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Antioxidant activity of water-soluble Maillard reaction products

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Abstract

Maillard reaction products (MRP) results from a condensation reaction between amino acids (or proteins) and reducing sugars or lipid oxidation products, and MRP exhibit in vitro antioxidant activities. The objective of this study was to determine antioxidant activities of water-soluble MRP from the reaction between histidine (His) and glucose (Glu) by using the oxygen radical absorbing capacity ($ORAC_{PE}$) assay with phycoerythrine. Heating His–Glu mixture at 100 °C up to 30 min did not generate MRP with antioxidant activity. However, significant formation of MRP with $ORAC_{PE}$ values of 0.25, 0.43, and 0.44 μmol Trolox equivalent/mg reaction mixture was observed when the mixture was heated at 120 °C for 10, 20, and 30 min, respectively. Heating the mixture at 120 °C over 30 min reduced the peroxyl radical scavenging activity of the MRP, possibly due to the degradation of antioxidant MRP formed in the earlier stages of the reaction. In conclusion, MRP obtained from His–Glu mixture possesses peroxyl radical scavenging activity, and this activity can be quantified by the $ORAC_{PE}$ assay.

Keywords: Maillard reaction; ORAC; Antioxidant activity; Peroxyl radical scavenging activity; Glucose; Histidine

1. Introduction

The Maillard reaction, a well-known non-enzymatic browning reaction involving a reducing sugar and an amino acid, may produce colored or colorless reaction products depending on the stage of the reaction as well as other factors such as pH, type of reactants, temperature, water activity, etc. Condensation reactions between amino acids and lipid oxidation products may also form MRP, and the role of lipids in the Maillard reaction is similar to the role of reducing sugars (see Hidalgo & Zamora, 2000). A group of compounds in the final products of the reaction includes high molecular weight melanoidins, which are furan ring and nitrogen containing brown compounds. Little is known about their physical, chemical and physiological properties because of their complex structures. This complexity in MRP structures limits the determination of antioxidant activity for each compound in the whole group of MRP. Therefore, the recently developed $ORAC_{PE}$ assay can be used to determine the total antioxidant capacity of MRP formed during thermal processing of foods.

The Maillard reaction plays an important role in the production of quality bakery products. Color of bread crust and pasta products is determined particularly by MRP. Dehydrated food, baked and grilled meat products, and thermally processed foods may contain various levels of MRP. Since various factors such as type

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; DPPH, 2,2-diphenyl-1-picrylhydrazyl; Fru, fructose; Glu, glucose; Gly, glycine; His, histidine; HMF, 5-hydroxymethyl-2-furaldehyde; Lac, lactose; Lys, lysine; MRP, Maillard reaction products; $ORAC_{PE}$, oxygen radical absorbing capacity with β-phycoerythrine; β-PE,β-phycoerythrine.

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of reactants (Wijewickreme, Kitts, & Durance, 1997), temperature, pH (Monti, Bailey, & Ames, 1998), water activity, intermediate products (Vasiliauskaite & Wedzicha, 1997) and availability of oxygen can strongly affect the formation and properties of the final reaction products, model systems have been studied more often than the actual food.

MRP, especially melanoidins, have been reported to have antioxidant activity through scavenging oxygen radicals or chelating metals (Table 1). MRP from histidine had the highest antioxidant activity determined by conjugated diene formation from peroxidation of linoleic acid among MRP from either dipeptides of histidine–phenylalanine or lysine–alanine, amino acids histidine, lysine, or ascorbic acid when glucose was used as a reducing sugar (Reische, 1994). Compounds in the MRP with amino reductone structures may have both antioxidant and pro-oxidant activities (Pischetsrieder, Rinaldi, Gross, & Severin, 1998) depending on the reaction conditions. MRP obtained from heated histidine and glucose exhibit copper ion binding ability in oil/water mixtures (Bersuder, Hole, & Smith, 2001).

The oxygen radical absorbance capacity assay can be used to quantify the antioxidant capacity of foods by measuring peroxyl radical scavenging activity of the compounds present (Cao & Prior, 1999). This assay is based on the chemical damage to β-PE caused by a peroxyl radical producing compound (i.e. 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) in this assay), reducing the fluorescence emission of β-PE. The presence of antioxidants in the medium prevents the damage and prolongs the fluorescence emission. Antioxidant capacity of foods can be quantified by measuring the area under the relative fluorescence intensity vs. time curves (Cao & Prior, 1999). Antioxidant activities of foods are calculated in terms of Trolox equivalents. This indication provides the means of comparison among the antioxidant activities of numerous foods.

The objective of this study was to determine the antioxidant activity of water-soluble MRP from glucose–histidine mixture by means of $ORAC_{PE}$ assay.

2. Materials and methods

2.1. Production of MRP

L-Histidine hydrochloride monohydrate and D-(+)-glucose were purchased from Sigma Chemical Co. (St. Louis, MO). Histidine and glucose were mixed (1:2 ratio), and approximately 30% (w/v) deionized water was added to the mixture in screw-capped tubes. The screw cap was kept loose to expose the reaction mixture to air while heating in a vegetable oil bath at either 100 °C for 10, 20, 30, and 60 min or 120 °C for 5, 10, 20, 30 and 60 min simulating conditions on baking. Then the screw cap was tightened, and tubes were kept at −18 °C until extraction. pH was not controlled during the production of MRP. Triplicates of the experiment were run.

2.2. Extraction of antioxidant MRP

Deionized water (40 ml/g mixture) was added to the mixture and vortexed until MRP dissolved. The mixture was centrifuged at 26,000*g* for 30 min at 4 °C. Supernatants were collected and subjected to $ORAC_{PE}$ assay. At least two $ORAC_{PE}$ assays were done on each replicate of the treatments.

Table 1
Antioxidative properties of Maillard reaction products obtained from model systems reported in the literature

Model system Sugar–amino acid	Mode of antioxidative property	Reference
Glu–His	Copper chelator	Bersuder et al. (2001)
	Oxygen radical scavenger	Lingnert et al. (1983)
	Peroxyl radical scavenger	Lingnert and Eriksson (1980a,b)
Glu–Lys	Copper chelator	Wijewickreme et al. (1997), Dittrich et al. (2003), Wijewickreme and Kitts (1998)
	DPPH radical scavenger	Morales and Jimenez-Perez (2001)
	Peroxyl radical scavenger	Bressa et al. (1996)
	Hydroxyl radical scavenger	Wijewickreme et al. (1999)
Glu–Gly	Copper chelator	Dittrich et al. (2003)
	Peroxyl radical scavenger	Wagner et al. (2002)
	Hydroxyl radical scavenger	Yoshimura et al. (1997)
	Fe^{2+} chelator	Yoshimura et al. (1997)
Fru–Lys	Copper chelator	Wijewickreme et al. (1997),Wijewickreme and Kitts (1998)
	Hydroxyl radical scavenger	Wijewickreme et al. (1999)
Lac–Lys	Peroxyl radical scavenger	Monti et al. (1999)

2.3. Color

Absorbances of appropriate dilutions of MRP solutions (to give absorbance values <2) were measured at 420 nm for melanoidins with a Spectronic® Genesys2 spectrophotometer (Rochester, NY).

2.4. $ORAC_{PE}$ assay

We adapted the $ORAC_{PE}$ assay developed by Cao and Prior (1999) to quantify antioxidant capacity of MRP. Our assay differed from the Cao and Prior (1999) assay primarily in the luminescence spectrometer used. β-Phycoerythrine (β-PE) was purchased from Cyanotech Co. (Kailua–Kona, HI) (Lot #0215100) and prepared according to the supplier-recommended reconstitution procedure for purification. Briefly, the vial (1 mg β-PE/0.2 ml buffer mixture) was rinsed with about 3.5 ml of phosphate buffer (Stock buffer/deionized water, 1:9, v/v) (Stock buffer; 0.75 M K_2HPO_4/0.75 M NaH_2PO_4, 61.6:38.9, v/v). Prior to passing the solution through the column, a Sephadex G-25 column was cleaned with about 20 ml phosphate buffer. The red band eluted from the column was collected, and washed off with buffer. The purity of the β-PE solution was determined according to the supplier-recommended procedure. β-PE was diluted further with phosphate buffer. 2,2′-Azobis(2-amidinopropane)dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA), and dissolved in phosphate buffer (320 mM). This stock solution of AAPH was prepared daily and kept in ice until used.

A LS-50B Luminescence Spectrometer (Perkin–Elmer, UK) was used for the analyses. 'A four position, motor driven, water thermostatted, stirred cell holder' (Perkin–Elmer, UK) was installed on the spectrometer, and the temperature of the water bath, which supplied hot water to the cell holder, was set to 37 °C. Emission and excitation wavelengths were 565 and 540 nm, respectively. The stirrer was set to low.

About 50 μl of β-PE in phosphate buffer was incubated at 37 °C for 5 min in the cuvettes located in the holder, and then appropriate aliquot sample (50–100 μl) was added to the cuvettes. The reaction was started by the addition of 150 μl of AAPH (final concentration 24 mM) to the cuvettes at 5 min, and initial reading was taken. Total volume in each cuvette was 2 ml. Blanks, which contained phosphate buffer, β-PE and AAPH only, were used for the area corrections. β-PE fluorescence intensities of samples and blanks were recorded every minute. The changes in β-PE fluorescence over time were displayed on the computer screen. The data were collected until the fluorescence intensity was reduced to 5% of the initial reading. Intensities were normalized by dividing with intensity at the time of AAPH addition. The areas under the normalized intensity were calculated using the software supplied by the spectrofluorometer manufacturer.

Trolox, a water-soluble analog of vitamin E, was purchased from Aldrich Chem. Co. (Milwaukee, WI). Trolox solution was prepared weekly and kept at refrigeration temperature. Standard curves were obtained using known concentrations of Trolox (0.5–3 μmol final concentration in cuvette). Corrected areas of relative fluorescence intensities versus Trolox concentrations were plotted. The Trolox equivalents of the samples were calculated by using the linear portion of the Trolox standard curve.

2.5. Statistical analysis

Significant differences among the treatment combinations were obtained using the statistical software (SAS Institute, NC). Separation of means for significant differences was conducted using Duncan's.

3. Results and discussion

The appearance of pre-melanoidins and the formation of melanoidins were monitored by the absorbance values of diluted MRP solutions at 420 nm (Wijewickreme et al., 1997). Corrected absorbances were obtained by multiplying absorbance values of diluted MRP solutions by their respective dilution factors. Corrected absorbances data revealed that formation of melanoidins increases as temperature and heating time increased (Fig. 1). However, 1 h heating at 120 °C reduced the absorbance values (Fig. 1(a)). In general, the color of MRP solutions was a darker brown at 120 °C compared to 100 °C; however, heating at high temperature for a long time degraded antioxidant MRP formed in the early stages of the reaction. This change was reflected by a reduction in the corrected absorbances. Melanoidin formation was affected by the 1 h heating at 120 °C. From Fig. 1(a) and (b), the appearance of complex melanoidins was faster at 120 °C than at 100 °C.

MRP protected β-PE from loss of fluorescence intensity caused by peroxyl radicals from AAPH (Fig. 2). Trolox, a water-soluble vitamin E analogue, was used as the standard to quantify the antioxidant activity per Cao and Prior (1999). A lag phase in inhibiting the oxidation of β-PE by AAPH is produced for Trolox because this antioxidant displays 100% inhibition over a period of time. Certain antioxidants like Trolox are reported to exhibit this lag phase (Cao & Prior, 1999). Area differences in the β-PE fluorescence intensity decay curves with and without Trolox were plotted against the Trolox concentrations to obtain the standard curve.

Histidine by itself showed peroxyl radical scavenging activity. The $ORAC_{PE}$ values of His ranged from 0.50 to 0.63 μmol of Trolox/mg His. However, neither heating

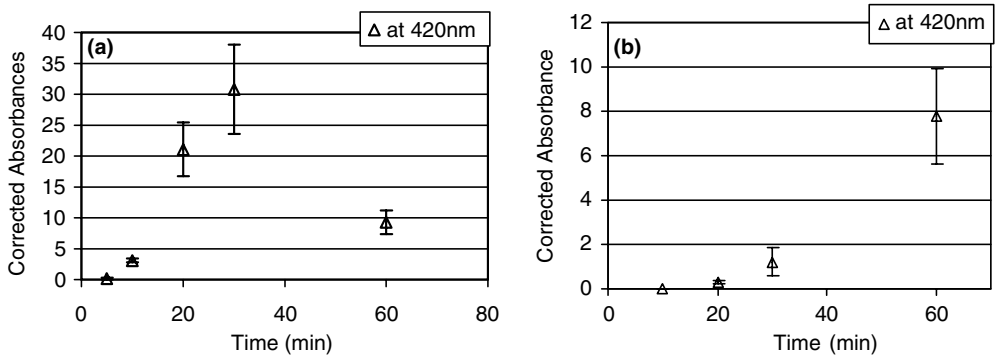


Fig. 1. Corrected absorbances (absorbance × dilution factor) and standard deviations of melanoidin formation in MRP solutions at 420 nm, which were obtained from a His–Glu model system heated for up to 1 h at 120 °C (a) and at 100 °C (b). Bars represent standard deviations.

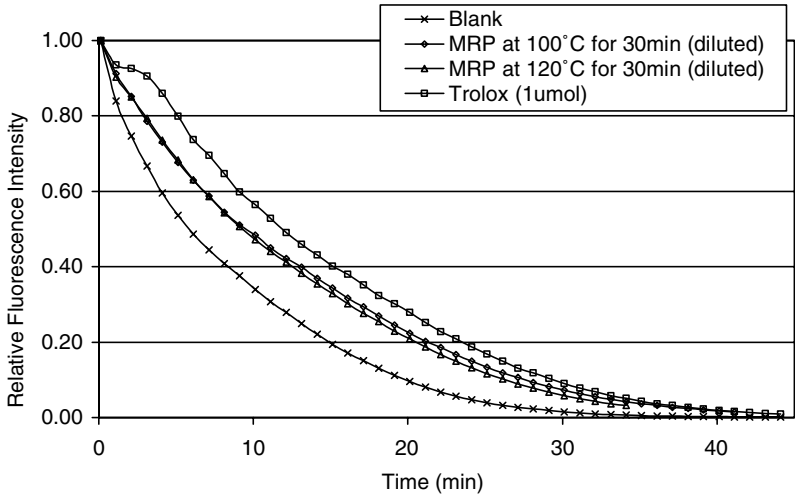


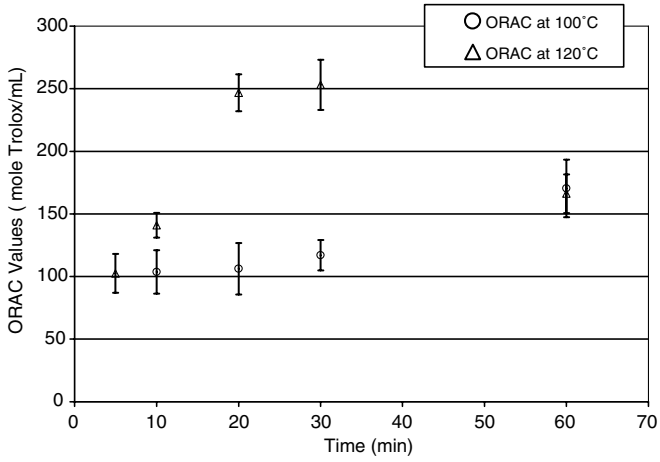
Fig. 2. Relative fluorescence intensity reduction of β-PE in phosphate buffer and in the presence of MPR and Trolox.

time nor temperature significantly affected $ORAC_{PE}$ values of His ($P > 0.05$). Glucose was also subjected to the $ORAC_{PE}$ assay, but it did not have antioxidant activity in $ORAC_{PE}$ assay.

$ORAC_{PE}$ values of MRP are shown in Fig. 3. $ORAC_{PE}$ values of MRP solutions per gram of mixtures did not significantly change when the mixtures were heated at 100 °C for 10, 20 or 30 min ($P > 0.05$). Although using DPPH (2,2-diphenyl-1-picrylhydrazyl), Murakami et al. (2002) revealed radical scavenging activities of bright color pigments from histidine and xylose heated at 30 °C up to 120 h, heating histidine–glucose mixture at 100 °C for 10, 20 or 30 min was insufficient to generate MRP with peroxyl radical scavenging activities. Heating the reaction mixture at 100 °C for 1 h, however, increased the $ORAC_{PE}$ significantly ($P < 0.05$) to about 0.3 mmol/g mixture. Statistical analyses showed that peroxyl radical scavenging activity of the mixture prepared at 120 °C for 5 min was similar to that of the mixture heated at 100 °C for either 10, 20 or 30 min ($P > 0.05$). Furthermore, the $ORAC_{PE}$ values of MRP heated <1 h at 100 °C was primarily from histidine. At 120 °C, $ORAC_{PE}$ values of MRP increased with heating time up to 30 min, then dropped after 1 h of heating. At 120 °C 30 min of heating approximately doubled the $ORAC_{PE}$ value of the MRP at 10 min.

Neither glucose nor histidine by themselves showed browning upon heating at either 100 or 120 °C for up to 60 min. Therefore, the brown color (from light to dark brown) that appeared upon heating glucose and histidine mixture was a result from the Maillard reaction. A study on the effect of caramelization on the Maillard browning reaction revealed that heating glucose at 100 °C at pH 6 or 8 showed little or no browning while the browning of glucose and lysine mixture increased at pH 8 (Ajandouz & Puigserver, 1999).

Yoshimura, Iijima, Watanabe, and Nakazawa (1997) reported that heating glucose and glycine at around 100 °C for 1–6 h produced compounds that are able to scavenge hydroxyl radicals and superoxide anion (O_2^-) and to chelate with Fe^{2+}. Our results showed that glucose and histidine also produce MRP with oxygen radical scavenging activity. When tested with $ORAC_{PE}$ assay, antioxidant activity of MRP from this model system



$ORAC_{PE}$ (μmol Trolox) MRP/g mixture

Time (min)	5	10	20	30	60
100°C	-	181.9[d]	186.4[d]	205.6[cd]	299.1[b]
120°C	180.1[d]	247.5[cb]	433.3[a]	444.3[a]	291.8[b]

Superscripts with different letters across the table show significant differences at α=0.05.

Fig. 3. Peroxyl radical scavenging activities of MRP per ml of solution in the graph and per g of initial mixture in the table below. Bars represent standard deviations.

was >400 μmol TE/g mixture upon heating at 120 °C for up to 30 min.

Wagner, Derkits, Herr, Schuh, and Elmadfa (2002) reported that MRP formed by heating glucose and glycine at 120 °C exhibit higher antioxidant activity in hydrophilic matrices than lipophilic matrices. The authors also found that addition of those MRP to coconut fat prior to frying at 200 °C was ineffective for extending the shelf life. In our research, results indicated that water soluble MRP from a model system of glucose and histidine also exhibit antioxidant activity, and further heating reduces the antioxidant activity of MRP.

Prior et al. (1998) reported that the estimated antioxidant intake in terms of $ORAC_{PE}$ ranges from 1.2 to 1.7 mmol TE/day in the US. Strawberry, plum, red grape (whole), and orange has $ORAC_{PE}$ values of 154, 80, 36 and 52 μmol TE/g dry matter, respectively (Wang, Cao, & Prior, 1996). Red raspberries have $ORAC_{PE}$ values ranged from 91 to 115 μmol TE/g dry matter (Wang & Lin, 2000). $ORAC_{PE}$ values of blueberries depend on variety but the range was reported to be from 63 to 282 μmol TE/g dry matter (Prior et al., 1998). $ORAC_{PE}$ values of MRP from histidine–glucose model system ranged from 250 to 450 mmol/g His–Glu mixture; therefore, MRP formed during the thermal processing of foods may have a beneficial effect on the total dietary antioxidant intake for humans. In our study, we demonstrated that antioxidant activity of MRP could be quantified by a relatively new $ORAC_{PE}$ assay compared with other methods. $ORAC_{PE}$ assay can be successfully used to determine the effect of dietary MRP on total antioxidant intake in the human diet.

MRP formed during baking can show antioxidant activity that inhibits the lipid oxidation. Lingnert (1980) successfully showed that addition of histidine and glucose to cookie dough inhibited the oxidation of lipids in cookies. In our study, MRP from heated glucose and histidine are able to scavenge peroxyl radicals produced by AAPH. On the other hand, undesired compounds like carcinogens or mutagens (Gazzani, Vagnarelli, Cuzzoni, & Mazza, 1987) can form at high temperatures from mixtures of amino acid or peptides and sugars in overheated products, and could be a problem for human health. Moreover, acrylamide, a carcinogen and neurotoxin for humans, can form when foods high in carbohydrate are fried, baked, or roasted at high temperatures (EC, 2002). Therefore, further research is needed in order to counteract the detrimental effects of excessively heated MRP on health while taking advantage of their in vitro antioxidant activities.

4. Conclusions

In this study, we showed that MRP from heated histidine and glucose have peroxyl radical scavenging activity, an indicator of antioxidative activity in vitro. $ORAC_{PE}$ assay makes the quantification of antioxidant activity of MRP in food products possible. This advantage of $ORAC_{PE}$ assay could be useful for future determinations of the contribution of non-enzymatic browning reaction products with antioxidant activity to total antioxidant intake in the human diet. However, precaution is needed since there is a possibility of the

formation of carcinogens, mutagens, or toxic substances at elevated temperatures during food processing.

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