Repeated Regeneration of Degraded Red Beet Juice Pigments in the Presence of Antioxidants

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ABSTRACT

Ascorbic, isoascorbic, metaphosphoric, and gluconic acids improved the regeneration of red beet juice pigments after heating, and resulted in greater retention of the pigments during processing and storage. Their effect varied depending on the pH of the juice solutions. Ascorbic and isoascorbic acids allowed for the greatest regeneration at pH 3.8. At pH 6.2, metaphosphoric acid and gluconic acid were more effective. Addition of ascorbic acid once prior to the first heating retained the initial concentration of pigments even after 5 cycles of heating (3 min at 100°C) and regeneration. Control solution lost red pigments completely.

Key Words: red beet, pigment, betanin, regeneration, antioxidant

INTRODUCTION

RED BEET COLOR EXTRACTS ARE OFTEN CONSIDERED AS AN ALternative to certain FD&C red dyes in foods for some consumers who have aversions to synthetic dyes in foods (Philip, 1978). Although red beet root (*Beta vulgaris*) is a good source of red pigments, their potential use may be somewhat limited to dried or refrigerated foods, and foods with a short shelf life because the pigments are subject to change by heat and light. Several factors affect the stability of beet pigments during preparation, processing, and storage. Many methods have been reported to prevent loss or improve the stability of pigments (Savolainen and Kuusi, 1978; Pasch and von Elbe, 1979; Saguy, 1979). The methods include degassing, addition of antioxidants and stabilizers, control of pH, minimal heat treatment, etc. These efforts have contributed to their application in food products. However, the pigments could not be used in moist foods or juice distributed at room temperature (Havlíková et al., 1985).

Von Elbe et al. (1981) first reported that degraded betanin was partially regenerated after thermal treatment. Studies have shown that this regeneration was affected by storage temperature, pH, type of buffer solution, and the presence or absence of oxygen (Bilyk and Howard, 1982; Huang and von Elbe, 1985, 1987). If the magnitude of degradation was limited, up to 92% of the degraded betanin could be regenerated.

Regeneration studies have largely focused on factors necessary for regeneration and kinetics of regeneration. Active regeneration with the aid of food additives could contribute to the increased retention of pigments during processing and storage, and one such study described the effect of isoascorbic acid (Bilyk et al., 1981). The objective of our study was to retain red beet color by repeated regeneration of fresh beet juice pigments with the aid of food additives such as ascorbic acid, isoascorbic acid, gallic acid, gluconic acid, and metaphosphoric acid, etc.

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MATERIALS & METHODS

Preparation of red beet juice

Fresh red beet roots (*Beta vulgaris*) were purchased from a commercial source. Samples (2 kg) were washed, peeled, cut into cubes about 1 cm × 1 cm, and then added in a one-to-one ratio to boiling water. After the mixture started to boil, it was blanched for 1 min. After rapid cooling, beet juice was extracted with an Angel green juicer (Hosan Co., Korea). The crude juice was heated to boiling, and maintained at 100°C for 3 min. After rapid cooling, it was clarified stepwise through cheesecloth and filter pads (3 mm thick) with average pore sizes of 18 µm and 2 µm. This heating removed some coagulating components. The clear beet juice was stored frozen at -70°C for further studies.

Thermal destruction and regeneration of red beet juice pigments

Frozen beet juice was thawed at room temperature, diluted 1:10 with distilled water, and then clarified through a Micro Filtration System (Millipore) with 0.45 µm membrane (Gelman Sciences). The juice sample was treated thus for every use. Various food additives such as antioxidants (ascorbic acid, isoascorbic acid, and propyl gallate), organic acids (acetic, citric, lactic, gallic, and gluconic acid), and inorganic acids (metaphosphoric acid, phosphoric acid, and Na_2EDTA) purchased from Sigma (St. Louis, MO) were added to the red pigment solution before heating at a concentration of 40 mM, and the pH was adjusted to 3.8 with 1.0N HCl. For degradation and regeneration tests in the presence of food additives, screw-cap test tubes with a capacity of 16 mL were filled with 13.5 mL of the pigment solutions. They were heated at 100°C for 5 min. Separate 2 sets of tubes were stored after heating for regeneration at 10°C for 24h in the dark.

For experiments in which pigments were degraded without food additives and regenerated with them, 1L of beet juice in a capped media bottle (1L) was placed in boiling water and heated for 5 min or 30 min, respectively, to reduce the pigments to 66.2% and 15.0% of the initial level. To each 100 mL of degraded pigment solutions, each additive (40 mM) was added. The pH was adjusted to 3.8 or 6.2 with 1.0N HCl or 1.0N NaOH. Pigment solutions containing the food additives were sterilized by filtration through disposable filters (0.45 µm membrane, PVDF syringe filter, Whatman), and each 13.5 mL were transferred into sterilized screw-cap tubes (empty volume, 16 mL). If not sterilized, solutions became hazy within a day, probably due to the growth of microorganisms. Sample pigment solutions prepared with and without food additives were stored at 10°C for 24h in the dark, for regeneration.

To study the stability of food additives during regeneration, juice solutions containing ascorbic acid, metaphosphoric acid, or gallic acid (40 mM each) was repeatedly heated at 100°C for 3 min, and regenerated at 10°C for 24h after each heating. Volume of pigment solution was 13.5 mL in 16 mL screw-cap test tubes. This heating and regeneration procedure was repeated 7 times, and pigment retention was analyzed after every heating and regeneration step. Pig-

ment solution without any additive was a control. Twenty-eight tubes were necessary for 7 heating and regeneration cycles and for analysis in duplicate. Once exposed to air for analysis, pigment solutions were discarded.

Color analysis

Absorbance measurement with a cell (light-path length, 1 cm) was made against a distilled water blank at 535 nm and between 400 and 650 nm with a Beckman DU-7 spectrophotometer. All measurements were conducted in duplicate and averaged. The percentage of pigment retention was calculated from absorbance measurements at the absorption maximum, 535 nm for betacyanins. The data were reproducible within ±7%.

RESULTS & DISCUSSION

FOR THE REGENERATION STUDY OF RED BEET PIGMENTS, BEET juice instead of pure pigments was chosen because it serves as a practical example of a real food, and it shortens time needed for purification of the pigments. In preparing the juice, beet cubes were blanched to prevent the action of a betacyanin decolorizing enzyme (Lashley and Wiley, 1979). Crude juice extracted from blanched beet cubes was boiled for 3 min to remove components which would otherwise coagulate during the thermal destruction experiments.

The degradation and regeneration of red beet pigments was compared in the presence of food additives (Table 1). The percentage of red pigment retention is based on absorbance at the betacyanin absorption maximum, 535 nm. With the addition of additives and pH adjustment to 3.8 with 1.0N HCl before heating, absorbance of each solution changed slightly ranging from 0.3% to 2.3%. Addition of phosphoric acid, however, reduced the absorbance by 8.7%.

Pigment retention increased in all samples to 109.5%-118.3% of initial value, if the solutions were stored at 10°C for 24h in the dark. Obviously this increase may result from regeneration of pigments degraded during blanching and boiling of the juice. Increase of pigment concentration even in the case of a control may be explained by the results of Huang and von Elbe (1987) in which the regeneration rate constant increased as the pH decreased. Thus the shift of pH from 6.2 (original pH of the prepared juice) to 3.8 seemed to result in higher regeneration of the beet juice pigments.

When pigment solutions were heated at 100°C for 5 min immediately following additive incorporation, the control lost 80.3% of the original red pigments. Presence of food additives, however, generally stabilized the pigments. Bilyk and Howard (1982) reported that isoascorbic acid contributed slightly to beet pigment retention during heating. On the contrary, in our result, addition of isoascorbic acid and ascorbic acid prior to heating resulted in a large increase in pigment retention during heating. This was more consistent with the work of Bilyk et al. (1981) in which isoascorbic acid acted as a stabilizer for red beet pigments. Citric acid, gallic acid, propyl gallate, phosphoric acid, and Na_2EDTA favorably influenced juice pigment retention as noted by Pasch and von Elbe (1979). Note that metaphosphoric acid was a better stabilizer for red beet pigments than citric acid which is widely used in various foods. In foods containing red beet pigments, replacement of citric with metaphosphoric acid may be desirable for red pigment retention. Gluconic acid had little influence on the stabilization and regeneration of the pigments.

Addition of various additives to the juice caused a regeneration of red pigments, though the effect varied depending on type of additive (Table 1). Ascorbic and isoascorbic acid caused the highest regeneration of pigments. Those two antioxidants are well known stabilizers of red beet pigments. Our data indicate that they also acted as a regeneration aid. Although citric, metaphosphoric, Na_2EDTA and gallic acid aided regeneration to a lesser degree than ascorbic and isoascorbic acid, their effects were identifiable. Czapski (1985) explained that the higher retention of red pigments during heating and regeneration in a citrate buffer than in a phthalate buffer might result from metal-chelating action of citrate ion. Our results indicate, however, that a part of the higher retention could have occurred as a result of aiding regeneration of citrate ion. Czapski (1990) also reported that EDTA at 100 mg/L slightly increased retention of red beet pigments during heating and regeneration. Other additives, e.g., acetic acid, lactic acid, propyl gallate, gluconic acid, and phosphoric acid did not affect regeneration of degraded red pigments.

To study the regeneration capacity of additives, similar tests were carried out with pigment solutions to which 40 mM of various additives were added after thermal destruction. The percentage of pigment retention after thermal destruction at 100°C and regeneration at 10°C for 24h in the dark was compared on different conditions (Table 2). Red pigments were degraded by 33.8% and 85.0% of the initial levels by heating 5 and 30 min, respectively. Solutions were than stored for regeneration after adjusting the pH to 3.8 or 6.2. Since the pH of commercially formulated juices is around 3.8, regeneration was studied at the two pHs. At pH 3.8, addition of food additives to the juice after heating generally resulted in less regeneration than addition prior to heating. This may be due to reduced regeneration as degradation of red pigments increased (Huang and von Elbe, 1985). Heating degraded beet juice pigments more in the absence of additives than in their presence. The effects of various additives on regeneration markedly differed as a function of solution pH. At pH 3.8, ascorbic acid and isoascorbic acid aided regeneration of red pigments. Citric, gallic, and gluconic acid had little effect at pH 3.8, but at pH 6.2 their effects were greater than ascorbic and isoascorbic acid. Regeneration was less at pH 6.2 than at pH 3.8 at the same level of pigment degradation, as found by Huang and von Elbe (1987). Metaphosphoric acid and gluconic acid were appropriate as regeneration aids at pH 6.2. If solutions were regenerated for a longer time, the order of maximum regeneration did not change, indicating 24h was adequate for maximum regeneration.

The regeneration data (Tables 1, 2) show that at a similar level of degradation, regeneration after pigment destruction in the presence of antioxidants was higher than that in the absence of antioxidants. According to the proposed mechanism of degradation (Schwarz and von Elbe, 1983; Huang and von Elbe, 1987), betanin in solution is hydrolyzed by heating into betalamic acid and cyclodopa-5-*o*-glycoside, which are then further degraded. It seems that ascorbic acid and isoascorbic acid prevent degradation not only of betanin but also of betalamic acid and cyclodopa-5-*o*-glycoside. The amount of betanin that regenerated after heating was a function of the presence of betalamic acid and cyclodopa-5-*o*-glycoside (Huang and von Elbe, 1985).

During storage, red beet pigments may be degraded and regenerated continuously because the reaction is reversible. Pigment retention, however, decreases, since degradation proceeds at a faster rate than regeneration. Pigment retention may be enhanced by reduction of degradation rate, for example by using a stabilizer or by regeneration. Upon repeated heating, a control solution continued to lose red pigments with no apparent regeneration, but in the presence of ascorbic acid, red pigments were lost upon heating but regenerated during storage (Fig. 1). In pigment solutions regenerated after each heating, addition of ascorbic acid retained initial concentration even after 5 cycles of heating (at 100°C for 3 min) and regeneration. When isoascorbic acid was added, the degradation and regeneration data (data not shown) were quite similar to those for ascorbic acid. Metaphosphoric acid and gallic acid also enhanced retention of red pigments, though to a lesser extent than the two antioxidants. Addition of ascorbic, isoascorbic, gallic, and metaphosphoric acid would retain more pigments during processing and storage by acting not only as stabilizers but also as regeneration aids for red beet pigments. The

Table 1—Degradation and regeneration of red beet juice pigments in the presence of food additives

Food additive[b] (40 mM each)	% Red pigment retention[a] After addition[c]	After storage at 10°C for 24h	After degradation	After regeneration at 10°C for 24h
Control	100.0	114.5	19.7	21.0
Ascorbic acid	100.7	118.2	73.3	115.6
Isoascorbic acid	100.9	118.3	73.0	114.8
Acetic acid	99.2	109.5	34.3	39.0
Citric acid	98.0	113.2	41.6	54.7
Lactic acid	97.7	115.3	36.1	38.5
Gallic acid	99.7	110.8	44.9	52.7
Propyl gallate	98.8	111.7	44.8	48.9
Gluconic acid	100.3	112.4	22.2	24.5
Metaphosphoric acid	99.7	114.6	58.5	82.7
Phosphoric acid	91.7	115.2	42.6	46.8
Na_2EDTA	98.9	109.8	58.4	77.0

[a]Based on the absorbance at the absorption maximum, 535 nm for betacyanins.
[b]They were added prior to heating, and pH was adjusted to 3.8 with 1.0N HCl.
[c]After addition and adjustment of pH from 6.2 to 3.8, absorbance changed slightly. If the pigment solutions were stored, regeneration of the pigments occurred.

Table 2—Regeneration of degraded red beet juice pigments in the presence of food additives at different pH and at different degradation levels

Food additive[b] (40 mM each)	% Red pigment retention after regeneration [a] When 15.0% remained after thermal degradation at pH[c] 3.8	at pH 6.2	When 66.2% remained after thermal degradation at pH 3.8	at pH 6.2
Control	39.6	20.4	85.4	65.0
Ascorbic acid	45.7	25.6	98.3	66.5
Isoascorbic acid	45.7	27.2	98.3	68.4
Acetic acid	39.9	23.4	88.4	67.2
Citric acid	40.5	30.5	85.2	66.9
Lactic acid	39.6	26.7	86.0	67.6
Gallic acid	40.0	31.2	85.2	71.2
Propyl gallate	32.8	21.6	83.5	61.8
Gluconic acid	41.7	34.1	86.6	81.7
Metaphosphoric acid	43.4	35.9	92.6	75.4
Phosphoric acid	40.0	32.4	83.3	66.6
Na_2EDTA	37.1	22.9	88.1	64.9

[a]Based on the absorbance at the absorption maximum, 535 nm for betacyanins.
[b]They were added prior to thermal degradation of pigment solution
[c]Beet juice was thermally degraded at pH 6.2, and then the pH (in case of pH 3.8) was adjusted after adding each additive.

degradation and regeneration patterns at pH 6.2 were quite different from those at pH 3.8. When a similar experiment of repeated degradation and regeneration was done at pH 6.2, the effect of various additives, as stabilizers or regeneration aids was weaker than that at pH 3.8 (Fig. 2). Gallic acid accelerated the degradation of beet pigments at pH 6.2.

Visible absorption spectra of prepared beet juice was compared (Fig. 3) with juice after the first and the fifth degradation, and juice immediately after adding ascorbic acid and after regeneration. The peaks at 481 nm and 535 nm in control juice were typically attributed to the yellow betaxanthine and the red betacyanin, respectively. After the first degradation of the control solution, the two peaks partially disappeared, and after the fifth degradation, no peaks remained. Immediately after adding ascorbic acid, the peak at 481 nm was slightly reduced. Spectrum E denotes juice which was degraded 5 times at 100°C for 3 min followed by regeneration after every degradation. A betacyanin peak clearly shows that the red pigments had been regenerated. Complete disappearance without any shoulder of the peak at 481 nm indicates that once degraded, betaxanthines did not regenerate as reported previously (Bilyk and Howard, 1982).

Thus addition of some food additives to red beet juice increased regeneration of degraded red pigments. This could improve retention of natural red pigments in foods with high moisture content. Upon drying, degraded beet juice pigments were not regenerated. The regeneration effects of additives varied depending on the pH of the solutions. For example, at pH 3.8 ascorbic and isoascorbic acid were the best choices for highest regeneration. At pH 6.2, metaphosphoric and gluconic acid were best. The amount of antioxidants we used was 40 mM (ca 7 6/L for ascorbic acid), which may be too high for practical usage. This would add considerably to the cost of a product, considering that 1 g/L of ascorbic acid is a usual level in drinks. Our test level (7 g/L) of ascorbic acid, however, is not a pro-

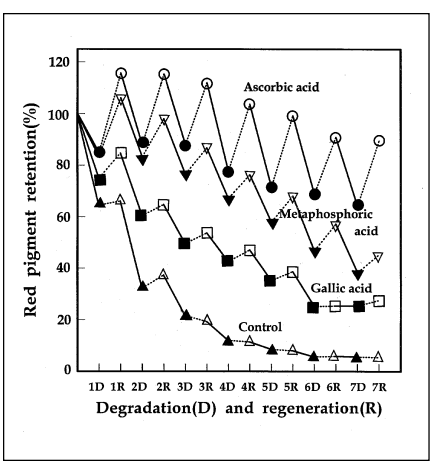

Fig. 1—Percentage red pigment retention of red beet juice after repeated degradation and regeneration at pH 3.8 in the presence of food additives. The additives were added once prior to first heating. Closed symbols and solid lines indicate degradation, and open symbols and dotted lines indicate regeneration.

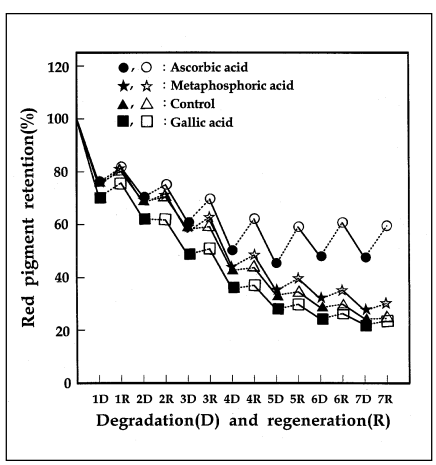

Fig. 2—Percentage red pigment retention of red beet juice after repeated degradation and regeneration at pH 6.2 in the presence of food additives. Refer to Fig. 1 for conditions and legend.

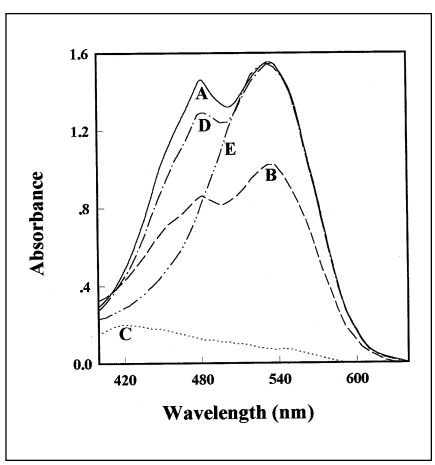

Fig. 3—Visible light absorption spectra of prepared juice: control (A); juice after the first (B) and the fifth degradation (C); and juice immediately after adding ascorbic acid (D) and after the fifth regeneration (E). The fifth regeneration means that the juice was degraded 5 times at 100°C for each 3 min followed by regeneration after every degradation.

posed guideline. The addition level should depend on a balance of cost, taste, acidity, and shelf life of food products which contain red beet pigments.

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