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Effect of Maillard Reaction Products on Inhibition of Burdock Polyphenol Oxidase and Their Antioxidant Activities

†GyeYeong Kim, Heesun Choi and Inshik Park

Dept. of Food Science and Nutrition, Dong-A University, Busan 49315, Korea

Abstract

This study was conducted in an effort to investigate the effect of Maillard reaction products (MRPs) on enzymatic browning of burdock and their anti-oxidant activity. The MRPs were prepared by heating glucose and amino acids at 90℃, which served to produce a strong inhibitory effect on burdock polyphenol oxidase. As the reaction time of the solution containing glucose and amino acid increased at 90℃, the production of MRPs increased and intensity of the brown color deepened. When MRPs were prepared by heating at 90℃ for five hours, the absorbance of MRPs from glucose and lysine was 6.44, while those of glucose and glycine was 1.95. The MRPs synthesized from the glucose and lysine also reduced the pH of MRPs from 5.60 to 4.51, but those from glucose and glycine decreased slightly from 5.57 to 5.33. The Michealis-Menten constant value (K_m) of burdock PPO with pyrocatechol as a substrate was 16.0 mM, and MRPs were a non-competitive inhibitor against burdock PPO. The anti-oxidant activity of MRPs was measured by evaluating its radical scavenging activities of DPPH radicals, ABTS radicals and reducing power. The color intensity of MRPs produced by lysine and glucose were deeper than that produced by glucose and glycine. It was also found that MRPs produced from glucose and lysine exhibited stronger anti-oxidant properties than those produced by glucose and glycine.

Key words: MRPs, polyphenol oxidase, burdock, antioxidant activity

Introduction

Browning reaction of plants and vegetables has been an interesting research area due to application in food stability and technology as well as for health and nutrition (Manzocco et al. 2001). 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 M 1998). The types of carbonyl compounds and amine affect the rate of reaction as well as the products formed, which ultimately become brown melanoidin pigments (Willits et al. 1958). The enzymatic browning of plants is catalyzed by polyphenol oxidase (PPO), which converts phenolic compounds to quinones and their subsequent condensation to become colored pigments (Vamos-Vigyazo L 1981). Polyphenol oxidase (PPO; EC 1.14.18.1) is an enzyme widely distributed in nature, which is responsible for enzymatic browning occurring during the handling, storage and processing of fruits and vegetables (Dincer et al. 2002). Natural agents proposed to have an inhibitory effect on PPO are cysteine (Kahn 1985), honey (Oszmianski & Lee 1990), natural aliphatic alcohols (Valero et al. 1990), alkaline electrolyzed water (Kim & Hung 2014), onion extract (Kim et al. 2005; Kim et al. 2007) and Maillard reaction products (MRPs). MRPs synthesized from sugar and amino acid were reported to inhibit PPO in apple (Nicoli et al. 1991; Tan & Harris 1995; Billaud et al. 2003; Mogol et al. 2010) and potato (Lee & Park 2005).

The development of non-enzymatic browning such as Maillard reaction was reported to be associated with antioxidant activity (Yilmaz & Toledo 2005; Vhangani & Van Wyk 2013; Morales & Jimenez-Perez 2001; Manzocco et al. 2001; Bell 1997; Billaud et al. 2003, Yoshimura et al. 1997). The relationship between color development and antioxidant capacity during Maillard re-

† Corresponding author: GyeYeong Kim, Dept. of Food Science and Nutrition, Dong-A University, Busan 49315, Korea. Tel: +82-52-200-5655, Fax: +82-51-200-7535, E-mail: tkrtmvls44@hanmail.net

action progress has been found to be either positive or complex depending on the composition of product. Various parameters affect mechanism of Maillard reaction, which leads to formation of different antioxidant properties (Manzocco et al. 2001).

Burdock (*Arctinum lappa* L.) has been cultivated and utilized as folk medicine, tea as well as culinary use (Lee & Kim 2017). In Korea, burdock root has been widely used as side dish or tea. During cooking process, it was often brown-colored due to Maillard reaction. Though there have been previous reports on inhibitory effect of MRPs on PPO activities from various sources, few reports have been reported on burdock PPO. There were few reports of MRPs prepared from glucose and glycine/lysine on antioxidant capacity such as DPPH radical scavenging activities, ABTS radical scavenging activity and reducing power of MRPs prepared at 90℃. The aim of this study was to investigate the comparison of MRPs on inhibitory effect of on burdock PPO and antioxidant capacities.

Materials and Methods

1. Materials

Pyrocatechol, Folin–Ciocalteu's phenol reagent, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2',2'-azinobis (3-ethylbenzothiazline-6-sulfonic acid) diammonium salt), pyrocatechol, gallic acid Trolox, glucose, lysine, and glycine were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Burdock was obtained from a local market in Busan, Korea.

2. Extraction and assay of burdock polyphenol oxidase

Burdock (5 g) was homogenized with 50 mL of 50 mM K-phosphate buffer at pH 6.6 for 2 min. The homogenate was centrifuged at 15,000 × g for 20 min, and the supernatant was collected. All steps were carried out at 4℃. Burdock PPO activity (Zauberman et al. 1991) was assayed with 0.2 M pyrocatechol as a substrate spectrophotometrically (Ultrospec 3000, Pharmacia Biotech). The assay mixture containing 0.1 mL of burdock PPO, 0.9 mL of 50 mM K-phosphate buffer at pH 6.6 and 1.0 mL of MRPs was incubated for 5 min at 25℃. After this incubation, 0.2 M pyrocatechol was added to the assay mixture, and the increase in absorbance at 420 nm and 25℃ was recorded automatically for 1 min. The total assay volume was 3.0 mL. The reaction rate was calculated from the initial slope of the progress curve and percentage PPO inhibition values were calculated as follows:

$$\text{PPO inhibition (PPO)} = 1 - \frac{\text{Initial rate (sample)}}{\text{Initial rate (control)}} \times 100$$

3. Synthesis of Maillard reaction products

MRPs were prepared following the procedure described by Kim and Lee (2008) with slight modifications. Equimolar (1.0 M) amount of each glucose and amino acid (lysine) was dissolved in 10 mL distilled water. Equal volumes (5 mL) of each sugar and an amino acid were mixed to make up 10 mL solutions at final concentrations of 0.5 M. The solutions were heated at 90℃ for 7 hrs. The resultant MRPs were rapidly cooled in an ice bath. A portion of the MRPs was retained for measurement of pH and absorbance at 420 nm.

4. DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity of MRPs

DPPH radical scavenging activity of MRPs was determined by the method described by Blois M (1958) with some modifications. A 0.2 mL aliquot of each MRPs was added to 0.8 mL DPPH solution in ethanol, which was 1.2 in absorbance at 520 nm. The mixture was vortexed and allowed to stand at room temperature in the dark for 10 min. The absorbance of the reaction mixture was measured at 520 nm in a spectrophotometer. The control was prepared in the same manner, with the MRPs solution substituted with distilled water. The DPPH-radical scavenging activity was calculated as following equation.

$$\text{DPPH RSA (\%)} = 1 - \frac{\text{Abs sample (520 nm)}}{\text{Abs control (520 nm)}} \times 100$$

5. ABTS radical scavenging activity of Maillard reaction products

The radical scavenging capacity of the samples for the ABTS (2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulfonate) was determined as described by Re et al. (1999). ABTS radical was generated by mixing 7 mM solution of ABTS at pH 7.4 (50 mM Na-phosphate buffer) with 2.5 mM of potassium persulfate (final concentration) and stored in the dark at room temperature for 16 h before use. The mixture was diluted with water to give an absorbance of 1.2 at 734 nm using spectrophotometry. Sample (100 μL) was kept with fresh ABTS solution (900 μL) for 10 min and the absorbance was measured at 734 nm after 10 min. Gallic acid or Trolox was used as a reference.

$$\text{ABTS RSA(\%)} = 1 - \frac{\text{Abs sample (734 nm)}}{\text{Abs control (734 nm)}} \times 100$$

6. Reducing power of Maillard reaction products

The reducing power of MRPs was determined by the method of Lertittikul et al. (2007) with slight modifications. One mL of MRPs was mixed with 2.5 mL of 0.2 M K-Phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixtures were kept in a water bath at 50℃ for 30 min, followed by the addition of 2.5 mL of 10% TCA. The reaction mixtures were centrifuged at 10,000 × g for 5 min at room temperature. A 2.5 mL aliquot of the supernatant was added to 1.0 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The control was prepared same as above, except that MRPs were replaced with distilled water. After incubation for 10 min, the absorbance of the reaction mixture was measured at 700 nm.

Results and Discussion

1. Effect of heating time on pH of Maillard reaction products

The Maillard reaction is characterized by reactants consumption, formation of initial, intermediate and complex brown polymers. The reaction rates and end-products of MRPs formation would be different depending on the set parameters. During the Maillard reaction, the pH is crucial since the initial step is facilitated by pH values (Lertittikul et al. 2007). In this study, the pH changes were monitored during Maillard reaction to evaluate its significance. Fig. 1 shows pH changes of MRPs produced between glucose and lysine or glycine as a function of heating time. Increased reaction time of glucose and amino acid resulted in higher pH reduction in MRPs. The pH drop during MRPs formation was higher in MRPs produced from glucose and lysine than those from glucose and glycine. When heating temperature and time of amino groups-glucose model systems were increased, similar result was also reported from Kim and Lee (2009), and Gu et al. (2010). Liu et al. (2008) also reported that amount of acids formed is highly dependent on the co-existence of an amino and a carbonyl group. It was reported that the decrease in pH during the MRP formation is due to the acetic and formic acid formation (Vhangani & Van Wyk 2012). Therefore, the type of amino acids participating in the Maillard reaction would decide the amount of organic acids formed.

2. Effect of heating time on color of MRPs

Development of a brown color is a non-specific index used to assess the extent and the rate to which the Maillard reaction has occurred (Laroque et al. 2008). In Maillard reaction, the carbonyl group reacts with the amino group giving rise to colorless compounds which do not absorb in the visible spectrum. Further progress of the Maillard reaction involves the production of high molecular weight compounds, termed melanoidins with a characteristic absorbance maximum at 420 nm (Delgado-Andrade et al. 2010). The brown color has been used as an indicator of the progress of Maillard reaction. Fig. 2 shows browning intensity as a function of reaction time. The browning of produced MRPs increased with an increase in reaction time. Significant differences among MRPs produced with different amino acids were observed as a function of reaction time at 90℃. MRPs synthesized from glucose and lysine exhibited significantly higher browning than their corresponding MRPs produced by glucose and glycine (Fig. 2). This further confirms that type of amino acid is an important in Maillard reaction.

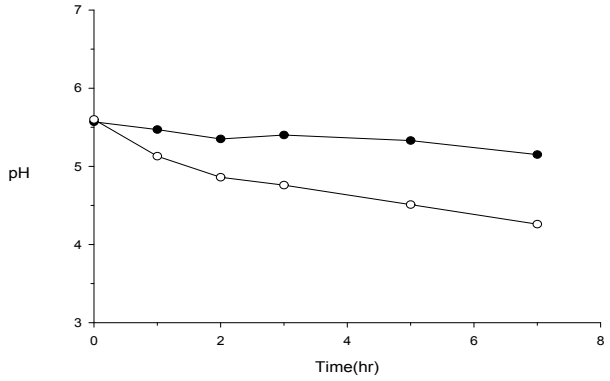

Fig. 1. Effect of reaction time on pH of Maillard reaction products. glucose+lysine (-○-); glucose+glycine (-●-).

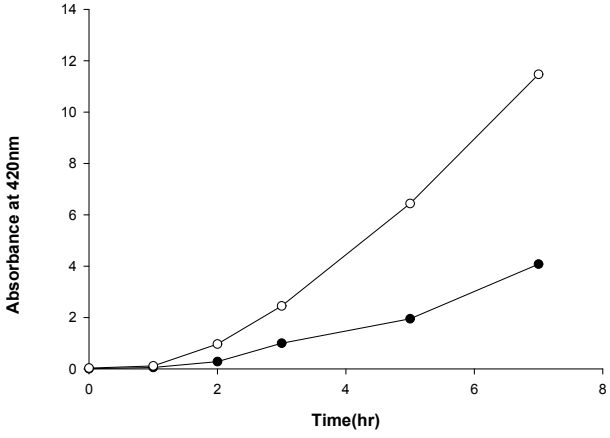

Fig. 2. Effect of reaction time on brown color of MRPs. glucose+lysine (-○-); glucose+glycine (-●-).

3. Effect of Maillard reaction products on burdock PPO

Fig. 3 shows the effect of varying pyrocatechol concentration on burdock PPO activity in the absence or presence of MRPs from glucose and lysine. The enzyme activity of burdock PPO was increased with increasing pyrocatechol concentration. As shown in Fig 3A, the enzyme activity of burdock PPO was inhibited in the presence of MRPs. With increasing substrate concentration, the enzyme activity in the presence of MRPs was not fully active suggesting non-competitive inhibition by MRPs. Fig 3B shows the Lineweaver-Burk plot of burdock PPO in the presence or absence of MRPs from glucose and lysine. Since plots of 1/v versus 1/[S] in the presence of MRPs intersect at the same point on the horizontal axis, which is $-1/K_m$, the MRPs are non-competitive inhibitor against burdock PPO. This result coincides well with previous reports, which suggests non-competitive inhibition of MRPs against potato PPO (Lee & Park 2005). The K_m value of burdock PPO with pyrocatechol as a substrate was 16.0 mM as shown in Fig. 3B. The rate of inhibition of burdock PPO activity was increased with increasing added MRPs from glucose and lysine (Fig. 4). Ames J (1992) reported that color formation during Maillard reaction is likely due both to the formation of low molecular weight compounds and to the presence of melanoidins with high molecular weight. Gomyo et al. (1972) explained that the browning intensities of melanoidins were directly related to degree of polymerization. Since neither glucose nor amino acid alone showed browning upon heating at 90℃, brown color that appeared upon heating glucose and amino acid mixture was a result from the Maillard reaction.

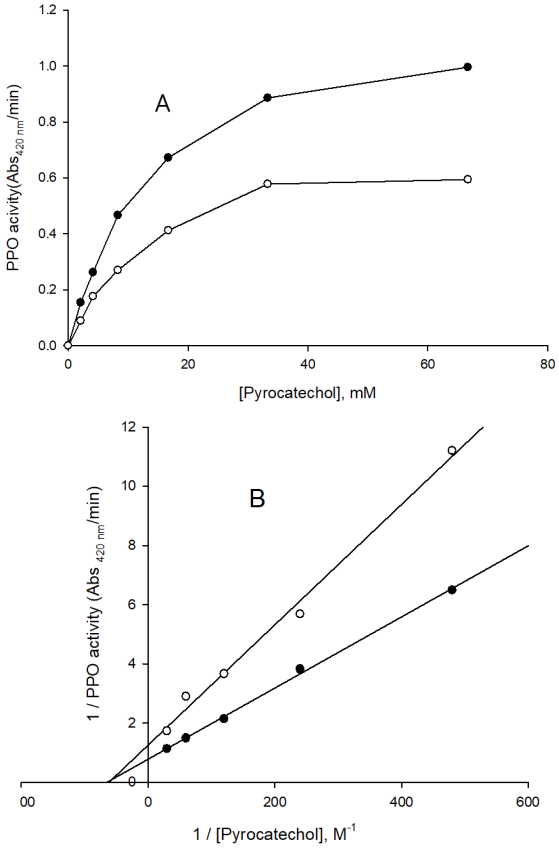

Fig. 3. Effect of pyrocatechol concentration on PPO activity of burdock extract in the presence or absence of MRPs (A), Lineweaver-Burk plots of burdock polyphenol oxidase in the presence of MRP (B). Pyrocatechol was used as a substrate. MRP was obtained by heating equal volumes of 1.0 M glycine and 1.0 M glucose at 90℃ for 5 h. Control (-○-); MRP (-●-).

4. Effect of MRPs on antioxidant activities

Since various MRPs were reported to have antioxidant activities, the MRPs prepared from glucose and glycine/lysine at 90℃ was evaluated and compared their antioxidant capacities. To evaluate the free radical scavenging properties of MRPs formed, various antioxidant capacities such as DPPH radical scavenging activity, ABTS radical scavenging activity as well as reducing power were determined. Fig. 5 shows the radical scavenging activity of MRPs such as DPPH radical scavenging activity, ABTS radical scavenging activity and reducing power. Fig. 5A illustrates the scavenging effect of MRPs synthesized from glucose/lysine or glucose/glycine on DPPH radical. DPPH is a stable free radical that accepts an electron to become a stable

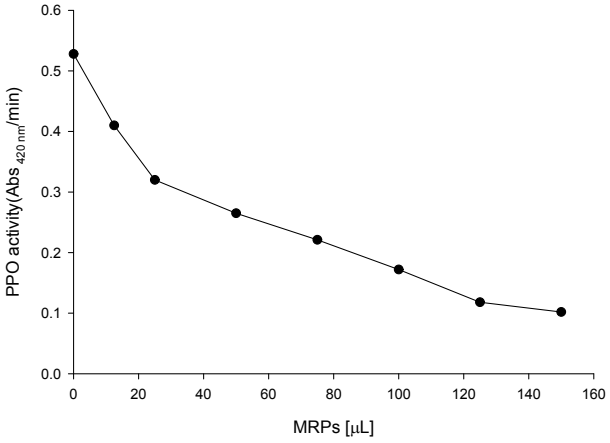

Fig. 4. Effect of added amount MRPs on polyphenol oxidase from burdock.

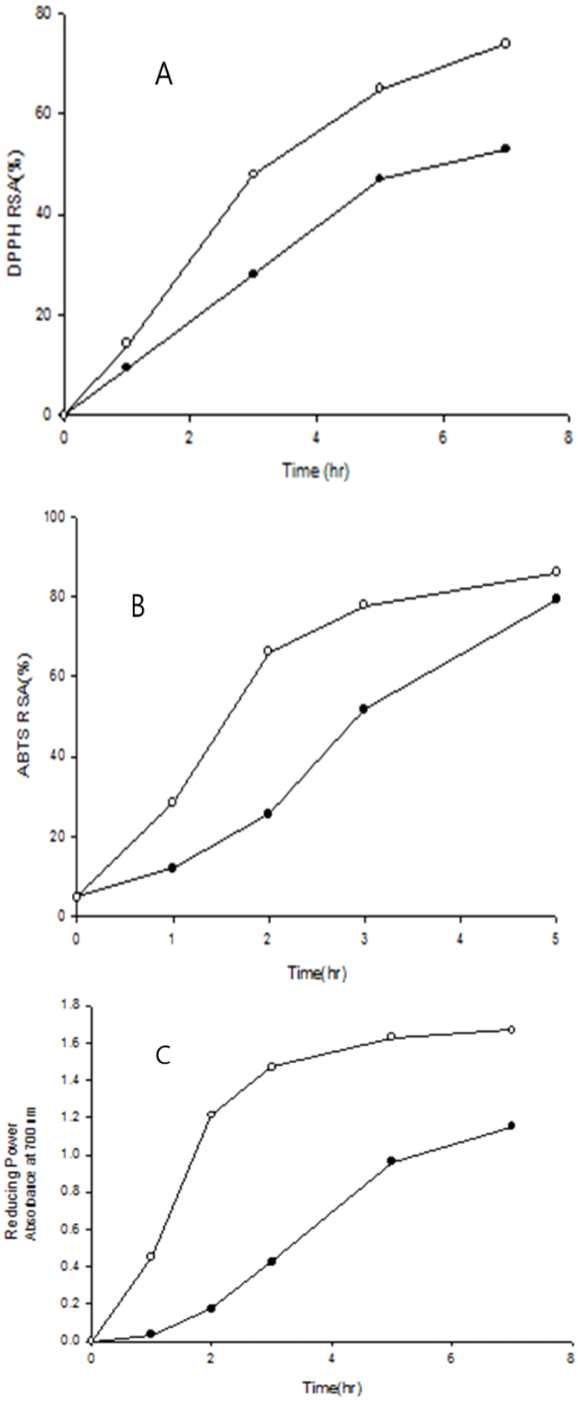

Fig. 5. Effect of MRPs on DPPH radical scavenging activity (A), ABTS radical scavenging activity (B) and reducing power (C). glucose+lysine (-○-) ; glucose+glycine (-●-).

molecule. The reduction in DPPH radical was measured by the change in its absorbance at 520 nm. This assay determines free radical scavenging activity of all antioxidants, including components of food products and plant and fruit extracts (Ak & Gulcin 2008). The degree of discoloration of the DPPH solution in the presence of MRPs indicated the radical scavenging capacity of MRPs. It manifested as a color change from purple to yellow on acceptance of a hydrogen atom from MRPs to form a stable DPPH-H molecule (Sharma & Bhat 2009). It has been found that both MRPs exhibited antioxidant effects. Fig. 5B shows the effect of heating time on ABTS radical scavenging activity of MRPs formed. Gallic acid was used as a positive standard for DPPH and ABTS radical scavenging activities. The MRPs prepared from glucose/lysine and glucose/glycine for 5 h exhibited 63% and 45% DPPH radical scavenging activities, which were obtained by 0.18 mM and 0.12 mM gallic acid. The MRPs from glucose/lysine and glucose/glycine for 5 h showed 78.2% and 51.0% ABTS radical scavenging activity, which corresponds to 0.68 mM and 0.40 mM gallic acid. The MRPs from glucose and lysine had higher gallic acid equivalent values in DPPH and ABTS radical scavenging activities than those from glucose and glycine.

The reducing power is an assay to determine the primary antioxidant activity of antioxidants obtained from components of food products and plant and fruit extracts. The antioxidant can reduce the ferric chloride/ferricyanide complex to its ferrous form. The ferrous form is monitored in a spectrophotometer by measuring the formation of blue color at 700 nm (Tafulo et al. 2010; Zeng et al. 2011). This assay particularly measures the antioxidant capacity of MRPs since the hydroxyl groups of MRPs play a role in the reducing activity through their redox potential of transferring electrons (Berker et al. 2010; Gu et al. 2010; Hwang et al. 2011). The MRPs from glucose and lysine had higher reducing power than those from glucose and glycine as shown in Fig. 5C.

Conclusion

From the results obtained in this research the author demonstrated that the MRPs produced from glucose and amino acid such as glycine or lysine had inhibitory effect on burdock PPO and antioxidant capacity such as DPPH radical scavenging activity, ABTS radical scavenging activity and reducing power. The MRPs synthesized from glucose and lysine had higher inhibitory effect on burdock PPO and antioxidant activity compared to those of glucose and lysine. The MRPs can inhibit burdock PPO by non-competitively. With increasing heating time, the production of MRPs was accordingly increased, which can be con-

firmed by determining color of MRPs spectrophotometrically at 420 nm. The inhibition of burdock PPO was higher with increasing added MRPs concentration. The antioxidant capacity of MRPs was dependent upon amino acid used to synthesize MRPs. The MRPs produced from lysine was more effective than those from glycine for antioxidant activities such as DPPH radical scavenging activity, ABTS radical scavenging activity and reducing power.

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