Enzymatic Browning Inhibited in Fresh and Dried Apple Rings by Pineapple Juice

PATRICIA G. LOZANO-DE-GONZALEZ, DIANE M. BARRETT, RONALD E. WROLSTAD and ROBERT W. DURST

ABSTRACT

Sliced apple rings were treated with water (control), canned pineapple juice, frozen pineapple juice, ion-exchanged pineapple juice, frozen orange juice, ascorbic acid, a commercial antibrowning preparation or sodium bisulfite. The rings were either left exposed to air, vacuum packaged, or dehydrated. Browning was measured colorimetrically and by visual examination over extended periods of time. Pineapple juice was an effective browning inhibitor in both fresh and dried apples. Pineapple juice was fractionated using various size and charge separation procedures. All fractions inhibited enzymatic browning of crude apple extracts by at least 26%. Results indicate that the inhibitor is a neutral compound of low molecular weight.

Key Words: apple, enzymatic browning, inhibition, pineapple juice

INTRODUCTION

POLYPHENOL OXIDASE-catalyzed browning occurs in fruits and vegetables upon bruising during handling or transportation, and when exposed to air in the cut, sliced or pulped state (Labuza and Schmidl, 1986). The prevalent use of sulfites as inhibitors of enzymatic browning in foods has been restricted by the Food & Drug Administration (Anon., 1986, 1987) due to allergic reactions sometimes exhibited by individuals with respiratory ailments.

Alternative browning inhibitors have been investigated by several researchers (Dudley and Hotchkiss, 1989; Sapers and Ziolkowski, 1987; Hsu et al., 1988; Santerre et al., 1988; Sapers et al., 1989 a,b; Friedman and Molnar-Perl, 1990; Labuza et al., 1990; Richard et al., 1991). Potential alternatives to sulfites include: ascorbic acid (AA), ascorbic acid phosphates, sodium ascorbate, citric acid, benzoic acid, sodium benzoate, sodium chloride, calcium chloride, zinc chloride, cinnamic acid, sodium cinnamate, cysteine, glutathione, β-cyclodextrins, Sporix (a commercial polyphosphate) and their various combinations. Ascorbic acid has received particular attention because of its multiple effects: it chelates copper, reduces o-quinones and acts as a competitive inhibitor of PPO.

In a recent study, Labuza et al. (1990) tested various proteases such as ficin, actinidin, papain, and bromelain for their relative effectiveness at inhibiting enzymatic browning. Bromelain, an enzyme present in pineapples, proved to be effective for inhibiting browning in refrigerated apple slices. Amino acids and peptides may contribute to enzymatic browning by reacting with o-quinones (Golan-Goldhirsh and Whitaker, 1984).

Sulfhydryl compounds react with o-quinones produced by PPO and form colorless products (Walker, 1975). Sulfhydryl compounds such as cysteine and glutathione have been effective for control of enzymatic browning in fruit products, however, neither is approved for that use in the U.S. (Labuza et al., 1990). Bennion (1990) stated that 3-(methylthio)propanoic acid methyl ester, a sulfhydryl compound present in pineapple juice, contributed to the characteristic aroma and may be responsible for the inhibition of browning.

Pineapple, papaya and lemon juices have been reported to prevent discoloration of cut surfaces of fruits and vegetables (Balls and Hale, 1935; Bennion, 1990). Pineapple juice compounds which may have potential as PPO inhibitors include organic acids, proteins, amino acids, certain metals and sulfhydryl compounds. No other published reports were found on the use of pineapple juice as an inhibitor of PPO.

The objectives of our study were to: evaluate the potential of pineapple juice as an inhibitor of enzymic browning in fresh and dried apple products; compare the effectiveness of various pineapple juice samples to other known inhibitors of PPO; fractionate pineapple juice and carry out compositional analysis and identify fractions most effective at inhibiting PPO in crude apple extracts.

MATERIALS & METHODS

Fruit

'Red Delicious' and 'Granny Smith' apples were picked in September and early October, 1990, at the Mid-Columbia Research and Extension Center in Hood River, Oregon and were kept at 3°C in air for up to 10 mo. Apple maturity was determined by penetrometer and soluble solids measurements. 'Red Delicious' apples have been used in many other browning inhibition studies due to their extreme sensitivity to browning (Toribio and Lozano, 1986; Sapers and Douglas, 1987; Sapers and Ziolkowski, 1987; Sapers et al., 1989 a,b; Molnar-Perl and Friedman, 1990; Richard et al., 1991). 'Granny Smith' apples are recommended for dried products due to their relatively limited browning and have been also used in numerous browning inhibition studies (Sapers and Douglas, 1987; Sapers et al., 1989a,b). In our investigation, 'Red Delicious' apples were used for the fresh apple studies and 'Granny Smith' apples were selected for the dried product study.

Evaluation of color changes in fresh and dried apple rings

Sample Preparation. Apples were screened with respect to size, shape and defect level, and then cored, peeled and sliced to obtain rings of 1 cm thickness. A manual corer-peeler-slicer (White Mountain Freezer Inc., Winchendon, MA) was used to prepare apple rings. The rings were dipped in each treatment solution for 2 min and drained.

Eight different inhibitors were evaluated for potential to inhibit enzymatic browning in both fresh and dried apple slices: distilled water (control), canned pineapple juice (PJ), frozen concentrated pineapple juice (FCPJ), diluted to 12.8° Brix, ion exchanged canned pineapple juice (IEPJ), 0.7% ascorbic acid (AA), frozen concentrated orange juice (OJ), diluted to 11.8° Brix, 0.7% EVER-FRESH (EF), and 0.1% sodium bisulfite (sulfite).

Both canned and frozen concentrated pineapple juices were obtained from Dole Packaged Foods, Co., San Jose, CA. The ion exchanged pineapple juice (IEPJ) was prepared by filtering canned juice through a 0.45 μm filter, then passing it through a BioRex-5 column (Bio-Rad Laboratories, Richmond, CA) and, finally, passing the juice through a SP Sephadex C-25 exchange column (Pharmacia LKB Biotechnology Inc, Pleasant Hill, CA). The BioRex-5 column was used to remove organic acids and absence of ascorbic acid was monitored with dip test strips (EM Industries, Inc., Gibbstown, NJ). The SP Sephadex C-25 column is a cation exchanger and would be expected to remove basic compounds such as amino acids and small peptides.

The approximate ascorbic acid (AA) concentration of the canned

Authors Wrolstad and Durst are affiliated with the Dept. of Food Science & Technology, Wiegand Hall, Oregon State Univ., Corvallis, Oregon 97331-6602. Author Lozano-de-Gonzalez is with the Dept. of Chemistry, Universidad Autonoma de Chihuahua, Chihuahua, Mexico. Author Barrett is with the Dept. of Food Science & Tehcnology, Univ. of California, Davis, CA 95616.

Table 1—Composition of dip treatments

Treatment	pH	Soluble solids (°Brix)	Ascorbic acid (mg/ml)
Control	7.0	0	0
Pineapple juice (PJ)	3.4	13.0	7.0
Frozen concentrate PJ	3.6	12.8	3.0
Ion exchange PJ	4.8	10.6	0
Ascorbic acid	3.0	0.9	7.0
Orange juice	3.9	11.8	5.0
EVER-FRESH	3.5	1.0	7.0
Sulfite	4.2	0	0

Table 2—Experiments performed on apple rings

	Storage temp	Storage times	Analyses
Fresh	21°C	0,0.5,1,2,8,10,18,24,48 hr	L*,a*,b* A_{420nm}
		1,24 hr	Visual scores
Fresh vacuum-packed	1°C	0,1,2,3,4,6 wk	L*,a*,b* A_{420nm}
		0,1,2,3,4 wk	Visual scores
Dried	21°C	0,1,2,3 mo	L*,a*,b* A_{420nm}

pineapple juice (PJ) was determined using dip test strips, and a solution of commercial AA was prepared at the same concentration (0.7%). Concentrated orange juice (Minute Maid, Coca Cola Foods, Houston, TX) was diluted to 11.8° Brix. EVER-FRESH, a commercial preparation containing sucrose, ascorbic acid, citric acid and calcium phosphate (MCP Foods, Anaheim, CA) was prepared as a 0.7% (w/v) solution in distilled water. Sodium bisulfite was made at a concentration typically used for sliced apples (0.1%). Table 1 lists the pH, soluble solids and ascorbic acid content of the treatment solutions.

Fresh and dried apple ring processing. Three separate experiments were carried out (Table 2), two with 'Red Delicious' and one with 'Granny Smith' apples. After experimental treatments, fresh 'Red Delicious rings were either immediately analyzed for color changes or vacuum packed in Cryovac bags (B900, W.R. Grace and Co., Duncan, SC) and stored at 1°C until evaluated. Dried 'Granny Smith' rings were treated and drained, then dried at 74°C in a tunnel dryer (Dept. Food Science and Technology, Oregon State University, Corvallis, OR). Drying was completed in 2 hr, with a final moisture content of ≈22%, after which the rings were allowed to equilibrate to room temperature, packed in Cryovac vags and stored at 21°C until evaluated.

Fresh apple rings. The first experiment involved evaluation of color of the fresh apples immediately after dipping in each treatment and draining. Color was measured at zero time (≈1 min), 0.5, 1, 2, 8, 10, 18, 24 and 48 hr after each treatment using the Hunter ColorQUEST colorimeter and after 1 and 24 hr by a sensory panel.

Three to four rings were placed into petri dishes and hand-held to the colorimeter reflectance port for determination of L*, a* and b* and absorbance at 420 nm. Percent inhibition was calculated using the change in L* value as compared to the control, which was defined as zero percent inhibition.

$$\%\ \text{Inhibition}_t = (\Delta L^*\ \text{control} - \Delta L^*\ \text{treatment})/\Delta L^*\ \text{control}_t \times 100$$

The sensory panel consisted of eight untrained individuals familiar with the product who examined the rings for color at 1 and 24 hr under fluorescent light at room temperature (21°C). Between measurements the samples were kept in closed petri dishes at room temperature (≈21°C).

Fresh vacuum-packed apple rings. Color changes in vacuum-packed rings stored at 1°C were measured with the Hunter ColorQUEST spectrophotometer immediately after opening. Bags were opened on the first day, and after 1, 2, 3, 4 and 6 wk. In addition, an untrained sensory panel of 30 persons was asked to evaluate browning of the vacuum-packed fresh apples on the first day and after 1, 2, 3 and 4 wk storage. The individuals evaluating browning were not always the same for each session. This evaluation was carried out at room temperature (21°C) under natural light in the OSU Food Science Pilot Plant. A visual scale from one (no browning) to five (highly browned) had been previously established using photographs of apple rings as references.

Dried apple rings. Color changes in the dried 'Granny Smith' rings that had been vacuum-packed and stored in Cryovac bags at 21°C were also measured. Hunter L*, a*, b* and absorbance at 420 nm were recorded immediately after drying and cooling the rings, and after 1, 2 and 3 mo storage.

Determining effectiveness of pineapple juice fractions

Apple extract. A crude apple extract was prepared by homogenizing 100g apple flesh with 100 mL cold sodium phosphate buffer, pH 6.5, filtering the homogenate through 4 layers of cheesecloth, then centrifuging 20 min (4°C) at 14,750 *xg* (Sorvall RC5 Referigerated Superspeed Centrifuge). The supernatant was decanted and used as a source of polyphenol oxidase (PPO).

Pineapple juice fractionation. Canned pineapple juice (PJ) from Dole Packaged Foods Co., San Francisco, CA, was fractionated in the following manner (Fig. 1):

Fraction 1. Centrifuged, filtered through Whatman #1 filter paper, followed by filtration through a 0.45 μm Millipore filter.

Fraction 2. Fraction 1 was passed through a BioRex-5 anion exchange column (Bio-Rad Laboratories, Richmond, CA).

Fraction 3. Fraction 1 was passed through a SP Sephadex C25 cation exchange column (Pharmacia LKB Biotechnology Inc., Pleasant Hill, CA).

Fraction 4. Fraction 2 was passed through a SP Sephadex C25 cation exchange column.

Fraction 5. Fraction 1 was ultrafiltered in an Amicon stir cell with a 10,000 molecular weight membrane cut-off.

Fraction 6. Fraction 5 was passed through a BioRex-5 anion exchange column.

Fraction 7. Fraction 5 was passed through a SP Sephadex C25 cation exchange column.

Fraction 8. Fraction 7 was passed through a BioRex-5 anion exchange column.

Fraction 9. The concentrated high molecular weight fraction obtained from ultrafiltration was diluted with sodium phosphate buffer, pH 6.5, and acetone was added. The sample was centrifuged and the supernatant was decanted, then the acetone was evaporated in a rotary evaporator and the sample was diluted again with buffer.

Fraction 10. The concentrated high molecular weight fraction obtained from ultrafiltration was diluted with sodium phosphate buffer, pH 6.5, and acetone was added. The sample was centrifuged and the precipitate was dried, pulverized in a mortar and redissolved in buffer.

Compositional analyses. L-Ascorbic acid was determined spectrophotometrically with an enzyme kit following manufacturer's recommended procedures (Boehringer Mannheim Biochemicals, Indianapolis, IN). Protein was analyzed by the BCA method (Pierce Co., Rockford, IL). Sugar and acid compositions were determined by HPLC using the method described by Spanos and Wrolstad (1987). For sugar analyses, a Varian 5000 liquid chromatograph equipped with a column heater (Varian Instrument Group, Walnut Creek, CA) and a refractive index detector, a Perkin Elmer LCI-100 laboratory computing integrator (Perkin Elmer Corp., Analytical Instruments, Norwalk, CT), and a Beckman 501 autosampler were used. Glucose, fructose and sucrose contents were determined, summed and reported as mg total sugar/100 mL. Organic acids were analyzed using a Perkin Elmer Series 400 liquid chromatograph (Perkin Elmer Corp., Analytical Instruments, Norwalk, CT) equipped with an HP 1040 diode array detector, and a Beckman 501 autosampler. Total free amino acids (Formol value) was determined by the AOAC procedure, 1984. Results are reported as meq NaOH/100 mL. Total phenolics were analyzed with the Folin-Ciocalteau reagent (Sigma Chemical Co., St. Louis) using the procedure described by Spanos and Wrolstad, 1990.

Inhibition. To test relative inhibition caused by each fraction, 20μL of crude extract was added to 20μL of that fraction and 2.96 mL of a 0.05M catechol (Sigma Chemical Co.) substrate solution in 0.2M phosphate buffer, pH 6.5 at 25°C. The change in absorbance at 420 nm was recorded for 1 min. As a control, 20 μL of crude extract was added to 2.98 mL of the same catechol solution. One unit of enzyme activity was defined as the quantity of enzyme responsible for a change in absorbance of 0.001 /min.

The percent inhibition was calculated using the polyphenol oxidase activity values (units/g) from the control and each fraction, defining the control as zero percent inhibition.

$$\%\ \text{Inhibition}_t = \frac{(A_{420nm}\text{control} - A_{420nm}\text{treatment})}{A_{420nm}\text{control}_t} \times 100$$

Statistical analyses

All treatments were conducted at least in duplicate, and each of the samples was analyzed in duplicate. A completely randomized design

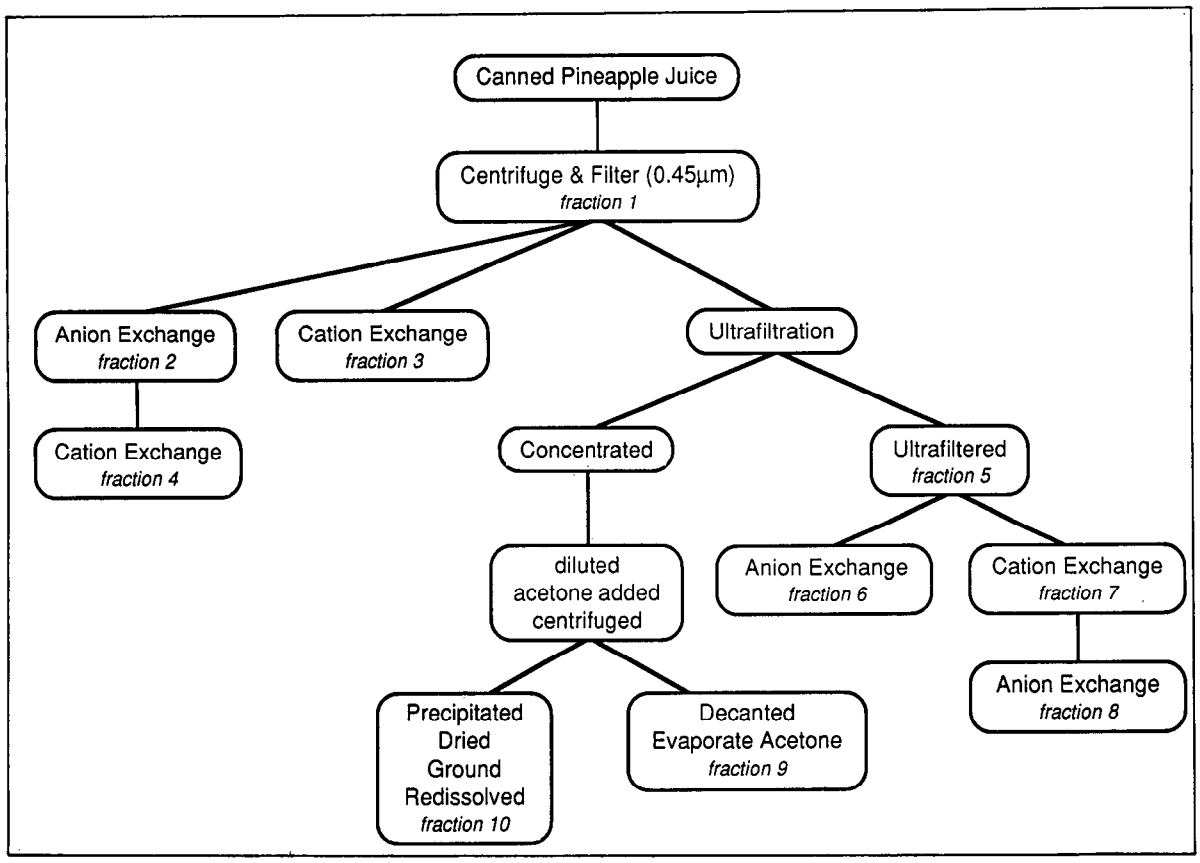

Fig. 1—Fractionation of pineapple juice.

experiment with a factorial arrangement to determine the time and treatment effect on response was carried out. The data were subjected to a one factor analysis of variance (ANOVA) to compare treatments at each specified time of color evaluation. A one factor ANOVA analysis was performed to compare percent inhibition among fractions.

RESULTS & DISCUSSION

Color changes in apple rings dipped in pineapple juice

Figure 2 illustrates browning trends in the various treatments of 'Red Delicious' apple rings with time. A two factor analysis of variance showed that in all color measurements (L*, a*, b* and absorbance at 420 nm), there was a highly significant difference ($p = 0.0001$) among treatments and times.

The L* value of the water-dipped control sample decreased sharply in the first hour, and continued to decline throughout the 48 hr period, as compared to other treatments. There was no significant difference ($p = 0.05$) in percent inhibition among treatments, after either 0.5 or 1.0 hr at 21°C (Table 3). The sensory panel, however, reported less browning in sulfite, PJ and AA treated samples after 1 hr at 21°C (results not shown).

After 2 hr in air at 21°C, significant differences in percent inhibition were apparent (Table 3). These differences varied with both type of solution and time, as shown.

Neither orange juice (OJ) nor frozen concentrated pineapple juice (FCPJ) were very effective inhibitors after the first hour for fresh apple rings in air. Both of these solutions had relatively high soluble solids and somewhat lower ascorbic acid contents (Table 1). Although OJ contained 5.0 mg/mL ascorbic acid (Table 1), which was more than that found in IEPJ, it was a relatively ineffective inhibitor of browning. The commercial mixture, EVER-FRESH, was an adequate inhibitor of enzymatic browning for the first 2 hr, but soon lost its effectiveness.

The ascorbic acid concentration used in the dip solution was

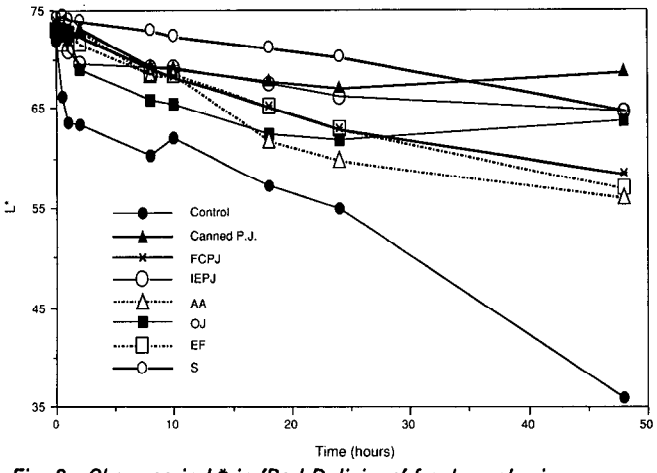

Fig. 2—Changes in L in 'Red Delicious' fresh apple rings.*

7.0 mg/mL (Table 1), the same as that found in commercial canned pineapple juice. This treatment seemed to be as effective as PJ in inhibiting the browning of 'Red Delicious' apple rings for at least the first 10 hr (Table 3). After 18 hr, however, AA treated rings browned very rapidly. This was presumably due to depletion of ascorbic acid, which inhibits brown color development by reducing oxidized quinones to o-diphenols.

From 18 hr to 48 hr, sulfite, PJ, and IEPJ were significantly better ($p = 0.05$) inhibitors than the other treatments. The inhibitory effect of PJ as compared to AA with time suggested that ascorbic acid was not the only factor responsible for inhibition. This also was demonstrated by the fact that IEPJ, which contained no AA, was more effective than AA for all periods longer than 18 hr. The BioRex and Sephadex ion exchange columns removed organic acids and basic compounds, respectively.

Table 3—Effect of various treatments on percent inhibition of browning in fresh 'Red Delicious' apple rings

Treatment	Time (hr) 0.5	1	2	8	10	18	24	48
PJ	104 a	95 a	108 b	72 ab	67 ab	68 c	67c	90 bc
FCPJ	93 a	92 a	82 ab	60 a	43 a	41 b	35 ab	57 a
IEPJ	91 a	75 a	56 a	66 a	60 ab	62 c	59 c	76 b
AA	90 a	95 a	96 b	70 ab	50 ab	22 a	21 a	54 a
OJ	93 a	95 a	46 a	73 ab	21 a	27 a	32 a	52 a
EF	72 a	96 a	83 b	48 a	57 ab	47 b	40 ab	55 a
Sulfite	91 a	98 a	95 b	46 a	79 b	79 cd	80 cd	80 b

[a-d] Values within a column, followed by different letters are significantly different ($p<0.05$).

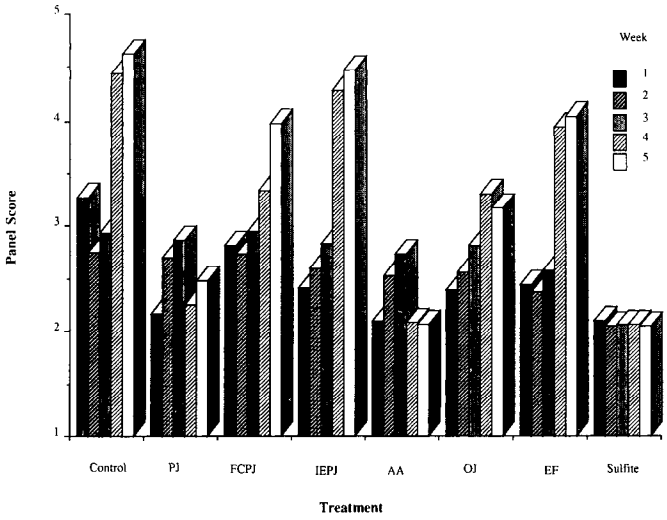

Fig. 3—Visual panel scores for vacuum-packed fresh apple rings.

Table 4—L values of dried 'Granny Smith' apple rings*

Treatment	Storage time 1 day	1 month	2 months	3 months
Control	60 a	65 a	65 b	63 b
Pineapple juice (PJ)	65 b	71 bc	66 bc	67 bc
Frozen concentrated PJ	59 a	63 a	61 a	64 b
Ion exchanged PJ	71 c	68 b	66 bc	65 b
Ascorbic acid	65 b	66 b	65 b	55 a
Orange juice	67 b	61 a	59 a	58 a
EVER-FRESH	66 b	65 ab	63 ab	63 a
Sulfite	73 d	74 c	66 bc	61 ab

[a-d] Values within a column, followed by different letters, are significantly different ($p<0.05$).

Prevention of enzymatic browning by sulfur containing amino acids and peptides in apples has been demonstrated by Friedman and Molnar-Perl (1990). Pineapple juice contains a sulfhydryl compound that contributes to its unique aroma (Collins, 1960) and may contribute to its ability to inhibit enzymatic browning (Bennion, 1990). The sulfite treatment was not significantly different from PJ and IEPJ at any point after 10 hr. The sensory panel found that, after 24 hr, sulfite and IEPJ treated apple rings were the least brown and therefore the most visually appealing. Thus PJ and IEPJ appear to be good potential alternatives to sulfites for prevention of browning in fresh apple rings.

Fresh vacuum-packed apple rings. Apple rings which were vacuum-packed in Cryovac bags were stored at 1°C and evaluated for color change weekly (results not shown). There was no significant change in color during the first 4 wk as shown by reflectance measurements made immediately after opening the bags. After 6 wk, the apples showed a slight change in color but were fermented, therefore measurements are not reported. The bags used had a low permeability to oxygen, which probably helped delay browning.

The sensory panel results for vacuum-packed 'Red Delicious' apple rings are shown in Fig. 3. Although analytical measurements were not significantly different, the sensory panel determined that the sulfite, ascorbic acid and pineapple juice treated rings were relatively lighter in color than other treatments. The scale used for evaluating browning ranged from 1 (no browning) to 5 (highly browned).

In most samples, the score given to samples increased with storage time, i.e. the samples became more brown. Note that many samples (control, IEPJ, EF) showed the greatest increase in color between weeks two and three. Panelists may have given the best scores to sulfite-treated samples in part because the sulfite appeared to bleach the apple rings. The texture of sulfite-treated samples declined dramatically toward the end of the storage period, with samples becoming translucent and somewhat slimy.

Sensory panel results indicate that sulfite, AA and PJ treated vacuum-packed rings rated lowest in browning. These results concur with those of the previous unpackaged study in that sulfite and PJ treated rings were lighter in color. In addition, the panel evaluating fresh unpackaged apple rings found IEPJ treated rings were less brown than most other samples.

The instrumental measurement in the unpacked study indicated that sulfite, PJ and IEPJ had greater percent inhibition than other treatments. These results were in reasonable agreement with sensory ratings in the vacuum packed study.

Dried apple rings. Time and treatment were highly significant effects ($p=0.0001$) on L*, a*, b* and absorbance at 420 nm measurements in dried 'Granny Smith' apple rings (Table 4). Some samples had significantly higher percent inhibition values at different times as indicated.

Dried apple ring packages were sampled destructively. Because of possible variance in maturity of initial apples, we could not assume that all samples within a treatment would brown progressively. Thus, results in Table 4 indicate that, within a treatment, L* value changes were somewhat erratic. For this reason, observations should be made across treatments at each particular time.

On day one there was a significant difference ($p=0.05$) between IEPJ and the other treatments. IEPJ was a more effective inhibitor than all other treatments, except sulfite. After 1 mo storage, there was no significant difference ($p=0.05$) between sulfite and PJ, but there was a significant difference between sulfite and the other treatments.

After 2 mo storage PJ and IEPJ were as effective as sulfite in inhibiting browning of dried apple rings. The PJ treatment was significantly more effective ($p=0.05$) than all other treatments, including the sulfite samples, after 3 mo storage. At that time there was a significant difference between all treatments, with AA and OJ samples browning more than the other treatments. The browning of OJ and AA treated samples with time could be due to non-enzymic browning, reactions involving reducing sugars, amino acids and ascorbic acid. Both pineapple juice and ion exchanged pineapple juice appeared to be effective browning inhibitors in both fresh and dried apple rings.

Effectiveness of pineapple juice fractions

Compositional analysis. Pineapple juice was fractionated using various size and charge separation methods (Fig 1) to concentrate and partially characterize the inhibitor. Anion ex-

Table 5—Composition of pineapple juice fractions

Fraction	Protein (mg/mL)	Formol value (meq/100mL)	Total sugars (mg/mL)	Citric acid (mg/mL)	Malic acid (mg/mL)	Ascorbic acid (mg/mL)	Phenolics (mg/mL)
1	17.1	1.27	3.3	2.6	0.7	0.1	0.59
2	13.0	0.00	4.4	0.0	0.0	0.0	0.00
3	16.2	0.64	3.0	2.2	0.6	0.1	0.41
4	3.2	0.00	4.4	0.0	0 0	0.0	0.00
5	15.8	1.49	3.0	2.4	0.6	0.1	0.50
6	21.6	1.32	4.1	0.1	0.4	0.0	0.30
7	20.2	1.38	3.6	3.0	0.7	0.0	0.48
8	12.1	0.50	2.1	0.0	0.0	0.0	0.07
9	46.3	2.50	5.4	4.5	0.1	0.1	0.90
10	0.6	0.00	0.0	0.0	0.0	0.0	0.00

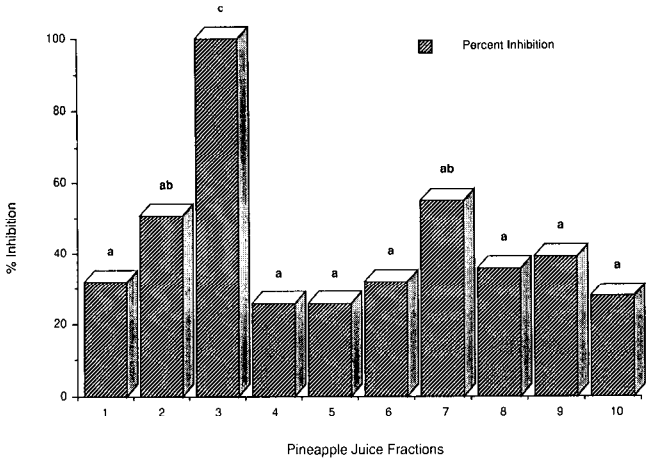

Fig. 4—Percent inhibition of enzymatic browning by pineapple juice fractions.

change resin was used to remove negatively charged compounds, e.g., organic acids (fractions 2, 4, 6 and 8). Cation exchange resin was expected to remove positively charged compounds such as metals, amino acids and small peptides from fractions 3, 4, 7 and 8. Ultrafiltration was used to remove compounds with molecular weights greater than 10,000 daltons. Therefore, fractions 5 through 8 contained only low molecular weight compounds. High molecular weight compounds isolated by ultrafiltration were further fractionated by acetone precipitation (fractions 9 and 10). Fractions 1 through 4 contained both low and high-molecular weight compounds.

Compositional analyses were performed on the different fractions to test the effectiveness of various fractionation procedures, and to give an indication of sample dilution (Table 5). Examination of fractions 9 and 10 shows that ultrafiltration concentrated proteins with essentially all of the proteins remaining in the supernatant rather than being precipitated with acetone.

The value for total sugars (Table 5) is the summation of glucose, fructose and sucrose values as determined by HPLC. The amounts of these neutral, low molecular weight compounds should not be affected by ion exchange and ultrafiltration procedures. Thus their levels could serve as an index for dilution and concentration effects inherent with fractionation procedures. The high sugar content of fraction 9 shows that there was a concentration effect with its isolation. No sugars were present in the acetone precipitate as would be expected (Fraction 10). Fraction 8 contained the lowest amount of sugars indicating a dilution of ≈33%. All other fractions contained between 3.0 and 4.4 mg/mL total sugars.

Anion exchange treatment (fractions 2, 4, 6 and 8) completely removed citric and malic acids, except for fraction 6 which contained a trace amount of citric and reduced (ca. 50%) amounts of malic acid. Anion exchange treatment was also effective in removing ascorbic acid (none was detected in fraction 2, 4, 6, and 8).

The major objective of the cation exchange treatment was to remove free amino acids. The results from the formol titration (Table 5) reveal that cation exchange treatment (fractions 3, 4, 7, and 8) was not always effective in removing or reducing the amounts of free amino acids.

Anion exchange treatment reduced total phenolics considerably (from 30 to 100 percent) while cation exchange treatment had less influence. Fraction 9 was concentrated in total phenolics.

Inhibition studies. The level of enzymatic browning inhibition achieved by the various pineapple juice fractions is shown in Fig. 4. All of the pineapple juice fractions tested with crude apple extracts resulted in at least 25% inhibition as compared to the control. Fraction 3, a cation exchanged fraction of the original juice, was a significantly better inhibitor than all other fractions and resulted in 100% inhibition for at least 12 hr. Fraction 3 contained higher amounts of ascorbic acid, total phenolics and free amino acids than did fractions 2 and 4 (Table 5).

While fractions 2 and 7 were not as effective inhibitors as fraction 3, they were better inhibitors than the other fractions. Both had been treated with cation exchange resin. Fraction 7 differed from 2 and 3 in that it had undergone ultrafiltration. The compositional profile of fraction 7 differed little from fraction 5 (Table 5). Fraction 2 contained no malic, citric, or ascorbic acid as expected from anion exchange treatment. No phenolics or free amino acids were found in that sample. This finding was not anticipated for anion exchange treatment, and the results were not consistent with those for the parallel sample, fraction 6. Few conclusions could be drawn from this sample as to the nature of the inhibitor present. Fractions 2 and 3 had a characteristic pineapple aroma, which suggested the possible presence of sulfhydryl compound.

There was no significant difference in inhibitory properties between fractions 1, 4, 5, 6, 8, 9 and 10. These fractions gave between 26 and 39% inhibition of enzymatic browning. There was no significant difference between the inhibitory properties of fractions 1 and 5 which differed in that fraction 5 had high molecular weight compounds removed by ultrafiltration. Fraction 9 which concentrated the proteins, was a relatively ineffective inhibitor. Bromelain, the proteolytic enzyme in pineapple has been reported to have browning inhibitory properties (Labuza et al., 1990). Our results indicate, however, that the inhibiting factor was not high molecular weight protein material.

Ascorbic acid is known to inhibit PPO and if present would be expected to contribute to the inhibitory effects of the fraction. Fractions 6 and 8 contained no ascorbic acid but still inhibited enzymatic browning by 32 and 36%, respectively. Compounds in addition to ascorbic acid must be responsible for inhibition.

CONCLUSIONS

BOTH PJ and IEPJ may be effective inhibitors of PPO catalyzed browning. While pineapple juice itself was not as effective as bisulfite in inhibiting browning in dried apple rings, it

still may be useful where use of bisulfite is to be avoided. All fractions imparted at least 25% inhibition of PPO. Compositional analyses indicated that ascorbic acid contributed to PPO inhibition but compounds in addition to ascorbic acid must be present to account for the measured inhibition. The inhibitor(s) was not a high molecular weight protein such as bromelain. Ion exchange fractionation indicated that the inhibitor(s) is a neutral compound. Further investigation is needed to characterize and concentrate the inhibitor.

REFERENCES

Anon. 1986. Sulfiting agents; revocation of GRAS status for use on fruits and vegetables intended to be served or sold raw to consumers. Fed. Reg. 51(131): 25021.

Anon. 1987. Sulfiting agents; proposal to revoke GRAS status for use on "fresh" potatoes served or sold unpackaged and unlabeled to consumers. Fed Reg. 52(237): 46968.

AOAC. 1984. *Official Methods of Analysis,* 14th ed. Association of Official Analytical Chemists, Arlington, VA.

Balls, A.K. and Hale, W.S. 1935. Process for inhibiting the discoloration of fruits and vegetables. U.S. Patent 2,011,465.

Bennion, M. 1990. Enzymatic oxidative browning. In *Introductory Foods,* p.168. McMillan Publishing Company, New York.

Collins, J.L. 1960. *The Pineapple: Botany, Cultivation and Utilization.* Leonard Hill Publishers, London.

Dudley, E.D. and Hotchkiss, J.H. 1989. Cysteine as an inhibitor of polyphenol oxidase. J. Food Biochem. 13: 65–75.

Friedman, M. and Molnar-Perl, I. 1990. Inhibition of browning by sulfur amino acids. 1. Heated amino acid-glucose systems. J. Agric Food Chem. 38: 1642-1647.

Golan-Goldhirsh, A. and Whitaker, J.R. 1984. Relation between structure of polyphenol oxidase and prevention of browning. In *Nutritional and Toxicological Aspects of Food Safety,* (Ed.) Friedman, M. p. 437. Plenum Press, New York.

Hsu, A.F., Shien, J.J., Bills, D.D., and White, K. 1988. Inhibition of mushroom polyphenoloxidase by ascorbic acid derivatives. J. Food Sci. 53(3): 765.

Labuza, T., Lillemo, J.H. and Taoukis, P.S. 1990. Inhibition of polyphenol oxidase by proteolytic enzymes, "Killer Enzymes" XX Symposium International Federation of Fruit Juice Producers. Scientific Technical Commission. Paris.

Labuza, T. and Schmidl, M.K. 1986. Advances in the control of browning reactions in foods. In *Role of Chemistry in the Quality of Processed Food,* (Ed.) O.R. Fennema, W. Chang and C. Lii. Food and Nutrition Press Inc., Westport, CT.

Molnar-Perl, I. and Friedman, M. 1990. Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. J. Agric Food Chem. 38: 1652-1656.

Ponting, J.D. and Joslyn, M.A. 1948. Ascorbic acid oxidation and browning in apple tissue extracts. Arch. Biochem. 19: 47–63.

Richard, F.C., Goupy, M., Nicolas, J.J., Lacombe, J.M. and Pavia, A.A. 1991. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. J. Agric. Food Chem. 39: 841.

Santerre, C.R., Cash, J.N., and Vannorman D.J. 1988. Ascorbic acid/citric acid combinations in the processing of frozen apple slices. J. Food Sci. 53(6): 1713.

Sapers, G.M. and Douglas, F.W. Jr. 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. J. Food Sci. 52(5): 1258.

Sapers, G.M., El-Atawy, Y.S, Hicks, K.B., and Garzarella, L. 1989a. Effect of emulsifying agents on inhibition of enzymatic browning in apple juice by ascorbyl palmitate, laurate and decanoate. J. Food Sci. 54(4): 1096.

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., and El-Atawy, Y.S. 1989b. Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenoloxidase inhibitors, and complexing agents. J. Food Sci. 54(4): 997-1002, 1012.

Sapers, G.M. and Ziolkowski, M.A. 1987. Comparison of erythorbic and ascorbic acids as inhibitors of enzymatic browning in apple. J. Food Sci. 52(6): 1732.

Spanos, G.A. and Wrolstad, R.E. 1987. Anthocyanin pigment, nonvolatile acid, and sugar composition of red raspberry juice. J. Assoc. Off. Anal. Chem. 70(6): 1036-1046.

Spanos, G.A. and Wrolstad, R.E. 1990. Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. J. Agric. Food Chem 38: 1565-1571.

Toribio, J.L., and Lozano, J.E. 1986. Heat induced browning of clarified apple juice at high temperature. J. Food Sci. 51(1): 172.

Walker, J.R.L. 1975. Enzymic browning in foods: A review. Enzyme Technol. Dig. 4(3): 89

Ms received 8/3/92; revised 11/4/92; accepted 11/19/92.

Technical Paper No. 9962 from the Oregon State Agricultural Experiment Station. Presented as paper #309 at the 52nd Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 20-24, 1992. The research fulfilled part of the requirements for the senior author's Ph.D. degree. We thank Dole Packaged Foods Inc. for financial support and for pineapple juice samples.

 From page 398

and functional properties. Enzymatic modification was also possible at low water content to obtain the required degree of degradation of protein and carbohydrate components. Good textural and functional properties of samples modified at 30% moisture may have implications for enzymatic treatment of HPF during preconditioning for extrusion. Enzymatic pretreatment of HPF may help develop new snack products from high temperature extrusion.

REFERENCES

Aguilera, J.M., Crisafuli, E.B., Lusas, E.W., Uebersax, M.A., and Zabik, M.E. 1984. Air classification and extrusion of navy bean fractions. J. Food Sci. 49: 543.

Anderson, R.A., Conway, H.F., and Pfeifer, Y.F. 1969. Gelatinization of corn grits by roll- and extrusion-cooking. Cereal Sci. Today 14: 4.

AACC. 1983. *Approved Methods of the American Association of Cereal Chemists,* 8th ed. The Association, St. Paul, MN.

Bahnassey, Y., Khan, K., and Harrold, R. 1986. Fortification of spaghetti with edible legumes. I. Physico-chemical, antinutritional, amino acid and mineral composition. Cereal Chem. 63: 210.

Bailey, L.N., Hauck, B.W., Sevatson, E.S., and Singer, R.E. 1990. Systems for manufacture of ready-to-eat breakfast cereals using twin-screw extrusion. Cereal Foods World 36:863.

Bjorck, J. and Asp, N.G. 1983. The effects of extrusion cooking on nutritional value - a literature review. J. Food Eng. 2: 281.

Cheetham, P.S.J. 1986. The applications of enzymes in industry. In *Handbook of Enzyme Biotechnology,* p. 274, A. Wiseman (Ed.), Ellis Horwood Limited Publishers, Chichester and New York.

Conway, H.F. and Anderson, R.A. 1973. Protein fortified extruded food products. Cereal Sci. Today. 18: 94.

Franzen, K.L. and Kinsella, J.E. 1976. Functional properties of succinylated and acetylated soy protein. J. Agric. Food. Chem. 24: 799.

Fretzdorf, B. and Seiler, K. 1987. The effects of twin-screw extrusion cooking on cereal enzymes. J. Cereal Sci. 5: 13.

Gomez, M.H. and Aguilera, J.M. 1983. Changes in starch fraction during extrusion cooking of corn. J. Food Sci. 48: 378.

Gujska, E. and Khan, K. 1990. Effect of temperature on properties of extrudates from high starch fractions of navy, pinto and garbanzo beans. J. Food Sci. 55: 466.

Gujska, E. and Khan, K. 1991. Functional properties of extrudates from high starch fractions of navy and pinto beans and corn meal blended with legume high protein fraction. J. Food Sci. 56: 431.

Han, J.-Y. and Khan, K. 1990. Physicochemical studies of pin-milled and air-classified dry edible beans. Cereal Chem. 67: 384.

Hsu, H.W., Varak, D.L. Satterlee, L.D., and Miller, G.A. 1977. A mulitenzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Jeunink, J. and Cheftel, J.C. 1979. Chemical and physico-chemical changes in field bean and soybean proteins texturized by extrusion. J. Food Sci. 44: 1322.

Linko, P. 1987. Enzymes in the industrial utilization of cereals. In *Enzymes and their Role in Cereal Technology,* J.E. Kruger, (Ed.). AACC Inc., St. Paul, MN.

Miller, G.L. 1959. Use of dinitrosalicylic reagent for determination of reducing sugar. Anal. Chem. 31: 426.

SAS Institute, Inc. 1985. *SAS© Users Guide: Statistics 5th Edition.* SAS Institute Inc., Cary, NC.

Sathe, S.K. and Salunkhe, D.K. 1981. Investigation of winged bean proteins and antinutritional factors. J. Food Sci. 46: 1389.

Seyam, A.A., Banasik, O.J., and Breen, M.S. 1983. Protein isolates from navy and pinto beans: their uses in macaroni products. J. Agr. Food Chem. 31: 499.

Sgarbieri, V.C. and Whitaker, J.R. 1982. Physical, chemical and nutritional properties of common bean proteins. Adv. Food Res. 28: 93.

Skierkowski, K., Gujska, E., and Khan, K. 1990. Instrumental and sensory evaluation of textural properties of extrudates from blends of high starch/high protein fractions of dry beans. J. Food Sci. 55: 1081.

Tovar, J., Bjorck, J.M., and Asp, N.G. 1990. Analytical and nutritional implications of limited enzymatic availability of starch in cooked red kidney beans. J. Agric. Food Chem. 38: 488.

VanDer Poel, A.F.B. 1990. Effect of processing on antinutritional factors and protein nutritional value of dry beans (phaseolus vulgaris). A review. Animal Feed Sci. Tech. 29: 179.

Zamost, B.L., Nielsen, H.K., and Starnes, R.L. 1991. Thermostable enzymes for industrial applications. J. Industrial Microbiol. 8: 71.

Ms received 5/4/92; revised 11/2/92; accepted 11/7/92.

Journal Series No. 2074 of the North Dakota Agricultural Experiment Station. Financial assistance from the North Dakota Agricultural Products Utilization Commission, Bismarck, ND and CSRS Special Funding for Alternate Crops, North Dakota State University, is gratefully acknowledged.