Stability of Betalain Pigments from a Cactacea Fruit

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The garambullo tree (*Myrtillocactus geometrizans*) which grows in the deserts of Mexico produces a purple fruit. The pigments were extracted, identified, and evaluated for their stability at different temperatures and pH values and in the presence of iron and chromium. One percent citric acid or ascorbic acid was added as a stabilizer, and a mixture of both was also used. On the basis of their visible light spectrum and chromatographic profile, the pigments were identified as betalains, which have a stability greater than that of red beet pigments and which are very stable at low temperatures. The pigment concentration was determined to be 214 mg/(100 g of dry weight). The energy of activation for bleaching of color was 87.09 ± 8.53 J K^{-1} mol^{-1} at pH 5.5. Ascorbic acid protects the red color even when it is exposed to drastic treatments such as sterilization. Metals decrease the stability of garambullo pigment; the effect of iron was greater than that of chromium. Garambullo pigment has the potential for use in food processing at low temperatures such as in the dairy industry for the production of ice cream and dairy drinks.

Keywords: *Stability; betalains; garambullo; pigments*

INTRODUCTION

In recent years, there has been a tendency to limit the use of synthetic colorants because of the new regulations of several countries. This is particularly true for red colors, and therefore, it becomes necessary to seek alternative natural sources that could be used by the food industry (Duxbury, 1990).

Garambullo (*Myrtillocactus geometrizans*) is a cactacea easily propagated by seed which grows in arid and semiarid zones of Mexico. It grows on mountainsides and lowlands where soils of good quality exist. Garambullo contains a purple fruit used as a commodity, and it is produced in the months of June, July, and August (Ballester, 1978). Garambullo represents a potential source of water-soluble betalain type pigments. Betalains are additives that belong to the five colorants most widely used in the food industry (Jackman and Smith, 1992). Betalains are compounds with a quaternary amino group with a range of molecular weights between 400 and 500 and with an attractive red color. However, the poor color stability of betalains represents an obstacle for its industrial use. It has been reported that the stability of betalains is strongly influenced by light, oxygen, water activity, pH, and temperature (von Elbe, 1975; Pasch and von Elbe, 1975; Saguy et al., 1978, 1984; Saguy, 1979; Attoe and von Elbe, 1981; Cohen and Saguy, 1983; Huang and von Elbe, 1987).

The susceptibility of betalains to the above factors restricts their use as food colorants. Their applications are therefore confined to products that are exposed to limited heat processing, that have a low water activity or a short storage time, and that do not contain SO_2. Betalains are particularly suited for use in powder mixes and most dairy and frozen products (von Elbe, 1975; Jackman and Smith, 1992).

The objectives of this study were to elucidate the composition of garambullo pigments and to determine the influence of pH, temperature, stabilizing agents, and metals on their color stability during storage.

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MATERIALS AND METHODS

Chemical Composition. Garambullo (*M. geometrizans*) fruits and red beet harvested in July and August from the Queretaro valley (Mexico) were stored at −15 °C until they were used, about 4 weeks. Extraction of the pigments was performed by chopping and grinding 50 g of fruit with 100 mL of distilled water, and a juice was obtained.

Pigments were dissolved in 0.1 M NaH_2PO_4 and adjusted to pH 5.5. This solution was then scanned in a Beckman DU-65 spectrophotometer to obtain its absorption spectrum. UV−vis spectra (200−700 nm) were determined for garambullo and red beet pigment; this was later used as the standard. High-performance liquid chromatography was used to separate the pigments and to cochromatograph the pigment preparations (1:1). For this purpose, a Perkin-Elmer model 4000 high-performance liquid chromatograph with automatic injection was used. A UV-VIS Perkin-Elmer model LC-95 detector and an LCI-100 Perkin-Elmer integrator were also used. Betanin was monitored at 535 nm; 100 μL samples were injected into a μ-Bondapack C18 column (10 μm; 3.9 × 150 mm; Waters Associates) through a 100 μL loop injector. The solvents were delivered at a flow rate of 1 mL/min. Betanin was eluted with a 1:5 (v/v) mixture of methanol and 0.05 M NaH_2PO_4 adjusted to pH 3.0 with H_3PO_4, using isocratic conditions (Attoe and von Elbe, 1981). Pure pigments were pooled at the end of the liquid chromatography for stability studies; the absorption spectrum was obtained.

Extraction of Betalains. The juices obtained as mentioned above were fermented with *Saccharomyces cerevisiae* at 37 °C for 6 h. Fermented extracts were then filtered through cheesecloth and centrifuged at 4000*g* and 4 °C for 15 min. The supernatant was concentrated 10 times and kept in sealed amber jars to avoid degradation of the pigment. The product was dehydrated by spray drying (at an inlet temperature of 140 °C and an outlet temperature of 80 °C) to avoid microbial spoilage and thus prolong its stability. Exactly the same procedure was used for the red beet pigment, which is equivalent to a commercial preparation.

Stability of the Pigment. Stability studies were carried out using 0.014% w/v betalains from fermented and dry garambullo (*M. geometrizans*) pigment. These samples were adjusted at pH values of 4, 5, and 6 with 0.25 N hydrochloric acid or 2% sodium hydroxide. These samples were compared

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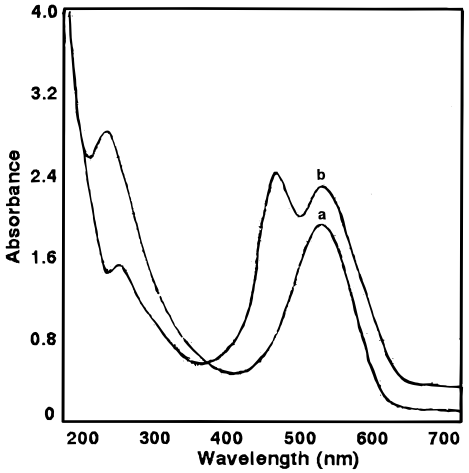

Figure 1. Absorption spectrum of freshly extracted (a) garambullo (*M. geometrizans*) pigment and (b) red beet pigment at pH 5.5.

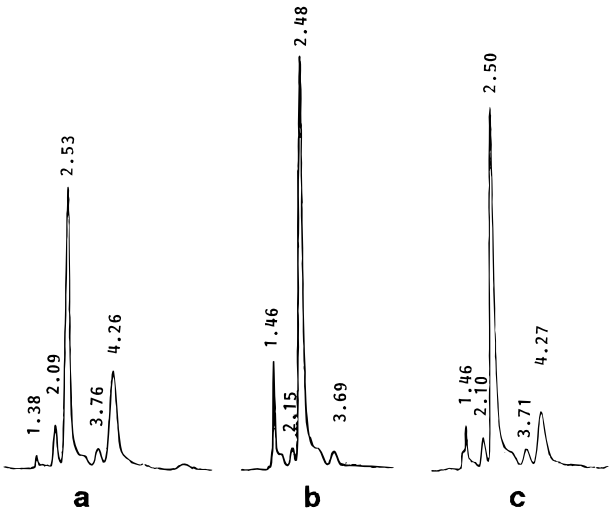

Figure 2. HPLC profile of freshly extracted (a) garambullo (*M. geometrizans)* pigment, (b) red beet pigment, and (c) a mixture (1:1) of both pigments at pH 5.5 and 535 nm.

against a pigment extracted in the same way from red beet. Both pigments were stored at room temperature over the course of 5 days. The percentage of pigment remaining [(milligrams of betanin after 5 days/milligrams of initial betanin)(100)] was evaluated during that period. Solutions of garambullo and red beet with and without ascorbic acid (1%) were stored over the course of 5 days and exposed to daylight with an intensity of 40 W. In order to evaluate the effect of temperature on stability, 0.014% w/v betalains from dry garambullo pigment were tested at 0 °C (90 days), 25 °C (90 days), 40 °C (10 h), 60 °C (6 h), 80 °C (8 min), and 100 °C (45 min) and at sterilization conditions (121 °C). A solution without a stabilizer was used as a control. Stabilizing solutions of 0.1% w/v ascorbic acid, 1% w/v citric acid, and a mixture of both additives (1:1) were also evaluated. To different solutions of garambullo pigment were added 2 ppm potasium chromate (chromium) and 15 ppm iron, with and without ascorbic acid at 0.1%. L^*, a^*, and b^* values were determined using a Hunterlab D-259 tristimulus colorimeter (Francis, 1989). Values of the Hunterlab standard of tristimulus color were as follows: Y_{CIE}, 84.03; X_{CIE}, 82.11; and Z_{CIE}, 100.58.

Statistical Analysis. Data were analyzed using Tukey's multiple-range test and linear regressions, with the Statgraphics software version 5.0 (Statgraphics Corp., 1992). For all the stability studies, at least triplicate values were used.

RESULTS AND DISCUSSION

Chemical Composition. As shown in Figure 1, the red beet pigment had three major peaks, at about 535, 480 and 280 nm. Two of those peaks coincided with those shown by garambullo (*M. geometrizans*) (535 and 280 nm) and are representative of the betacyanin. The other peak in the beet pigment (477 nm) corresponded to betaxanthin; it did not show up in the freshly extracted garambullo pigment. Exactly the same composition was obtained for the fermented and spray-dried samples of garambullo. However, the composition of the red beet pigment changed during fermentation as well as during spray drying. According to Piatelli (1976), the absorption at 280 nm indicates that the betalains in both pigments are acylated.

Garambullo and red beet pigment HPLC chromatograms at 535 nm are shown in Figure 2. In the red beet, three main peaks were observed. Collection of the first peak (1.46 min) using the mobile phase as a blank revealed an absorption spectrum with a maximum absorption band at 477 nm and a yellow color characteristic of the betaxanthins. This peak corresponds to the vulgaxanthins mixture (vulgaxanthin I and vulgaxanthin II), as described by Pourrat et al. (1988). The total area percentage of the betaxanthins was 18.68%. The main peak at 2.48 min, as well as the one at 3.69 min, had a red hue with a 535 nm maximum absorption. Such absorption signals correspond to the betanin (71.60%) and isobetanine (3.09%), respectively (Pourrat et al., 1988). Garambullo pigments were treated similarly by HPLC chromatography, and peaks corresponding to the betacyanins and to the betaxanthins were observed. After analysis of the garambullo HPLC chromatogram, four main absorption signals were found. A first peak (1.38 min), with a total area of 2.92%, was a compound corresponding to the betaxanthins on the basis of the spectra results of maximum absorbance at 477 nm. Another of the peaks, which corresponded to those found in red beet, had a retention time of 2.53 min (betanin), comprising 57.22% of the total area. There was a signal at 3.76 min (isobetanins) with an area of 4.42%. An additional peak found in garambullo (4.26 min), which did not correspond to any of the peaks found in red beet, had an area of 9.77% on the basis of the spectrum of the pure compound (535 nm maximum absorbance). Most probably, this pigment is a filocactin found in cactacea fruit and flowers (Piatelli and Minale, 1964). In both chromatograms, a small peak at 2.09 min (garambullo) and 2.15 (red beet) appeared; we do not know its identity. The mixture (1:1) of both pigments showed an additive result for the three peaks at 1.46, 2.50, and 3.71 min and nonadditive results for the peak at 4.27 min. This implies that the garambullo pigment has a composition similar to that of the beet pigment.

Extraction of the Pigment. Since 4−6% of the solids of garambullo (*M. geometrizans*) juice are fermentable carbohydrates and nitrogen compounds, it was necessary to ferment the juice in order to decrease the total solid content and increase the concentration of betalains (Adams et al., 1976). During fermentation of the garambullo juice, there was a slight increase in the betalain content (Table 1). The reduction of sugars also contributed to the purification of the pigments; the sugar contents changed from 6 to 4 °Brix due to the fermentation procedure. This process eliminated disagreeable odors that could be produced later by the natural fermentation of the fruit. Dry pigment contained 214 mg of betalains/(100 g), and the drying

Table 1. Betalain Content of Different Garambullo Pigment Presentations

	betalain content[a] [mg/(100 g)]	water content (w/w)
fresh fruit	2.30 ± 0.35	57.0
nonfermented juice	7.02 ± 0.26	97.0
fermented juice	7.98 ± 0.30	96.0
dry pigment	214.13 ± 3.15	2.0

[a] Mean + SD.

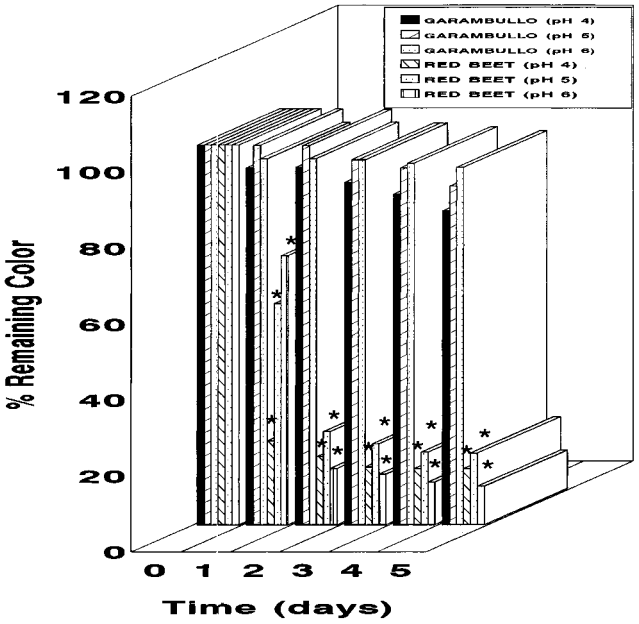

Figure 3. Effect of pH on the stability of fermented and concentrated garambullo (*M. geometrizans*) pigment in comparison with that of concentrated red beet pigment. An * indicates statistical difference with respect to garambullo (*M. geometrizans*) ($p < 0.05$).

process did not degrade the red color of the pigment. However, it was necessary to apply arabic gum and maize starch to the garambullo concentrate in order to avoid caramelization of the remaining sugars present in the extract of the pigment and to avoid its sticking to the walls of the drier (Altamirano et al., 1992; Saguy et al., 1980).

Stability of the Pigment. Figure 3 shows the lower stability of the concentrated red beet pigment in relation to the fermented and concentrated preparation of the pigment from garambullo (*M. geometrizans*) at the pH values tested (4, 5, and 6). When the fermented and spray-dried red beet pigment was studied, the degradation was too fast for accurate measurement, and that was why a concentrated sample was used for stability studies. On the other hand, after fermentation of the red beet pigment, new compunds were formed; these compounds were not oberved with garambullo. The stability of the pigment was affected on the first day of storage with a pigment loss of 70% at pH 4, 35% at pH 5, and 20% at pH 6 for red beet. Garambullo had a pigment loss of 7% at pH 4, 1% at pH 5, and 4% at pH 6. This difference is probably due to the greater concentration of labile betaxanthins in red beet (18.68). Singer and von Elbe (1980) found a half life ($T_{1/2}$) of 100 min for vulgaxanthines of red beet and 1100 min for betanin. These data indicate the high susceptibility of vulgaxanthine to degradation and the fact that it requires an additional technique to separate yellow pigments and to prevent loss of color during storage. This leads to additional expense and a higher price for the use of red beet pigment. The low concentration of betaxanthine (2.92%) in garambullo pigment increases the potential for its utilization as a colorant. When stability studies were performed with pure pigments from garambullo and red beet, it was clear that the cactacea pigment was 15% more stable after 15 min at pH 5.5 and 40 °C than the red beet pigment. A higher degradation of the pigment was observed after 30 min (36%). These data were similar to those obtained for the crude extract of garambullo pigment. However, the red beet pigment was more stable in the crude extract (24%) than in the pure state.

When solutions of the garambullo pigment (pH 5.5) were heated for varying lengths of time, the red color diminished and, at the same time, a light brown-yellowish color appeared. A linear behavior was shown for the depletion of the garambullo pigment after it was exposed to different temperatures. When the percentage of remaining pigments versus time was plotted on a semilogarithmic scale, it followed a first-order reaction (Figure 4). This is in accordance with other studies that showed that the thermal degradation of betalains follows first-order kinetics over a pH range of 3.0−7.0 under aerobic conditions (Saguy, 1979). In our data, we observed a greater percentage of remaining pigment (15.65%) in solution at 4 °C in the fifth week than at 25 °C, with complete degradation of the pigment at 100 °C after 30 min. During degradation of the betalains by temperature, the primary steps involve nucleophilic attack by water at the C-11 position on the betanin molecule, yielding cyclodopa-5-*O*-glycoside and betalamic acid (Huang and von Elbe, 1987). It is possible that betalamic acid and cyclodopa-5-*O*-glycoside may undergo Schiff base condensation to regenerate betanin, especially at lower temperatures. However, betalamic acid is heat sensitive; it may undergo aldol condensation or participate in Maillard reactions, making the pigment unavailable for the regeneration reaction. Similarly, the glycosidic moiety of cyclodopa-5-*O*-glycoside may be cleaved at high temperatures. It is also very susceptible to oxidation reactions, initiating polymerization to melanin type compounds. Thus, as the temperature increases, particulary in the presence of oxygen, irreversible betanin degradation is promoted. The quantity of betanin degradation and regeneration after thermal treatment depends not only on temperature and pH but also on the initial betanin concentration (von Elbe et al., 1981). As the initial betanin concentration increases, so does the color stability. Betanin may also yield isobetanin or decarboxylated betanin; their formation is favored upon heating at pH 3.0−4.0 (Huang and von Elbe, 1985).

The loss of color was greater when the temperature was increased to 100 °C. This is confirmed by the increasing values of the kinetic parameter k with respect to temperature (higher values of k mean greater velocities of degradation), and also by the stability ($T_{1/2}$) (von Elbe et al., 1974). The color of the pigment decreased when the temperature increased, with a k of 1.18×10^{-5} min^{-1} and a $T_{1/2}$ of 58 650 ± 1980 min at 0 °C and a k of 1.54×10^{-1} min^{-1} and a $T_{1/2}$ of 4.49 ± 0.28 min at 100 °C (Figure 5). The rate of degradation of betanin is more rapid in a model system compared with that in beet juice, suggesting a protective effect conferred by other constituents in the natural system (e.g. by polyphenols, antioxidants, etc.). The energy of activation (E_a) was calculated for the garambullo pigment using a semilogarithmic plot of k versus $1/T$; it

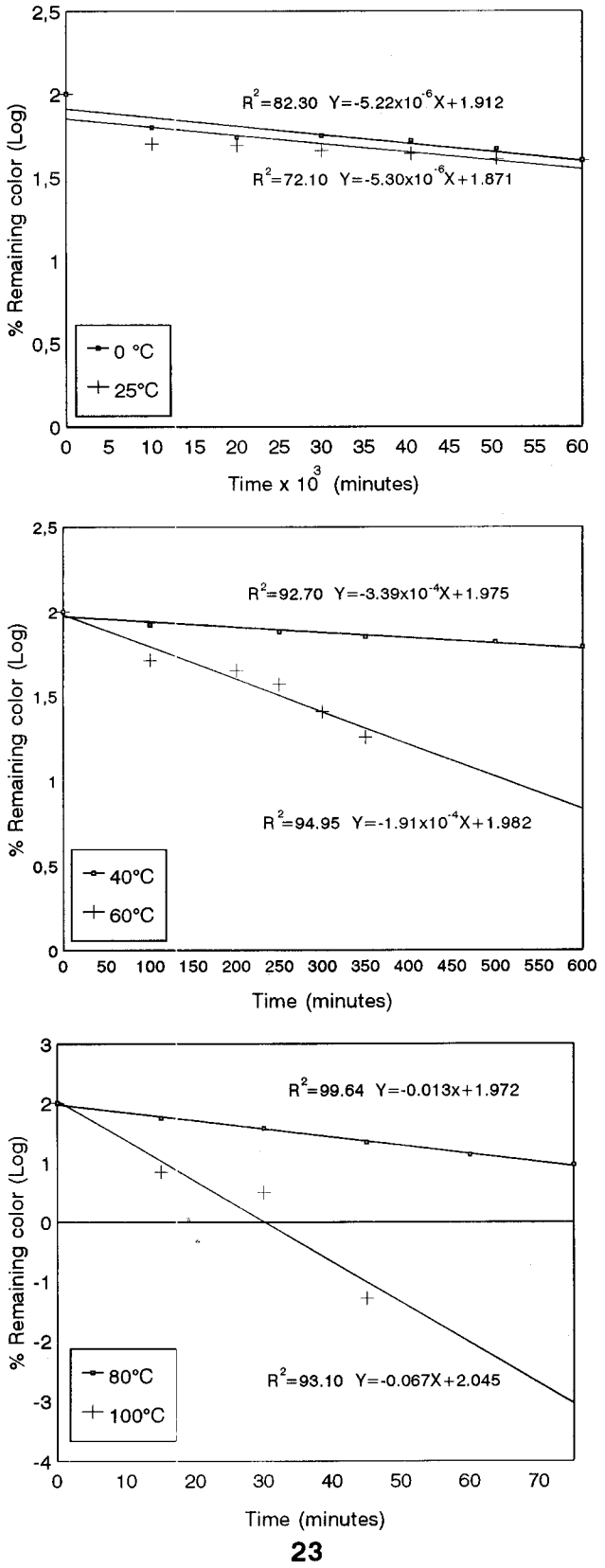

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Figure 4. Effect of temperatures on garambullo (*M. geometrizans*) pigment with respect to time.

was found to be 87.09 ± 8.53 J K^{-1} mol^{-1}. The garambullo pigment was more stable in comparison with the red beet one (E_a = 68.24 ± 7.68 J K^{-1} mol^{-1}), at the same temperature.

Betalains are known to be sensitive to oxidation, which has an impact on their color stability. Therefore, compounds such as ascorbic and citric acids have been

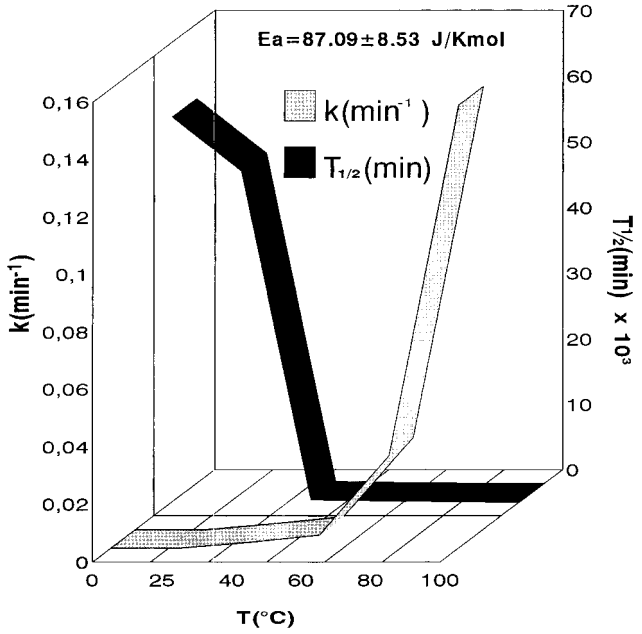

Figure 5. Effect of temperature on reaction rate and half-life times of the garambullo (*M. geometrizans*) pigment.

used to counteract this phenomenon due the fact that ascorbic acid is a good stabilizer for its scavenger oxygen capacity in a closed system and citric acid can chelate metal ions such as iron which promote oxidation. In our study, it was found that the addition of ascorbic acid to the pigment extract protected their color stability. In the solution of garambullo pigment after 5 weeks at 25 °C, the parameter *a** was 29 for the sample with ascorbic acid, 19 for citric acid, and only 22 for the control (data not shown). A reaction between betanin and molecular oxygen appeared to cause the most pigment loss in air-saturated solutions. Citric acid damaged the red tonality, probably due to the lower pH. When there is no control of the pH of the pigment solution, stability alterations caused by the change of pH cannot be easily differentiated from the direct influences of the chemical additives (Attoe and von Elbe, 1985). Pasch and von Elbe (1979) reported that citric acid can increase the half-life 1.5 times when the sequestrant agent is added to a 1% solution of betalains. This solution was readjusted at pH 5.5 with crystals of Na_2HPO_4. No differences were observed in color parameter *a** between the control (22) and the pigment when it was added to a mixture of 1:1 ascorbic acid/citric acid (23). This sample was slightly more degraded that those with ascorbic acid alone. There is not a synergistic relationship for these two protective compounds. This is probably due to the fact that citric acid inhibits the oxygen scavenger activity of ascorbic acid in betanin solutions.

Figure 6 shows a small increase in the stability under light conditions of the red beet and the garambullo pigments in the presence of 0.1% ascorbic acid. The garambullo pigment can be seen to be more stable. There was not a difference between the control of garambullo and the pigments with antioxidants up to day 5 of storage. Red beet was degraded more than 70% by day 2 of its preparation in the absence of ascorbic acid. Ascorbic acid (0.1%) was proven to be a good treatment for preserving the red hue of the colorant.

Even when the stability in ascorbic acid is greater at low pHs and in the presence of oxygen, ascorbic acid also protected color at sterilization temperatures, al-

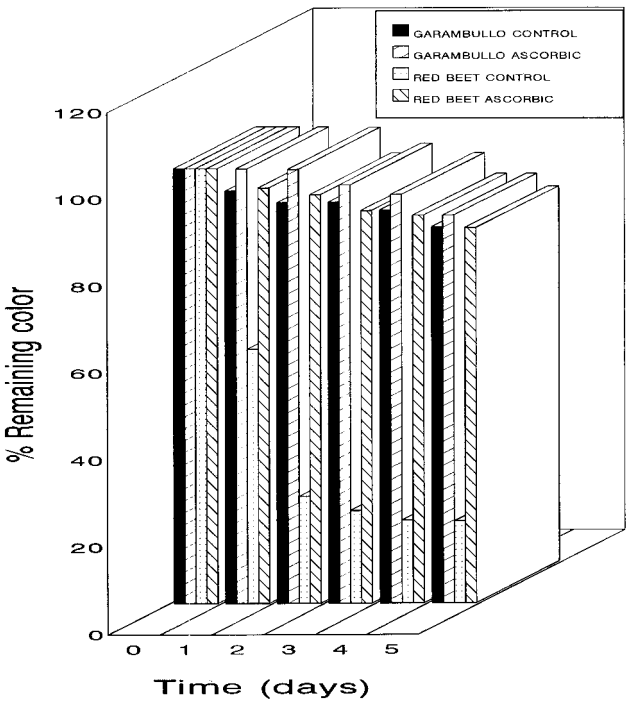

Figure 6. Comparison of the stability of garambullo (*M. geometrizans*) pigment and red beet pigment under light conditions at 25 °C and pH 5.5.

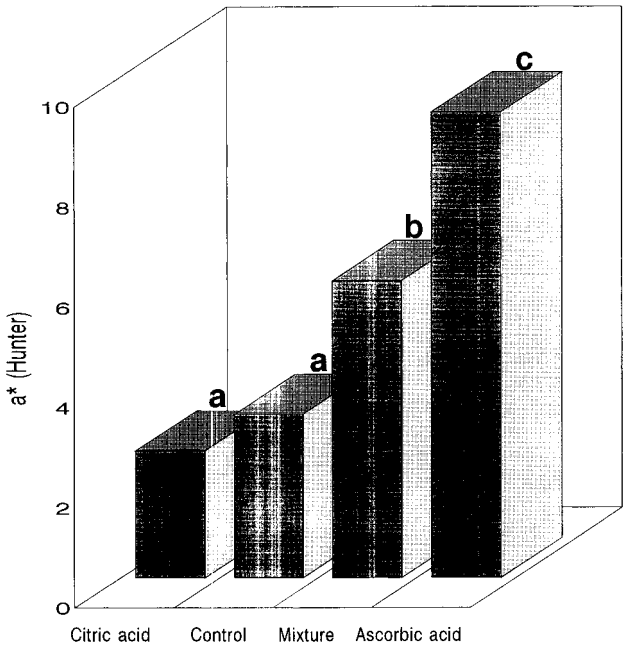

Figure 7. Changes in the *a** value of garambullo (*M. geometrizans*) pigment with stabilizing agents under autoclaving conditions. a−c indicate statistical difference ($p < 0.05$).

though the color decrease was considerable (Figure 7). Consequently, utilizing garambullo pigment in foods that need sterilization during their processing is not recommended.

During the processing and storage of foods, metal ions are present as they are in the equipment. Metals, particularly those that contain two or more valence states, decrease the induction time and increase the oxidation rate of compounds. Metals can be prooxidant by transferring electrons, thereby releasing and forming free radicals. Figure 8 shows that iron had a greater effect (52%) on the degradation of garambullo pigment than chromium (32%) after 4 days of storage. Iron

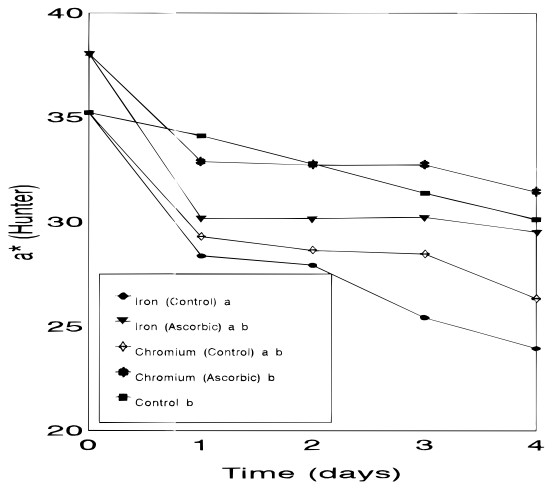

Figure 8. Stability of garambullo (*M. geometrizans*) pigment affected by metals and stabilizing agents at pH 5.5 and 25 °C.

attacks the electrophilic center of the betalains, causing a loss of color by destruction of the chromophore group.

Ascorbic acid protects the pigment when iron and chromium are present. However, the metals have a major impact on the oxidation rate of ascorbic acid, therefore decreasing its efficacy to scavenge oxygen.

In summary, the garambullo (*M. geometrizans*) pigment as well as other cactecea (Domínguez, 1995) has potential as a source of natural pigment for the food industry, especially for those food products that do not require high-temperature processing such as dairy products.

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