Color Tone of Various Melanoidins Produced from Model Systems

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The color tone of melanoidins prepared from model systems by heating mixtures of 1 mmole amino acid or peptide and 1 mmole sugar in aqueous solution was investigated using ΔA (change of log absorbance per 100 $m\mu$) as a parameter of color tone.

Amino acids and peptides were responsible for the color tone of melanoidins while sugar had no effect on the color tone. In addition, the color tone of melanoidin varied as the concentration ratio of amino acid to sugar changed. Melanoidins from tryptophan- and proline-xylose systems exhibited the lightest color tone. Melanoidins from peptides generally exhibited dark tones compared with those from amino acids.

Using the distribution pattern of color components fractionated by DEAE-cellulose chromatography, melanoidins have been classed into 4 groups based on the percentage of P1 (non-adsorbed fraction on DEAE-cellulose): group 1, melanoidins containing 90~100% P1; group 2, 70~80% P1; group 3, 30~50% P1; group 4, less than 20% P1. Melanoidins derived from most amino acids examined, those from basic amino acids and proline, those from glutamic acid and glycine, and those from peptides and amino terminal monocarboxylic acids belonged to groups 2, 1, 3, and 4, respectively.

The color tone of each color component except for P1 was generally very similar in most melanoidins. However, the color tone of P1 was variable. Therefore, melanoidin is composed of various color components having intrinsic color tones from yellowish-brown to dark brown and their color tone depends upon the variation of the amount of color components and upon the color tone of P1.

Non-enzymatic browning by the Maillard reaction between carbonyl and amino groups is known as typical browning in various foods. Furthermore, it can be an indicator for many types of deteriorative changes in foods. Although the mechanism of browning and degree of browning in model system under various conditions have been intensively investigated, little is known about the color tone of melanoidins and the color tone of foods formed by the Maillard reaction, which is closely related to food quality.

In the previous paper,¹⁾ it was proposed that the change of log absorbance per 100 $m\mu$ (designated as ΔA) could be used as a parameter of color tone, since a linear relationship between the above change and wavelength (450~650 $m\mu$) was observed in the color of shoyu (soy sauce) and of melanoidins prepared using model systems.

To clarify the browning mechanism and

properties of the browning material, the color tone of melanoidins prepared with model systems and the distribution of color components of melanoidins fractionated with DEAE-cellulose have been investigated using ΔA as a parameter of color tone.

MATERIALS AND METHODS

Chemicals. Amino acids and sugars were purchased from WAKO Pure Chemical Industries Ltd. and peptides were obtained from the Protein Research Foundation, Osaka, Japan. These chemicals were commercially available guaranteed reagents.

Model system. The model system contained 1 mmole amino acids or peptides and 1 mmole sugars in 5 ml of 0.2 M acetate buffer, pH 5.0. The mixtures in glass tubes were frozen in acetonedry ice. The tubes were sealed under vacuum to prevent the oxidation of melanoidins as far as possible and were heated at 120°C for 60 min, followed by immediate cooling in water. The seals of the tubes were broken and the samples were taken for measurements of absorbance

and fractionation of the color components.

Estimation of ΔA as a parameter of color tone. The absorbance of melanoidins was measured at every 25 m μ between 450 and 650 m μ . Then ΔA was determined by plotting log absorbance against wavelength. The larger the value of ΔA , the brighter the color tone and the smaller ΔA , the darker the color tone.

Fractionation of color components. The above reaction mixtures containing browning materials formed by heating were diluted 20-fold with distilled water, and then subjected to chromatography on DEAE-cellulose equilibrated with 0.01 M acetate buffer, pH 5.0 (column size; 1 $\times$ 20 cm, flow rate; 100 ml/hr). Stepwise elution was performed with the buffer, and with 0.025, 0.05, 0.10, 0.15, 0.20, 0.30 and 0.40 M NaCl in the same buffer. These color component fractions were designated as P1, P2, P3, P4, P5, P6, P7, and P8 in order of elution, respectively. Each component was a single peak on DEAE-cellulose chromatography by gradient elution.

Estimation of amount of browning. The amount of browning was estimated from the absorbance at 450 m μ . The relative amount of browning is indicated as a percentage against total absorbance at 450 m μ of melanoidin applied to the DEAE-cellulose.

FIG. 1. Effect of pH on the Color Development of Melanoidins.

Melanoidins were prepared by heating a mixture of 1 mmole glycine and 1 mmole xylose in 5 ml of 0.06 M sodium citrate solution at 120°C for 60 min in a tube sealed under vacuum. The pH of the solution was adjusted with 1 N NaOH and 1 N HCl.

RESULTS AND DISCUSSION

Effect of pH on the color of melanoidin

A linear relationship between log absorbance and wavelength was observed at pH 4.0 to 5.5 (Fig. 1). These pH values represent limits which might naturally occur in various foods. The color tone of melanoidins was unchanged at pH 4.0 to 5.5. Above pH 6.0 and below pH 3.0, the linear relationship between log absorbance and wavelength was lost in the vicinity of 550 m μ . More browning was found at higher pH values of the reaction mixture.

Effect of amino groups on the color tone of melanoidins

The amount of browning and the color tone of melanoidins formed by heating mixtures of amino acids or peptides and xylose is recorded in Fig. 2. The amount of browning

FIG. 2. Color of Melanoidins Formed in Model Systems.

1: Lys, 2: His, 3: Ala, 4: Thr, 5: Gly, 6: Asp, 7: Glu, 8: Gln, 9: Asn, 10: Pro, 11: Arg, 12: Ser, 13: Val, 14: Leu, 15: Ile, 16: Met, 17: Phe, 18: Tyr, 19: Trp, 20: Gly₂, 21: Gly-Leu, 22: Gly₃, 23: Gly-Phe, 24: Leu-Gly, 25: Leu-Gly₂.

Melanoidins were prepared by heating a mixture of 1 mmole amino acids or peptides and 1 mmole xylose in 5 ml of 0.2 M acetate buffer (pH 5.0) at 120°C for 60 min in a tube sealed under vacuum.

and the color tone of melanoidins varied widely depending on the type of amino compound used. In general, peptides caused a significant increase in the amount of browning compared to amino acids. Amino acids such as Lys, His, Glu, and Asp resulted in the largest increase in the amount of browning of all amino acids tested.

On the other hand, the color tone of melanoidins from peptides generally was dark and those from Pro and Tyr exhibited the lightest tone of all the amino acids tested. Color tone of melanoidins from amides such as Gln and Asn and basic amino acids such as His and Lys exhibited a dark tone compared to those from other amino acids.

As shown in Fig. 3, which illustrates the distribution patterns of color components fractionated by DEAE-cellulose chromatography, melanoidins were grouped into 4 groups based on the content of P1: group 1, melanoidins containing 90~100% P1, group 2, 70~80% P1, group 3, 30~50% P1; group 4, less than 20% P1. Melanoidins from basic amino acids and Pro belong to group 1. Melanoidins from most amino acids and amides belong to group 2. Melanoidins from Gly and Glu belong to group 3. Melanoidins from peptides belong to group 4.

As shown in Table I, ΔA values of each color component of melanoidins derived from most amino compounds were similar. However, P1 from His and Lys showed somewhat lower ΔA , and P1 from Pro gave a somewhat higher ΔA than those from other amino compounds. Therefore, the dark tone of melanoidins from Lys and His can be explained by the low ΔA value of P1, which is the major component. Similarly, the light tone of melanoidins from Pro and Trp is due to the high ΔA value of each color component.

A linear relationship between the log absorbance and wave length was not found in the color components P1 and P2 from Tyr, Trp, Leu-Gly and Leu-Gly₂.

Effect of sugars on the color tone of melanoidins

Amount of browning and color tone of melanoidin from individual sugar—glycine systems are shown in Table II. The browning intensity of melanoidins was in the following order: Ribose>Xylose>Arabinose>Mannose>Eructose>Glucose. With lactose, maltose and saccharose, browning did not occur. The color tones of melanoidins formed by heating a solution of glycine with various sugars were almost the same and each color component showed a similar ΔA value. How-

FIG. 3. Grouping of Melanoidins Based on Content of P1 Component.

TABLE I. THE COLOR TONE OF COLOR COMPONENTS OF VARIOUS MELANOIDINS DERIVED FROM DIFFERENT AMINO ACIDS AND PEPTIDES

Group	Amino acid or peptide	ΔA							
		P1	P2	P3	P4	P5	P6	P7	P8
1	Arg	0.77	0.66	—	—	—	—	—	—
	His	0.66	—	—	—	—	—	—	—
	Lys	0.65	0.58	—	—	—	—	—	—
	Pro	0.97	—	—	—	—	—	—	—
2	Ala	0.78	0.65	0.57	0.55	0.48	0.45	—	—
	Val	0.76	0.63	0.58	0.56	0.48	0.45	—	—
	Leu	0.75	0.62	0.57	0.55	—	—	—	—
	Ile	0.79	0.65	0.57	0.53	0.47	0.44	0.40	—
	Thr	0.76	0.63	0.58	0.54	—	—	—	—
	Ser	0.72	0.61	0.55	0.51	0.48	0.41	0.36	—
	Asp	0.80	0.68	0.58	0.54	—	—	—	—
	Asn	0.72	0.63	0.60	0.56	0.49	0.43	0.39	—
	Gln	0.68	0.64	0.56	0.52	0.48	0.44	0.38	—
	Met	0.79	0.68	0.60	0.54	0.47	0.45	—	—
	Tyr	N.E	N.E	0.58	0.55	0.45	0.43	—	—
	Phe	0.84	0.60	0.54	0.48	0.43	0.35	—	—
	Trp	N.E.	N.E.	0.75	0.66	0.62	0.55	—	—
3	Glu	0.75	0.66	0.62	0.60	0.54	0.51	0.47	0.41
	Gly	0.80	0.66	0.60	0.55	0.50	0.46	0.44	0.40
4	Gly ₂	0.80	0.70	0.62	0.54	0.50	0.46	0.43	0.38
	Gly-Leu	0.77	0.68	0.65	0.57	0.48	0.42	0.41	0.38
	Gly-Phe	0.72	0.63	0.57	0.55	0.50	0.44	0.37	0.34
	Leu-Gly	N.E	N.E	0.63	0.54	0.48	0.43	0.37	0.34
	Leu-Gly ₂	N.E	N.E	0.66	0.55	0.47	0.45	0.40	0.34
	Gly ₃	0.80	0.70	0.60	0.58	0.51	0.46	0.42	0.38

N.E: ΔA could not be estimated, since a linear relationship was not observed between log absorbance and wavelength. —: not detectable.

TABLE II. COLOR TONE OF MELANOIDINS FROM VARIOUS SUGAR—GLYCINE SYSTEMS

Sugars	ΔA	E_{450}/ml	ΔA							
			P1	P2	P3	P4	P5	P6	P7	P8
Ribose	0.74	20.06	0.82	0.66	0.60	0.55	0.51	0.46	0.44	0.40
Xylose	0.74	18.07	0.80	0.66	0.60	0.55	0.50	0.46	0.44	0.40
Arabinose	0.75	11.91	0.81	0.67	0.60	0.54	0.50	0.45	0.43	0.40
Fructose	0.77	2.88	0.77	0.69	0.59	0.56	0.52	0.45	0.44	0.40
Mannose	0.76	4.57	0.76	0.66	0.60	0.55	0.49	0.44	0.42	0.40
Glucose	0.75	2.85	0.79	0.65	0.58	0.55	0.50	0.45	0.44	0.39

ever P1 from pentose gave a considerably higher ΔA value than that from hexose. Figure 4 illustrates the color distribution of melanoidins with various sugars. Carbonyl compounds such as sugars seemed to have no effect on the color tone of melanoidins, since melanoidins from various sugars showed al-

most the same color tone. On the other hand, the color tone of melanoidins formed in model systems varied remarkably depending on the nature of the amino acids or peptides. Therefore, it is apparent that the color tone of melanoidins is governed by amino compounds but not by carbonyl compounds.

TABLE III. EFFECT OF CONCENTRATION OF XYLOSE AND GLYCINE ON THE COLOR TONE OF MELANOIDINS

Concentration (mmole)		ΔA	E_{450}/ml	ΔA							
Glycine	Xylose			P1	P2	P3	P4	P5	P6	P7	P8
4.0	1.0	0.73	35.76	0.76	0.66	0.60	0.56	0.50	0.46	0.42	0.40
2.0	1.0	0.74	22.41	0.80	0.67	0.62	0.58	0.51	0.46	0.41	0.40
1.0	1.0	0.74	16.48	0.80	0.66	0.60	0.55	0.50	0.46	0.44	0.40
0.5	1.0	0.78	5.53	0.80	0.66	0.58	0.53	0.50	0.47	0.42	0.39
0.1	1.0	0.81	0.65	0.82	0.66	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
1.0	4.0	0.66	58.55	0.72	0.66	0.61	0.56	0.50	0.47	0.44	0.40
1.0	2.0	0.69	34.88	0.72	0.67	0.62	0.57	0.51	0.47	0.45	0.40
1.0	0.5	0.77	4.08	0.80	0.65	0.60	0.58	0.52	0.47	0.44	0.41
1.0	0.1	N.E.	1.37	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.

N.E.: ΔA could not be estimated, since a linear relationship was not observed between log absorbance and wavelength.

FIG. 4. Color Distribution of Melanoidins from Various Sugar—Glycine Systems.

Effect of concentration of xylose and glycine on the color tone of melanoidins

As seen in Table III, the color tone of melanoidins became lighter as the amount of sugar or amino acid became smaller. Each color component except P1 gave almost the same ΔA but ΔA for P1 was somewhat variable depending on the amounts of glycine and xylose.

Similarly, color components showing a light tone increased when the amounts of sugar

FIG. 5. Effect of Concentration of Xylose and Glycine on the Color Distribution of Melanoidins.

or amino acid decreased (Fig. 5).

Effect of position of the amino group in amino acid on the color tone of melanoidins

Table IV shows the effect of the position of the amino group of amino acids on the color tone of melanoidins with 3 different series of amino acids. The amount of browning on

TABLE IV. EFFECT OF THE POSITION OF THE AMINO GROUP IN AMINO ACIDS ON THE COLOR TONE OF MELANOIDINS

Amino acid	ΔA	E_{450}/ml	ΔA							
			P1	P2	P3	P4	P5	P6	P7	P8
Glycine	0.74	16.48	0.80	0.66	0.60	0.55	0.50	0.46	0.44	0.40
β -Alanine	0.67	35.13	0.80	0.72	0.62	0.55	0.50	0.46	0.43	0.40
γ -Aminobutyric acid	0.66	38.40	0.76	0.71	0.65	0.54	0.50	0.45	0.42	0.39
δ -Aminovaleric acid	0.66	30.33	0.82	0.70	0.60	0.53	0.48	0.44	0.41	0.39
ϵ -Aminocaproic acid	0.68	28.98	0.82	0.68	0.64	0.55	0.48	0.44	0.42	0.41
L-Alanine	0.77	8.38	0.78	0.65	0.57	0.55	0.48	0.45	0.44	0.40
DL- α -Aminobutyric acid	0.76	12.10	0.81	0.65	0.59	0.52	0.47	0.43	0.43	0.40
DL-Norvaline	0.80	12.60	0.82	0.65	0.59	0.54	0.51	0.44	0.42	0.39
DL-Norleucine	0.83	11.60	0.86	0.67	0.58	0.52	0.48	0.45	0.44	0.40
DL-Diaminobutyric acid $\cdot 2\text{HCl}$	0.66	29.00	0.67	0.58	—	—	—	—	—	—
DL-Ornithine-HCl	0.64	33.40	0.65	0.56	—	—	—	—	—	—
L-Lysine	0.65	42.78	0.65	0.56	—	—	—	—	—	—

—: not detectable.

heating a mixture of xylose and each of these amino acids was almost the same as that found by Lento *et al.*²⁾ with glucose and amino acids.

The color tone of melanoidins from amino-terminal monocarboxylic acids was darker than that from glycine. However, chain length (distance between amino group and carboxyl group) had no effect on the color tone of melanoidins. The color tone of melanoidins from monoamino acids with amino groups at the alpha position became lighter when the side-chain length of the molecule increased. Melanoidins from diamino acids showed a dark tone compared to those from other amino acids, but these gave a similar tone independent of chain length.

Color components of melanoidins except for P1 from monoamino acids with an amino group at the end of the chain and monoamino acids with an amino group in the alpha position showed similar ΔA values. However P1 of melanoidins from monoamino acids with an amino group at the alpha position showed higher ΔA with increasing side chain length. Melanoidins from diamino acids were composed of only P1 and P2. Each color component showed almost similar ΔA values and low ΔA values compared to those from other amino acids.

According to the grouping proposed with

the data of Fig. 3, the distribution of color components of melanoidins from diamino acids belong to group 1, those from monoamino monocarboxylic acids belong to group 4, and those from monoamino acids with an amino group at the alpha position belong to group 2 (Fig. 6).

As regards the distribution pattern of color components, melanoidins could be classed into 4 groups based on the percentage of P1. From the distribution pattern of the color component, the degree of stability to oxidation of each group may be estimated, since the color components of melanoidins formed in model systems were found to change on oxidation in the following way; P1→P2→P3→P4→P5→P6→P7→P8.³⁾ Therefore, it is thought that the degree of stability to oxidation of melanoidins is in following order; group 1 > group 2 > group 3 > group 4. Actually, light color components in the melanoidins produced from peptides and terminal amino monocarboxylic acids were easily changed into dark color components.

P1 showed somewhat variable ΔA values in most melanoidins. In general, P1 is unstable to oxidation compared to the other color components. On exposure to air, P1 is liable to become dark compared to the other color components. These facts may explain the variable ΔA values of P1 component.

FIG. 6. Effect of the Position of the Amino Group in Amino Acids on the Color Distribution of Melanoidins.

In most melanoidins produced in the model systems, the color tone of each color component darkened according to the order of elution and each color component from P2 to P8 except for P1 gave similar ΔA values. Therefore, the dark tone of melanoidins belonging to group 4 can be explained by the presence of a large amount of color components showing dark tone and the light tone of melanoidins belonging to groups 2 and 3 can be explained by the large amount of color components showing light tone.

It is concluded that melanoidins are composed of various color components having intrinsic color tones from yellowishbrown to dark brown and that their color tone depends upon the amount of color components and

upon the color tone of P1.

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