ELSEVIER

Food Chemistry 53 (1995) 267–273
Elsevier Science Limited
Printed in Great Britain.
0308-8146/95/$9.50

Maillard reaction products inhibit apple polyphenoloxidase

B. K. Tan & N. D. Harris*

Department of Nutrition, Food and Movement Science, Florida State University, Tallahassee, FL 32306, USA

(Received 18 January 1994; accepted 23 August 1994)

The effects of Maillard reaction products (MRP) on apple polyphenol oxidase (PPO) were evaluated spectrophotometrically at 400 nm after oxidation of 4-methylcatechol. MRP, synthesized under different conditions including heating time, type of amino acids, pH and concentration of both amino acid and glucose, showed different effects on apple PPO. The inhibitory effects of MRP, heated for 7 h at 90°C and synthesized from various amino acids with glucose, were as follows: arginine = histidine > cysteine > lysine. The MRP synthesized from glutamic acid or valine enhanced rather than inhibited PPO activity. Increasing the heating time of histidine–glucose solutions also enhanced the inhibitory effect. However, a slight decrease in the inhibitory effect was observed in the cysteine–glucose solutions after 3 h of heating. The MRP synthesized at low pH levels was a more effective inhibitor than the MRP synthesized at higher pH levels.

INTRODUCTION

The storage life of fresh fruits and vegetables may be limited by the onset of enzymatic browning during post-harvest handling and processing. The consequences of enzymatic browning are discoloration, off-flavors and nutritional damage. The browning of fruits and vegetables is a result of the oxidation catalyzed by polyphenol oxidase (PPO) of phenolic compounds to quinones and their subsequent condensation to colored pigments (Vamos-Vigyazo, 1981; Sapers & Miller, 1992). Two types of oxidative reactions that are catalyzed by PPO are the hydroxylation of monophenols to *o*-diphenols due to cresolase activity and the oxidation of *o*-diphenols due to catecholase activity.

PPO is an enzyme widely distributed in nature, and it is responsible for most of the browning reactions and discoloration in fresh fruits and vegetables. The development of new anti-browning agents has become crucial in order to preserve or minimize the loss of fresh fruits and vegetables, since the use of sulfur dioxide (SO_2), an effective browning inhibitor, has been restricted by the Food and Drug Administration because of its role in the initiation of reactions in asthmatic patients (Vamos-Vigyazo, 1981; Anon., 1986, 1987; Sapers *et al.*, 1989).

After the ban of sulfite, numerous compounds have been reported to be capable of reducing enzymatic browning. Among these compounds were 4-hexylresorcinol (Monsalve-Gonzalez *et al.*, 1993) aromatic acids (Kermasha *et al.*, 1993) and halide ions (Janovitz-Klapp *et al.*, 1990). Due to the consumer's demand for natural food additives, a few studies have been devoted to the search for natural inhibitors of enzymatic browning (Namiki, 1990; Nicolas *et al.*, 1994). Some of the natural agents that were proposed to have had the inhibitory effect on PPO were honey (Oszmianski & Lee, 1990), natural aliphatic alcohols (Valero *et al.*, 1990), cysteine (Kahn, 1985) and Maillard reaction products (MRP) synthesized from glucose and lysine (Pitotti *et al.*, 1990; Nicoli *et al.*, 1991). The inhibitory effects of honey on apple slices and grape juice were attributed to the small peptides with the molecular weight of 600 (Oszmianski & Lee, 1990). Kahn (1985) suggested that the amino acid cysteine can form a stable complex with copper, thus retarding enzymatic browning. Valero *et al.* (1990) found that the hydrophobic chains of aliphatic alcohols have a direct effect on grape PPO as well as its substrates, thereby inhibiting the browning.

Recently, Italian workers found that MRP synthesized from glycine and glucose inhibited PPO in Golden Delicious apples (Pitotti *et al.*, 1990; Nicoli *et al.*, 1991). There are several properties of the MRP structure that make it a potential inhibitor. The key

*To whom correspondence should be addressed.

intermediate of the early stage of the Maillard reaction is the Amadori rearrangement product, which is a type of amino reductone that has chelating, reducing and oxygen-scavenging properties. The reductone content in MRP has chelating effects on Fe, Zn, and Cu in humans (Johnson *et al.*, 1983). A common reductone, ascorbic acid, retards enzymatic browning by reducing CU^{2+} to CU^{+} in the PPO. Ascorbic acid is also capable of converting the quinone back to the diphenol and thus preventing browning (Eskin, 1990). Namiki (1988) reported that amino reductones such as Amadori compounds have better antioxidative activities than ascorbic acid or triose reductones. Thus, it is reasonable to expect that MRPs may inhibit enzymatic browning.

The objective of this study was to investigate the inhibitory effect of MRP synthesized from various amino acids and glucose solutions on PPO activity. The factors, including pH, heating time, type of amino acid and concentration of both amino acid and glucose, were examined for their effectiveness in producing MRP that inhibited the oxidation of 4-methylcatechol.

MATERIALS AND METHODS

Enzyme extraction

PPO was extracted from fresh Red Delicious apples purchased from a local supermarket. The apples were washed, cored and peeled. The preparation of crude PPO was carried out by using the methodology described by Galeazzi *et al.*, (1981) and Pitotti *et al.*, (1990). Apples were blended in a 1:1 ratio with 0·5 M phosphate buffer at pH 7 in the presence of 5% polyvinylpyrrolidone. The crude juice was squeezed though four layers of cheese cloth and the juice containing the enzyme was centrifuged for 30 min at 12000 *g*. The samples were divided into small portions and frozen at −18°C. The frozen samples were used within 4 h of thawing.

Synthesis of MRP/inhibitors

MRP of different amino acids were obtained by heating equal volumes of 1·5 M amino acid solution and 1·5 M glucose solution at 90°C for 7 h. The amino acids used were L-arginine, L-histidine, L-cysteine, L-glutamic acid, L-lysine, L-valine and D(+)-glucose (Sigma Chemical Co., St Louis, MO, USA). The reacted mixture of valine–glucose, cysteine–glucose and histidine–glucose solutions contained precipitates. Only the soluble fraction of the reaction mixtures were used in all studies.

The MRP synthesized from arginine, cysteine, histidine and lysine with glucose showed a strong inhibitory effect on PPO. Therefore, the browning intensity of the above MRPs were studied. The browning intensity of samples was determined by measuring the absorbance at 420 nm with a UV-Vis Perkin–Elmer Lambda 3 spectrophotometer after 1000-fold dilution with distilled water.

The MRP synthesized by the heating for different time periods was obtained by heating an equal volume 1·5 M of cysteine or histidine and 1·5 M of glucose solutions at 90°C up to 6 h. The MRP synthesized from various concentrations of amino acids was obtained by heating a mixture of 0·5, 1·0 or 1·5 M of cysteine solution with a 0·5 M of glucose solution at 90°C for 2 h. The MRP containing various concentrations of glucose was obtained by heating a mixture of 0·5, 1·0 or 1·5 M of glucose with 0·5 M of cysteine solution at 90°C for 2 h.

The MRP synthesized at various pH levels was obtained by adjusting the pH value of a mixture of 1·5 M cysteine and glucose solutions to pH levels of 3·0, 5·0, 7·0, 9·0 and 11·0 using 0·1 M H_3PO_4 or 0·1 M NaOH. All of the solutions were heated for 2 h at 90°C.

Substrate reactivity

The reactivity of PPO toward catechol, 4-methylcatechol and L-dopa was examined under each substrate's optimum pH and wavelength as reported by Zhou *et al.* (1993) and Nicolas *et al.* (1994). The most reactive substrate was 4-methylcatechol; thus, it was used for the remainder of the studies.

PPO activity

The PPO activity was assayed spectrophotometrically according to Pitotti *et al.* (1990), Nicoli *et al.* (1991) and Zhou *et al.* (1993). The reaction was initiated by

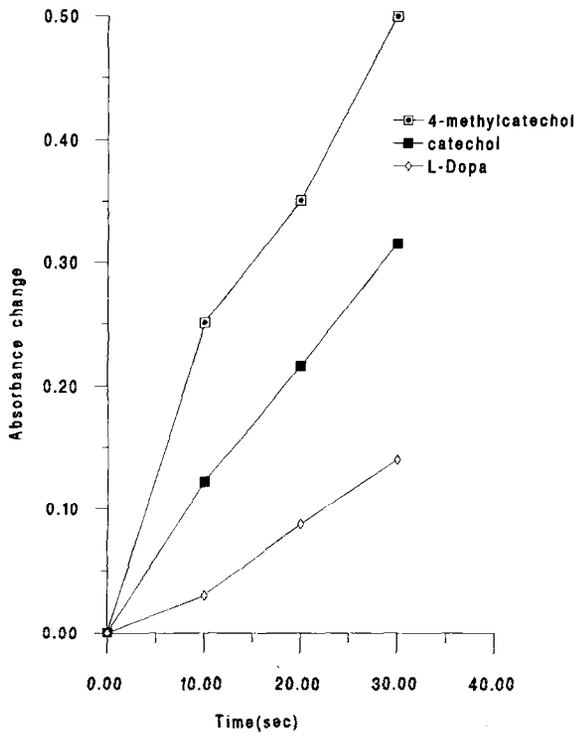

Fig. 1. The reactivity of PPO toward 0·001 M of catechol, 4-methylcatechol and L-dopa prepared in a buffer of 0·1 M citric and 0·2 M-phosphate. The PPO activity of catechol and 4-methycatechol were assayed at 400 nm, while L-dopa was assayed at 480 nm. The optimum pH for catechol, 4-methylcatechol and L-dopa were pH 5, pH 4·6 and pH 4, respectively.

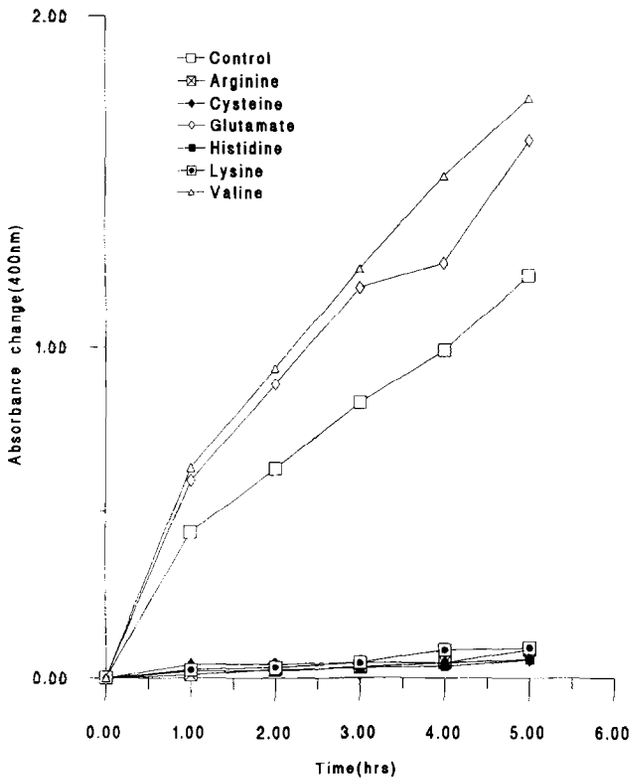

Fig. 2. The effect of 500 μl MRP synthesized from various amino acids (1·5 M) with constant amount of glucose (1·5 M) on apple PPO. The MRP solutions were heated for 7 h at 90°C. The oxidation rate of 0·001 M 4-methylcatechol was assayed at 400 nm spectrophotometrically. Readings were taken on solutions held at 25°C at 1 h intervals.

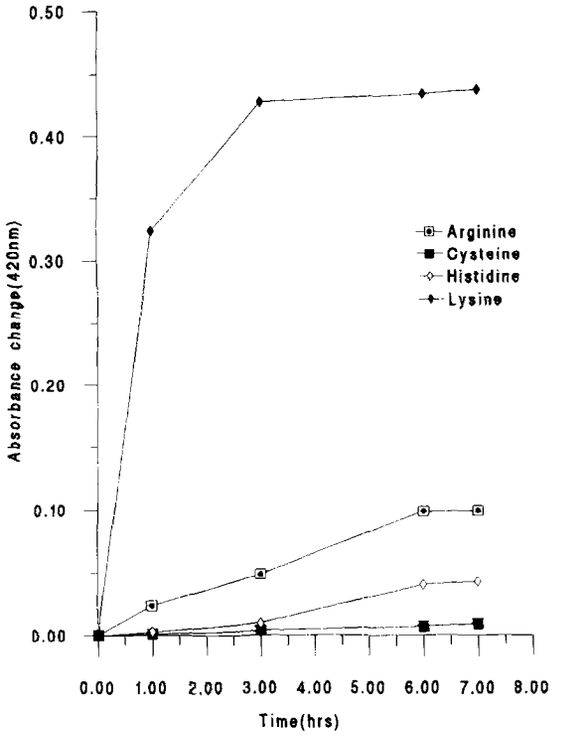

Fig. 3. The color intensity of MRP synthesized from various solutions of amino acid and glucose heated at 90°C up to 6 h were assayed spectrophometrically at 420 nm. The assay was performed after 1000-fold dilutions.

adding 100 μl of juice sample to a final volume of 3·0 ml incubation medium containing 0·001 M 4-methycatechol as substrate added to a buffer of 0·1 M citrate and 0·2 M phosphate at pH 4·6 and MRP inhibitor. The same incubation medium in the absence of the inhibitor was used as a reference in order to subtract the contribution of the inhibitor to the absorbance. The rate of oxidation of 4-methylcatechol was followed at 400 nm using a UV-Vis Perkin–Elmer Lambda 3 spectrophotometer. A low absorbance reading indicated low enzyme activity.

Statistical analysis

An analysis of variance (ANOVA) was performed to test the treatment effect at $P = 0{\cdot}05$ level, and the Duncan's multiple-range test was used to compare the treatment means. The analysis was done by using SPSSPC+.

RESULTS

The reactivity of PPO toward catechol, 4-methylcatechol and L-dopa assayed under each substrate optimum pH and wavelength are presented in Fig. 1. The maximum activity was detected toward 4-methylcatechol followed by catechol and L-dopa. Significant differences were found between 4-methylcatechol and L-dopa at the $P = 0{\cdot}05$ level.

The effects of different solutions of amino acid and glucose on PPO activity are reported in Fig. 2. Surprisingly, the MRP synthesized from both valine and glutamic acid had an enhancing effects on PPO rather than an inhibitory effect. In contrast, the MRP synthesized from arginine, cysteine, histidine and lysine

Table 1. The color of various solutions of amino acid (1·5 M) and glucose (1·5 M) heated at 90°C

	Time			
	1 h	3 h	5 h	7 h
Valine	Clear	Clear	Light pale yellow	Very light pale orange
Cysteine	Clear	Clear	Clear	Clear
Glutamate	Clear	Clear	Clear	Clear
Histidine	Very pale yellow	Pale yellow	Light pale orange	Light tan orange
Arginine	Light orange brown	Dark orange brown	Dark orange brown	Dark orange brown
Lysine	Dark brown	Very dark brown	Very dark brown	Very dark brown

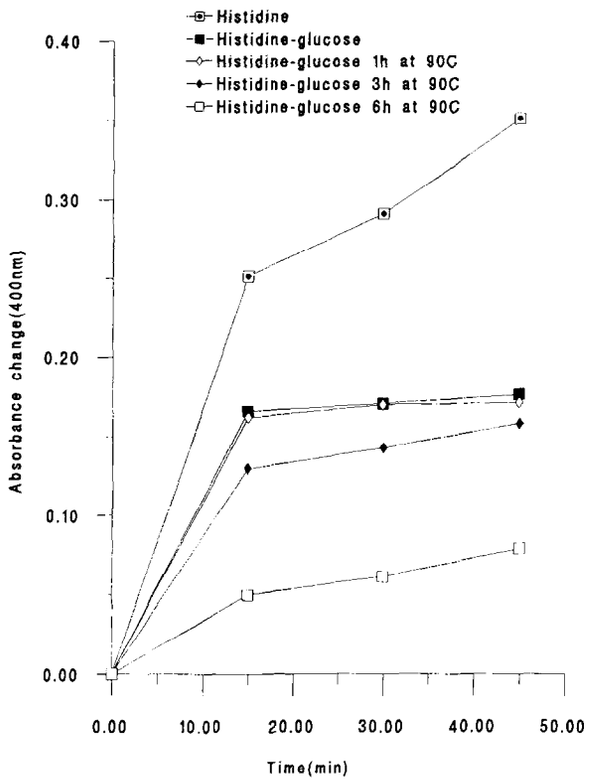

Fig. 4. The inhibitory effect of 25 μl of histidine and a solution of 1·5 M histidine and 1·5 M glucose heated for 0, 1, 3 and 6 h at 90°C on apple PPO.

significantly reduced the PPO activity. The inhibitory effects of MRP heated for 7 h at 90°C were as follows: arginine = histidine > cysteine > lysine. Although the MRP synthesized from arginine and lysine are effective inhibitors, it is not practical to use them on fresh fruits and vegetables because of their dark color. The pigment formation of MRP synthesized from lysine was significantly higher than all of the other amino acids (Fig. 3). The visual color of MRP is reported in Table 1. There was no direct correlation between the inhibitory effects and the browning intensity of MRP.

Increasing the reaction time of the glucose–histidine solution improved the inhibitory effects of MRP on apple PPO (Fig. 4). These results agree with Pitotti *et al.* (1990) and Nicoli *et al.* (1991). The inhibitory effects of the histidine–glucose solution improved significantly after 6 h of heating when compared with the amino acid histidine alone. The MRP synthesized from the cysteine–glucose solution heated for 1 h was a slightly better inhibitor of enzymatic browning than the non-heated cysteine–glucose solution and the amino acid cysteine (Fig.5). Both heated and unheated MRP as well as cysteine were significantly better inhibitors than the glucose alone. The increase in heating time did not improve the inhibitory effects of MRP synthesized from the cysteine–glucose solution. A slight decrease in the inhibitory effect was observed after 3 h of heating (Fig. 6).

The inhibitory effects of MRP synthesized from various ratios of cysteine to 0·5 M glucose are reported in Fig. 7. Using a constant amount of glucose 0·5 M, the inhibitory effects of MRP improved as the amount of

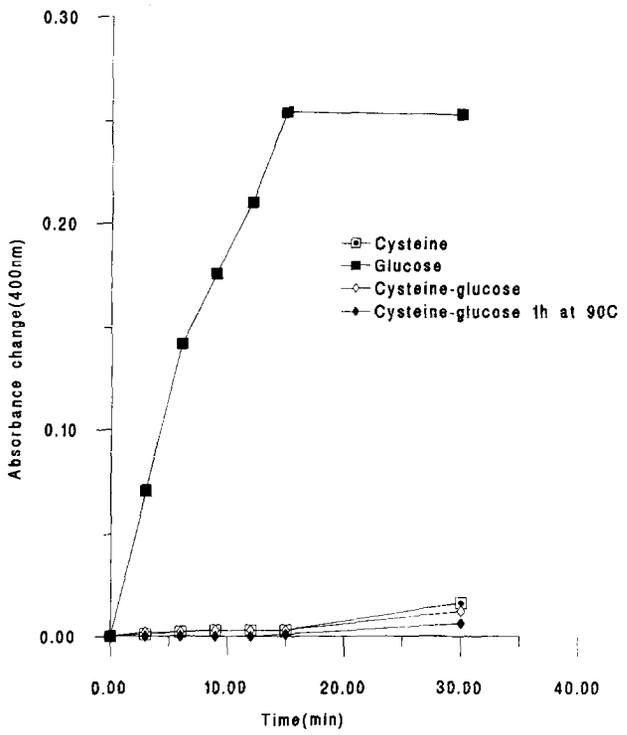

Fig. 5. The effect of 25 μl cysteine, glucose and cysteine–glucose solutions heated for 0 and 1 h on apple PPO. All the solutions were synthesized from the initial concentration of 1·5 M glucose and 1·5 M of cysteine solutions; the solution was heated at 90°C.

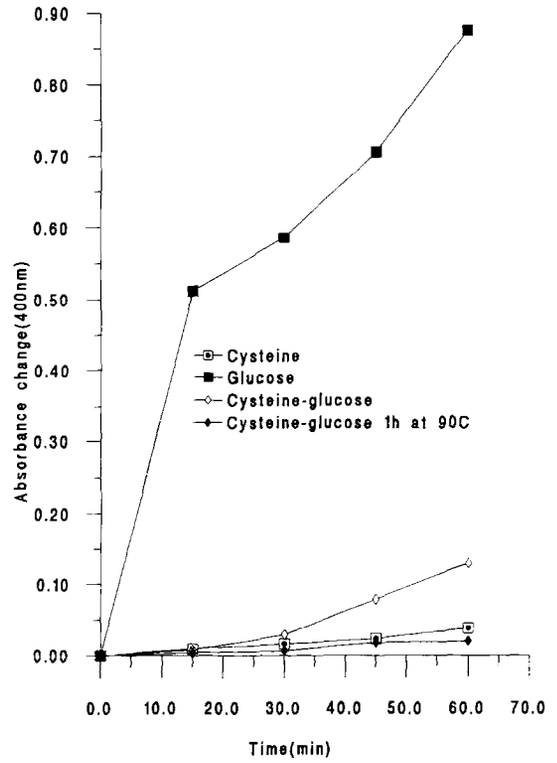

Fig. 6. The inhibitory effect of 25 μl of solution of 1·5 M cysteine and 1·5 M glucose heated at 90°C for 0, 1, 3 and 6 h on PPO activity.

cysteine increased. When 25 μl of 1·5 M cysteine was added to the medium, the PPO activity approached zero.

The MRP synthesized at lower pH levels were more effective inhibitors than the MRP synthesized at higher pH levels (Fig. 8). The inhibitory effects of MRP synthesized at pH 3 and 5 are significantly better than those synthesized at pH 11.

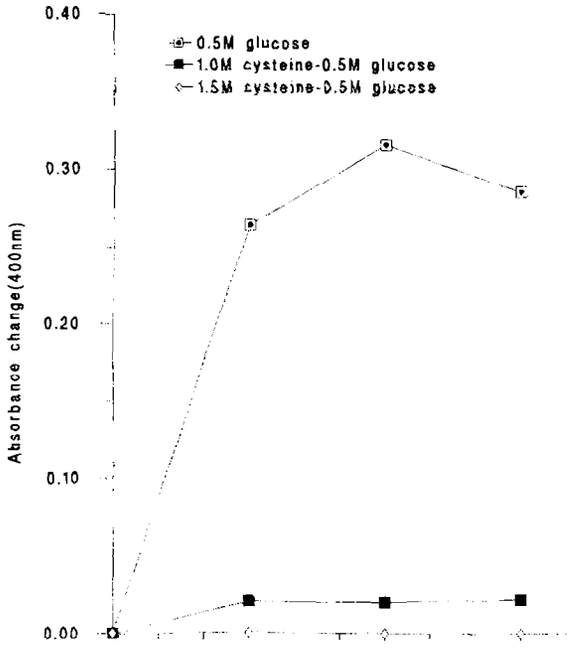

Fig. 7. The inhibitory effect of 25 μl of 1·0 M and solutions of 1·5 M cysteine and 0·5 M glucose on PPO activity. The solutions were heated for 2 h at 90°C.

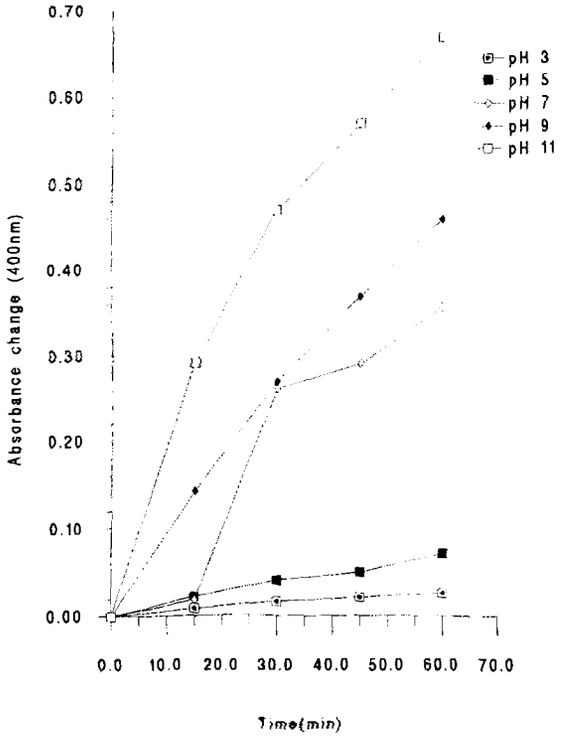

Fig. 8. The inhibitory effect of 25 μl of cysteine–glucose solutions synthesized at various initial pH levels. All of the MRP were heated at 90°C for 2 h.

DISCUSSION

After 7 h of heating, basic amino acid and glucose solutions caused a strong inhibitory effect on apple PPO, and this may be attributed to the melanoidins that are present in the Maillard reaction system. The colored pigment formation in the Maillard reaction system indicates the formation of the melanoidins (Saltmarch & Labuza, 1982). The reductone moiety present in the melanoidin structure has been reported to exhibit both reducing and chelating properties (Namiki, 1988), in addition to oxygen scavenging properties (Hayase *et al.*, 1989). The reductone moiety in the melanoidin's structure may also prevent browning by reducing the copper of PPO. Another possibility is that the reductone in the MRP may be capable of converting the quinone back to diphenol, thus preventing the polymerization of quinone.

The high metal-binding capacity of melanoidins has been reported by Johnson *et al.* (1983) as they studied the effect of browned and unbrowned corn products on the absorption of Zn, Fe and Cu in humans. Melanoidins may also inhibit enzymatic browning by preventing oxygen contact with the PPO, thus retarding the enzymatic reaction. In addition, pyrrole-like structures and the free radicals present in the melanoidins may contribute to the scavenging properties of melanoidins.

The degree of polymerization of melanoidins for the histidine–glucose solution appears to have an impact on the PPO activity. The browning intensity of melanoidins has been found to be directly related to its degree of polymerization (Gomyo *et al.*, 1972). The inhibitory effects of the cysteine–glucose solution appear to be attributed to both the melanoidin's structure and the SH group present in the cysteine–glucose system. The cysteine–glucose solution, when heated for 1 h, appears to be the most effective inhibitor. Moreover, there is only half the amount of cysteine present in the MRP system when compared with cysteine solution alone. The slight decrease in the inhibitory effect of the cysteine–glucose solution after 3 h of heating may be due to the thermal degradation of the cysteine that results in the evaporation of cysteine from the MRP system.

Low pH levels not only have an effect on the PPO catalyzed reaction, but also are involved in the polymerization of the quinone to brown melanins. Low pH inhibitors may retard the browning by reducing the copper in the PPO enzyme or by preventing the oxidation of the substrate in the presence of oxygen. The instability of the PPO substrate at high pH levels has been confirmed by Muneta (1977). Muneta found that PPO substrates incubated at low pH levels are less susceptible to autoxidation in the presence of oxygen than those incubated at high pH levels.

Anet (1964) reported that under acidic conditions the main products produced in the MRP system are furfural and hydroxymethylfurfural. His finding was confirmed by Feather and Russell (1969) by studying the decomposition of the Amadori products under acidic and basic conditions. They found that, in the

presence of acetic acid or HCl, the Amadori compound produced high yields of furfural and hydroxymethylfurfural when compared with sugar. In acidic conditions the nitrogen atom in the Amadori compound is nearly completely protonized, thus enhancing the 1,2 enolization reaction and facilitating the production of hydroxymethylfurfural. The additional products produced by the 1,2 enolization are pyrrole-like structures which result from the interaction of the amino compounds and furfural (Namiki, 1988). Pyrrole-like structures have been reported to have strong oxygen scavenging effects (Hayase *et al.*, 1986).

The sugar fragments present in the MRP system appear not to have much effect on PPO. Sugar fragments present in the MRP system have been found to contribute to browning in the MRP systems with pH levels at or above neutral in the early stages of the Maillard reaction (Hayashi & Namiki, 1986). The color intensity of the cysteine–glucose solution increased as the pH increased after 2 h of heating at 90°C. However, the inhibitory effect of MRP decreased as the pH increased. Hayashi and Namiki (1986) quantified C2 and C3 carbonyl products in an alanine–glucose reaction mixture and showed that the production of C2 and C3 carbonyl products was negligible at acidic pH levels, observable at neutral pH levels and greatly increased at alkaline pH levels.

CONCLUSIONS

This study showed that MRPs have an inhibitory effect on apple PPO. The MRP synthesised under different conditions, such as pH, heating time, and type and concentration of amino acids, exhibited different effects on apple PPO. Among the amino acid and glucose solutions, cysteine–glucose and basic amino acids and glucose solutions appear to be especially good inhibitors. However, the arginine or lysine–glucose solutions will be limited in their use as PPO inhibitors due to the high color intensity associated with them. Although there is little difference in the effectiveness of cysteine alone and cysteine–glucose MRP, the use of cysteine–glucose MRP is more economical than the use of cysteine alone. Additional studies are needed to compare the effectiveness and cost of MRP with that of other antibrowning agents.

REFERENCES

Anet, E. F. L. J. (1964). 3-Deoxyglucosones and the degradation of carbohydrates. *Adv. Carbohydr. Chem.*, **19**, 181–218.

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**, 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.

Eskin, N. A. M. (1990). Biochemistry of food spoilage: enzymatic browning. In *Biochemistry of Foods*, ed. N. A. M. Eskin, 2nd edn. Academic Press, New York, USA, pp. 401–32.

Feather, M. S. & Russell, K. R. (1969) Deuterium incorporation during the conversion of 1-amino-1-deoxy-D-fructose derivatives to 5-(hydroxy, ethyl)-2-furaldehyde. *J. Org. Chem.*, **34**, 2650–2.

Galeazzi, M. A. M., Sgarbieri, V. & Constantantinides, S. M. (1981). Isolation, purification and physicochemical characterization of polyphenol oxidase from a Dwarf variety of banana (*Musa cavendishii*). *J. Food Sci.* **46**, 150–5.

Gomyo, T., Kato, H., Udaka, K., Horikoshi, M. & Fujimaki, M. (1972). Chemical studies on melanoidins: Part III. Effects of heating on chemical properties of melanoidin prepared from Glycine-Xylose system. *Agr. Biol. Chem.*, **36**, 125–32.

Hayase, F., Hirashima, S., Okamoto, G. & Kato, H. (1989). Scavenging of active oxygens by Melanoidins. *Agric. Biol. Chem.*, **53**, 3383–5.

Hayashi, T. & Namiki, M. (1986). Role of sugar fragmentation in an early stage browning of amino-carbonyl reaction of sugar with amino acids. *Agric. Biol. Chem.*, **50**, 1965–70.

Janovitz-Klapp, A. H., Richard, F. C., Goupy, P. M. & Nicolas, J. J. (1990). Inhibition studies on apple polyphenol oxidase. *J. Agric. Food Chem.*, **38**, 926–31.

Johnson, P. E., Lykken, G., Mahalko, J., Milne, D., Inman, L., Gracia, W. J. & Inglett, G. E. (1983). The effect of browned and unbrowned corn products on absorption of zinc, iron, and copper in humans. *ACS Symp. Ser.*, **215**, 3349–60.

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**, 111–15.

Kermasha, S., Goetghebeur, M., Monfette, A., Metche, M. & Rovel, B. (1993). Studies on inhibition of mushroom polyphenol oxidase using chlorogenic acid as substrate. *J. Agric. Food Chem.*, **41**, 526–31.

Monsalve-Gonzalez, A., Barbosa-Canovas, G. V., Cavalieri, R. P., MxEvily, A. J. & Iyengar, R. (1993). Control of browning during storage of apple slices preserved by combined methods. *J. Food Sci.*, **58**, 797–800.

Muneta, P. (1977) Enzymatic blacking in potatoes: influence of pH on dopachrome oxidation. *J. Am. Potato.*, **54**, 387–93.

Namiki, M. (1988). Chemistry of Maillard reactions: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. In *Advances in Food Research*, ed. C. O. Chichester & B. S. Schweigert. Academic Press, New York, USA, pp. 1115–84.

Namiki, M. (1990). Antioxidants/antimutagens in food. *CRC Crit. Rev. Food Sci. Nutr.*, **29**, 273–99.

Nicolas, J., Richard-Forget, S. C. . Goupy, P. M., Amiot, M. J. & Aubert, S. Y. (1994). Enzymatic browning reactions in apple and apple products. *CRC Crit. Rev. Food Sci. Nutr.*, **34**, 109–57.

Nicoli, M. C., Elizalde, B. E., Pitotti, A. & Lerici, C. R. (1991). Effect of sugar and Maillard reaction products on polyphenol oxidase and peroxidase activity in food. *J. Food Biog.*, **15**, 169–84.

Oszmianski, J. & Lee, C. Y. (1990). Inhibition of polyphenol oxidase activity and browning by honey. *J. Agric. Food Chem.*, **38**, 1892–5.

Pitotti, A., Nicoli, C., Sensidon, A. & Lerici, C. R. (1990). Control of enzymatic browning in food: effects of some substances and their mechanism action. In *Engineering and Food* (Vol. 1 — *Physical Properties and Process Control*), eds W. E. L. Spiess & H. Schuberts. Elsevier Sciences Publisher, London, UK, pp. 671–81.

Saltmarch, M. & Labuza, T. P. (1982). Non-enzymatic browning via the Maillard reaction in foods. *Diabetes*, **31**(Suppl. 3), 29–36.

Sapers, G. M. & Miller, R. L. (1992). Enzymatic browning control in potato with ascorbic acid-2-phosphates. *J. Food Sci.*, **57**, 1132–5.

Sapers, G. M., Hicks, K. B., Phillips, J. G., Garzarella, L., Pondish, D. L., Matulaitis, R. M., McCormack, T. J., Sondey, S. M., Seib, P. A. & El-Atawy, Y. S. (1989). Con-

trol of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *J. Food Sci.*, **54**, 997–1012.

Valero, E., Varon, R. & Gracia-Carmona, F. (1990). Inhibition of grape polyphenol oxidase by several aliphatic alcohols. *J. Agric. Food Chem.*, **38**, 1097–1100.

Vamos-Vigyazo, L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr.*, **15**, 49–127.

Zhou, P., Smith, N. L. & Lee, C. Y. (1993). Potential purification and some properties of Monroe apple peel polyphenol oxidase. *J. Agric. Food Chem.*, **41**, 532–6.