Sulfated Polysaccharides Inhibit Browning of Apple Juice and Diced Apples

5646

Cindy B. S. Tong and Kevin B. Hicks*

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118

Browning of fresh Granny Smith apple juice was inhibited almost 100% for 24 h at room temperature by the addition of ι-, κ-, or λ-carrageenan, alone (0.25%) or in combination (0.05%) with 0.5% citric acid. The combination of 0.1% of any of the carrageenans and 0.5% sodium hexametaphosphate was slightly less effective. Browning was also inhibited by amylose sulfate (0.025%) or xylan sulfate (0.025%) combined with 0.5% citric acid. Under the assay conditions, citric acid alone inhibited browning approximately 34%, but sodium hexametaphosphate alone did not inhibit browning. The inhibition of browning by these compounds in combination with the carbohydrate polymers was synergistic. The combination of 0.1% of any of the carrageenans and 0.5% citric acid was able to inhibit browning of unpasteurized apple juice containing 0.1% sodium benzoate for up to 3 months at 3 °C. The combination of 0.5% carrageenan and 0.5% citric acid also inhibited browning in Granny Smith and Red Delicious diced apple fruit. These combinations may have practical application in the prevention of enzymatic browning in fresh, raw apple juice or diced apples.

INTRODUCTION

When certain fruits and vegetables are cut or bruised, their injured surfaces brown rapidly. This browning can be caused enzymatically, upon oxidation of polyphenols by polyphenol oxidase [see review of browning by Vámos-Vigázó (1981)], or through nonenzymatic means (Bolin and Steele, 1987; Cilliers and Singleton, 1989). In both cases, quinones can form and polymerize, yielding brown pigments. Nonenzymatic autoxidation of phenolics into quinones can occur within minutes under alkaline conditions but is much slower (months) under acidic conditions (Cilliers and Singleton, 1989). Nonenzymatic browning can also be due to the Maillard reaction between sugars and amines, amino acids, peptides, or proteins (Hodge, 1953).

Various compounds have been used to control browning in cut, unblanched fruits and vegetables and in juices, including sulfites (Anderson and Zapsalis, 1957; Embs and Markakis, 1965), cysteine (Walker and Reddish, 1964), sulfur amino acids (Molnar-Perl and Friedman, 1990), ascorbic acid (Makower, 1964; Tate et al., 1964; Markakis and Embs, 1966; Pierpoint, 1966; Janovitz-Klapp et al., 1990), and ascorbic acid derivatives and phosphate-based blends (Sapers and Hicks, 1989; Sapers et al., 1989). Of these, sulfites are the most efficacious, but they have been banned by the U.S. Food and Drug Administration for use on fresh fruits and vegetables because they are potentially fatal to asthmatics.

Sapers et al. (1989) have shown that β-cyclodextrins (cycloheptaamylose) inhibit browning of raw apple juice. It was hypothesized that β-cyclodextrin inhibited browning by binding polyphenol oxidase substrates in its hydrophobic core. On the basis of this work, we decided to examine other carbohydrates that may have the proper conformation to bind polyphenol oxidase substrates.

Under the right conditions, carrageenans, algal galactose polysaccharides, can form helical structures that may possess hydrophobic domains. They are generally recognized as safe for food use and are used in many products, including puddings, toothpaste, air fresheners, and pet food. The three types of carrageenans generally used in food and industrial applications are ι-, κ-, and λ-carrageenan. Both ι- and κ-carrageenan contain 3,6-anhydrogalactose, while λ-carrageenan does not. These three carrageenans also differ in their degree of sulfation. ι- and κ-carrageenan form helices in the presence of certain ions at temperatures below their melting points and in solutions of pH above 3.3. λ-carrageenan does not appear to form helices and therefore does not form gels.

In view of the need for safe and effective alternatives to sulfites, we report novel formulations using carrageenans and other sulfated polysaccharides to prevent browning in fresh, raw apple juice and dice.

MATERIALS AND METHODS

Chemicals. Amylose sulfate and larch xylan sulfate were purchased from V-Labs, Covington, LA. The larch xylan sulfate was molecular weight fraction II of the synthetic batch. Carrageenans were purchased from Sigma Chemical Co. Food grade sodium hexametaphosphate (SHMP) was provided by FMC Corp., Philadelphia, PA. Other chemicals used were of reagent grade.

Juice Preparation. Apples (*Malus domestica* Borkh. cv. Granny Smith) were purchased at local supermarkets and kept at 3 °C until needed. Juicing was performed at room temperature. To prepare juice, apples were rinsed with H_2O, sectioned with a household apple slicer, and juiced with an Acme Supreme Model 6001 Juicerator lined with Whatman No. 1 filter paper. Juice was collected in a beaker containing ascorbic acid (5 mg/100 mL of juice) and kept stirred until decanted. The ascorbic acid was used to prevent instantaneous browning, providing a short lag time to allow test materials (polysaccharides, with or without citric acid or sodium hexametaphosphate) to be completely solubilized. The amount of ascorbic acid used was not enough to prevent browning alone for more than 1 h (Sapers et al., 1989). Aliquots (50 mL) of juice were poured into beakers containing test material and stirred. Test materials were not predissolved by heating or sonication; aggregates in juice were mechanically dispersed with spatulas. After approximately 1 min, the mixtures were poured into optical cells for reflectance measurements at room temperature. For long-term juice experiments, 25-mL aliquots of juice were mixed with test materials, kept in closed vials at 3 °C, and removed periodically for reflectance measurements. Juice pH was measured with an Orion Model 611 pH meter and typically ranged from 3.2 to 3.5.

Browning Measurements. Changes in pigmentation were followed with a Byk-Gardner Color Machine spectrophotometer

Table I. Inhibition of Browning of Fresh, Raw Granny Smith Apple Juice by Sulfated Polysaccharides, with or without the Addition of 0.5% Citric Acid or Sodium Hexametaphosphate at Room Temperature[a]

type and % used of polysaccharide	% inhibition by polysaccharide alone	polysaccharide + 0.5% citric acid	polysaccharide + 0.5% SHMP
none		34.1 ± 4.1	1.34 ± 1.5
ι-carrageenan			
0.05	14.0 ± 3.6[b]	95.2 ± 7.8	20.7 ± 10.2[b]
0.1	40.3 ± 11.9	100	93.6 ± 8.7
0.25	100	100	100
κ-carrageenan			
0.05	34.4 ± 3.4[b]	95.7 ± 2.4	39.1 ± 16.2[b]
0.1	53.4 ± 9.7	97.7 ± 4.4	84.7 ± 3.0
0.25	91.3 ± 12.7	98.9 ± 2.2	95.2 ± 7.0
λ-carrageenan			
0.05	28.9 ± 2.4	100	33.5 ± 7.5
0.1	24.5 ± 7.5	87.0 ± 4.5	93.1 ± 4.7
0.25	88.0 ± 2.9	100	88.7 ± 3.8
amylose sulfate			
0.025	9.0 ± 9.8[b]	94.7 ± 6.3	0[b]
0.25	27.0 ± 12.1	98.1 ± 4.7	23.7 ± 10.4
xylan sulfate			
0.025	11.7 ± 12.4[b]	91.5 ± 4.5	0.4 ± 0.8[b]
0.25	2.3 ± 3.8	88.6 ± 6.5	55.7 ± 30.5

[a] Data are listed as percent inhibition of browning as measured by reflectance at 440 nm (mean ± standard error of six or more samples), 24 h after the start of treatment (unless otherwise noted). [b] Data recorded 2 h after treatment.

system (Pacific Scientific, Silver Spring, MD) according to the method of Sapers and Douglas (1987). Reflectance (R) at 440 nm was recorded at specific intervals. For these measurements, samples were illuminated at 45° and the reflected light at 0° was collected and detected. Specular reflectance was not collected. Samples were kept at 25 °C throughout the measurement period, for up to 24 h. R_{440} values were used to calculate percent inhibition of browning in the following way:

$$\% \text{ inhibition} = \frac{\Delta R_{\text{control}} - \Delta R_{\text{sample}}}{\Delta R_{\text{control}}} \times 100$$

Two or three replicates of each treatment were used per experiment. Each experiment was done at least twice.

Dice Experiments. Apples (cv. Granny Smith or Red Delicious) were sliced into transverse sections which were then cut into cubes (approximately 1 cm^3). Epidermis remaining on the cubes was removed and discarded. Mesocarp immediately surrounding the core was also discarded. Cubed mesocarp was kept in a container on ice until all apples were diced. When dicing was completed, the cubes were weighed, placed in jars, and then mixed with test solutions in the jars. After 10 min, the cubes were drained of test solution and measured for reflectance at 440 nm. Samples were then covered and stored at 3 °C. Reflectance measurements were taken daily for up to 10 days afterward. Significance of difference between the means of control and treated samples was determined by using a one-tailed t-test of independent samples.

RESULTS AND DISCUSSION

Inhibition of Browning. The sulfated polysaccharides tested included ι-, κ-, and λ-carrageenans, amylose sulfate, and larch xylan sulfate. Addition of these carbohydrates did not alter pH of the juice. ι-Carrageenan used alone at 0.25% (w/v) inhibited browning 100%, while 0.25% κ- and λ-carrageenan inhibited browning 91% and 88%, respectively (Table I). Concentrations of less than 0.25% of the carrageenans generally produced more variable inhibition results. Neither amylose sulfate nor xylan sulfate was effective alone at concentrations up to 0.25%.

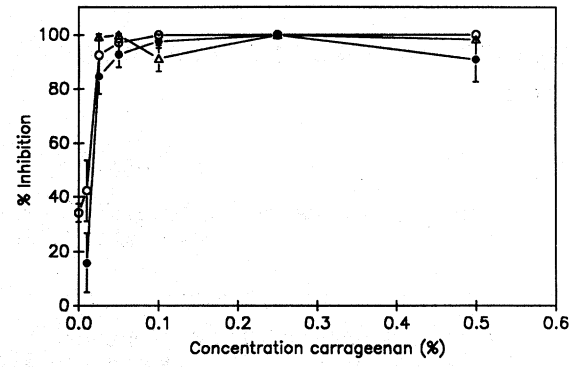

Figure 1. Dose–response curves for ι- (open circles), κ- (solid circles), or λ- (open triangles) carrageenan in the presence of 0.5% citric acid. Response shown is the inhibition of browning of apple juice as change in reflectance at 440 nm compared to controls (means of six or more replicates ± 95% confidence interval).

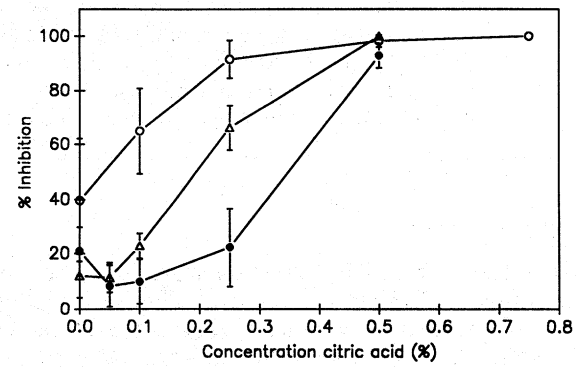

Figure 2. Dose–response curves for citric acid in the presence of 0.05% ι-, κ-, or λ-carrageenan. Data are as in Figure 1.

To determine if possible chelators of copper in polyphenol oxidase could be used to prevent apple juice browning in combination with carrageenan and if, therefore, lower concentrations of carrageenan could be used, citric acid and sodium hexametaphosphate were tested in combination with the carrageenans. Browning was inhibited 95–100% by the combination of 0.05% of any of the carrageenans and 0.5% citric acid. Addition of 0.5% citric acid decreased juice pH by approximately 0.2 unit, to 3.0–3.3. Inhibition by 0.5% citric acid alone was approximately 35%, indicating that decrease of pH alone was not sufficient to inhibit browning. Inhibition by 0.05% of any of the carrageenans alone was less than 35%, demonstrating that the combination of the carrageenan and citric acid resulted in a synergistic effect (Table I). Concentrations of ι- or κ-carrageenan of less than 0.05% in combination with 0.5% citric acid were less effective in preventing browning. However, even 0.025% λ-carrageenan combined with 0.5% citric acid inhibited browning approximately 100% (data not shown). Figure 1 shows dose–response curves for ι-, κ-, and λ-carrageenan when the citric acid concentration was kept constant at 0.5%.

Decreasing the concentration of citric acid to less than 0.5% decreased the percent inhibition when used in combination with 0.05% of any of the carrageenans. This effect was most pronounced with κ-carrageenan. Figure 2 shows dose–response curves for which the concentration of citric acid was varied in the presence of 0.05% carrageenan.

Amylose sulfate and xylan sulfate were very effective inhibitors of enzymatic browning when they were used in combination with citric acid. The combination of only 0.025% amylose sulfate or xylan sulfate with 0.5% citric acid inhibited browning 91.5–95%. This was also a

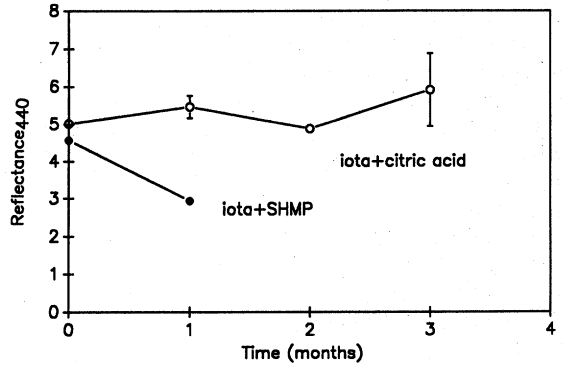

Figure 3. Reflectance at 440 nm of juice containing 0.1% ι-carrageenan, 0.1% sodium benzoate, and 0.5% citric acid or sodium hexametaphosphate stored for 1–3 months at 3 °C. Data shown are means of three replicates ± 95% confidence interval.

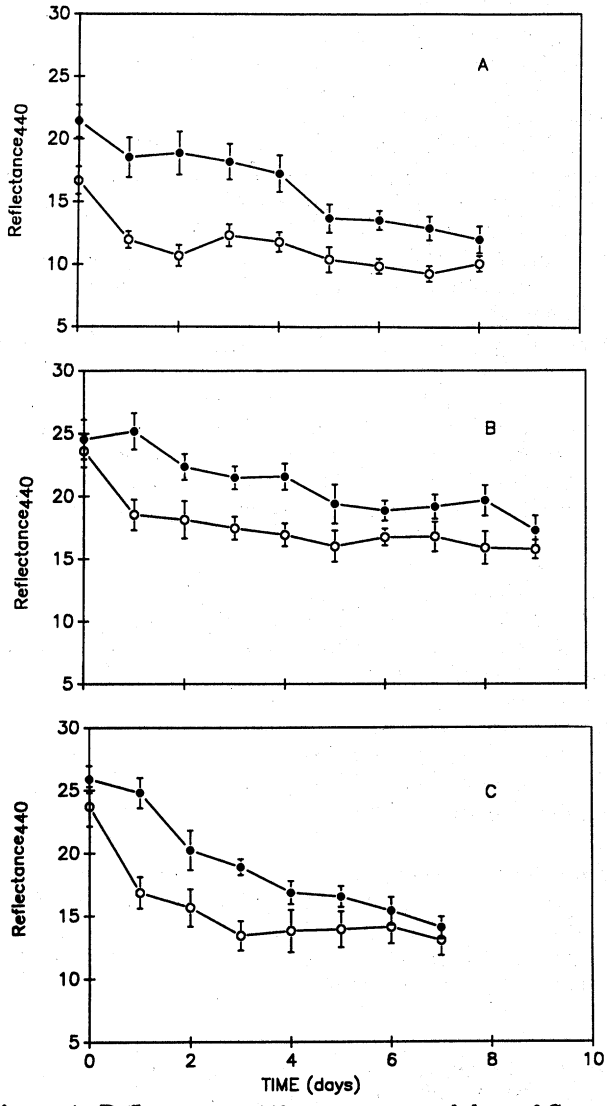

Figure 4. Reflectance at 440 nm over several days of Granny Smith dice dipped in distilled water (control, open circles) or in a solution containing 0.5% ι- (A), κ- (B), or λ- (C) carrageenan and 0.5% citric acid (solid circles). Data shown are means of 10 or more replicates ± 95% confidence interval.

synergistic effect, as 0.025% amylose sulfate or xylan sulfate alone was a poor inhibitor of browning (Table I).

Browning was also inhibited approximately 90–100% with the combination of 0.1% ι-, κ-, or λ-carrageenan and 0.5% sodium hexametaphosphate (SHMP). Alone, 0.5% SHMP did not inhibit browning, indicating that its combination with carrageenan also produced a synergistic

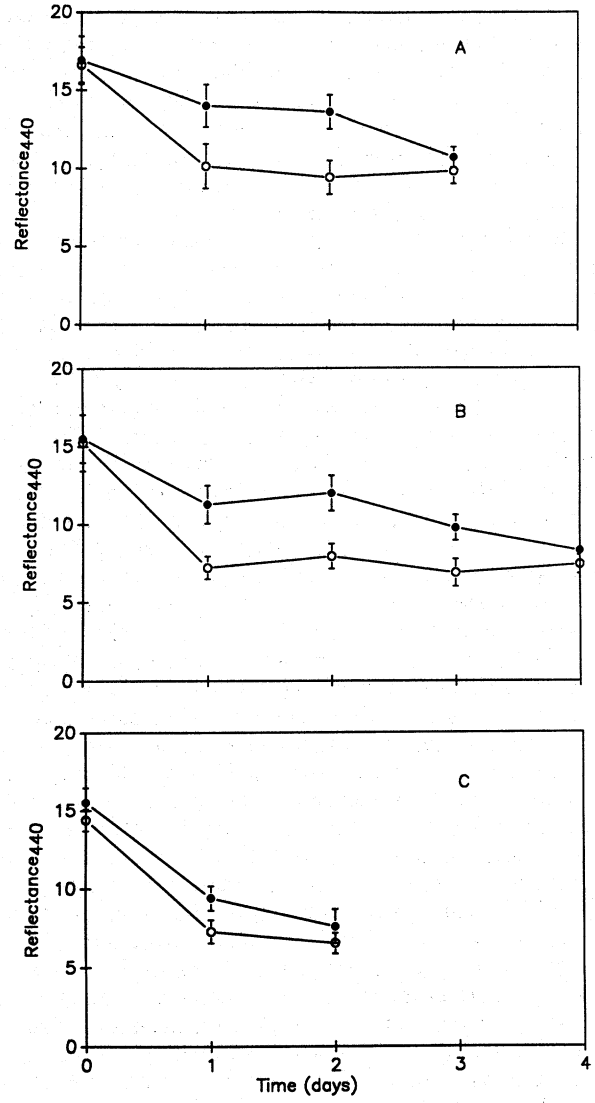

Figure 5. Reflectance at 440 nm over several days of Red Delicious dice dipped in distilled water or in solutions containing 0.5% carrageenan and 0.5% citric acid. Symbols are as in Figure 4.

effect (Table I). Addition of 0.5% SHMP increased juice pH 0.5 to 1.0 unit. The dose–response curve for ι-carrageenan in the presence of 0.5% SHMP was similar to that shown in Figure 1, but 0.5% SHMP with 0% or 0.01% ι-carrageenan produced 0% inhibition. The combinations of SHMP and amylose sulfate or xylan sulfate were ineffective.

Variability of results was most noticeable when treatments were marginally effective (Table I). Variation was most likely due to variability of the apples used, which differed by source (e.g., New Zealand, Chile, United States) and storage conditions. Juice made from apples purchased in autumn sometimes did not brown within 24 h at room temperature even with nothing added. On the other hand, juice from apples purchased in late spring sometimes would brown immediately, even in the presence of 0.005% ascorbic acid. Generally, when browning was inhibited more than 80%, variation in results was low, indicating that such treatments were effective enough to overcome the apple variability.

Long-Term Inhibition of Browning. The color of unpasteurized juice containing 0.1% ι-carrageenan, 0.5% citric acid, and 0.1% sodium benzoate (as a preservative) remained unchanged (Figure 3) for at least 3 months at 3 °C. Similar results were produced when κ- or λ-carra-

geenan was used instead of ι-carrageenan. No other juice treatment in this study prevented browning for more than 3 days. For example, the combination of ι-carrageenan and SHMP was ineffective at preventing browning over long-term storage (Figure 3). After 3 or 4 months the carrageenan/citric acid/sodium benzoate-treated juice darkened slightly (data not shown), suggesting that nonenzymatic browning may have occurred.

Diced-Apple Experiments. The carrageenan and citric acid combination was also tested on diced Granny Smith and Red Delicious apples. Amylose sulfate and xylan sulfate were not used for dice experiments due to lower availability of these materials. Carrageenans or citric acid alone did not inhibit browning. However, the mixture of ι-, κ-, or λ-carrageenan and citric acid delayed browning for up to 7 days with Granny Smith apple dice (Figure 4) and up to 3 days with the faster browning (Sapers and Douglas, 1987) variety Red Delicious (Figure 5). There seemed to be no significant difference in effectiveness of the three carrageenans. Hence, the mixture of 0.5% ι-, κ-, or λ-carrageenan and 0.5% citric acid may be used to prevent browning in some minimally processed fruit. However, at these concentrations, ι-carrageenan forms a slightly stickly gel and κ-carrageenan forms a brittle gel that may not be attractive to consumers. λ-Carrageenan does not form a gel. Wyss et al. (1990) recommended the use of xanthan gum over carrageenan as a thixotropic agent in their formulation for reducing deterioration of salad bar items because carrageenan has a slimy mouth feel. We did not perform formal taste tests to determine the effect of the carrageenans on apple dice flavor or consumer acceptability. However, informal taste tests of apple juice treated with carrageenans indicated that juice flavor and mouth feel was not altered compared to untreated juice. We speculate that mouth feel of apple dice may be unacceptable to some consumers. More carrageenan was necessary to prevent browning in diced apples than in juice. This may be because polyphenol oxidase and its substrates are more readily accessible to carrageenan in juice than on the cut surface of diced apples.

Our current research is focusing on the mechanism of inhibition by the carbohydrate polymers. Initially, it was hypothesized that the conformation of the ι- and κ-carrageenans in the gel state was important, perhaps capturing phenolics in a hydrophobic core. However, λ-carrageenan does not form gels but is as effective as ι- and κ-carrageenans at inhibiting browning in apple juice. Also, at the conditions present in apple juice, ι- and κ-carrageenans do not form gels. Other possible mechanisms of action could include chelation of the copper cofactor of polyphenol oxidase by sulfate groups of the carrageenans, amylose sulfate, and xylan sulfate or direct binding of the polyphenol oxidase protein, inhibiting its activity.

ACKNOWLEDGMENT

We thank Gerald Sapers for the use of the Byk-Gardner Color Machine spectrophotometer and for helpful discussion and Rebecca Haines for technical assistance.

LITERATURE CITED

Anderson, E. E.; Zapsalis, C. Technique ups quality, shelf-life of pre-peeled potatoes. *Food Eng.* **1957**, *29*, 114–116.

Bolin, H. R.; Steele, R. J. Nonenzymatic browning in dried apples during storage. *J. Food Sci.* **1987**, *52*, 1654–1657.

Buera, M. P.; Chirife, J.; Resnik, S. L.; Wetzler, G. Nonenzymatic browning in liquid model systems of high water activity: kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. *J. Food Sci.* **1987**, *52*, 1063–1067.

Cilliers, J. J. L.; Singleton, V. L. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. *J. Agric. Food Chem.* **1989**, *37*, 890–896.

Embs, R. J.; Markakis, P. The mechanism of sulfite inhibition of browning caused by polyphenol oxidase. *J. Food Sci.* **1965**, *30*, 753–758.

Hodge, J. E. Chemistry of Browning Reactions in Model Systems. *J. Agric. Food Chem.* **1953**, *1*, 928–943.

Janovitz-Klapp, A. H.; Richard, F. C.; Goupy, P. M.; Nicolas, J. J. Inhibition studies on apple polyphenol oxidase. *J. Agric. Food Chem.* **1990**, *38*, 926–931.

Makower, R. U. ATP-induced inhibition of potato browning. Effect of ascorbic acid oxidase and of reducing substances. *Plant Physiol.* **1964**, *39*, 520–522.

Markakis, P.; Embs, R. J. Effect of sulfite and ascorbic acid on mushroom phenol oxidase. *J. Food Sci.* **1966**, *31*, 807–811.

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. *J. Agric. Food Chem.* **1990**, *38*, 1648–1651.

Pierpoint, W. S. The enzymic oxidation of chlorogenic acid and some reactions of the quinone produced. *Biochem. J.* **1966**, *98*, 567–580.

Sapers, G. M.; Douglas, F. W., Jr. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *J. Food Sci.* **1987**, *52*, 1258–1262, 1285.

Sapers, G. M.; Hicks, K. B. Inhibition of enzymatic browning in fruits and vegetables. In *Quality Factors of Fruits and Vegetables*; Jen, J. J., Ed.; ACS Symposium Series 405; American Chemical Society: Washington, DC, 1989; pp 29–43.

Sapers, G. M.; Hicks, K. B.; Phillips, J. G.; Garzarella, L.; Pondish, D. L.; Matulaitis, R. M.; McCormack, T. J.; Sondey, S. M.; Seib, P. A.; El-Atawy, Y. S. Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *J. Food Sci.* **1989**, *54*, 997–1002, 1012.

Tate, J. N.; Luh, B. S.; York, G. K. Polyphenoloxidase in Bartlett pears. *J. Food Sci.* **1964**, *29*, 829–836.

Vámos-Vigyázó, L. Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 49–127.

Walker, J. R. L.; Reddish, C. E. S. Note on the use of cysteine to prevent browning in apple products. *J. Sci. Food Agric.* **1964**, *15*, 902–904.

Wyss, C. R.; McGuire, M. T.; Frost, R. Composition for extending shelf life of fruits and vegetables. U.S. Patent 4,959,230, 1990.

Received for review February 11, 1991. Revised manuscript received May 6, 1991. Accepted June 3, 1991. Use of a company or product name does not imply approval or recommendation of the product to the exclusion of others that may also be available.

Registry No. ι-Carrageenan, 9062-07-1; κ-carrageenan, 11114-20-8; λ-carrageenan, 9064-57-7; amylose sulfate, 37251-17-5; xylan sulfate, 37300-21-3; citric acid, 77-92-9.