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Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time

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ABSTRACT

The purpose of this study was to evaluate the antioxidant activity of Maillard reaction products (MRPs) derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. The pH of MRPs derived from the Glu-G model system decreased markedly as the heating time increased, while MRPs derived from the Glu-Di model system showed the highest increase in absorbance at 420 nm. MRPs derived from the Glu-G model system showed the highest cupric ion chelating ability, while MRPs derived from the Glu-Di model system had the highest ferrous ion chelating activity. MRPs derived from the Glu-Di model system were found to be effective antioxidants in different in vitro assays with regard to the 2,2′-azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activities and ferric reducing/antioxidant power (FRAP).

1. Introduction

The Maillard reaction involved in the formation of brown pigments comprises the condensation between a carbonyl group of reducing sugars, aldehydes or ketones and an amine group of free amino acids (such as amino acids, peptides and proteins) or any nitrogenous compound. The Maillard reaction is a complicated reaction that produces a large number of the so-called Maillard reaction products (MRPs) such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds named melanoidins (Wijewickreme, Kitts, & Durance, 1997). The MRPs produced from an amino acid-sugar model system have been associated with the formation of compounds with strong antioxidant activity (Yen & Hsieh, 1995; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997).

Oligopeptides have been extensively isolated and identified in both natural and artificial protein hydrolysates of foods such as seafood, coffee beans, soy, and wheat gluten (Aaslyng et al., 1998; Ludwig, Lipke, Raczek, & Jäger, 2000). They have been recognised as precursors of the Maillard reaction, which leads to the colour of processed foods (Aaslyng et al., 1998). Moreover, these oligopeptides, as a final product of food hydrolysis (mostly milk, cheese, and wine), are known to act as very good antioxidants, anticoagulants, and blood pressure reducers (Hartman & Meisel, 2007).

Although a wide range of peptides has been reported in considerable quantity in many food systems such as aged sake, meat, and hydrolysed vegetable protein, the only a few studies investigated the Maillard reaction products form from oligopeptides (Oh, Hartman, & Ho, 1992; Oh, Shu, & Ho, 1991). In addition, the antioxidant activity of Maillard reaction products from peptides and on peptide degradation is still unknown. Therefore, the objective of this study was to evaluate the antioxidant activity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time.

2. Materials and methods

2.1. Materials

D-Glucose, glycine, diglycine, triglycine, ferrous chloride, 1, 1-diphenyl-2-picryl-hydrazil (DPPH), (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) Trolox, 2,2′-azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS), pyridine, pyrocatechol violet and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ)were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless mentioned otherwise.

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2.2. Preparation of Maillard reaction products (MRPs)

D-Glucose (0.2 M) and 0.2 M each of glycine, diglycine, and triglycine were dissolved in 90 ml of deionized water. The pH of the solution was adjusted to 7.8 with 6 N NaOH. The solution was then brought to a final volume of 100 ml with deionized water. The solutions (10 ml) were then transferred to 25 ml screw-sealed tubes (Pyrex®, USA), tightly capped and heated in a thermostatic oil bath at 100 °C. The samples were taken out after heating for 10, 60, 120, 180, and 240 min. After the heating period, the tubes were immediately cooled in ice-water. Part of the MRP samples was used directly for final pH measurements, while the remainder was kept at 4 °C until analysed. All model systems were prepared in triplicate.

2.3. Measurement of pH

The pH was measured using Corning 440 pH metre (Corning Life Sciences, New York, USA) calibrated with buffer solution of pH 4.0 and 10.0, respectively.

2.4. Measurements of browning

The browning of MRPs with 5-fold dilution was measured using a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan) at 420 nm, as an index of the brown polymers formed in more advanced stages.

2.5. Metal ions chelating activity

The ability of MRPs to chelate the prooxidative transitional metal ions Cu^{2+} and Fe^{2+} was investigated according to respectively, Wang and Xiong (2005) and Dinis, Madeira, and Almerida (1994) with slight modifications. For the Cu^{2+} chelation experiment, 1 ml of 2 mM $CuSO_4$ was mixed with 1 ml of pyridine (pH 7.0) and 20 μl of 0.1% pyrocatechol violet. After the addition of 1 ml of MRP samples with 5-fold dilution, the disappearance of the blue colour, due to dissociation of Cu^{2+}, was recorded by measuring the absorbance at 632 nm with a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan) at 5 min of the reaction. For the Fe^{2+} chelation experiment, 100 μl of MRP samples with 5-fold dilution was added with 600 μl of distilled water and 100 μl of 0.2 mM $FeCl_2 \cdot 4H_2O$. The mixture was allowed to rest at room temperature for 30 s. The reaction mixture thus obtained was later added with 200 μl of 1 mM ferrozine and changes in colour were monitored at 562 nm with a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan), after a 10 min resting time at room temperature. The Cu^{2+} and Fe^{2+} chelating activity were calculated as [1 – (sample solution absorbance/blank solution absorbance)] × 100.

2.6. DPPH radical scavenging activity

The free radical scavenging activity of MRPs were determined by the 1, 1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$). This activity was measured by the procedure described by Yen and Hsieh (1995) wherein the bleaching rate of a stable free radical, $DPPH^{\cdot}$ is monitored at a characteristic wavelength in the presence of the sample. In its radical form, $DPPH^{\cdot}$ absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.12 mM solution of $DPPH^{\cdot}$ in methanol was prepared daily and protected from light. An aliquot of 2 ml of this solution was added to 80 μl of MRP samples with 5-fold dilution and 320 μl of distilled water. The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of mixtures was measured at 517 nm using a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan). The antiradical activity of sample was expressed as percentage of disappearance of the initial purple colour. Aqueous solutions of trolox at various concentrations were used to perform the calibration curves (0.15–1.15 mM).

2.7. Antioxidant capacity by ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was done according to the modified Benzie and Strain method (1996) with some modifications. Briefly, 900 μl of FRAP reagent, freshly prepared and warmed at 37 °C, were mixed with 90 μl distilled water and either 30 μl of MRP samples with 5-fold dilution or standard or appropriate reagent blank. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$, plus 25 ml 0.3 mM acetate buffer pH 3.6. Readings at the absorption maximum (595 nm) were taken every 15 s using a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan). Temperature was maintained at 37 °C. The readings at 30 min were selected for calculation of FRAP values. Calibration was performed, as described previously, with trolox stock solution.

2.8. ABTS radical cation decolourization assay

The spectrophotometric analysis of $ABTS^{\cdot+}$ radical scavenging activity of MRPs was determined according to the method described by Re et al. (1999) with slight modifications. This method is based on the reaction between ABTS and potassium persulfate giving blue/green ABTS radical ($ABTS^{\cdot+}$). With the addition of the antioxidants, decolourization is attained and measured spectrophotometrically at 734 nm. The results were expressed as mM trolox per gram of melanoidins. Briefly, $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature during 12–16 h before use. The $ABTS^{+\cdot}$solution (stable for two days) was diluted with 5 mM phosphate buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. For the photometric assay, 3 ml of the $ABTS^{\cdot+}$ solution and 30 μl of MRP samples were mixed for 45 s and measured immediately after 5 min at 734 nm (absorbance did not change significantly up to 10 min). Calibration was performed, as described previously, with trolox stock solution.

2.9. Statistical analysis

All experimental data were analysed by analysis of variance (ANOVA) and significant differences among means from triplicate analysis at ($P < 0.05$) were determined by Duncan's multiple range tests using the statistical analysis system (SPSS 12.0 for windows, SPSS Inc, Chicago, IL).

3. Results and discussion

3.1. Changes in the pH

The changes in the pH of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time are shown in Fig. 1. The pH of MRPs derived from the Glu-Di and Glu-Tri model systems with the same initial pH decreased markedly from their initial values within the first 10 min. Thereafter, the pH decreased slightly until 240 min of the heating time ($P < 0.05$). However, the pH of MRPs derived from the Glu-G model system decreased gradually as the heating time increased up to 240 min. Among the peptides tested, glycine rendered a high-

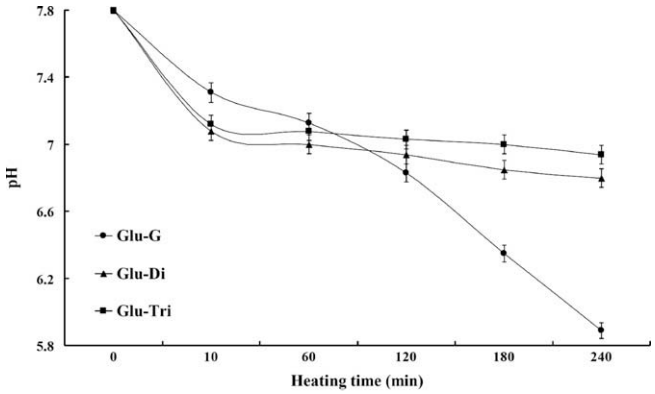

Fig. 1. The changes in the pH of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. Bars indicate the standard deviation from triplicate determinations.

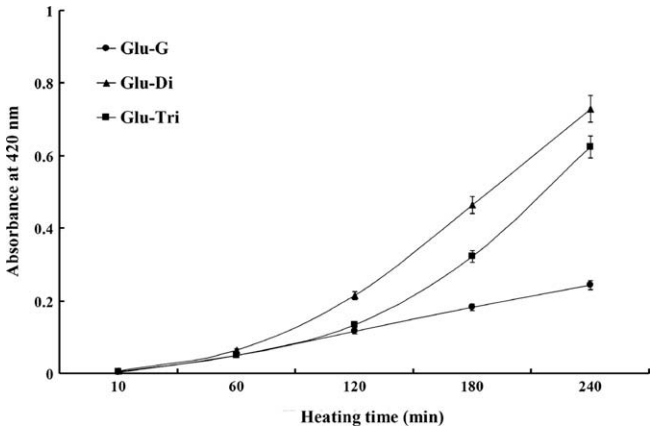

Fig. 2. The changes in the browning intensity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. Bars indicate the standard deviation from triplicate determinations.

er pH of MRPs than did diglycine and triglycine, up to 60 min of the heating time. After 60 min, triglycine rendered the highest pH of MRPs. The result was in accordance with the result of studies conducted by Morales and Jimenez-Perez (2001) and Benjakul, Lertittikul, and Bauer (2005). They found that during the Maillard reaction, the pH frequently decreases as the heating time increases. In the Maillard reaction, the pH reduced due to the formation of organic acids such as formic and acetic acid (Brands & Van Boekel, 2002). Ogura, Nakayama, Nakaoka, and Nishihata (2000) reported that formation of the acid form of glycine or glycil–glycine was attributed to the lowering of pH which occurred because oxygen evolution occurs in further positive polarisation. Huber and Wächtershäuser (1998) reported that the lowering of the pH can be explained by the formation of acids from CO during the heating process of peptide. In addition, some authors reported that during the reaction of peptides with carbonyl compounds, peptide bond breaking occurred through an anionic reaction (Chuyen, Kurata, & Fujimaki, 1973). However, for pH values above the isoelectric point of the amino compound (5.57 for glycine, 5.59 for diglycine and 5.58 for triglycine), the concentration of the anionic form are not a limiting factor (Reynols, 1969, chap. 12). Besides, it is expected that the molecule size could play a role in the smaller reactivity of peptides as compared to glycine (Buera, Chirife, Resnik, & Lozano, 1987). Therefore, the result suggested that the difference in the pH of MRPs was might be due to hydrolysis of the peptide bond and molecule size of peptides.

3.2. Changes in the browning intensity

Brown colour development (A 420 nm) is the easiest measurable consequence of the Maillard reaction because it offers a visual estimate. Its intensity is often used as an indicator of the extent to which the Maillard reaction took place in foods and it symbolizes an advanced stage of the Maillard reaction (Morales and Jimenez-Perez, 2001). The changes in the browning intensity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time are shown in Fig. 2. From the result, a sharp increase in A_{420} of all MRP samples was observed for up to 240 min of the heating time ($P < 0.05$). The MRPs derived from the Glu-Di model system showed the highest increase in absorbance at 420 nm, followed by those derived from the Glu-Tri and Glu-G model systems. This result was in agreement with that of Lu, Hao, Payne, and Ho (2005), who reported that diglycine–glucose reaction mixtures have a higher degree of browning, followed by glycine–glucose, and triglycine–glucose reaction mixtures. Moreover, the UV absorbing and colourless compounds formed at the intermediate stages contributed to the brown pigment formation in both the Maillard and the caramelization reactions (Benjakul et al., 2005). Buera et al. (1987) found that peptides promote more browning than related amino acids. In addition, Ivanov, Ivanov, Simeonova, and Mirkova (1983) reported that the hydrolyzability of peptides increases with the lengths of the peptide chin. The result suggested that the stability of peptide bond for triglycine was higher than those of diglycine. Moreover, the degree of browning was might be due to hydrolyzability of the peptides.

3.3. Changes in the metal ion chelating activity

MRPs are known metal chelators (Delgado-Andrade, Seiquer, & Navarro, 2004), and their metal ion binding affinity has been proposed as a possible mechanism for their antioxidant activity (Jing & Kitts, 2004) because transition metals, especially iron and copper, are implicated in the generation of free radicals by Fenton reactions. The changes in the metal ion chelating activity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time are shown in Fig. 3. The cupric ion chelating ability of all MRP samples increased and then decreased as a function of heating time. MRPs derived from the Glu-G model system showed a higher cupric ion chelating ability than those derived from the Glu-Di and Glu-Tri model systems. However, all MRP samples showed a cupric ion chelating effect below 15%. In contrast, the ferrous ion chelating activity of all MRP samples was much higher than the cupric ion chelating ability. This

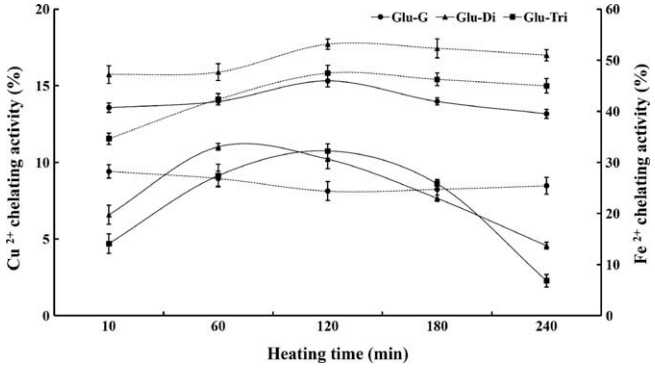

Fig. 3. The changes in the metal ion chelating activity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. Bars indicate the standard deviation from triplicate determinations (solid line: Cu^{2+} chelating ability, dotted line: Fe^{2+} chelating ability).

result is consistent with the findings of Ruiz-Roca, Navarro, and Seiquer (2008), who reported that MRPs had a stronger iron-chelating ability than copper- chelating ability. MRP samples showed a high ferrous ion chelating ability, ranging from 24% to 53% as a function of heating time. The ferrous ion chelating activity of MRPs derived from the Glu-Di and Glu-Tri model systems increased up to 120 min of heating time. Thereafter, the chelating activity slightly decreased up to 240 min of heating time ($P < 0.05$). However, the ferrous ion chelating ability of MRPs derived from the Glu-G model system decreased up to 120 min of heating time and then increased slightly. Moreover, the ferrous ion chelating activity of MRPs derived from the Glu-Di model system was the highest, followed by the Glu-Tri and the Glu-G model systems. There is now strong evidence that MRPs have the ability to bind transition metals, including copper (O'Brien and Morrissey, 1997; Wijewickreme et al., 1997). Previously, other studies have shown the ability of different sugar-amino acid model MRPs to chelate iron (Yoshimura et al., 1997), and equations for the effect of melanoidins on the chelation of iron have been proposed (Morales, Fernandez-Fraguas, & Jiménez-Pérez, 2005). In the study of Morales et al. (2005), no relationship was found between the browning and iron-binding ability of MRPs. This observation was in agreement with the present study (Figs. 2 and 3). The specific affinity of MRPs to chelate copper has been described in several studies (O'Brien and Morrissey, 1997). Seifert, Krause, Gloe, and Henle (2004) studied the complex formation of some MRPs (Nε-fructoselysine and Nε-carboxymethyllysine) with copper and zinc and reported the formation of moderately stable copper complexes, but no complex formation with zinc was observed. Thus, it was concluded that new copper-binding centres are formed on the glycation of proteins. Moreover, the chelation of copper has been shown to be related to the antioxidant activity in model MRPs (Wijewickreme & Kitts, 1998) and in other compounds such as hydrolysed potato protein (Wang and Xiong, 2005). Therefore, this result indicated that MRPs had different chelating activities according to the heating time and peptide chain length. Specifically, MRPs had a stronger iron-chelating ability than copper- ion chelating ability. The chelating ability might be related to the degree of hydrolysis of the peptide bond and the stability of this peptide bond with an increase in heating time. Furthermore, this result suggested that the peptide chain length might be one of the main factors that affect the chelating ability of MRPs derived from the Maillard reaction.

3.4. Changes in the DPPH radical scavenging activity

DPPH is a chromogen-radical-containing compound that can directly react with antioxidants. When the DPPH radical is scavenged by antioxidants through the donation of hydrogen to form a stable DPPH-H molecule, the colour is changed from purple to yellow (Shon, Kim, & Sung, 2003). Stable radical DPPH has been widely used for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials (Shih, Lai, & Jen, 2006). The changes in the DPPH radical scavenging activity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time are shown in Table 1. The DPPH radical scavenging activity of all MRP samples was increased as a function of heating time. This activity of MRPs derived from the Glu-Di (11.66–56.49) and Glu-Tri (10.89–55.25) model systems was markedly increased as a function of heating time ($P < 0.05$). In contrast, the activities of the MRPs derived from the Glu-G (10.58–27.37) model system were only slightly increased as a function of heating time, and the difference as a function of heating time was not significant ($P > 0.05$). The MRPs derived from the Glu-Di model system showed a higher DPPH radical scavenging activity than those derived from the Glu-G and Glu-Tri model systems; however, the difference between Glu-Di and Glu-Tri model systems was not significant ($P > 0.05$). This result indicates that MRPs have different free radical scavenging activities according to the heating time and peptide chain length. The result was also in agreement with those of Yen and Hsieh (1995), Morales and Jimenez-Perez (2001) and Benjakul et al. (2005) who found that MRPs had DPPH radical scavenging activity. Rufián-Henares and Morales (2007) reported a statistically significant linear relationship between the antioxidant activity measured with the DPPH assay and the iron-binding capability of melanoidins. In addition, Chen, Muramoto, Yamauchi, Fujimoto, and Nokihara (1998) proved that the size of the peptides could affect the antioxidant ability. Peptides are potentially better food antioxidants than amino acids due to their increased free radical scavenging activity, metal chelation, and aldehyde adduction activity (Zhou & Decker, 1999). Di- and tri-peptides show greater antioxidative activity than their constituent amino acids (Yamaguchi, Yokoo, & Fujimaki, 1975). Therefore, it was concluded peptide chain length was the most important factor that determines the antioxidant activity of MRPs.

Table 1
Antioxidant activity determined by the DPPH method.[a]

Heating time (min)	Samples		
	Glu-G[b]	Glu-Di[c]	Glu-Tri[d]
10	$10.58 \pm 1.23^{c,A}$	$11.66 \pm 1.22^{e,A}$	$10.89 \pm 2.08^{e,A}$
60	$13.64 \pm 2.49^{c,A}$	$20.31 \pm 2.21^{d,B}$	$18.92 \pm 1.43^{d,B}$
120	$18.25 \pm 2.59^{b,A}$	$32.66 \pm 2.34^{c,B}$	$30.77 \pm 1.91^{c,B}$
180	$22.23 \pm 2.00^{b,A}$	$45.65 \pm 1.28^{b,B}$	$43.32 \pm 2.15^{b,B}$
240	$27.37 \pm 2.77^{a,A}$	$56.49 \pm 0.93^{a,B}$	$55.25 \pm 1.64^{a,B}$

Different small superscripts (a–e) indicate significant as a function of the heating time in the same sample at $P < 0.05$ level.
Different capital superscripts (A–B) indicate significant differences among samples in the same heating time at $P < 0.05$ level.
[a] Data expressed as μM equivalents of trolox per 1 ml of MRPs.
[b] Glu-G, glycine with glucose.
[c] Glu-Di, diglycine with glucose.
[d] Glu-Tri, triglycine with glucose.

3.5. Changes in the FRAP assay

The FRAP assay measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. This assay is also commonly used for the routine analysis of single antioxidants and total antioxidative activity. The changes in the FRAP of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time are shown in Table 2. The FRAP of all MRP samples was increased as a function

Table 2
Antioxidant activity determined by the FRAP method.[a]

Heating time (min)	Samples		
	Glu-G[b]	Glu-Di[c]	Glu-Tri[d]
10	$37.32 \pm 1.87^{e,A}$	$37.59 \pm 1.88^{e,A}$	$36.23 \pm 1.81^{e,A}$
60	$44.42 \pm 2.22^{d,B}$	$51.52 \pm 2.58^{d,A}$	$45.24 \pm 2.27^{d,B}$
120	$59.43 \pm 2.97^{c,B}$	$70.90 \pm 3.55^{c,A}$	$62.98 \pm 3.15^{c,B}$
180	$75.27 \pm 3.77^{b,B}$	$103.11 \pm 5.16^{b,A}$	$83.82 \pm 4.20^{b,B}$
240	$87.28 \pm 4.37^{a,C}$	$134.23 \pm 6.72^{a,A}$	$114.03 \pm 5.70^{a,B}$

Different small superscripts (a–e) indicate significant as a function of the heating time in the same sample at $P < 0.05$ level.
Different capital superscripts (A–C) indicate significant differences among samples in the same heating time at $P < 0.05$ level.
[a] Data expressed as μM equivalents of trolox per 1 ml of MRPs.
[b] Glu-G, glycine with glucose.
[c] Glu-Di, diglycine with glucose.
[d] Glu-Tri, triglycine with glucose.

of heating time. The FRAP of MRPs derived from the Glu-Di model system was the highest as a function of heating time, followed by those derived from the Glu-Tri and Glu-G model systems; however, the difference between Glu-G and Glu-Tri model systems was not significant ($P > 0.05$), an exception being the 240 min of the heating time. The FRAP of MRPs derived from the Glu-Di (37.59–134.23) and Glu-Tri (36.23–114.03) model systems was markedly increased as a function of heating time ($P < 0.05$). In contrast, the FRAP of MRPs derived from the Glu-G (37.32–87.28) model system was only slightly increased as a function of heating time, and the difference was not significant. These results are in agreement with those obtained for the antioxidant activity determined by the DPPH radical scavenging assay (Table 1). Rufián-Henares and Morales (2007) pointed out that the ferric reducing ability of melanoidins was in parallel with the data from the DPPH method. Compounds responsible for reducing activity are formed during the thermolysis of Amadori products in the primary phase of Maillard reactions (Hwang, Shue, & Chang, 2001) or they could be formed as heterocyclic products of the Maillard reaction or caramelization of sugars (Charurin, Ames, & Castiello, 2002). The result revealed that MRPs could function as electron donors. The hydroxyl groups of MRPs play an important role in reducing activity (Yoshimura et al., 1997). Additionally, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of a hydrogen atom (Eichner, 1981). The strong reducing power of hydrolysed protein may be attributed to the increased availability of hydrogen ions due to peptide cleavages (Kong & Xiong, 2006). Besides, changes in size, level and small peptides affect the antioxidative activity (Wu, Chen, & Shiau, 2003). This result suggested that the peptide chain length might be one of the main factors that affect the reducing power of MRPs derived from the Maillard reaction.

3.6. Changes in the TEAC assay

The generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that has been applied for the measurement of the total antioxidative activity of solutions of pure substances, aqueous mixtures, and beverages. The method for the screening of antioxidative activity is reported as a decolourization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. A more appropriate format for the assay is the decolourization technique, in which the radical is directly generated in a stable form prior to the reaction with putative antioxidants. An improved technique for the generation of $ABTS^{\cdot+}$, which is described here, involves the direct generation of a blue/green $ABTS^{\bullet+}$ chromophore by the reaction between ABTS and potassium persulfate. The changes in the ABTS radical scavenging activity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time are shown in Table 3. The ABTS radical scavenging activity of all MRP samples was increased as a function of heating time. The ABTS radical scavenging activity of MRPs derived from the Glu-Di model system was the highest as a function of heating time, followed by those derived from the Glu-Tri and Glu-G model systems. The ABTS radical scavenging activity of MRPs derived from the Glu-Di (50.48–575.16) and Glu-Tri (60.57–484.35) model systems was markedly increased as a function of heating time ($P < 0.05$). The difference between Glu-G, Glu-Di, and Glu-Tri model systems was significant ($P < 0.05$), an exception being the 10 min of the heating time. In contrast, the activity of MRPs derived from the Glu-G (40.23–191.74) model system was only slightly increased as a function of heating time, and the difference was not significant. These results are in agreement with those obtained for the antioxidant activity determined by the FRAP assay (Table 2). However, the values obtained with the ABTS assay were higher than those obtained with the FRAP assay in each sample analysed. The difference in the antioxidant activity obtained with the FRAP and ABTS assays could be due to the different reaction mechanisms involved. The FRAP assay detects compounds that act only by the single electron transfer (SET) mechanism, whereas the ABTS assay detects compounds that act either by direct reduction via electron transfer or by radical quenching via the hydrogen atom transfer (HAT) mechanism (Prior, Wu, & Schaich, 2005). In addition, it is assumed that the difference in the radical scavenging activity is due to the different reaction media; aqueous and methanolic for ABTS and DPPH, respectively (Rufián-Henares and Morales, 2007).

Table 3
Antioxidant activity determined by the TEAC method.[a]

Heating time (min)	Samples		
	Glu-G[b]	Glu-Di[c]	Glu-Tri[d]
10	$40.23 \pm 10.10^{c,A}$	$50.48 \pm 5.05^{e,A}$	$60.57 \pm 6.06^{e,A}$
60	$50.48 \pm 5.49^{c,C}$	$141.29 \pm 14.13^{d,A}$	$111.02 \pm 11.10^{d,B}$
120	$111.02 \pm 22.21^{b,C}$	$282.55 \pm 14.91^{c,A}$	$232.10 \pm 23.21^{c,B}$
180	$151.38 \pm 30.28^{ba,C}$	$433.90 \pm 21.70^{b,A}$	$373.36 \pm 18.67^{b,B}$
240	$191.74 \pm 38.35^{a,C}$	$575.16 \pm 28.76^{a,A}$	$484.35 \pm 24.22^{a,B}$

Different small superscripts (a–e) indicate significant as a function of the heating time in the same sample at $P < 0.05$ level.
Different capital superscripts (A–C) indicate significant differences among samples in the same heating time at $P < 0.05$ level.
[a] Data expressed as μM equivalents of trolox per 1 ml of MRPs.
[b] Glu-G, glycine with glucose.
[c] Glu-Di, diglycine with glucose.
[d] Glu-Tri, triglycine with glucose.

4. Conclusion

The purpose of this study was to evaluate the antioxidant activity of MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. The pH of all MRP samples decreased considerably as the heating time increased. In particular, the pH of MRPs derived from the Glu-G model system decreased markedly as the heating time increased up to 240 min. However, MRPs derived from the Glu-Di model system showed the highest increase in absorbance at 420 nm, followed by those derived from the Glu-Tri and Glu-G model systems. MRPs derived from the Glu-G model system showed the highest cupric ion chelating ability; however, the cupric ion chelating ability of all MRP samples were below 15%. The ferrous ion chelating activity of all MRP samples were higher than the cupric ion chelating ability. Moreover, the ferrous ion chelating activity of MRPs derived from the Glu-Di model system was the highest. MRPs derived from the Glu-Di model system were found to be effective antioxidants in different in vitro assays with regard to the ABTS and DPPH radical scavenging activities, and FRAP. Therefore, the results have shown that the antioxidant activity of MRPs is greatly affected by the peptide chain length. The antioxidant activity of MRPs might be related to both the peptide chain length and the stability of the peptide bond as the heating time increases. Furthermore, these results suggest that the peptide chain length may be one of the main factors that affect MRP formation via the Maillard reaction. The results obtained here cannot be generalised, but they might be of considerable interest to predict the behaviour of peptides when they are used in food formulations.

References

Aaslyng, M. D., Martens, M., Poll, L., Nielsen, P. M., Flyge, H., & Larsen, L. M. (1998). Chemical and sensory characterization of hydrolyzed vegetable protein, a savory flavoring. *Journal of Agricultural and Food Chemistry, 46*, 481–489.

Benjakul, S., Lertittikul, W., & Bauer, F. (2005). Antioxidant activity of Maillard reaction products from a porcine plasma protein–sugar model system. *Food Chemistry, 93*, 189–196.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Brands, C. M. J., & Van Boekel, M. A. J. S. (2002). Kinetics modeling of reactions in heated monosaccharide–casein system. *Journal of Agricultural and Food Chemistry, 50*, 6725–6739.

Buera, M. D. P., Chirife, J., Resnik, S. L., & Lozano, R. D. (1987). Nonenzymatic browning in liquid model systems of high water activity: Kinetics of colour changes due to reaction between glucose and glycine peptides. *Journal of Food Science, 52*, 1059–1062.

Charurin, P., Ames, J. M., & Castiello, M. D. (2002). Antioxidant activity of coffee model systems. *Journal of Agricultural and Food Chemistry, 50*, 3751–3756.

Chen, H. M., Muramoto, K., Yamauchi, F., Fujimoto, K., & Nokihara, K. (1998). Antioxidant properties of histidine-containing peptides designed from peptide fragment found in the digests of a soybean protein. *Journal of Agricultural and Food Chemistry, 46*, 49–53.

Chuyen, N. V., Kurata, T., & Fujimaki, M. (1973). Studies on the reaction of dipeptides with glyoxal. *Agricultural and Biological Chemistry, 37*, 327–334.

Delgado-Andrade, C., Seiquer, I., & Navarro, P. (2004). Bioavailability of iron from a heat treated glucose-lysine model food system: Assays in rats and in Caco-2 cells. *Journal of the Science of Food and Agriculture, 84*, 1507–1513.

Dinis, T. C. P., Madeira, V. M. C., & Almerida, L. M. (1994). Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavenges. *Archives of Biochemistry and Biophysics, 315*, 161–169.

Eichner, K. (1981). Antioxidant effect of Maillard reaction intermediates. *Progress in Food and Nutrition Science, 5*, 441–451.

Hartman, R., & Meisel, H. (2007). Food-derived peptides with biological activity: From research to food applications. *Current Opinion in Biotechnology, 18*, 163–169.

Huber, C., & Wächtershäuser, G. (1998). Peptides by activation of amino acids with CO on (Ni, Fe)S surfaces: Implications for the origin of life. *Science, 281*, 670–671.

Hwang, J. Y., Shue, Y. S., & Chang, H. M. (2001). Antioxidative activity of roasted and defatted peanut kernels. *Food Research International, 34*, 639–647.

Ivanov, Ch. P., Ivanov, O. Ch., Simeonova, R. A., & Mirkova, G. D. (1983). A study of the interaction of glycine and its oligohomopeptides with formaldehyde and acetaldehyde under possible primitive earth conditions. *Origins of Life, 13*, 97–108.

Jing, H., & Kitts, D. D. (2004). Antioxidant activity of sugar-lysine Maillard reaction products in cell free and cell culture systems. *Archives of Biochemistry and Biophysics, 429*, 154–163.

Kong, B. H., & Xiong, Y. L. (2006). Antioxidant activity of zein hydrolysates in a liposome system and the possible mode of action. *Journal of Agricultural and Food Chemistry, 54*, 6059–6068.

Lu, C. Y., Hao, Z., Payne, R., & Ho, C. T. (2005). Effects of water content on volatile generation and peptide degradation in the Maillard reaction of glycine, diglycine, and triglycine. *Journal of Agriculture and Food Chemistry, 53*, 6443–6447.

Ludwig, E., Lipke, U., Raczek, U., & Jäger, A. (2000). Investigation of peptides and proteases in green coffee beans. *European Food Research Technology, 211*, 111–116.

Morales, F. J., Fernandez-Fraguas, C., & Jiménez-Pérez, S. (2005). Iron binding ability of melanoidins from food and model systems. *Food Chemistry, 90*, 821–827.

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.

O'Brien, J. O., & Morrissey, P. A. (1997). Metal ion complexation by products of the Maillard reaction. *Food Chemistry, 58*, 17–27.

Ogura, K., Nakayama, M., Nakaoka, K., & Nishihata, Y. (2000). Spectroelectrochemical and EQCM studies on the oxidation of glycil-peptides in alkaline medium. *Journal of Electroanalytical Chemistry, 482*, 32–39.

Oh, Y. C., Hartman, T. G., & Ho, C. T. (1992). Volatile compounds generated from the Maillard reaction of Pro-Gly, Gly-Pro, and a mixture of glycine and proline with glucose. *Journal of Agriculture and Food Chemistry, 40*, 1878–1880.

Oh, Y. C., Shu, C. K., & Ho, C. T. (1991). Some volatiles compounds formed from thermal interaction of glucose with glycine, diglycine, triglycine, and tetraglycine. *Journal of Agriculture and Food Chemistry, 39*, 1553–1554.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized method for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agriculture and Food Chemistry, 53*, 4290–4302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Reynols, T. M. (1969). Nonenzymatic browning. Sugar-amine interaction. In H. W. Shultz (Ed.), *Carbohydrates and their roles*. Westport, CT.: AVI Pub. Co.

Rufián-Henares, J. A., & Morales, F. J. (2007). Functional properties of melanoidins: In vitro antioxidant, antimicrobial and antihypertensive activities. *Food Research International, 40*, 995–1002.

Ruiz-Roca, B., Navarro, M. P., & Seiquer, I. (2008). Antioxidant properties and metal chelating activity of glucose-lysine heated mixtures: Relationships with mineral absorption across caco-2 cell monolayers. *Journal of Agriculture and Food Chemistry, 56*, 9056–9063.

Seifert, S. T., Krause, R. M., Gloe, K., & Henle, T. (2004). Metal complexation by the peptide-bound Maillard reaction products N(ε)-fructoselysine and N(ε)-carboxymethyllysine. *Journal of Agriculture and Food Chemistry, 52*, 2347–2350.

Shih, P. W., Lai, P. L., & Jen, H. W. K. (2006). Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry, 99*, 775–783.

Shon, M. Y., Kim, T. H., & Sung, N. J. (2003). Antioxidants and free radical scavenging activity of *Phellinus baumii* extracts. *Food Chemistry, 82*, 593–597.

Wang, L., & Xiong, Y. L. (2005). Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *Journal of Agriculture and Food Chemistry, 53*, 9186–9192.

Wijewickreme, A. N., & Kitts, D. D. (1998). Metal chelating and antioxidant activity of model Maillard reaction products. *Advances in Experimental Medicine and Biology, 36*, 543–553.

Wijewickreme, A. N., Kitts, D. D., & Durance, T. D. (1997). Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*, 4577–4583.

Wu, H. C., Chen, H. M., & Shiau, C. Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International, 36*, 949–957.

Yamaguchi, N., Yokoo, Y., & Fujimaki, M. (1975). Studies on antioxidative activities of amino compounds on fats and oils. Part II. Antioxidative activities of dipeptides and their synergistic effects on tocopherol. *Nippon Shokuhin Kogyo Gkkaishi, 22*, 425–430.

Yen, G., & Hsieh, P. (1995). Antioxidative activity and scavenging effects on active oxygen of xylose–lysine Maillard reaction products. *Journal of the Science of Food and Agriculture, 67*, 415–420.

Yoshimura, Y., Iijima, T., Watanabe, T., & Nakazawa, H. (1997). Antioxidative effect of Maillard reaction products using glucose–glycine model system. *Journal of Agricultural and Food Chemistry, 45*, 4106–4109.

Zhou, S., & Decker, E. A. (1999). Ability of amino acids, dipeptides, polyamines, and sulfhydryls to quench hexanal, a saturated aldehydic lipid oxidation product. *Journal of Agricultural and Food Chemistry, 47*, 1932–1936.