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. DUNST

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 apple rings. 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 Schmidt, 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 Hootkiiss, 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 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 (FCOJ), 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 BioRad 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 BioRad 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...
Pineapple juice inhibition of browning in apple rings.

Table 1—Composition of dip treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Soluble solids (°Brix)</th>
<th>Ascorbic acid (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pineapple juice (PJ)</td>
<td>3.4</td>
<td>13.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Frozen concentrate PJ</td>
<td>3.6</td>
<td>12.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Ion exchange PJ</td>
<td>4.8</td>
<td>10.6</td>
<td>0</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>3.0</td>
<td>0.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Orange juice</td>
<td>3.9</td>
<td>11.8</td>
<td>5.0</td>
</tr>
<tr>
<td>EVER-FRESH</td>
<td>3.5</td>
<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Sulfite</td>
<td>4.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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 bisulfitie 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., Houston, TX) 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 bags and stored at 21°C until used.

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's 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.

% Inhibition, = (ΔL* control − ΔL* treatment)/ΔL* control × 100

The sensory panel consisted of eight untrained individuals familiar with the product who examined the rings for color at 1 and 24 hr after experimental treatments, fresh 'Red Delicious' rings for color at 1 and 24 hr after experimental treatments, 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 x g (Sorvall RCV Refigerated 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 Whamman #1 filter paper, followed by filtration through a 0.45 μm Millipore filter.

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

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

Fraction 4. Fraction 2 was passed through a Sephacryl 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 Bio-Rex-5 anion exchange column.

Fraction 7. Fraction 5 was passed through a Bio-Rex-5 anion exchange column.

Fraction 8. Fraction 7 was passed through a Bio-Rex-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 dialysis 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 redeisolated 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 Sapos and Wroclaw (1987). For sugar analyses, a Varian 5000 liquid chromatograph equipped with a column heater (Varian Instrument Group, Walnut Creek, CA) and a mass spectrometric detector (Perkin Elmer SCI-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 mg NaOH/100 mL. Total phenolics were analyzed with the Folin-Ciocalteau reagent (Sigma Chemical Co., St. Louis) using the procedure described by Sapos and Wroclaw, 1987. Inhibition. To test relative inhibition caused by each fraction, 20μL of crude extract was added to 20μL of that fraction and 2.06 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.

% Inhibition, = (A200nm control − A200nm treatment)/A200nm control × 100

Statistical analyses

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

Table 2—Experiments performed on apple rings

<table>
<thead>
<tr>
<th>Storage temp</th>
<th>Storage times</th>
<th>Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh 21°C</td>
<td>0.05, 1, 2, 8, 10, 18, 24, 48 hr</td>
<td>L*, a*, b* A420nm</td>
</tr>
<tr>
<td>Fresh vacuum-packed 1°C</td>
<td>0.1, 2, 3.4, 6 wk</td>
<td>L*, a*, b* A420nm</td>
</tr>
<tr>
<td>Dried 21°C</td>
<td>0.1, 2, 3.4 wk</td>
<td>Visual scores</td>
</tr>
</tbody>
</table>
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 content (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 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.
**PINEAPPLE JUICE INHIBITION OF BROWNING IN APPLE RINGS.**

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>8</th>
<th>10</th>
<th>18</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>PJ</td>
<td>104 a</td>
<td>95 a</td>
<td>108 b</td>
<td>72 ab</td>
<td>67 ab</td>
<td>68 c</td>
<td>67 c</td>
<td>90 bc</td>
</tr>
<tr>
<td>FCPJ</td>
<td>93 a</td>
<td>92 a</td>
<td>92 ab</td>
<td>60 a</td>
<td>43 a</td>
<td>41 b</td>
<td>95 ab</td>
<td>57 a</td>
</tr>
<tr>
<td>IEPJ</td>
<td>91 a</td>
<td>75 a</td>
<td>56 a</td>
<td>66 a</td>
<td>60 ab</td>
<td>62 c</td>
<td>59 c</td>
<td>76 b</td>
</tr>
<tr>
<td>AA</td>
<td>90 a</td>
<td>95 a</td>
<td>96 b</td>
<td>70 ab</td>
<td>50 ab</td>
<td>22 a</td>
<td>21 a</td>
<td>54 a</td>
</tr>
<tr>
<td>OJ</td>
<td>95 a</td>
<td>95 a</td>
<td>48 a</td>
<td>73 ab</td>
<td>71 a</td>
<td>77 a</td>
<td>32 a</td>
<td>52 a</td>
</tr>
<tr>
<td>EF</td>
<td>72 a</td>
<td>96 a</td>
<td>83 b</td>
<td>48 a</td>
<td>57 ab</td>
<td>47 b</td>
<td>40 ab</td>
<td>55 a</td>
</tr>
<tr>
<td>Sulfite</td>
<td>91 a</td>
<td>98 a</td>
<td>95 b</td>
<td>46 a</td>
<td>79 b</td>
<td>79 cd</td>
<td>80 cd</td>
<td>80 b</td>
</tr>
</tbody>
</table>

* * 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 (1980).** 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 sulfite 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 apple rings showed a slight change in color but were fermented, therefore measurements were 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-
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 ultrafiltration increased the amount of 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 sulphydryl compound.

There was no significant difference in inhibitory properties between fractions 1, 3, 4, 5, 6, 8, 9 and 10. These fractions gave between 26 and 35% 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 (Labuzza 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

---

**Table 5—Composition of pineapple juice fractions**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg/mL)</th>
<th>Formol value (meq/100mL)</th>
<th>Total sugars (mg/mL)</th>
<th>Citric acid (mg/mL)</th>
<th>Malic acid (mg/mL)</th>
<th>Ascorbic acid (mg/mL)</th>
<th>Phenolics (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.1</td>
<td>1.27</td>
<td>3.3</td>
<td>2.6</td>
<td>0.7</td>
<td>0.1</td>
<td>0.59</td>
</tr>
<tr>
<td>2</td>
<td>13.0</td>
<td>0.00</td>
<td>4.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>16.2</td>
<td>0.64</td>
<td>3.0</td>
<td>2.2</td>
<td>0.6</td>
<td>0.1</td>
<td>0.41</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
<td>0.00</td>
<td>4.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>15.8</td>
<td>1.49</td>
<td>1.9</td>
<td>2.4</td>
<td>0.6</td>
<td>0.1</td>
<td>0.50</td>
</tr>
<tr>
<td>6</td>
<td>21.6</td>
<td>1.32</td>
<td>4.1</td>
<td>0.1</td>
<td>0.4</td>
<td>0.0</td>
<td>0.39</td>
</tr>
<tr>
<td>7</td>
<td>20.2</td>
<td>1.38</td>
<td>3.6</td>
<td>3.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.48</td>
</tr>
<tr>
<td>8</td>
<td>12.1</td>
<td>0.50</td>
<td>2.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.07</td>
</tr>
<tr>
<td>9</td>
<td>46.3</td>
<td>2.50</td>
<td>5.4</td>
<td>4.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.30</td>
</tr>
<tr>
<td>10</td>
<td>0.6</td>
<td>0.00</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

---

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

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.
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 acidic amino acids contributed to PPO inhibition but compounds in addition to acidic amino acid must be present to account for the measured inhibition. The inhibitor was not a high molecular weight protein such as bromelain. Ion exchange fractionation indicated that the inhibitor is a neutral compound. Further investigation is needed to characterize and concentrate the inhibitor.

REFERENCES


EXTRUSION OF PINTO BEAN HIGH PROTEIN FRACTION... 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 50% 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

Bjorck, J. and Asp, N.G. 1983. The effects of extrusion cooking on nutri-