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Influence of pH on the Antioxidant Activity of Melanoidins Formed from Different Model Systems of Sugar/Lysine Enantiomers

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Abstract This study was to investigate the influence of pH on the antioxidant activity of melanoidins formed from glucose (Glc) and fructose (Fru) with lysine enantiomers in the Maillard reaction. Melanoidins formed from D-isomers were found to be effective antioxidants in different *in vitro* assays with regard to the ferrous ion chelating activity, 1, 1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activities, ferric reducing/antioxidant power (FRAP), and 2,2'-azinobis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity. In particular, the chelating activity of these melanoidins at a pH of 7.0 was greater than those with pH of 4.0 and 10.0. The chelating activity and DPPH radical scavenging activity of the melanoidins formed from the Glc systems were higher than those of the melanoidins formed from the Fru systems. However, the FRAP and ABTS radical scavenging activity of these melanoidins were not different according to pH level, with exceptions being the Fru systems.

Keywords: amino acid enantiomer, antioxidant activity, Maillard reaction, melanoidin

Introduction

The reaction between reducing sugars and amino acids is known as the Maillard reaction or non-enzymic browning reaction. 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 (UV) absorbing intermediates, and dark-brown polymeric compounds named melanoidins. Melanoidins are widely distributed in foods and have different functional properties such as antioxidant, antimicrobial, and metal-binding activities (1-3). Recently, immobilized metal affinity chromatography (IMAC) has been proposed as a useful tool for the fractionation of melanoidins in homogeneous fractions according to their metal binding ability (4).

The Maillard reaction can also explain the formation of D-amino acids in food. Brückner *et al.* (5) have recently pointed out that D-amino acids are formed on heating aqueous solutions of L-amino acids (2.5 mM) together with an excess (278 mM) of saccharides (glucose, fructose, and saccharose) at 100°C for 24-96 hr in aqueous solutions of pH 2.5 (AcOH) or pH 7.0 (NaOAc). Thus, the formation of D-amino acids in many foods of plant and animal origin are the results of nonenzymic browning since the presence of amino acids together with saccharides is common. As for the racemization mechanism, it is postulated that the reaction of amino acids with glucose or fructose starts with the reversible formation of Schiff bases. The degree of racemization depends in particular on steric and electronic properties of the amino acid side chains. It should be noted that the early stages of the Maillard reaction proceeds already under mild conditions (5) and do not require alkaline or acidic condition. This new racemization mechanism based on the relatively stable Amadori compounds has been used to explain the generation of free D-amino acid in foods such as dried fruits, concentrated plant juices, and fortified wines (6). Recently, heating experiments of synthetic Amadori compounds proved that they are sources of amino acid enantiomers (7-9). Furthermore, convincing evidence has been recently established that D-amino acids are formed in the course of the Maillard reaction (5,7,8).

Therefore, the objective of this study is to evaluate the influence of pH on the antioxidant activity of melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction. For this, antioxidant activities were evaluated by using ferrous ion chelating activity, free radical scavenging activity includes DPPH and ABTS, and ferric reducing/antioxidant power (FRAP). Melanoidins were, rather arbitrarily, defined as being a high molecular weight (HMW) with a lower limit of 3.5 kDa, which was the nominal cut-off value in the dialysis system used.

Materials and Methods

Materials D-Glucose, D-fructose, L-lysine, D-lysine, ferrous chloride, 1, 1-diphenyl-2-picryl-hydrazil (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-azinobis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and ethylene diamine tetraacetate (EDTA, 2 Na-salt) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydrogen carbonate ($NaHCO_3$) was purchased from Shimakyu Chemical Co. (Osaka, Japan). Iron(III) chloride 6-hydrate, iron(II) sulfate 7-hydrate, and acetic acid were purchased from Merck (Darmstadt, Germany). All reagents were of highest reagent grade and used without any further purification.

Preparation of MRPs Glucose, fructose and amino acids were dissolved in 100 mL of 0.5 M sodium acetate buffer (pH 4.0), 0.5 M phosphate buffer (pH 7.0) or 0.5 M

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sodium carbonate buffer (pH 10.0) to obtain a final concentration of 1 M. Four model systems were prepared, being glucose-L-lysine (Glc/L-Lys), glucose- D-lysine (Glc/D-Lys), fructose-L-lysine (Fru/L-Lys), and fructose-D-lysine (Fru/D-Lys). The reaction mixtures were then distributed over glass, screw-capped, Schott tube (16×160 mm), each containing a minimum of 10 mL. Model solutions were heated at 100°C for 2 hr without pH control in at least duplicate. The heating was carried out in a silicone oil bath and the proper safety measures are taken. After heated, model solutions were withdrawn, immediately cooled in ice water and then dialyzed.

Dialysis Approximately 2 mL of the reaction mixture were injected into Slide-Alyzer dialysis cassettes (Mr> 3,500) (3.5K MWCO; Pierce, Rockford, IL, USA) and dialyzed against distilled water. A batch dialysis was performed against 1,500 mL of double distilled water for 168 hr at 4°C. Water was changed every 3 hr for the first 12 hr, and then every 10-12 hr for the rest of the dialysis time. After dialysis, samples were freeze-dried and stored in a desiccator at 4°C until analysis. MRPs samples after dialysis were dissolved in water before use, and their concentration were related to the concentration of the parent melanoidins, 200 μg/mL.

Ferrous (Fe^{2+}) metal ions chelating activity The chelating of ferrous ions (Fe^{2+}) by melanoidins and standards are estimated by the method of Dinis *et al.* (10). A 100 μL of melanoidins solution (200 μg/mL) 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 sec. The reaction mixture thus obtained was later added with 200 μL of 1 mM ferrozine and changes in color were monitored at 562 nm with a spectrophotometer (UV 160A; Shimadzu Co., Kyoto, Japan), after a 10 min resting time at room temperature. EDTA (2 Na-salt) was used as the reference antioxidant compounds. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated in the following equation (1):

$$\text{Ferrous ion chelating activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

where, $A_{control}$ was the absorbance of the control and A_{sample} was the absorbance with the sample.

DPPH radical scavenging activity The free radical scavenging activity of melanoidins was determined by the DPPH$^{\bullet}$. This activity was measured by the procedure described by Yen and Hsieh (11) wherein the bleaching rate of a stable free radical, DPPH$^{\bullet}$ is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH$^{\bullet}$ absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.12 mM solution of DPPH$^{\bullet}$ in methanol was prepared daily and protected from light. An aliquot of 2 mL of this solution was added to 80 μL of melanoidins solution in water at concentrations (200 μg/mL) 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 (UV 160A; Shimadzu Co.). The antiradical activity of sample was expressed as percentage of disappearance of the initial purple color. Aqueous solutions of Trolox at various concentrations were used to perform the calibration curves (0.15-1.15 mM).

Antioxidative capacity by FRAP assay The FRAP assay was done according to the modified Benzie and Strain method (12) 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 melanoidins solution (200 μg/mL) or standard or appropriate reagent blank. The FRAP reagent contained 2.5 mL of a 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) 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 sec, using a spectrophotometer (UV 160A; Shimadzu Co.). 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.

ABTS radical cation decolorization assay The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging activity of melanoidins was determined according to the method described by Re *et al.* (13) with slight modifications. This method is based on the reaction between ABTS and potassium persulfate giving blue/green ABTS radical ($ABTS^{\bullet+}$). With the addition of the antioxidants, decolorization is attained and measured spectrophotometrically at 734 nm. The results were expressed as mM Trolox/g 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 hr before use. The $ABTS^{\bullet+}$ solution (stable for 2 days) was diluted with 5 mM phosphate buffered saline (PBS, 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 $ABTS^{\bullet+}$ solution and 30 μL of melanoidins solution (200 μg/mL) were mixed for 45 sec 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.

Statistical analysis All experimental data was analyzed by one-way analyses of variance (ANOVA) and significant differences among the means from triplicate analysis at ($p<0.05$) was determined by Duncan's multiple-range tests using the statistical analysis system (SPSS 12.0 for windows, SPSS Inc, Chicago, IL, USA).

Results and Discussion

Ferrous (Fe^{2+}) ion chelating activity The production of highly reactive oxygen species (ROS) - such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals - was also catalyzed by free iron through the Haber-Weiss reaction:

$$O_2^{\bullet-} + H_2O_2 \; O_2 + OH^- + OH^{\bullet}$$

Transition metals, such as ferrous ion, can stimulate lipid

peroxidation by generating hydroxyl radicals through the Fenton reaction and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, therefore drive the chain reaction of lipid peroxidation (14). The Fenton reaction was:

$$Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+ OH^-+\cdot OH$$

Ferrous (Fe^{2+}) ion is the most powerful pro-oxidant among the various species of metal ions. The method of chelating activity is based on chelating of Fe^{2+} by the reagent ferrozine (10). In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. The ferrous ion chelating activities of melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction are shown in Fig. 1. These melanoidins showed different ferrous ion chelating activities, according to pH level. In the Glc systems, the ferrous ion chelating activities of the melanoidins formed from the Glc/L-Lys and Glc/D-Lys systems were increased and then decreased with pH level. In particular, the ferrous ion chelating activities of the melanoidins formed from the Glc/D-Lys system at a pH of 7.0 was the higher compared to those of other systems. The ferrous ion chelating activities of melanoidins formed from the D-isomers were significantly ($p<0.05$) higher than those from the L-isomers. On the other hand, in the Fru systems, the ferrous ion chelating activities of the melanoidins formed from the Fru/L-Lys and Fru/D-Lys were increased as pH increased; however, the differences in the ferrous ion chelating activities on the basis of the type of amino acid enantiomers were only statistically different in the case of at a pH of 10.0 ($p<0.05$). In particular, the ferrous ion chelating activities of melanoidins formed from the Fru/L-Lys system was the highest, while the melanoidins were much weaker than those of EDTA, was used as the reference antioxidant compound. In both the Glc and Fru systems, the melanoidins formed from the Glc systems showed higher chelating activities than those formed from the Fru systems. The ferrous ion chelating activities of the melanoidins formed from the Glc/D-Lys system was the highest with increasing pH. In this sense, the products of the Maillard reaction (glucose-casein) were reported to be capable of chelating divalent cations, thereby resulting in a decrease in the absorption Fe^{2+} from foods in animal models (15). Fe solubility in the presence of glucose-lysine heated mixtures is affected by the lower initial pH and destruction of free lysine in the model system (16,17). The formation of melanoidins in the later stage of the Maillard reaction was responsible for the chelating ability (18), which was dependent on the sugar sources as well as on heating conditions, e.g., glucose was the most favorable sugar for achieving copper chelating affinity (19). This observation was similarities with the structure of melanoidins proposed by Cämmerer *et al.* (20). Chelating activity occurs by the chelation of Fe^{2+} by the reagent ferrozine (10). Metal chelating capacity was significant since it reduced the concentration of the transition metal catalyst in lipid peroxidation. It was reported that chelating agents were effective as secondary antioxidants because they reduced the redox potential, thereby stabilizing the oxidized form of metal ions.

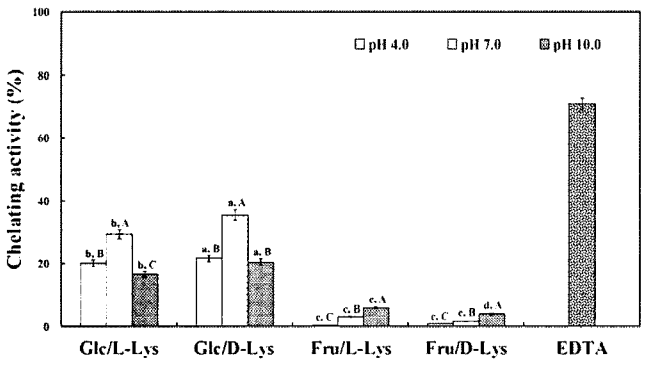

Fig. 1. Ferrous ions (Fe^{2+}) chelating activity of the melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction. Different small letters compare to the samples within the same pH level (Duncan's multiple-range test, $p<0.05$). Different capital letters compare to the pH level within the sample (Duncan's multiple-range test, $p<0.05$). Concentration of EDTA is 200 μg/mL.

DPPH radical scavenging activity The results obtained for the DPPH assay in melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction are shown in Table 1. The DPPH is a chromogen radical compound that can directly react with antioxidants. When the DPPH radical was scavenged by antioxidants through donation of hydrogen to form a stable DPPH-H molecule, the color changed from purple to yellow (21). The 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. In the Glc systems, the DPPH radical scavenging activity of the melanoidins formed from the Glc/D-Lys systems were increased and then decreased according to pH level, with exceptions being the Glc/L-Lys system. In particular, the DPPH radical scavenging activity of the melanoidins formed from the Glc/D-Lys system at a pH of 7.0 was the higher compared to those of other systems. The DPPH radical scavenging activity of melanoidins formed from the L-isomers were significantly ($p<0.05$) higher than those of melanoidins formed from the D-isomers, in the case of at a pH of 4.0 and 10.0, and not at a pH of 7.0. On the other hand, in the Fru systems, the DPPH radical scavenging activity of the melanoidins formed from the Fru/L-Lys systems was decreased as pH increased. Especially, the differences in the DPPH radical scavenging activity, on the basis of the type of amino acid enantiomers, were only not significant in the case of at a pH of 4.0 ($p<0.05$). The DPPH radical scavenging activity of melanoidins formed from the D-isomers were significantly ($p<0.05$) higher than those of melanoidins formed from the L-isomers, in the case of at a pH of 7.0 and 10.0, and not at a pH of 4.0. In both the Glc and Fru systems, the melanoidins formed from the Glc systems showed higher radical scavenging activity than those formed from the Fru systems. The DPPH radical scavenging activity of the melanoidins formed from the Fru/L-Lys system was the highest with increasing pH. Rufián-Henares and Morales (22) reported that a statistically significant linear relationship was found between the antioxidant activity measured with the DPPH

Table 1. Antioxidant activity determined by the DPPH method as affected by pH[1)]

Sample	pH 4.0	pH 7.0	pH 10.0
Glc/L-Lys	83.33±0.65$^{aA2)}$	83.26±0.76^{aA}	80.66±1.19^{aB}
Glc/D-Lys	80.44±0.76^{bB}	82.39±0.54^{aA}	69.67±1.30^{bC}
Fru/L-Lys	75.67±0.98^{cA}	71.91±1.53^{cB}	65.70±0.98^{cC}
Fru/D-Lys	75.74±1.00^{cA}	74.73±0.87^{bA}	71.48±1.09^{bB}

[1)]Data expressed as μM equivalents of Trolox/melanoidins released from 1 g of melanoidins.
[2)]$^{a-c,A-C}$Different superscripts within a column and row, respectively, indicate significant differences at $p<0.05$ level.

Table 2. Antioxidant activity determined by the FRAP method as affected by pH[1)]

Sample	pH 4.0	pH 7.0	pH 10.0
Glc/L-Lys	280.45±13.90$^{A2)}$	280.24±13.25^{bA}	269.94±12.31^{aA}
Glc/D-Lys	276.66±13.90^{aA}	280.03±14.21^{bA}	267.83±12.01^{aA}
Fru/L-Lys	295.80±12.62^{aB}	330.49±1.33^{aA}	275.82±11.36^{aC}
Fru/D-Lys	294.75±13.57^{aB}	330.61±1.36^{aA}	287.60±12.31^{aB}

[1)]Data expressed as μM equivalents of Trolox/melanoidins released from 1 g of melanoidins.
[2)]$^{a-c,A-C}$Different superscripts within a column and row, respectively, indicate significant differences at $p<0.05$ level.

assay and the iron-binding capability of melanoidins. However, the data obtained in this study differ on those reported by Rufián-Henares and Morales (22). Therefore, the results show that the melanoidins formed from the D-isomers has exhibited radical scavenging activities similar to those exhibited by melanoidins formed from the L-isomers. In a recent paper, Del Castillo *et al.* (23) have pointed out that bread-derived melanoidins has a stronger peroxyl radical scavenging activity than low molecular weight (LMW) compounds, which has been previously reported to be bound to the melanoidins skeleton (24) and contributes to the final antioxidant activity. This observation is in agreement with the hypothesis formulated by Delgado-Andrade and Morales (1) with regards to the existence of melanoidins in a dynamic equilibrium in the food matrix, where LMW compounds are non-covalently linked to the core structure. In any case, the mechanism of the antioxidant effect of the melanoidins is still unclear, and under study basically due to chemical structure of the melanoidins is unknown; this mechanism is presently being studied. It is assumed that the mechanism is based on the ability of melanoidins to trap positively charged electrophilic species, scavenge oxygen radicals, or carry out metal chelation to form inactive complexes (24).

Antioxidative capacity by FRAP assay Several assays have been introduced to measure of the antioxidant activity of single compound and/or complex mixtures (12). 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+}. The FRAP assay measures the reduction of Fe^{3+} to Fe^{2+} in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. From a mechanistic standpoint, FRAP is an electron transfer (ET)-based assay like Folin, ABTS/TEAC, or cupric ion reducing antioxidant capacity (CUPRAC), in the sense that the oxidant probe accepts an electron from the antioxidant analyte, to be converted into the reduced probe that is colored (25). In the presence of a chromogenic ligand like tripyridyltriazine (TPTZ) that is rather selective for Fe (II), Fe (III) acts as an oxidant toward the antioxidants in the sample, and is itself reduced to Fe (II), which readily chelates with the chromogenic ligand to form a colored species. The increase in absorbance at 593 nm due to a Fe (II)-TPTZ complex formation is proportional to the combined (total) FRAP of the antioxidants in the sample (26). The FRAP assay has been claimed to be a robust and potentially useful test, using inexpensive reagents and equipment and a speedy reaction applicable over a wide concentration range (12). The FRAP value of the melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction are shown in Table 2. In the Glc systems, the FRAP value of these melanoidins showed no difference according to pH level. In addition, the differences in the FRAP, on the basis of the type of amino acid enantiomers, were not significant ($p<0.05$). On the other hand, in the Fru systems, the FRAP value of these melanoidins were increased and then decreased with increases to pH. In particular, the FRAP value of the melanoidins formed from the Fru/L-Lys and Fru/D-Lys systems was the highest, at a pH of 7.0, however, the differences in the FRAP, on the basis of the type of amino acid enantiomers, were not significant ($p<0.05$). In both the Glc and Fru systems, the FRAP value of the melanoidins formed from the Glc systems showed only higher than those formed from the Fru systems, at a pH of 7.0. Rufián-Henares and Morales (22) have pointed out that the ferric reducing ability of melanoidins were in parallel with data from the DPPH method. It was reported that compounds responsible for reducing activity were formed during the thermolysis of Amadori products in the primary phase of Maillard reactions (27) or they could be formed as the heterocyclic products of the Maillard reaction or caramelization of sugars (28). The following exhibited reducing power: heat-induced MRPs produced from the xylose-lysine reaction (11), glucose-glycine reaction (29), and the sugar-lysine reaction (30); a porcine plasma protein-glucose model (31) could also be used to obtain reducing power. The hydroxyl groups of MRPs played an important role in the reducing activity (29).

ABTS radical scavenging activity The generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that has been applied to measuring the total antioxidant activity of solutions of pure substances, aqueous mixtures, and beverages (14). The method for screening antioxidant activity has been reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxy-cinnamates, carotenoids, and plasma antioxidants; a more appropriate format for the assay is a decolorization technique in that the radical is generated directly in a stable form prior to reaction with putative antioxidants. The improved technique for the generation of $ABTS^{\cdot+}$ described here involves the direct production of the blue/green

Table 3. Antioxidant activity determined by the ABTS method as affected by pH[1)]

Sample	pH 4.0	pH 7.0	pH 10.0
Glc/L-Lys	831.67±40.46$^{bA2)}$	827.53±34.25^{aA}	759.12±34.25^{aA}
Glc/D-Lys	846.18±31.30^{bA}	810.94±40.78^{aAB}	759.12±40.46^{aB}
Fru/L-Lys	943.61±3.80^{aA}	839.97±3.75^{aB}	775.71±2.18^{aC}
Fru/D-Lys	761.19±3.73^{cC}	827.53±3.43^{aA}	790.22±1.90^{aB}

[1)]Data expressed as μM equivalents of Trolox/melanoidins released from 1 g of melanoidins.
[2)]$^{a-c,A-C}$Different superscripts within a column and row, respectively, indicate significant differences at $p<0.05$ level.

$ABTS^{\bullet+}$ chromophore through the reaction between ABTS and potassium persulfate. The ABTS radical scavenging activities of melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction are shown in Table 3. The ABTS radical scavenging activities of these melanoidins showed no difference according to pH level, with exceptions being the Glc/D-Lys system. In addition, the differences in the ABTS radical scavenging activities, on the basis of the type of amino acid enantiomers, were not significant ($p<0.05$). On the other hand, in the Fru systems, the ABTS radical scavenging activities of the melanoidins formed from the Fru/L-Lys system was decreased, while the melanoidins formed from the Fru/D-Lys system was increased and then decreased as pH increased. Especially, the differences in the ABTS radical scavenging activities, on the basis of the type of amino acid enantiomers, were only significant in the case of at a pH of 4.0 ($p<0.05$). In both the Glc and Fru systems, the ABTS radical scavenging activities of the melanoidins formed from the Glc and Fru systems showed no difference. Results differ on those obtained with the DPPH assay. It is assumed that the difference of radical scavenging activity is due to de different reaction media; aqueous and methanolic for ABTS and DPPH, respectively (22).

In conclusion, this study was to investigate the influence of pH on the antioxidant activity of melanoidins formed from glucose and fructose with lysine enantiomers in the Maillard reaction. For this purpose, antioxidant activities were evaluated on the basis of FRAP and free radical scavenging activity includes DPPH and ABTS, and ferrous ion chelating activity. EDTA and Trolox, a water-soluble analog of tocopherol, were used as the reference antioxidant compounds. Melanoidins formed from D-isomers were found to be effective antioxidants in different *in vitro* assays with regard to the ferrous ion chelating activity, ABTS and DPPH radical scavenging activities, and FRAP. In particular, the chelating activity of these melanoidins at a pH of 7.0 was greater than those with a pH of 4.0 and pH 10.0. The chelating activity and DPPH radical scavenging activity of the melanoidins formed from the Glc systems were higher than those of the melanoidins formed from the Fru systems. However, the FRAP and ABTS radical scavenging activity of these melanoidins were no difference according to pH level, with exceptions being the Fru systems. Therefore, the melanoidins have the different antioxidant activities on the basis of the type of sugars, pH level, and different antioxidant assays, but not amino acid enantiomers.

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