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Effect of Stem Bromelain on the Browning of Apple Juice

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Abstract: The effectiveness of pineapple stem protease (bromelain) in enzymatic browning inhibition was evaluated on apple juice and compared with that of L-cysteine and ascorbic acid at 25±1°C. The relative effectiveness of these anti-browning agents was determined in terms of color (L*) and enzymatic activity measurements with respect to time. L-cysteine at 0.7 and 1.0 mM concentrations gave the best results though the latter is associated with undesirable odor and bleaching effect. Ascorbic acid seemed to be only effective within the first 5 h after which its effectiveness dropped sharply. Stem bromelain, as compared to both L-cysteine and ascorbic acid, was found weakest in enzymatic browning inhibition hence considered ineffective.

Key words: Pineapple stem bromelain, enzymatic browning, polyphenol oxidases, L-cysteine, ascorbic acid, apple juice, browning inhibition, protease enzyme

INTRODUCTION

Enzymatic browning occurs in fruits and vegetables upon bruising during handling or transportation and when exposed to air in cut, sliced or pulped states (Labuza and Schmidl, 1986). Basically, enzymatic browning can be defined as the initial enzymatic oxidation of phenols into slightly colored quinones (Nicolas *et al.*, 1994). These quinones are then subjected to further reactions, enzymatically catalyzed or not leading to the formation of pigments (Özoğlu and Bayindirli, 2002; Wen and Wrolstad, 2001). Polyphenol oxidases (PPO) which are able to act on phenols in the presence of oxygen have been associated with enzymatic browning (Vámos-Vigyázó, 1981; Sapers and Miller, 1992). Nicolas *et al.* (1994) has classified two kinds of enzymes; Catechol oxidases (E.C.1.10.3.1) and Laccases (E.C.1.10.3.2) under this trivial name. Catechol oxidases catalyzes two distinct reactions: the hydroxylation of monophenols into o-diphenols and the oxidation of o-diphenols into o-quinones (Wen and Wrolstad, 2001). These two reactions use oxygen. Laccases oxidizes o-diphenols as well as p-diphenols and is not common in fruits, although it has been reported to be present in peach and apricot (Walker, 1995).

Enzymatic and nonenzymatic browning reactions may adversely affect the quality, nutritional value and safety of foods (Molnar-Perl and Friedman, 1990; Tan and Harris, 1995; Friedman, 1996; Rigal *et al.*, 2001; Laurila and Ahvenainen, 2002; Billaud *et al.*, 2003) and just washing with water is not effective in preventing discoloration (Willey, 1994; Mattila *et al.*, 1995). Friedman (1996) has identified reactions of amines, amino acids, peptides and proteins with reducing sugars and vitamin C (nonenzymatic browning) and quinones (enzymatic browning) to cause deterioration of foods during storage and commercial or domestic processing. The control of browning in order to maintain their quality, nutritional value and safety is of great importance just at the start of their processing procedures.

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One approach to the prevention of enzymatic browning in fruit juices has been the use of anti-browning agents such as sulphites. However, due to health concerns, sulphites use has been restricted (Anon, 1991). On the other hand, due to consumer's demand for natural food additives, studies have been devoted to the search for natural inhibitors of enzymatic browning (Naimiki, 1990; Nicolas *et al.*, 1994). Laurila and Ahvenainen (2002) have noted that the most attractive way to inhibit browning would be by natural methods. Some of the natural agents that have been proposed to have inhibitory effect on PPO include honey (Oszmianski and Lee, 1990), natural aliphatic alcohols (Valero *et al.*, 1990) cysteine (Kahn, 1985) and Millard Reaction Products (MRP) synthesized from glucose and lysine (Pitotti *et al.*, 1990; Nicoli *et al.*, 1991) and pineapple fruit protease-bromelain (Labuza *et al.*, 1990; Lozano-de-Gonzales *et al.*, 1993; Meza *et al.*, 1995; Wen and Wrolstad, 2001). Many inhibitors of PPO are known, but only a few have been considered as potential alternatives to sulphites (Vámos-Vigyázó, 1981). Lozano-de-Gonzales *et al.* (1993), Meza *et al.* (1995) and Wen and Wrolstad (2001) demonstrated pineapple juice as an effective enzymatic browning inhibitor in fresh apple slices. According to Wen and Wrolstad (2001), the inhibitory effect of fresh pineapple juice sub-type was inherent in a high molecular weight fraction and a protease enzyme (fruit bromelain), added ascorbic acid in the canned sub-type while in the concentrate sub-type, a polar organic acid (neither ascorbic, citric, malic nor oxalic acid).

To our knowledge, there are no published reports on the effectiveness of pineapple stem protease (bromelain) as an inhibitor of enzymatic browning in apple fruit juice. Available publications have zeroed in the use of PPO or fruit slices. The objectives of this study were to assess the effectiveness of pineapple stem bromelain as an enzymatic browning inhibitor and compare its efficiency to widely used anti-browning agents such as L-cysteine and ascorbic acid on apple juice.

MATERIALS AND METHODS

Golden delicious apples were obtained from the local fruit store in Wuxi, Jiangsu Province of People's Republic of China and stored at 4°C before use during the spring of 2008. The anti-browning agents, Ascorbic Acid (AA), L-Cysteine (LC) (Sinopharm chemical reagents Co.) and Stem Bromelain (SB) (Sigma) were evaluated.

Apples were peeled, de-stoned and juiced using Warring blender No. DS-1(Shanghai model company-China). The 20 cm³ of juice samples were transferred into beakers containing anti-browning agents and stirred with a magnetic stirrer for 10 sec (Özoğlu and Bayindirli, 2002). The concentration of the anti-browning agents in the runs were 0.3, 0.7 and 1 mM for AA and LC, while concentrations of 0.175, 0.350 and 0.700 g L⁻¹ were chosen for SB. As a control, 20 cm³ of the juice sample was treated and measured the same way but without anti-browning agents (Taukis *et al.*, 1990).

The first color measurements L* (lightness), a* (red to green) and b* (yellow to blue) dimension values of the juice samples were done using a color difference meter (WSC-S, Shanghai Model Company-China) 60 sec after juicing and repeated at different time intervals at a temperature of 25±1°C. The instrument was calibrated using the standard white reflector plate (L = 91.32, a = 0.03, b = 18.62). All experimental runs were in triplicates. Equation 1, was used to estimate % inhibition based on L* values (where, ΔL was defined as the difference in L measurement at time zero-t₀ (defined as any time between 0 and 60 sec) and corresponding value at time-t (defined as subsequent times when measurements were done). Normalised L (Table 1) was calculated as per Eq. 2, with L₀ being an initial L measurement (Özoğlu and Bayindirli, 2002).

$$\text{Inhibition (\%)} = (\Delta L_{\text{control}} - \Delta L_{\text{treatment}}) \times 100 / \Delta L_{\text{control}} \quad (1)$$

$$\text{Normalised L} = L / L_0 \quad (2)$$

Table 1: Normalised L values for different treatments at different concentrations over time

Treatments	L/L ₀ at various times (h)					
	0.5	1.0	3.0	5.0	7.0	10.0
0.3 mM AA	0.932	0.847	0.765	0.733	0.634	0.588
0.7 mM AA	0.953	0.916	0.832	0.746	0.669	0.609
1.0 mM AA	0.977	0.954	0.925	0.911	0.822	0.761
0.3 mM LC	0.943	0.888	0.806	0.775	0.730	0.674
0.7 mM LC	0.996	0.992	0.981	0.975	0.964	0.951
1.0 mM LC	1.012	1.025	1.034	1.038	1.025	1.000
0.175 g L ⁻¹ SB	0.931	0.860	0.772	0.729	0.644	0.604
0.350 g L ⁻¹ SB	0.928	0.865	0.776	0.728	0.669	0.615
0.700 g L ⁻¹ SB	0.929	0.863	0.772	0.737	0.664	0.604
Blank	0.864	0.746	0.627	0.576	0.491	0.457

Apple juice at 25°C±1

Polyphenol Oxidase Activity

Apple extract was prepared using the method of Lozano-De-Gonzalez *et al.* (1993) with a slight modification. Briefly, 100 g apple flesh was homogenized with 100 mL cold sodium phosphate buffer, pH 6.5, filtered through 2 layers of cheesecloth, then centrifuged (10,000 g) (CL-20B-Shanghai Technical Apparatus Factory, China) at 4°C for 20 min. The supernatant was used as a source of PPO. The anti-browning agents used for PPO activity were prepared in 0.1 M citric buffer (pH 4.5) in the concentrations previously mentioned.

Inhibition of PPO Extract

To test relative inhibition, 20 µL of the crude apple extract was added to 20 µL of anti-browning agents (test fraction) and 2.96 mL of 0.2 M Catechol (Sinopharm Chemical Reagent Co.), substrate solution in McIlvaine's buffer solution (McIlvaine, 1921) pH 6.5 at 25±1°C. The change in absorbance at 420 nm (UV-spectrophotometer model WFZUV-2102C/PC/PCS, Unico-Shanghai) was recorded for 1 min. As a control, 20 µL of the extract was added to 2.96 mL of the substrate solution to which 20 µL of 0.1 M of citric buffer (pH 4.5) had been added. One unit of enzyme activity was defined as the quantity of enzyme responsible for change in absorbance of 0.001 min⁻¹ under the assay conditions. The percent inhibition was calculated using the polyphenol oxidase activity values from the control and each test fraction, defining the control as zero percent inhibition (Eq. 3).

$$\text{Inhibition (\%)} = \frac{\Delta_{A420 \text{ nm}} \text{ Control} - \Delta_{A420 \text{ nm}} \text{ Treatment}}{\Delta_{A420 \text{ nm}} \text{ Control}} \times 100 \quad (3)$$

Statistical Analysis

The SPSS (Chicago, IL) statistical analysis system was used for analysis of the data. The statistical significance difference was assessed by one-way analysis of variance. Significant differences (p<0.05) among treatments were detected using Duncan's multiple range test.

RESULTS

The effectiveness of pineapple stem protease (bromelain) as an enzymatic browning inhibitor in apple juice was evaluated and compared with that of two other commonly used inhibitors (LC and AA). The results (Fig. 1) indicated that L* values of apple juice for all treatments except 1 mM LC decreased over time. At 1 mM concentration, LC showed inhibition extent to exceed 100% throughout the experimental period. At slightly lower concentration (0.7 mM), LC was still good enough to inhibit enzymatic browning up to 90% level during the 10 h study period. In this study, the use of 0.3 mM

LC concentration showed an average inhibition of 40% after 10 h of treatment (Table 2). Compared to percentage of average inhibition estimates (107, 94.5 and 49.3%) corresponding to 1, 0.7 and 0.3 mM LC, respectively from changes in L* values, inhibition (%) estimates (70, 67 and 53%) corresponding to the same LC concentrations from changes in absorbance at 420 nM were much lower except at 0.3 mM level which was higher in the latter. This trend was observed with all the three anti-browning agents (Table 3).

Ascorbic acid on the other hand was found to be more effective an inhibitor in all concentrations used except 0.3 mM, than SB. At 1 mM concentration, AA showed better results than 0.3 mM LC, with a 15 % point high (Fig. 1). Stem bromelain when compared with both AA and LC at the studied concentrations (0.175, 0.350 and 0.700 g L⁻¹) was far less effective in inhibiting browning in apple juice (Fig. 1). The first measurements of inhibition by bromelain at 0.5 h gave percent inhibition of 49, 47 and 48% corresponding to 0.175, 0.350 and 0.700 g L⁻¹ SB concentrations, respectively. Over, the 10 h experimental period, these percentages dipped to 27, 29 and 27% (Table 4). Statistical analysis of the L* values measured among the different bromelain concentrations by one-way analysis of variance, were insignificant (p = 0.05) as detected using Duncan's multiple range test. An examination

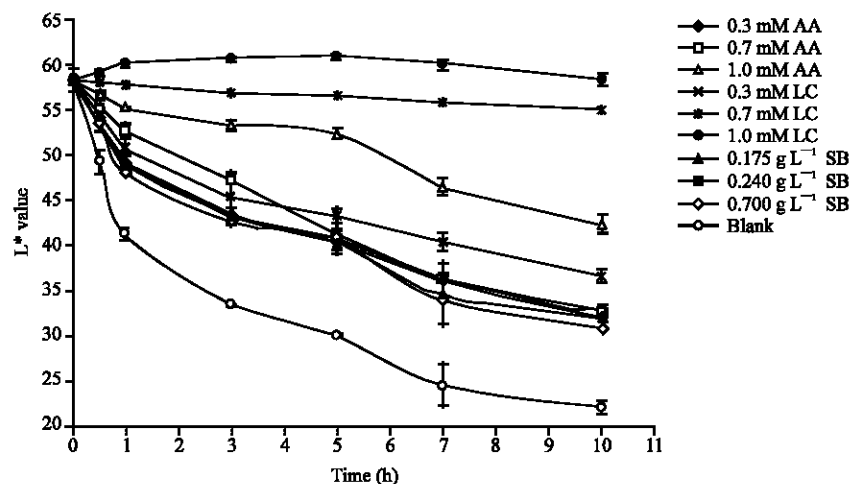


Fig. 1: The trends in L* values for apple juice treated with anti-browning agents at various concentrations

Table 2: Estimates of inhibition (%) in browning of apple juice by different LC concentrations

Concentrations (mM)	Time (h)					
	0.5	1	3	5	7	10
0.3	58±0.5	56±0.3	48±1.4	47±0.4	47±0.3	40±0.9
0.7	97±1.0	97±0.5	95±0.4	94±0.2	93±0.6	91±0.4
0.1	109±1.0	110±1.0	109±0.5	109±0.9	105±0.3	100±0.5

Table 3: Inhibition (%) of the PPO extract

Agents	Concentration levels		
	A	B	C
Ascorbic acid	52±0.4	63±0.6	65±0.5
L-cysteine	53±0.4	67±0.7	70±1.0
Stem bromelain	32±0.3	34±1.0	35±0.9

A: 0.3 mM (ascorbic acid and L-cysteine), 0.175 g L⁻¹ (stem bromelain). B: 0.7 mM (ascorbic acid and L-cysteine), 0.350 g L⁻¹ (stem bromelain). C: 1.0 mM (ascorbic acid and L-cysteine), 0.700 g L⁻¹ (stem bromelain)

Table 4: Inhibition (%) in browning of apple juice by pineapple stem protease (bromelain) at various concentrations

Concentration (g L ⁻¹)	Time (h)					
	0.5	1	3	5	7	10
0.175	49±1.0	45±0.2	39±1.5	36±0.4	30±1.0	27±0.3
0.350	47±0.5	47±0.6	40±1.0	36±1.8	35±0.3	29±0.9
0.700	48±1.5	46±0.7	39±0.4	38±0.5	34±0.5	27±0.5

of normalised L values (Table 1) associated with bromelain treatments, also show a close association. This is also evident from Fig. 1 where, the graphs for the different bromelain concentration treatments show somewhat kind of cluster.

DISCUSSION

The inhibition of browning to beyond 100% by LC is a well recognized phenomenon in browning inhibition studies (Sapers and Douglas, 1987) and may be attributed to the standard errors and/or bleaching effect associated with the treatment (Özoğlu and Bayindirli, 2002). At slightly lower concentration (0.7 mM), LC was still good enough to inhibit enzymatic browning up to 90% level during the 10 h study period. These results are compatible with those reported by Molnar-Perl and Friedman (1990) where 0.568 mM concentration effectively arrested browning to extent of 83% after 24 h of treatment. Although, LC appears to be more effective an inhibitor at 1 mM, such high concentration has been reported to produce undesirable odour and a bleaching effect (Özoğlu and Bayindirli, 2002; Molnar-Perl and Friedman, 1990). We ascribe these differences to either experimental errors and/or instrumental differences. The mechanism for the inhibition of enzymatic browning by LC has been demonstrated. The o-quinone, which is an oxidation product of PPO, reacts with LC non-enzymatically to form colorless conjugates (Nicolas *et al.*, 1994; Sapers, 1993; Richard-Forget *et al.*, 1992). Sanada *et al.* (1972) isolated one conjugate between cysteine and catechol and Richard *et al.* (1991) found at least two conjugates between cysteine and phenolic compounds such as 4-methylcatechol, chlorogenic acid, -epicatechin and -catechin. Also, Ito and Prota (1977) obtained four cysteine adducts from DOPA quinones. Kahn (1985) and Veleró *et al.* (1991) suggested the formation of a stable complex with copper, thus retarding enzymatic browning.

The action of AA in the prevention of enzymatic browning is to reduce the intermediate o-quinones to the original phenolic compounds before they can undergo further reaction to form pigments (Wen and Wrolstad, 2001). It may also reduce Cu²⁺ to Cu⁺ in the PPO thus retarding enzymatic browning (Tan and Harris, 1995). However, the effectiveness of AA as an anti-browning agent is temporary (Komthong *et al.*, 2007) as it can be easily oxidized by endogenous enzymes, as well as decomposed by iron or copper-catalyzed auto oxidation (McEvily *et al.*, 1992; Özoğlu and Bayindirli, 2002). The enzymatic browning can therefore regenerate after the AA has been completely reduced to dehydroascorbic acid.

We have shown in the results that bromelain had only minimal inhibition effect on PPO which is responsible for browning in apple juice and that effect is not concentration dependent. Rigal *et al.* (2001) compared protease enzyme extracts (papaya latex, actinidin, bromelain and ficin) on their efficiency in PPO inactivation. They observed that actinidin and ficin had no significant inactivation effect on endive PPO. Bromelain according to their results, showed slight inactivation in PPO activity, 10 fold lower than that associated with papaya latex crude extract. Taoukis *et al.* (1990) reported that bromelain did not inhibit melanosis in shrimp at 4°C and Labuza *et al.* (1990) also reported lack of bromelain to inhibit mushroom PPO activity in an aqueous model system, though it was effective in the browning inhibition of refrigerated apple slices. Recently, Chaisakdanugull *et al.* (2007) using pineapple juice fractions containing different bromelain activities on banana PPO showed the fraction with the highest enzyme activity was least in PPO inhibition. In conclusion, present

results have shown that LC is a more effective browning inhibitor as compared to either AA or SB. 0.7 mM LC was found effective to inhibit browning to slightly over 90% which was quite higher than that achieved by 1 mM AA. The inhibitory effect ascribed to SB was very minimal. This suggests that the results reported about pineapple juice effectiveness in anti-browning may not be inherent in bromelain.

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REFERENCES

- Anon, 1991. Sulphites banned. *Food Ingredients Process. Int.*, 11: 11-11.
- Billaud, C., E. Roux, Brun-Me'rimee, S. Maraschin and C.J. Nicolas, 2003. Inhibitory effect of unheated and heated d-glucose, d-fructose and l-cysteine solutions and Millard reaction product model systems on polyphenol oxidase from apple. I. Enzymatic browning and enzyme activity inhibition using Spectrophotometric and polarographic methods. *Food Chem.*, 81: 35-50.
- Chaisakdanugull, C., C. Theerakulkait and R.E. Wrolstad, 2007. Pineapple juice and its fractions in enzymatic browning inhibition of banana [Musa (AAA Group) Gros Michel]. *J. Agric. Food Chem.*, 55: 4252-4257.
- Friedman, M., 1996. Food browning and its prevention: An overview. *J. Agric. Food Chem.*, 44: 631-653.
- Ito, S. and G. Prota, 1977. A facile one-step synthesis of cysteinyl dopas using mushroom tyrosinase. *Cell. Mol. Life Sci.*, 33: 1118-1119.
- Kahn, V., 1985. Effect of protein, protein hydrolyzate and amino acids on dihydroxyphenolase activity of polyphenol oxidase of mushroom, avocado and banana. *J. Food Sci.*, 50: 111-115.
- Komthong, P., N. Igura and M. Shimoda, 2007. Effect of ascorbic acid on the odours of cloudy apple juice. *Food Chem.*, 100: 1342-1349.
- Labuza, T. and M.K. Schmidl, 1986. Advances in the Control of Browning Reactions in Foods. In: *Role of Chemistry in the Quality of Processed Food*, Fennema, O.R., W. Chang and C. Lii (Eds.). Food and Nutrition Press Inc., Westport, pp: 65-94.
- Labuza, T.P., J.H. Lillemo and P.S. Taoukis, 1990. Inhibition of polyphenol oxidase by proteolytic enzymes Killer Enzymes. *Proceedings of the Symposium on the 20th International Symposium International Federation of Fruit Juice Producers, 1990, Paris*, pp: 1-15.
- Laurila, E. and R. Ahvenainen, 2002. Minimal Processing of Fresh Fruits and Vegetables. In: *Fruits and Vegetable Processing -Improving Quality*, Wim, J. (Ed.). CRC Press, Washington, DC., pp: 1-22.
- Lozano-de-Gonzales, P.G., D.M. Barrett, R.E. Wrolstad and R.W. Durst, 1993. Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *J. Food Sci.*, 58: 399-404.
- Mattila, M., R. Ahvenainen and E. Hurme, 1995. Prevention of browning of pre-peeled Potato. *Proceedings of Workshop on Systems and Operations for Post-Harvest Quality, COST 94 Post-Harvest Treatment of Fruit and Vegetables, 1995, Belgium*, pp: 225-234.
- McEvily, A.J., R. Iyengar and W.S. Otwell, 1992. Inhibition of enzymatic browning in foods and beverages. *Crit. Rev. Food Sci.*, 32: 253-273.
- McIlvaine, T.C., 1921. A buffer solution for colorimetric comparison. *J. Biol. Chem.*, 49: 183-186.
- Meza, J., P. Lozano-de-Gonzales, A. Anzaldúa-Morales, J.V. Torres and J. Jiménez, 1995. Addition of pineapple juice for the prevention of discoloration and textural changes of apple slices. *Institute of Food Technologists Annual Meeting, Book of Abstracts*.

- Molnar-Perl, I. and M. Friedman, 1990. Inhibition of browning by sulphur Amino Acids. 2. Fruit juices and protein containing foods. *J. Agric. Food Chem.*, 38: 1648-1651.
- Naimiki, M., 1990. Antioxidants/antimutagens in food. *CRC Critical Rev. Food Sci. Nutr.*, 29: 273-300.
- Nicoli, M.C., B.E. Elizalde, A. Pitotti and C.R. Lericci, 1991. Effect of sugar and Millard reaction products on polyphenol oxidase and peroxidase activity in food. *J. Food Biochem.*, 15: 169-184.
- Nicolas, J.J., F.C. Richard-Forget, P.M. Goupy, M.J. Amiot and S.Y. Aubert, 1994. Enzymatic browning reactions in apple and products. *Crit. Rev. Food Sci. Nutr.*, 34: 109-157.
- Oszmianski, J. and C.Y. Lee, 1990. Inhibition of polyphenol oxidase activity and browning by honey. *J. Agric. Food Chem.*, 38: 1892-1895.
- Özoğlu, H. and A. Bayindirli, 2002. Inhibition of enzymic browning in cloudy apple juice with selected antibrowning agents. *Food Contr.*, 13: 213-221.
- Pitotti, A., C. Nicoli, A. Sensidon and C.R. Lericci, 1990. Control of Enzymatic Browning in Food: Effects of Some Substances and their Mechanism Action in Engineering and Food. In: *Physical Properties and Process Control*, Spiess, W.E.L. and H. Schuberts (Eds.). Elsevier Sciences Publisher, London, UK.
- Richard, F.C., P.M. Goupy, J.J. Nicolas, J.M. Lacombe and A.A. Pavia, 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: 841-847.
- Richard-Forget, F.C., M.A. Rouet-Mayer, P.M. Goupy, J. Philippon and J.J. Nicolas, 1992. Oxidation of chlorogenic acid, catechins and 4-methylcatechol in model solutions by apple polyphenol oxidase. *J. Agric. Food Chem.*, 40: 2114-2122.
- Rigal, D., M. Cerny, F. Richard-Forget and P. Varoquaux, 2001. Inhibition of endive (*Cichorium endivia* L.) polyphenoloxidase by a *Carica papaya* latex preparation. *Int. J. Food Sci. Technol.*, 36: 677-684.
- Sanada, H., R. Suzue, Y. Nakashima and S. Kawada, 1972. Effect of thiol compounds on melanin formation. *Acta Biochem. Biophys. Sin.*, 261: 258-266.
- Sapers, G.M. and F.W. Douglas Jr., 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *J. Food Sci.*, 52: 1258-1262.
- Sapers, G.M. and R.L. Miller, 1992. Enzymatic browning control in potato with ascorbic acid-2-phosphates. *J. Food Sci.*, 57: 1132-1135.
- Sapers, G.M., 1993. Browning of foods: Control by sulfites as antioxidants and other means. *Food Technol. Chicago*, 47: 75-84.
- Tan, B.K. and N.D. Harris, 1995. Millard reaction products inhibit apple Polyphenoloxidase. *Food Chem.*, 53: 267-273.
- Taoukis, P.S., T.P. Labuza, J.H. Lillemo and S.W. Lin, 1990. Inhibition of shrimp melanosis (black spot) by ficin. *Lebensmittel-Wissenschaft und-Technologie*, 23: 52-54.
- Valero, E., R. Varon and F. Gracia-Carmona, 1990. Inhibition of grape polyphenol oxidase by several aliphatic alcohols. *J. Agric. Food Chem.*, 38: 1097-1100.
- Valero, E., R. Varon and F. Garcia-Carmona, 1991. A kinetic study of irreversible enzyme inhibition by an inhibitor that is rendered unstable by enzymatic catalysis. The inhibition of polyphenol oxidase by L-cysteine. *Biochem. J.*, 277: 869-874.
- Vámos-Vigyázó, L., 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. *Crit. Rev. Food Sci.*, 15: 49-127.

- Walker, J.R.L., 1995. Enzymatic Browning in Fruits: Its Biochemistry and Control. In: Enzymatic Browning and its Prevention, Lee, C.Y. and J.R. Whitaker (Eds.). American Chemical Society, Washington, DC., pp: 8-22.
- Wen, L. and R.E. Wrolstad, 2001. Pineapple juice: Phenolic composition and enzymatic browning inhibition. Ph.D Thesis, Oregon State University.
- Willey, R.C., 1994. Minimally Processed Refrigerated Fruits and Vegetables. Chapman and Hall, New York.