jicama studies, quality and chilling injury symptoms were evaluated only at the end of the storage period (Barile and Esquerra, 1984; Bergsma and Brecht, 1992; Paull and Chen, 1988). In other studies, changes were documented both after storage and after a transfer period to warmer temperature (Cantwell et al., 1992; Mercado-Silva and Cantwell, 1998). A transfer period to warmer temperature typically accentuates or aggravates the changes induced by storage at chilling temperatures (Saltveit and Morris, 1990). The separate effects of the storage and the transfer period on the important color and textural attributes of jicama have not been clearly described.

The discoloration that occurs with chilling injury development in fruits and vegetables is often associated with metabolism of phenolic compounds (Saltveit and Morris, 1990). Increased concentrations of soluble phenolic compounds are associated with color changes in minimally processed jicama pieces (Aquino-Bolaños et al., 2000). The development of brown discoloration in jicama root during the onset of chilling injury is presumably also associated with changes in phenolic metabolism, but this has not been demonstrated.

Jicama roots were stored at chilling (10 °C) and nonchilling (13, 15 or 20 °C) temperatures to determine: (1) the time course changes in texture, color and concentrations of phenolic compounds and within-root variation in these attributes; (2) the impact of a transfer period to 20 °C after storage at low temperature on changes in texture, color and phenolic metabolism; and (3) the time course of changes in phenolic concentrations and enzyme activity (phenylalanine ammonia lyase [PAL]) in relation to chill induced discoloration.

## 2. Materials and methods

### 2.1. Raw material

Jicama root selections produced in Nayarit,

Mexico for export to the U.S. were used, and these are more chilling tolerant than varieties grown in other production areas (Mercado-Silva et al., 1998). For Experiment 1 (February, 1997), roots were obtained from a wholesaler in Querétaro, Mexico within 1 day of harvest. On the same day roots were stored in carton boxes at 10 (RH = 80–90%) or 13 °C (RH = 70–80%) at the lab in Querétaro, and evaluated for quality and phenolic concentrations after 0, 7, 14 and 21 days and again after transfer to 20 °C (RH = 60–70%) for 7 days. Other roots from this lot were stored for up to 6 weeks at 13 or 20 °C. For Experiment 2 (July, 1997), roots were obtained from Frieda's Produce (Los Angeles, CA) within 2–4 weeks of harvest. These roots had been stored in a commercial warehouse in Tijuana, Mexico at about 18 °C with night air ventilation, and then held at 15–20 °C for 2–3 days during transport to Frieda's and truck shipment to Davis. Roots were stored at 10 (RH = 80–90%) or 15 °C (RH = 70–80%) at the lab in Davis, and evaluated for quality, phenolic concentrations and PAL activity after 0, 5, 10, 15, 20 and 25 days. For Experiment 3 (August, 1997), roots were obtained within a few days of harvest from a wholesaler in Querétaro, Mexico, stored at 10 °C at the lab in Querétaro, and evaluated for sugar and phenolic concentrations after 15, 17, 19, 21 and 23 days before and after transfer to 20 °C for 7 days. All roots used were of sound quality with no decay and had minimal visual defects or mechanical damage. None of the roots had been stored at chilling temperatures prior to conducting the experiments.

### 2.2. Subjective evaluations

Visual quality was evaluated on a 9–1 scale, where 9 = excellent, no defects, 7 = good, minor defects, 5 = fair, moderate defects, 3 = poor, major defects, 1 = unuseable. A score of 6 was considered the limit of salability. Browning was evaluated on a scale of 1–5, where 1 = none, 2 = slight, 3 = moderate, 4 = severe, and 5 = ex-

treme browning. Decay were evaluated on scale of 1–5, where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5–20% surface affected), 4 = moderately severe (20–50%), and 5 = extreme (> 50% surface affected). These scales were applied as previously reported (Cantwell et al., 1992; Mercado-Silva et al., 1998).

### 2.3. Objective evaluations

Exterior and interior pulp refers to tissue cut from the outer 25% and the inner 50% of a root in cross section. Color was determined with a Minolta CR-200/300 spectrophotometer, with illuminant A and a 10° viewing angle and calibrated on a white tile.  $L^*$ ,  $a^*$  and  $b^*$  values were recorded and chroma ( $C^* = (a^{*2}b^{*2})^{1/2}$ ) and hue ( $h^\circ = \tan^{-1}(b^*/a^*)$ ) calculated.

Texture was measured as described by Mercado-Silva and Cantwell (1998) with modification. Maximum rupture force and distance to rupture point were determined on halved roots with a TA-HD texture analyzer (Texture Technologies Corp., Scarsdale, NY) using a flat cylindrical 3 mm probe at a penetration rate of 1 mm s<sup>-1</sup> to a depth of 8 mm.

The concentration of soluble solids was determined on expressed juice from four mid-section root pieces (1.8 × 4 cm) per determination on a refractometer at 20 °C. For analysis of total soluble sugars, a 10 g sample of fresh jicama was homogenized in 75 ml 95% ethanol, held 12 h at –20 °C and then filtered. After dilution with deionized water, 100 µl 80% phenol and 5 ml concentrated H<sub>2</sub>SO<sub>4</sub> were added to a 1 ml aliquot, the mixture was incubated 20 min at 30 °C and absorbance was read at 490 nm (Dubois et al., 1956). A glucose standard curve was used for quantification and the results were expressed as g kg<sup>-1</sup>. Other pieces from the mid-sections of the roots were used for percent dry weight determined from initial and final weights after drying chopped samples at 70 °C for 48 h.

For PAL activity, 4 g of tissue was homogenized with 0.4 g insoluble polyvinyl polypyrrolidone, 16 ml borate buffer (50 mM,

pH 8.5) and 14 µl 2-mercaptoethanol (Ke and Saltveit, 1986). After filtering and centrifugation at 17 000 × g at 4 °C for 20 min, PAL activity in the supernatant was determined at 40 °C using 100 mM L-phenylalanine as substrate and measuring absorbance at 290 nm. One unit of PAL activity corresponded to the formation of 1 µmol of cinnamic acid in 1 h.

For total soluble phenolics, 8 g of finely chopped jicama was homogenized with 15 ml 80% ethanol, filtered through four layers of cheesecloth and let stand 30 min before taking a 0.25 ml aliquot for spectrophotometric assay with Folin–Ciocalteu reagent (Folin and Ciocalteu, 1927). A standard curve of *p*-coumaric acid was used for quantification.

HPLC analysis was conducted on selected samples from Experiment 1. About 15 g of samples were extracted in 30 ml 100% methanol and centrifuged. A known portion of this sample was concentrated under a nitrogen flow at about 40 °C, taken to a known volume and passed through a 0.45 µm nylon filter. Samples were also extracted in 10% methanol and compared to the 100% methanol extracts. Analysis was done on a Waters C-18 column using gradient elution and a photodiode array detector. Solvent A was 5% acetic acid and solvent B was 100% methanol with 5% acetic acid, and the ratio at different times was: 3 min 100:0 A:B, 20 min 90:10, 35 min 60:40, 40 min 10:90, and 45 min 0:100. UV spectra were run on all peaks and compared with spectra of known catechins in green and black teas, and with commercial sources of *p*-coumaric acid, catechin, ferulic acid, caffeic acid and chlorogenic acid.

### 2.4. Experimental design

Experiments were conducted in a completely randomized design with a minimum of four repetitions per storage treatment per sampling time (1 root = 1 replication) unless otherwise specified. Data were calculated as averages ± standard deviation or analyzed by ANOVA with calculation of the LSD at  $P \leq 0.05$  (SigmaStat 2.0, Jandel Scientific, San Rafael, CA).

### 3. Results

#### 3.1. Changes in visual quality, decay, weight loss, dry weight and soluble solids

The loss in overall visual quality of jicama roots stored at 10 °C was associated with an increase in external decay and weight loss, especially after the transfer period at 20 °C (Fig. 1A and B). No decay was observed on roots stored at 13 °C although visual quality scores decreased one unit over 21 days (Fig. 1C). During storage weight loss rates were 0.4 and 0.6% per day at 10 and 13 °C,

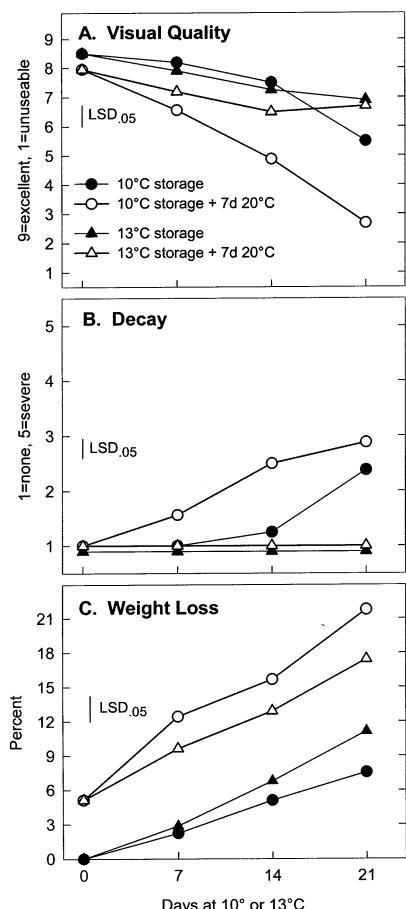


Fig. 1. External visual quality (A), macroscopic decay (B), and weight loss (C) of jicama roots stored at 10 or 13 °C for 21 days. Roots were evaluated after storage and after transfer to 20 °C for 7 days. Data are the averages from eight roots per determination.

respectively (Fig. 1C). However, after transfer to 20 °C, 10 °C-stored roots had a higher weight loss rate (>1% per day) than the roots previously stored at 13 °C (0.85% per day). The percent dry weight at 13 °C remained constant at 8.3–8.5% over 21 days + transfer period, while it decreased to 7.5% at 10 °C followed by transfer to 20 °C. The soluble solids content of the roots stored at both 10 and 13 °C for 21 days decreased from an initial 6.8% to 5 and 5.5%, respectively.

#### 3.2. Changes in texture at chilling and nonchilling temperatures

The force to rupture the pulp did not change in roots stored at 13 °C, and increased slightly in roots stored at 10 °C (Fig. 2A and B). Penetration forces for exterior and interior pulp were similar. The distance to rupture point, however, increased in the 10 °C-stored roots but did not change in the roots stored at 13 °C (Fig. 2C and D). Distances to rupture point were generally similar between exterior and interior tissues except after transfer of 10 °C-roots to 20 °C at 14 and 21 days. Roots in Experiment 2 required 20 days at 10 °C to show a chill-induced increase in rupture distance (Fig. 5), but the magnitude of the change was similar to that in Experiment 1 in which an increase was found after 14 days (Fig. 2).

#### 3.3. Changes in color and phenolic concentrations at chilling and nonchilling temperatures

No internal discoloration was observed in the jicama roots stored at 13 °C for 21 days as reflected in unchanged  $L^*$  and chroma values (Fig. 3). The pulp of the 10 °C-stored roots had a brown discoloration that progressed from the exterior towards the interior of the root. These changes were associated with lower  $L^*$  and higher chroma values in the exterior than in the interior tissues (Fig. 3). Transfer of the roots to 20 °C for 7 days increased the color changes in 10 °C-stored roots, but did not result in important changes in color values of the roots stored at 13 °C.

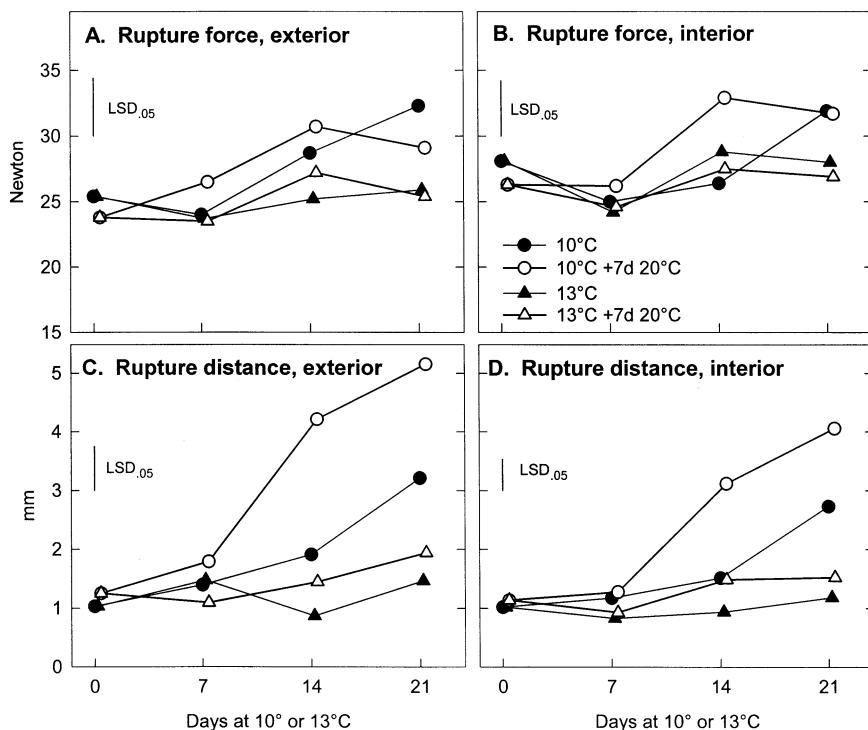


Fig. 2. Rupture force (A, B) and distance to rupture (C, D) of exterior (A, C) and interior (B, D) tissue of jicama roots stored at 10 or 13 °C for 21 days. Roots were evaluated after storage and after transfer to 20 °C for 7 days. Data are the averages from eight roots per determination.

In roots stored at 10 °C, phenolic concentrations increased after 14 days storage, whereas at 13 °C concentrations remained at initial levels (Fig. 4A and B). Transfer to 20 °C resulted in large increases in phenolic concentrations of roots stored at 10 °C (110–175 mg kg<sup>-1</sup> at 14 days for example) (Fig. 4A). Little change in phenolic concentrations occurred in the roots stored at 13 °C (Fig. 4B), nor were there large differences in concentrations between the exterior and interior tissues (Fig. 4D). In the roots stored at 10 °C, however, the concentrations of soluble phenolics in the exterior tissues were much higher than in the interior tissues after 14 and 21 days (Fig. 4C).

In Experiment 3, roots stored at 10 °C for 15–23 days had average phenolic concentrations of 117 and 165 mg kg<sup>-1</sup> before and after transfer to 20 °C for 7 days, respectively (a 42% increase after transfer) (data not shown). Over the same storage period, sugar concentrations of 10 °C-

stored roots increased 15% after transfer to 20 °C (65 and 75 mg kg<sup>-1</sup> before and after transfer, respectively) (data not shown).

#### 3.4. Changes after storage for 6 weeks at 13 or 20 °C

Following 6 weeks storage, roots held at 13 or 20 °C showed no differences in external visual quality or decay scores (data not shown). Total weight loss averaged 16 and 20% for the 13 and 20 °C-stored roots, respectively. However, after 6 weeks at 13 °C, some yellowing of the pulp was observed and this was associated with lower *L*\* and higher chroma values (Table 1). Chroma values were higher in the exterior than interior tissue of the 13 °C-stored roots, but *L*\* values did not vary within the root. Phenolic concentrations in exterior tissue were 134 and 80 mg kg<sup>-1</sup> in roots stored at 13 and 20 °C roots, respec-

tively. Average concentrations, however, differed less (109 vs 93 mg kg<sup>-1</sup> for the 13 and 20° roots), but were slightly higher than initial values (90 mg kg<sup>-1</sup>) for the 13 °C-stored roots. No differences were observed in texture (rupture force) between the roots stored at 13 and 20 °C (data not shown). Roots stored at both temperatures showed small increases in distance to rupture point (Table 1). Storage at 13 °C resulted in no sugar loss over the 6 weeks, whereas sugar content decreased about 25% in roots stored at 20 °C (Table 1).

### 3.5. Association of phenolic changes with phenolic enzyme activities

Chill-induced texture and color changes were evaluated in conjunction with changes in phenolics and PAL activity (Experiment 2). Pulp color in roots stored at 10° was different from that of

15 °C-roots after day 15 and this was preceded by increased concentrations of phenolics (Fig. 5C). Phenolic concentrations in exterior pulp averaged 10% higher than those of interior tissue. PAL activity was not detected on day 0, was measurable on day 5, and increased by day 10 to similar rates in roots stored at 10 or 15 °C. After day 10, however, PAL activity in the 10 °C-stored roots continued to increase (Fig. 5D), whereas the activity in the 15 °C-stored roots declined to undetectable levels.

### 3.6. Qualitative characterization of phenolics

Based on HPLC retention time and UV spectra, there were two groups of compounds observed from tissue of roots stored at both 10 and 13 °C. There were three compounds in the first group with retention times of 8.5, 12.5 and 13.5 min and each of these compounds had UV spectra similar

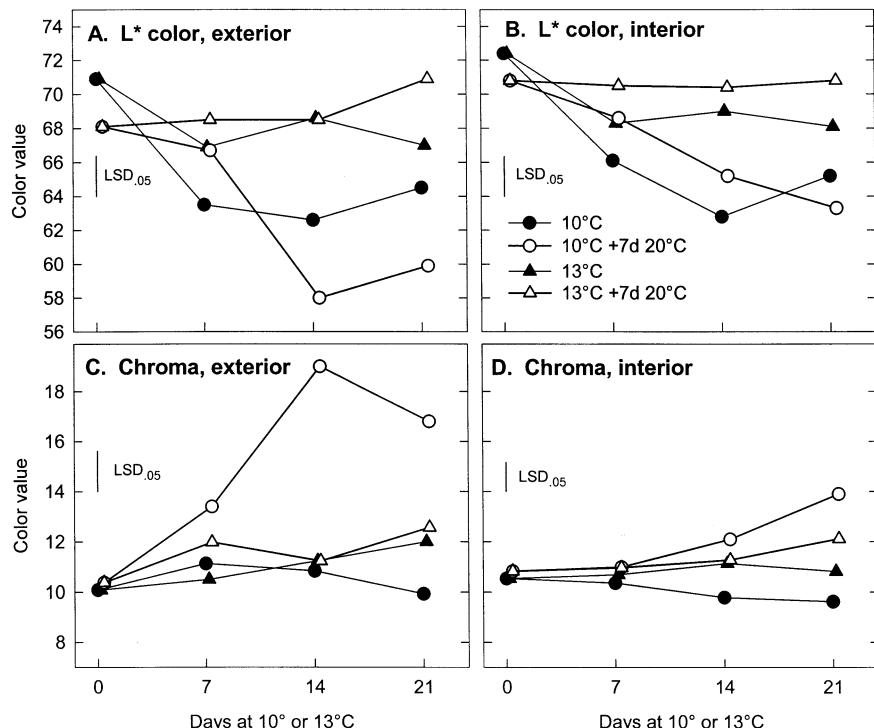


Fig. 3.  $L^*$  (A, B) and chroma (C, D) color changes in exterior (A, C) and interior (B, D) tissues of jicama roots stored at 10 or 13 °C for 21 days. Color values were determined in triplicate on eight roots per evaluation after storage and after transfer to 20 °C for 7 days.

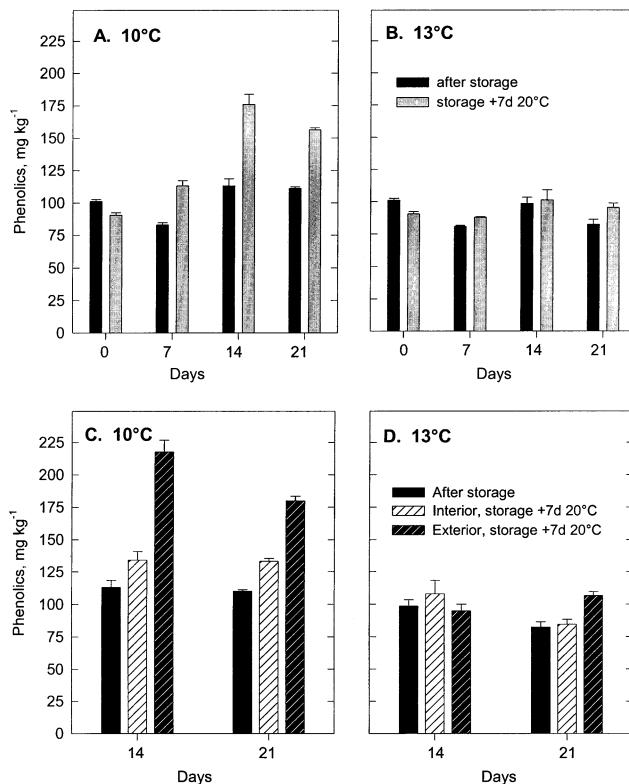


Fig. 4. Concentrations of total soluble phenolics in jicama roots stored at 10 or 13 °C for up to 21 days (A and B) and total phenolics in exterior and interior tissues (C and D). Roots were evaluated after storage and after transfer to 20 °C for 7 days. Data are the averages of three analyses for each composite sample of 2–3 roots  $\pm$  standard deviation.

to that of (+)-catechin with absorption maxima at 233 and 276 nm (Fig. 6). Yet none of these compounds had retention times of (+)-catechin (6 min) or (-)-epicatechin (16 min). The other group of compounds had retention times between 25 and 45 min and had an absorption maximum only at 233 nm. None of the jicama peak retention times coincided with those from hot water tea extracts. Extraction of tissue samples in 100% methanol or 10% methanol/90% water gave no major qualitative or quantitative differences in the peaks.

The area of the peak with retention time of 12.5 min increased about three-fold in tissue from roots stored at 10 °C for 14 days + 7 days at 20 °C compared with roots stored 14 days at 13 °C + 7 days at 20 °C. There were no consistent changes in the other peaks. Although, there were increases in the peak with retention time of

12.5 min from roots stored at 10 °C, the increases were only 2–4 mg kg<sup>-1</sup> (in terms of catechin equivalents) while the increases in total phenolics estimated by the spectrophotometric method were 50–100 mg kg<sup>-1</sup> (Figs. 4 and 5).

#### 4. Discussion

A temperature of 13 °C is usually recommended as the minimum safe temperature for storage of jicama roots (Cantwell et al., 1992). In roots stored at 10 °C, chill-induced changes in color occurred coincidentally with or before changes in texture (loss of crispness indicated by increased distance to rupture). Changes in  $L^*$  and chroma were notable within 7 days at 10 °C followed by transfer to 20 °C in one experiment and within 15 days in another. Another indication

that color changes are equally or more sensitive to storage temperature than textural changes are results from storage at 13 °C. Although, no browning was found in pulp of roots stored 6 weeks at 13 °C, measurable yellowing was observed. There were no corresponding changes in texture. Paull and Chen (1988) reported that concentrations of phenolics increased in jicama stored 3 months at either 12.5 or 20 °C. They did not, however, relate changes in phenolic compounds to changes in pulp color. There were large differences in discoloration between exterior and interior pulp tissues due to chilling exposure, and these were associated with differences in concentrations of phenolic compounds. Higher phenolic concentrations occurred in exterior tissues where there was greater discoloration. Gradients of phenolic concentrations have been reported in unstored potato tubers (Friedman, 1997). In jicama roots, low temperature storage was necessary to cause a concentration gradient. The within-root gradients in color and phenolic concentrations (Fig. 3) of jicama were large compared to the small corre-

Table 1  
Changes in color, sugar concentration and texture of jicama roots stored for 6 weeks at 13 or 20 °C

Attribute	Storage	Exterior tissue	Interior tissue
<i>L</i> *	Initial	70.9	72.4
	13 °C	66.1	67.5
	20 °C	75.5	76.5
	LSD 0.05	2.4	
Chroma	Initial	10.1	10.5
	13 °C	14.5	13.0
	20 °C	10.1	10.0
	LSD 0.05	1.1	
Sugar (g kg <sup>-1</sup> )	Initial	74.2	—
	13 °C	77.0	79.1
	20 °C	54.7	57.5
	LSD 0.05	4.0	
Texture (mm to rupture)	Initial	1.0	1.0
	13 °C	1.6	1.3
	20 °C	1.5	1.4
	LSD 0.05	0.4	

Data are averages of eight roots per evaluation.

sponding differences in texture (Fig. 2).

Transfer from 10 to 20 °C increased pulp discoloration, phenolic concentrations and loss of crispness. Rupture distance increased two- and three-fold after 14 days at 10 °C but more than four- and five-fold after the transfer period (Fig. 2). Similarly in the study by Mercado-Silva and Cantwell (1998), much of the reported increase in rupture distance likely occurred during the transfer period and not during low temperature storage. The increase in rupture distance is not related to water loss but rather to previous low temperature injury. Roots stored 2 weeks at 13 or 20 °C + 1 week at 20 °C lost a total of 17 and 19% weight, respectively, but had no change in rupture distances (Mercado-Silva and Cantwell, 1998). Roots stored 2 weeks at 10 °C + 1 week at 20 °C had 15% weight loss, but a doubling of rupture distance (Mercado-Silva and Cantwell, 1998). Similarly in the present study, the small difference in weight loss between the 10 and 13 °C-stored roots cannot explain the large increase in rupture distance in the 10 °C roots. The transfer period is also important for its effect on chroma values (Fig. 3) and increased concentrations of phenolic compounds (Fig. 4). Phenolic concentrations in exterior tissues increased 75–100% after transfer from 10 to 20 °C. The effect of the transfer period in exacerbating chilling symptoms may help explain some of the poor internal quality of jicama displayed in supermarkets at ambient temperatures (Cantwell et al., 1992).

Estimates of phenolic concentrations from HPLC analyses were substantially lower than the concentrations calculated from spectrophotometric analyses, which varied from about 100 to 200 mg kg<sup>-1</sup> and are similar to concentrations reported in fresh-cut jicama (Aquino-Bolaños et al., 2000). A simple phenolic (*p*-coumaric acid) was used as a standard in the spectrophotometric assay, but wavelength maxima and molar absorptivities may vary greatly for different phenolic compounds and lead to errors in estimates of concentrations of unknown phenolics in extracts (Swain and Goldstein, 1964). The Folin–Ciocalteu reagent may overestimate phenolic concentrations due to reaction with other reducing

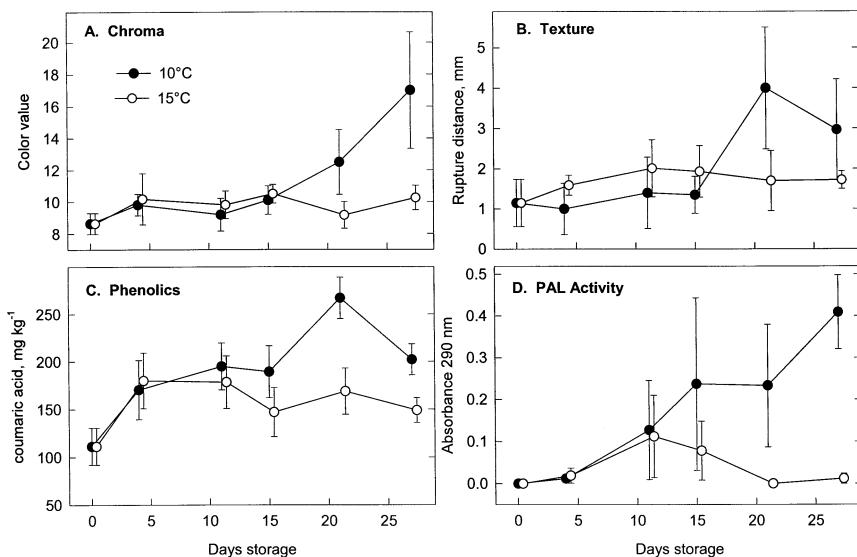


Fig. 5. Changes in chroma (A), texture (B), phenolic concentrations (C) and PAL activity (D) of jicama roots stored at 10 or 15 °C for up to 27 days. Roots were analyzed at the end of the storage period. Data are averages of eight determinations on two roots per sample period and are averages of analyses from exterior and interior tissues.

compounds, as shown in sweetpotato (Walter and Purcell, 1979). In potatoes, however, the spectrophotometric assay provided a more reproducible and accurate estimate of phenolic concentrations than HPLC analysis due to light- and time-dependent conversions (Friedman, 1997). Although, accurate estimates of phenolic concentrations are fraught with potential errors, concentrations in jicama root did increase due to storage at 10 °C.

HPLC analyses revealed that the phenolic compounds in jicama tissue were catechin-like compounds since their UV spectra were similar to those of (+)-catechin and its stereoisomer (−)-epicatechin with absorption maxima at 233 and 276 nm (Fig. 6) (Harborne, 1989). Depending on the complexity of the specific compound, absorption maxima for catechins in methanol can vary from 271 to 280 nm (Swain and Goldstein, 1964). Recent HPLC work on jicama and its more chilling tolerant relative ahipa (*P. ahipa*) has demonstrated differences in phenolic concentrations during low temperature storage, but has not yet led to identification of the compounds involved in chill-induced discoloration (E.N. Aquino-Bolaños, personal communication). The identifi-

cation of specific phenolics in jicama is important since some compounds may contribute to browning more than others. In apples, for example, chlorogenic acid is the predominant phenolic acid,

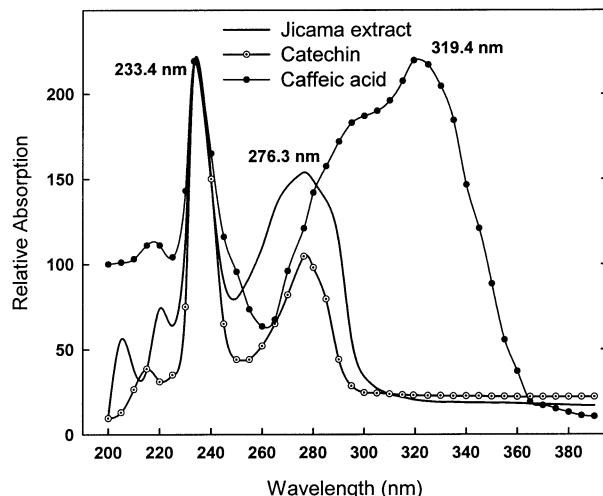


Fig. 6. UV spectra of caffeic acid, (+)-catechin, and jicama compounds with retention times on HPLC of 8.5, 12.5 and 13.5 min (see text for gradient conditions). The UV spectra of each of these compounds were recorded by the photodiode array detector during separation on HPLC.

but catechin is the main contributor to discoloration (Murata et al., 1995).

## 5. Conclusions

Measureable changes in color (decreased  $L^*$  and increased chroma) occurred before changes in texture (increased distance to rupture) when jicama roots were held at a chilling temperature (10 °C). No significant within-root gradient was found in texture measurements, but pulp discoloration progressed from exterior to interior tissues with chilling exposure. Increased discoloration was associated with increased phenolic synthesis. Some compounds, from methanol extracts of jicama separated by HPLC, had UV spectra similar to those of catechins, but no simple catechins were found. Transfer from 10 to 20 °C greatly enhanced chill-induced changes of color, texture and phenolic concentrations in jicama roots.

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