J Food Sci Nutr
Vol 13, p 306～312 (2008)
DOI: 10.3746/jfn.2008.13.4.306

J Food Science
and Nutrition

The Influence of pH on the Color Development of Melanoidins Formed from Fructose/Amino Acid Enantiomer Model Systems

Ji-Sang Kim and Young-Soon Lee†

Department of Food and Nutrition, Kyung Hee University, Seoul 130-170, Korea

Abstract

This study investigated the influence of pH on the color development of melanoidins formed from amino acid enantiomer model systems. For this, the color development was evaluated by measuring browning at 420 nm and color measurements by spectrophotometry and colorimetry. The browning and browning index showed no difference according to the type of amino acid enantiomers, while that formed from the D-Asn system was the only difference according to pH level. The tristimulus value of melanoidins formed from all model systems was located on a dominant wavelength of 475 nm, the blue zone of the diagram. In addition, the L^*, a^*, b^*, C^*_{ab} values, and ΔE^* index on the basis of the type of amino acid enantiomers, the differences were markedly found at pH 4.0. At pH 7.0, significantly differences were found in the L^*, a^*, b^* values, and ΔE^* index and not in the case of the lysine enantiomers. In addition, at pH 10.0, the differences were found in the a^* and b^* values from the lysine enantiomers and C^*_{ab} value from the asparagine enantiomers. Therefore, the color development of melanoidins was influenced by the type of amino acid enantiomers and pH levels. Especially, it is thought that the a^* and b^* values can be used to explain the differences among the amino acid enantiomers in the color development of melanoidins.

Key words: amino acid enantiomers, color development, Maillard reaction, melanoidins

INTRODUCTION

Non-enzymatic browning reactions occurring in food during processing and storage are known to contribute to food quality and acceptability. The Maillard reaction is a complicated reaction that produces a large number of Maillard reaction products (MRPs) such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds as melanoidins (1). From the point of view of color, Ames and Nursten (2) grouped the colored compounds into two general classes: low molecular weight compounds, which typically possess two-to-four linked rings containing extended double-bond conjugation (3,4), and melanoidins which are brown polymers and possess molecular weights of several thousand daltons and discrete chromophore groups (2,5). Some structural information on melanoidins isolated from model systems has been published (6,7) and it has been suggested that melanoidins possess a backbone of repeating units, but with different degrees of polymerization (8,9).

The Maillard reaction can also explain the formation of D-amino acids in food. Brückner et al. (10) recently pointed out that D-amino acids are formed upon 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. Furthermore, convincing evidence has been recently established that D-amino acids are formed in the course of the Maillard reaction, i.e. the condensation reaction of reducing sugars and amino compounds such as amino acids (11).

The color of foods has been measured usually in units L^*, a^*, b^* using either a colorimeter or specific data acquisition and image processing systems. L^*, a^*, b^* is an international standard for color measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976. L^* is the luminance or lightness component, which ranges from 0 to 100, and parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components, which range from -120 to 120 (12). In the L^*, a^*, b^* space, the color perception is uniform which means that the Euclidean distance between two colors corresponds approximately to the color difference perceived by the human eye (13).

†Corresponding author. E-mail: yysllee@khu.ac.kr
Phone: +82-2-961-0881, Fax: +82-2-968-0260

The objective of this study was, therefore, to elucidate the influence of pH on the color development of melanoidins formed from amino acid enantiomers model systems. For this, the color development was evaluated by measuring browning at 420 nm and color measurements by spectrophotometry and colorimetry. Melanoidins were, rather arbitrarily, defined as high-molecular-weight by a lower limit of 3,500 Da, which was the nominal cut-off value in the dialysis system used.

MATERIALS AND METHODS

Materials

D-fructose, L-asparagine, D-asparagine, L-lysine and D-lysine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate and sodium hydrogen phosphate were purchased from Merck Co. (Darmstadt, Germany). HPLC-grade water was purchased from J.T. Baker (Phillipsburg, USA). All reagents were of highest reagent grade and used without any further purification.

Preparation of Maillard reaction products (MRPs)

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 sodium carbonate buffer, (pH 10.0) to obtain a final concentration of 1 M. Four model systems were prepared: fructose/L-asparagine (Fru/L-Asn), fructose/D-asparagine (Fru/D-Asn), fructose/L-lysine (Fru/L-Lys) and fructose/D-lysine (Fru/D-Lys). All model solutions were refluxed for 2 hrs at 100°C without pH adjustment, in duplicate or more. The heating was carried out in a silicone oil bath and the proper safety measures were taken. After heating, model solutions were withdrawn and immediately cooled in ice water.

Dialysis

Approximately 2 mL of the reaction mixture was injected into dialysis cassettes (Mr> 3,500) (Slide-Alyzer Dialysis Cassette, 3.5K MWCO, Pierce, 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 concentrations were related to the concentration of the parent melanoidins, 200 μg/mL.

Measurement of browning

Browning indices of MRPs samples were recorded by their absorbance at 420 nm on a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan) using a 1 cm pathlength cell after appropriate dilution with distilled water.

Color measurements by spectrophotometer

Color measurements at different times were performed to follow the progress of non-enzymatic browning in the model systems over time. Huidrobo and Simal (14) proposed a simplified method of four selected coordinates to evaluate the color parameters of yellow to yellow-brown samples, which was employed to obtain the CIE (Commission Internationale de I'Eclairage) tristimulus values X, Y and Z, according to the following equations:

$$X = T_{625} \cdot 0.42 + T_{550} \cdot 0.35 + T_{445} \cdot 0.21,$$
$$Y = T_{625} \cdot 0.2 + T_{550} \cdot 0.63 + T_{495} \cdot 0.17,$$
$$Z = T_{495} \cdot 0.24 + T_{445} \cdot 0.94,$$

where T_{625}, T_{495}, T_{445}, T_{550} are the transmittance values at 625, 495, 445, 550 nm, respectively. Transmittance values at 445, 495, 550 and 625 nm were measured on a spectrophotometer. The function browning index "Br", which has been proposed as a suitable measure of visual browning, was calculated according to the following equations (15):

$$Br = 100\ (x - 0.31)/0.172,$$
Where $x = X/(X+Y+Z)$

Color measurements by colorimeter

The color measurements of melanoidins formed from amino acid enantiomers according to pH level were carried out using a colorimeter JS-555 (Color Techno system Co., Ltd., Japan), according to the CIE Lab scale (16). The system provides the values of three color components; L^* (black-white component, luminosity), and the chromaticness coordinates, a^* (+red to −green component) and b^* (+yellow to −blue component), taking as standard values those of the white background (L= 113.29; a=−0.22; b=−0.15). Samples were pipetted into a 5cm diameter glass petri dish, as described by Morales and van Boekel (17). The following equations were employed (18).

Metric chroma: C^*_{ab}
$$C^*_{ab} = (a^{*2}+b^{*2})^{1/2}$$

Color difference CIE 1976: ΔE^*_{ab}
$$\Delta E^*_{ab} = [(\Delta L^*_{ab})^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$$

Statistical analysis

All experimental data were analyzed by analysis of variance (ANOVA) and significant differences among the means from triplicate analysis at ($p < 0.05$) were determined by Duncan's multiple range test using the stat-

istical analysis system (SPSS 12.0 for windows, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Measurement of browning

The final stage of the browning reaction was monitored by the increase in absorbance at 420 nm (19). The browning development of melanoidins formed from amino acid enantiomers according to pH level are shown in Fig. 1. The differences in the browning development on the basis of the type of amino acid enantiomers, were not statistically significant ($p > 0.05$). The differences in the browning development on the basis of the pH level were only statistically different for asparagine enantiomers ($p < 0.05$). The browning development was increased and then decreased according to pH level, except in the D-Lys system. In particular, the browning development of the melanoidins formed from the lysine enantiomers was higher than that of melanoidins formed from the asparagine enantiomers. In addition, the browning development of the melanoidins for all model systems was the highest at a pH of 7.0, except in the D-Lys system. Leong and Wedzicha (20) observed that the high-molecular-weight fraction (>3,500 Da) contributed only up to 10% of the absorbance of the glucose/glycine reaction mixture heated in acetate buffer at 55°C and pH 5.5. Also, Hofmann (21) reported that in both glucose/glycine and glucose/alanine systems heated in phosphate buffer for 4 hr at 95°C, pH 7, only trace amounts of compounds with molecular weights greater than 3,000 Da were formed. These results are in contrast with sugar/protein reactions. A much higher percentage of color was detected in the high-molecular-weight fraction (≥70%), which is as expected since the melanoidins are attached to a protein that is high-molecular-weight by itself (22). Color in the fructose/amino acid reaction mixtures is almost exclusively due to the low-molecular-weight fraction.

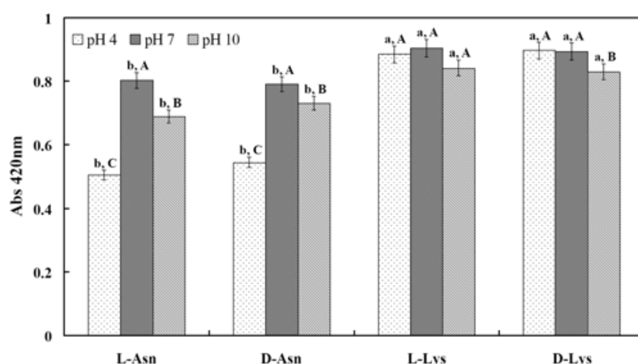

Fig. 1. The browning development (as measured by absorbance at 420 nm) of melanoidins formed from amino acid enantiomers according to pH level. Data are expressed as mean±standard deviation of three experiments. Different small superscripts means significant differences among samples in the same pH level at $p<0.05$ level. Different capital superscripts means significant differences according to pH level in the same sample at $p<0.05$ level.

Color measurements by spectrophotometer

The CIE chromaticity diagram, in which the tristimulus values are limited to a plane, determined by the x and y chromatic coordinates of melanoidins formed from amino acid enantiomers according to pH level are shown in Fig. 2 and Table 1. It is known that the color change of maillard browning is developed through several steps, as such: uncoloured→ yellow→ golden→ cinnamon→ reddish brown (23). In this study, the differences in the tristimulus values on the basis of the type of amino acid enantiomers and pH level, were not statistically significant ($p > 0.05$). Most of the melanoidins were located on a dominant wavelength of 475 nm, in contrast with those observed for melanoidins of yellow to light-brown colors obtained through non-enzymic browning reactions (24). It can be seen that a placement of the dominant wavelength occurred for all model systems, to the blue zone of the diagram. Gomyo et al. (25) have reported the development of colored compounds formed in the Maillard reaction between D-xylose and glycine. These had been postulated to be important intermediates in the generation of melanoidins. Hayase et al. (26) have recently reported the identification of a novel blue pigment designated as Blue-M1. This consists of four molecules of D-xylose and glycine, and has a methane proton between two pyrrolopyrrole rings. It is thought that Blue-M1 was polymerized by many Maillard reaction intermediates and generated higher-molecular-weight (HMW) compounds leading to melanoidins.

The browning index of melanoidins formed from amino acid enantiomers according to pH level are shown in Fig. 3. The differences in the browning index on the basis of the type of amino acid enantiomers were only statistically different in the case of the asparagine enantiomers at a pH of 10.0 ($p < 0.05$). The differences in the browning index on the basis of the pH level were only statistically different in the case of the D-Asn system ($p < 0.05$). The browning index of the melanoidins formed from the asparagine enantiomers decreased according to pH level, while in the case of lysine enantiomers, the index showed the highest value at pH 7.0. In particular, the browning index of the melanoidins formed from the asparagine enantiomers was higher than that of melanoidins formed from the lysine enantiomers. The formation of melanoidins is affected by the reactants and

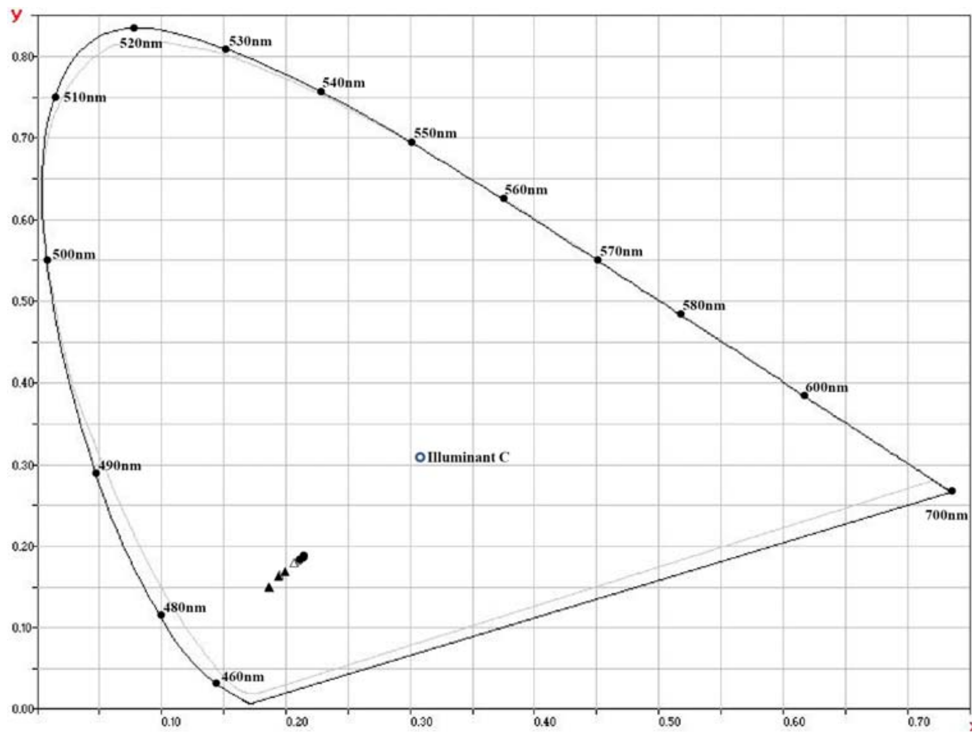

Fig. 2. CIE chromatic changes of melanoidins formed from amino acid enantiomers according to pH level. The numbers on the side of the diagram indicate the wavelength (in nm) of the pure chromatic colors.

Table 1. CIE chromatic changes of melanoidins formed from amino acid enantiomers according to pH level

Samples	pH 4.0		pH 7.0		pH 10.0	
	x	y	x	y	x	y
L-Asn	$^{A}0.19\pm0.01^{a}$	$^{A}0.15\pm0.01^{b}$	$^{A}0.20\pm0.01^{a}$	$^{A}0.17\pm0.02^{a}$	$^{A}0.21\pm0.01^{a}$	$^{A}0.18\pm0.02^{a}$
D-Asn	$^{A}0.19\pm0.01^{a}$	$^{B}0.15\pm0.01^{b}$	$^{A}0.20\pm0.01^{a}$	$^{AB}0.17\pm0.02^{a}$	$^{A}0.21\pm0.01^{a}$	$^{A}0.19\pm0.02^{a}$
L-Lys	$^{A}0.22\pm0.02^{a}$	$^{A}0.20\pm0.02^{a}$	$^{A}0.22\pm0.02^{a}$	$^{A}0.20\pm0.02^{a}$	$^{A}0.22\pm0.02^{a}$	$^{A}0.20\pm0.02^{a}$
D-Lys	$^{A}0.22\pm0.02^{a}$	$^{A}0.20\pm0.02^{a}$	$^{A}0.22\pm0.02^{a}$	$^{A}0.20\pm0.02^{a}$	$^{A}0.22\pm0.02^{a}$	$^{A}0.20\pm0.02^{a}$

Data are expressed as mean ± standard deviation of three experiments.
Different small superscripts (a,b) indicate significant differences among samples in the same pH level at $p<0.05$ level.
Different capital superscripts (A,B) indicate significant differences according to pH level in the same sample at $p<0.05$ level.

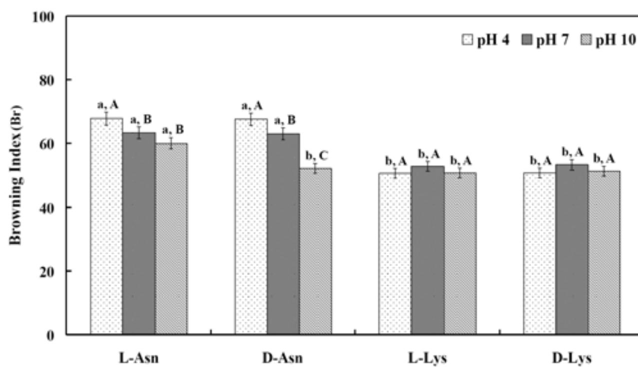

Fig. 3. Browning index (Br) of melanoidins formed from amino acid enantiomers according to pH level. Data are expressed as mean ± standard deviation of three experiments. Different small superscripts indicate significant differences among samples in the same pH level at $p<0.05$ level. Different capital superscripts indicate significant differences according to pH level in the same sample at $p<0.05$ level.

their concentrations, types of catalysts and buffers, reaction temperature, time, pH value, water activity, presence of oxygen, metal ions, etc. Therefore, the browning index of melanoidins formed in model systems might be related to their molecular weight and pH level.

Color measurements by colorimeter

The changes in color parameters of melanoidins formed from amino acid enantiomers according to pH level are shown in Table 2. The L^* value from the Lab color scale was used to measure the color differences indicative of melanoidins produced as a result of Maillard browning (27,28). The L^* value indicates the level of lightness or darkness on a 0~100 scale. For L^* value, the differences in the luminosity (L^*) on the basis of the type of amino acid enantiomers were statistically significant ($p<0.05$), at pH 4.0 and 7.0 and not

Table 2. Changes in color parameters of melanoidins formed from amino acid enantiomers according to pH level

Samples		Color parameters				
		L^*	a^*	b^*	ΔE^*	C^*_{ab}
pH 4.0	L-Asn	$^B6.38\pm0.32^b$	$^A2.47\pm0.12^a$	$^C0.12\pm0.01^c$	$^B6.84\pm0.21^c$	$^B2.47\pm0.07^a$
	D-Asn	$^A10.59\pm0.53^a$	$^B1.23\pm0.06^d$	$^B1.19\pm0.06^a$	$^A10.73\pm0.32^b$	$^C1.71\pm0.05^c$
	L-Lys	$^A11.34\pm0.57^a$	$^B1.67\pm0.08^c$	$^B0.57\pm0.03^b$	$^A11.47\pm0.34^a$	$^C1.76\pm0.05^c$
	D-Lys	$^C6.64\pm0.33^b$	$^A2.00\pm0.10^b$	$^C-0.31\pm0.02^d$	$^C6.94\pm0.21^c$	$^{AB}2.02\pm0.06^b$
pH 7.0	L-Asn	$^A11.21\pm0.56^a$	$^B1.68\pm0.08^a$	$^B0.99\pm0.05^d$	$^A11.38\pm0.34^{ab}$	$^C1.94\pm0.06^a$
	D-Asn	$^B9.35\pm0.47^b$	$^B1.15\pm0.06^d$	$^A1.57\pm0.08^a$	$^B9.55\pm0.29^c$	$^B1.95\pm0.06^a$
	L-Lys	$^A11.72\pm0.59^a$	$^B1.54\pm0.08^b$	$^A1.27\pm0.06^c$	$^A11.88\pm0.36^a$	$^B1.99\pm0.06^a$
	D-Lys	$^A10.73\pm0.54^a$	$^C1.34\pm0.07^c$	$^A1.45\pm0.07^b$	$^A10.91\pm0.33^b$	$^B1.98\pm0.06^a$
pH 10.0	L-Asn	$^A11.07\pm0.55^a$	$^C1.40\pm0.07^c$	$^A1.68\pm0.08^a$	$^A11.29\pm0.34^a$	$^A2.82\pm0.08^a$
	D-Asn	$^A10.77\pm0.54^a$	$^A1.40\pm0.07^c$	$^A1.69\pm0.08^a$	$^A10.99\pm0.33^a$	$^A2.19\pm0.07^b$
	L-Lys	$^B9.04\pm0.45^b$	$^A2.03\pm0.10^a$	$^B0.62\pm0.03^c$	$^B9.29\pm0.28^b$	$^A2.13\pm0.06^b$
	D-Lys	$^B9.31\pm0.47^b$	$^B1.77\pm0.09^b$	$^B1.14\pm0.06^b$	$^B9.55\pm0.29^b$	$^A2.10\pm0.06^b$

Data are expressed as mean±standard deviation of three experiments.
Different small superscripts (a~d) indicate significant differences among samples in the same pH level at $p<0.05$.
Different capital superscripts (A~C) indicate significant differences according to pH level in the same sample at $p<0.05$.

in the lysine enantiomers at pH 7.0. The L^* value of the melanoidins formed from the L-isomers were higher than that of the melanoidins formed from the D-isomers, at pH 4.0 and 7.0, but not in the case of the asparagine enantiomers at pH 4.0. Moreover, the differences in the luminosity (L^*) on the basis of the pH level were only statistically different in the case of the D-Lys system ($p<0.05$). The luminosity (L^*) of the melanoidins formed from the lysine enantiomers showed the highest value at pH 7.0.

The a^* value is a measure of redness or greenness and the b^* value is a measure of yellowness or blueness respectively. For a^* value, the differences in the a^* value on the basis of the type of amino acid enantiomers were statistically different ($p<0.05$) according to pH level and not in the asparagine enantiomers at pH 10.0. The a^* value of the melanoidins formed from the L-isomers were higher than that of the melanoidins formed from the D-isomers, at pH 4.0 and 7.0, but not in the case of the lysine enantiomers at pH 4.0. Moreover, the differences in the a^* value on the basis of the pH level, were only statistically different in the case of the L-Asn and D-Lys systems ($p<0.05$). In particularly, the a^* value of the melanoidins formed from both the L-Asn and D-Lys systems showed the highest value at pH 4.0.

The differences in the b^* value on the basis of the type of amino acid enantiomers were statistically significant ($p<0.05$) except in the case of the asparagine enantiomers at a pH of 10.0. On the other hand, the differences in the b^* value on the basis of the pH level, were only statistically different in the case of the L-Asn and D-Lys systems ($p<0.05$). In particular, the b^* value of the melanoidins formed from the L-Asn system showed the highest value at pH 10.0, while that formed from the D-Lys system showed the highest value at pH 7.0. Similar behaviors were observed in many products subjected to non-enzymatic browning (29). These results suggest that blue-green pigment of nondialysable melanoidins results from low molecular weight (Mr<3,500) compounds. It is thought that low molecular weight compounds are polymerized by many Maillard reaction intermediates and generate higher molecular weight compound of yellow-red pigment leading to melanoidins.

The CIE Lab color space or CIELAB system established a system of numerical coordinates to locate individual colors in a uniform visual color spacing (16). The ΔE^* index (color difference) is mainly influenced by the color lightness (L^*); a decrease in ΔE^* index is related to a loss of lightness. Briefly, the ΔE^* index value describes how far apart two colors or samples are in the color space. Differences in the ΔE^* index on the basis of the type of amino acid enantiomers were only statistically significant ($p<0.05$) at pH 4.0 and 7.0. The ΔE^* index of the melanoidins formed from the L-isomers were higher than that of the melanoidins formed from the D-isomers, at pH 4.0 and 7.0, except in the case of asparagine enantiomers at pH 4.0. In addition, the differences in the ΔE^* index on the basis of the pH level, were only statistically significant ($p<0.05$) in the D-Lys system. The ΔE^* index of the melanoidins formed from the D-Lys system showed the highest value at pH 7.0. Color formation is likely due to both the formation of low molecular weight compounds and to the presence of melanoidins with high molecular weight (3).

C^*_{ab} (chroma) indicates the degree of saturation, purity of intensity of visual color and is defined as degree of departure from grey (a^* and $b^*=0$, achromatic) toward pure chromatic color (30). According to the equation of

C^*_{ab}, when either a^* and/or b^* increases (i.e. in either a positive or negative direction), chromaticity increases. The differences in the C^*_{ab} value on the basis of the type of amino acid enantiomers were only statistically significant ($p<0.05$) at pH 4.0 and 10.0 and not in the case of the lysine enantiomers at pH 10.0. The differences in the C^*_{ab} value on the basis of the pH level, were statistically significant ($p<0.05$), except for the D-Lys system. In addition, the C^*_{ab} value of the melanoidins formed from the all model systems showed the highest value at pH 10.0. Low reducing sugar and asparagine contents in food are required to minimize color development by the Maillard reaction (31). The increase in the chroma value denotes that the heated model systems have more red and yellow characteristics until they reach a maximum, where the visual color of the system becomes much more complicated to define and where the yellow component starts to decrease.

In conclusion, on the basis of the data obtained in this study, the browning and browning index showed no difference according to the type of amino acid enantiomers, while that formed from D-Asn system was the only difference according to pH level. The tristimulus value of melanoidins formed from all model systems was located on a dominant wavelength of 475nm, the blue zone of the diagram. In addition, the L^*, a^*, b^*, C^*_{ab} value, and ΔE^* index on the basis of the type of amino acid enantiomers, exhibited marked differences at pH 4.0. At pH 7.0 and significantly differences were found in the L^*, a^*, b^* value, and ΔE^* index, but not in the case of the lysine enantiomers. In addition, at pH 10.0, the differences were found in the a^* and b^* values from the lysine enantiomers and C^*_{ab} value from the asparagine enantiomers. Therefore, these results suggested that the color development of melanoidins was influenced by the type of amino acid enantiomers and pH level. Especially, it is thought that the a^* and b^* values were influenced by the type of amino acid enantiomer and pH in the color development of melanoidins.

ACKNOWLEDGMENT

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-511-C00133).

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(Received September 20, 2008; Accepted November 14, 2008)