Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 91 (2005) 57–61
Food Chemistry
www.elsevier.com/locate/foodchem

Inhibition of potato polyphenol oxidase by Maillard reaction products

Min-Kyung Lee, Inshik Park *

Division of Food Science, Dong-A University, Busan 604-714, Korea

Received 10 November 2003; received in revised form 25 May 2004; accepted 25 May 2004

Abstract

The inhibitory effect of MRP (Maillard reaction product) on potato polyphenol oxidase (PPO) was investigated. As the reaction time of glucose/glycine was increased at 90 °C, the inhibitory effect of the produced MRP toward potato PPO was increased, whereas the amounts of glycine and glucose were decreased. The MRP synthesized from arginine, cysteine, histidine and lysine significantly inhibited potato PPO. The MRP prepared from glucose and glycine inhibited potato PPO non-competitively. The MRPs synthesised from monosaccharides, such as fructose and glucose, and glycine were more inhibitory against potato PPO than those from disaccharides. The MRP formed by increasing glucose and glycine concentration exhibited a stronger inhibitory effect on potato PPO. The MRP formed from glucose and glycine showed strong inhibition toward potato PPO with (+)catechin, catechol, 4-methylcatechol and L-DOPA as substrates among tested polyphenols.

Keywords: Polyphenol oxidase; Potato; Maillard reaction product

1. Introduction

Browning reactions, which are some of the most important phenomena occurring in food during processing and storage, represent an interesting research area due to implications for food stability and technology, as well as for nutrition and health (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). In general, there are five basic types of browning reactions that occur in foods: Maillard reaction, caramelization, ascorbic acid oxidation, enzymatic browning of phenols, and formation of browned polymers by oxidized lipids (Langdon, 1987; Pizzocaro, Torreggiani, & Gilardi, 1993). The Maillard reaction, a chemical reaction between amino groups and reducing sugars, is very significant for foods because it strongly affects food quality (van Boekel, 1998). The type of amine and carbonyl compound influence the rate of reaction as well as the products formed, which ultimately are brown melanoidin pigments (Willits, Underwood, Lento, & Ricciuti, 1958). The browning of raw fruits and vegetables is a result of the oxidation, catalyzed by polyphenol oxidase, of phenolic compounds to quinones and their subsequent condensation to coloured pigments (Vamos-Vigyazo, 1981). Polyphenol oxidase (PPO; EC 1.14.18.1) is an enzyme widely distributed in nature, and it is responsible for enzymatic browning reaction occurring during the handling, storage and processing of fruits and vegetables (Dincer, Colak, Aydin, Kadioglu, & Guner, 2002). Some of the natural agents proposed to have an inhibitory effect on PPO are honey (Oszmianski & Lee, 1990) natural aliphatic alcohols (Valero, Varon, & Gracia-Carmona, 1990), cysteine (Kahn, 1985) and Maillard reaction products synthesized from glucose and lysine (Jing & Kitts, 2000). Kahn (1985) suggested that the amino acid cyteine can form a stable complex with copper, thus retarding enzymatic browning. MRP synthesized from

* Corresponding author. Fax: +82-51-200-7535.
E-mail address: ispark@donga.ac.kr (I. Park).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.05.046

glycine and glucose inhibited PPO in Golden Delicious apples (Nicoli, Elizalde, Pitotti, & Lerici, 1991). The objective of this study was to investigate the inhibitory effect of MRP, synthesized from various amino acids and sugar solutions, on potato PPO activity. The factors, including heating time, type of amino acid and sugar, and concentration of both amino acids and sugar, were examined for their effectiveness in producing MRP that inhibited the oxidation of catechol.

2. Materials and methods

2.1. Materials

Potato (*Solanum tuberosum* L.) was purchased from a local market in Busan, Korea. All amino acids, sugars, catechol, 4-methylcatechol, pyrogallol, chlorogenic acid, (+)catechin, tyrosine, L-DOPA, glucose oxidase and peroxidase were obtained from Sigma Chemical Co.

2.2. Extraction and assay of potato PPO

Potato (200 g) was homogenized with 200 ml of 50 mM phosphate buffer at pH 6.6 for 3 min. The homogenate was centrifuged at 15,000*g* for 20 min, and the supernatant was collected. All steps were carried out at 4 °C. Potato PPO activity (Zauberman et al., 1991) was assayed, with 0.2 M catechol as a substrate, by a spectrophotometric procedure (Ultrospec 3000, Pharmacia Biotech). The assay mixture contained 0.1 ml of potato PPO, 0.9 ml of 50 mM phosphate buffer at pH 6.6, 1 ml of MRP and was incubated for 5 min at 25 °C. After this incubation, 0.2 M catechol was added to the assay mixture, and the increase in absorbance at 420 nm and 25 °C was recorded automatically for 1 min. The total assay volume was 3 ml.

2.3. Synthesis of MRPs

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 MRP formation was evaluated by measuring absorbance at 420 nm. MRP formed from various amino acids and sugars were obtained by heating equal volumes of 1.5 M amino acids and 1.5 M sugars at 90 °C for 7 h. The MRP synthesized from varying concentrations of glycine was obtained by heating 0.05–1.5 M glycine solution with a 1.5 M glucose solution at 90 °C for 7 h. The MRP containing varying concentrations of glucose was obtained by heating 0.05–1.5 M glucose with a 1.5 M glycine solution. The inhibitory effect of MRP synthesized from 1.5 M glucose and 1.5 M glycine toward various substrates was measured at each substrate's optimum wavelength (Zhou, Smith, & Lee, 1993).

2.4. Glucose and glycine determination

The glucose was quantitatively determined by enzymatic assay using glucose oxidase/peroxidase (Park, Kho, & Nam, 1989). The assay mixture contained 2.6 ml of 0.1 M acetate buffer at pH 5.5, 0.1 ml of MRP synthesized from glucose/glycine mixture and 0.1 ml of 0.45 M guaiacol. The assay was initiated by adding 0.2 ml of glucose oxidase/peroxidase. The total assay volume was 3 ml. The increase in absorbance at 460 nm was recorded automatically for 2 min. The glycine was determined by the ninhydrin method (Rosen, 1957).

3. Results and discussion

3.1. Inhibitory effect of MRP produced by glucose/glycine mixture on potato PPO

Fig. 1 shows the inhibitory effect of MRP produced by glucose/glycine mixture on potato PPO. As colour formed by heating the glucose/glycine mixture increased, the inhibitory effect of produced MRP also increased against potato PPO. However, the amounts of glucose and glycine were decreased with increasing MRP production. The formed MRP, dialyzed with a cellulose membrane (Sigma Chemical Co.) with molecular weight cutoff of 12,000, lost its activity completely, which suggests that the MRP responsible for the inhibitory effect would have been of low molecular weight. It was also found that the MRP inhibited the potato polyphenol oxidase non-competitively (Fig. 2). Ames (1992) reported that colour formation is likely due both to the formation of low molecular weight compounds and to the presence of melanoidins with high molecular weight. Gomyo, Kato, Udaka, Horikoshi, and Fujimaki (1972) explained that the browning intensities of melanoidins are directly related to degree of polymerization. Mauron (1981) reported that the rate of formation of brown pigments increases with the square of the time. It has been reported that the rates of glycine loss and browning increased with increasing phosphate buffer concentration (Bell, 1997). Potman and van Wijk (1985) demonstrated that glucose was consumed more rapidly when the phosphate concentration increased, but did not provide a clear reason for this phenomenon.

3.2. Inhibitory effects of MRPs synthesized from different amino acids and glucose on potato PPO

The inhibitory effects of MRP synthesized from different amino acids with a constant amount of glucose (1.5 M) on potato PPO activity are shown in Table 1. The MRPs synthesized from arginine, cysteine, histidine and lysine significantly reduced the PPO activity. When MRP synthesized from glucose/arginine solution was

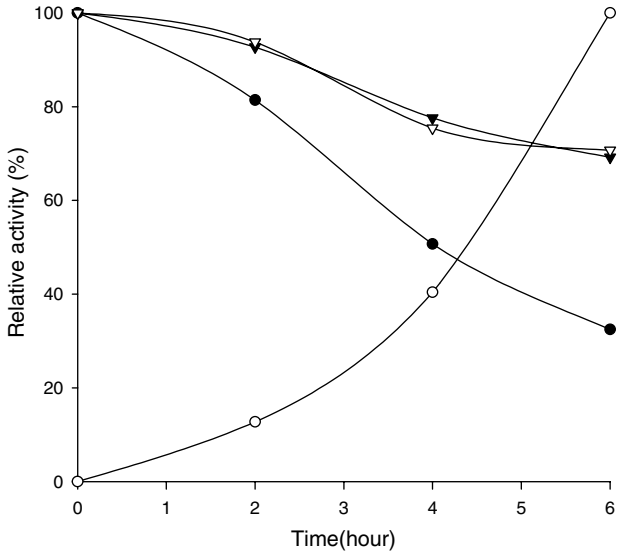

Fig. 1. The time dependence of production of MRPs. The MRPs were obtained by heating equal volumes of 1.5 M glycine and 1.5 M glucose at 90 °C for various time periods. The PPO activity was assayed with 0.2 M catechol as a substrate by a spectrophotometric procedure. The formation of MRP colour produced by glucose and glycine was evaluated by measuring absorbance at 420 nm. The amount of glycine was determined by the ninhydrin test. The amount of glucose was quantitatively determined by the glucose oxidase/peroxidase method. PPO activity (●-●); colour (○-○); glycine (▼-▼); glucose (▽-▽).

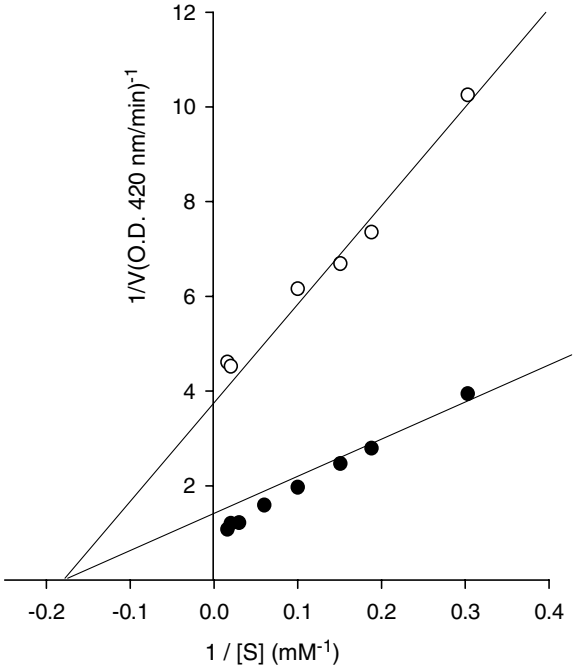

Fig. 2. Lineweaver–Burk plots of potato polyphenol oxidase in the presence of MRP. Catechol was used as a substrate. MRP was obtained by heating equal volumes of 1.5 M glycine and 1.5 M glucose at 90 °C for 7 h. Control (•-•); MRP (○-○).

added to the assay mixture, the PPO activity was completely inhibited. Tan and Harris (1995) demonstrated that the inhibitory effects of the cysteine/glucose solution

Table 1
The inhibitory effects of MRP produced by various amino acids with 1.5 M glucose on potato polyphenol oxidase

Compounds	Relative activity (%)
Control	100
Valine	30.8
Glycine	20.6
Serine	34.7
Cysteine	2.2
Asparagine	43.5
Lysine	12.5
Arginine	0.1
Histidine	8.9

The MRP was obtained by heating equal volumes of each 1.5 M amino acid and 1.5 M glucose at 90 °C for 7 h.

appear to be due to both the melanoidin's structure and the SH group present in the cysteine/glucose system.

3.3. Inhibitory effects of MRPs synthesized from different sugars and glycine on potato PPO

The inhibitory effects of MRPs synthesized from different sugars with constant amount of glycine (1.5 M) on potato PPO activity are shown in Table 2. The enzyme was most inhibited by addition of fructose. It was found in previous studies that fructose is more reactive than glucose because the former is easily dehydrated to intermediates leading to the HMF at low pH (Kato, Yamamoto, & Fugimaki, 1969). This could be explained by the higher ring opening rate of fructose than that of glucose in acidic medium (McWeeny, 1973). Wu, Russel, and Powrie (1987) used glucose and fructose as selected sugar reactants due to the different (aldose and ketose) structures and the fact that the sugars produce different amounts and types of MRP compounds. Morales and Jimenez-Perez (2001) demonstrated that, in browning, glucose was more reactive than lactose independently of the type of amino acid used, but lysine was more reactive than glycine and alanine. The partial inhibition of the enzyme activity by MRP prepared from sucrose, a non-reducing sugar, might be consequence of a rise in viscosity as well as a drop in dissolved oxygen concentration in the reaction vessel due to high sucrose concentration,

Table 2
The inhibitory effects of MRPs produced by various sugars with 1.5 M glycine on potato polyphenol oxidase

Compounds	Relative activity (%)
Control	100
Glucose	20.6
Lactose	38.5
Sucrose	55.9
Fructose	11.5
Maltose	44.2

The MRPs of various sugars were obtained by heating equal volumes of each 1.5 M sugar and 1.5 M glycine at 90 °C for 7 h.

Table 3
The inhibitory effects of MRPs produced by varying glycine concentration on potato polyphenol oxidase

Compounds	Relative activity (%)
None	100
0.05 M Glycine	85.4
0.1 M Glycine	84.5
0.5 M Glycine	57.9
1 M Glycine	28.3
1.5 M Glycine	20.6

The MRPs were produced by varying glycine concentration with 1.5 M glucose at 90 °C for 7 h.

as described by Billaud, Roux, Brun-Merimee, Maraschin, and Nicolas (2003).

3.4. Inhibitory effects of MRPs, synthesized from varying concentrations of glycine and glucose, on potato PPO

The inhibitory effects of MRP produced from varying concentrations of glycine and 1.5 M glucose on potato PPO are shown in Table 3. With a constant glucose amount used, the inhibitory effect of MRP increased as glycine amount increased. The inhibitory effects of MRPs synthesized from varying glucose concentrations with 1.5 M glycine are shown in Table 4. With a constant glycine amount used, the inhibitory effect of MRP increased as the amount of glucose increased.

3.5. Inhibitory effects of MRP produced from glucose/glycine toward various substrates

The inhibitory effects of MRP synthesized from glucose/glycine mixture toward various substrates are shown in Table 5. The MRP exhibited the strong inhibition toward potato PPO with (+)catechin, catechol, and 4-methylcatechol as substrate. Since these substrates are *o*-dihydroxyphenols, the MRP seems to be an efficient inhibitor against enzymatic browning of various *o*-dihydroxyphenols. Lee and Lee (1997) demonstrated that addition of CP (caramelization product) to the PPO inhibited catechol browning completely and decreased the caffeic acid reaction rate to 57% and that of DL-DOPA to 33%.

Table 4
The inhibitory effects of MRPs produced by varying glucose concentrations on potato polyphenol oxidase

Compounds	Relative activity (%)
None	100
0.05 M Glucose	84.0
0.1 M Glucose	80.1
0.5 M Glucose	43.9
1 M Glucose	31.7
1.5 M Glucose	20.6

The MRP solutions were produced by various concentrations of glucose with 1.5 M glycine at 90 °C for 7 h.

Table 5
The inhibitory effects of MRPs produced by 1.5 M glucose and 1.5 M glycine on potato polyphenol oxidase with various substrates

	Wavelength (nm)	Relative activity (%)
Catechol	420	19.8
4-Methylcatechol	410	20.2
Pyrogallol	334	50.1
Chlorogenic acid	400	62.5
(+)Catechin	475	16.2
L-DOPA	475	29.8

The MRP was produced by heating 1.5 M glucose and 1.5 M glycine at 90 °C for 7 h. The potato polyphenol oxidase activity was assayed at each wavelength by a spectrophotometric procedure.

Acknowledgement

This paper was supported by the Dong-A University Research Fund, in 2002.

References

Ames, J. M. (1992). The Maillard reaction. In Hudson, B. J. F., (Ed.). *Biochemistry of food proteins* (Vol. 4) (pp. 99–153). London: Elsevier Applied Science.

Bell, L. N. (1997). Maillard reaction as influenced by buffer type and concentration. *Food Chemistry, 59*, 143–147.

Billaud, C., Roux, E., Brun-Merimee, S., Maraschin, C., & Nicolas, J. (2003). Inhibitory effect of unheated and heated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction product model systems on polyphenoloxidase from apple. I. Enzymatic browning and enzymatic activity inhibition using spectrophotometric and polarographic methods. *Food Chemistry, 81*, 35–40.

Dincer, B., Colak, A., Aydin, N., Kadioglu, A., & Guner, S. (2002). Characterization of polyphenol oxidase from medlar fruits. *Food Chemistry, 77*, 1–7.

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. *Agricultural and Biological Chemistry, 36*, 125–132.

Jing, H., & Kitts, D. D. (2000). Comparison of the antioxidative and cytotoxic properties of glucose–lysine and fructose–lysine Maillard reaction products. *Food Research International, 33*, 509–516.

Kahn, V. (1985). Effect of protein, protein hydrolyzate and amino acids on dihydroxyphenolase activity of polyphenol oxidase of mushroom, avocado and banana. *Journal of Food Science, 50*, 111–115.

Kato, H., Yamamoto, M., & Fugimaki, M. (1969). Mechanisms of browning degradation of D-fructose in special comparison with *d*-glucose–glycine reaction. *Agricultural and Biological Chemistry, 33*, 939–940.

Langdon, T. T. (1987). Preventing browning in freshly prepared potatoes without the use of sulfiting agents. *Food Technology, 41*, 64–67.

Lee, G. C., & Lee, C. Y. (1997). Inhibitory effect of caramelisation products on enzymic browning. *Food Chemistry, 60*, 231–235.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology, 11*, 340–346.

Mauron, J. (1981). The Maillard reaction in foods; a critical review from the nutritional standpoint. *Progress in Food and Nutritional Science, 5*, 5–35.

McWeeny, D. J. (1973). The role of carbohydrates in non-enzymatic browning. In . In G. Birch & L. Green (Eds.), *Molecular structure and function of food carbohydrates* (pp. 21–32). New York: Wiley.

Morales, F. J., & Jimenez-Perez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chemistry, 72*, 119–125.

Nicoli, M. C., Elizalde, B. E., Pitotti, A., & Lerici, C. R. (1991). Effects of sugars and maillard reaction products on polyphenol oxidase and peroxidase activity in food. *Journal of Food Biochemistry, 15*, 169–184.

Oszmianski, J., & Lee, C. Y. (1990). Inhibition of polyphenol oxidase activity and browning by honey. *Journal of Agricultural and Food Chemistry, 38*, 1892–1895.

Park, I. S., Kho, S., & Nam, I. (1989). Korean-Radish peroxidase for enzymatic determination of glucose. *Journal of Korean Biochemistry, 22*, 411–416.

Pizzocaro, F., Torreggiani, D., & Gilardi, G. (1993). Inhibition of apple polyphenol oxidase by ascorbic acid, citric acid and sodium chloride. *Journal of Food Processing and Preservation, 17*, 21–30.

Potman, R. P., & van Wijk, T. A. (1985). Mechanistic studies of the Maillard reaction with emphasis on phosphate mediated catalysis. In . In T. H. Parliament R. J. McGorrin & C. T. Ho (Eds.), *Thermal generation of aromas* (pp. 182–195). Washington, DC: American Chemical Society.

Rosen, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. *Archives of Biochemistry and Biophysics, 67*, 10–15.

Tan, B. K., & Harris, N. D. (1995). Maillard reaction products inhibit apple polyphenol oxidase. *Food Chemistry, 53*, 267–273.

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

Vamos-Vigyazo, L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Critical Review of Food Science and Nutrition, 15*, 49–127.

van Boekel, M. A. J. S. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry, 62*, 403–414.

Willits, C. O., Underwood, J. C., Lento, H. G., Jr., & Ricciuti, C. (1958). Browning of sugar solutions: Part I. Effect of pH and type of amino acid in dilute sugar solutions. *Food Research, 23*, 61–67.

Wu, C. H., Russel, G., & Powrie, W. D. (1987). Paramagnetic behaviour of model system melanoidins. *Journal of Food Science, 52*, 813–816.

Zauberman, G., Ronen, R., Akerman, M., Weksler, A., Rot, I., & Fuch, Y. (1991). Postharvest retention of the red color of litchi fruit pericarp. *Scientia Horticulturae, 47*, 89–97.

Zhou, P., Smith, N. L., & Lee, C. Y. (1993). Potential purification and some properties of Monroe apple peel polyphenol oxidase. *Journal of Agricultural and Food Chemistry, 41*, 532–536.