

Viscosity of Melanoidins Formed by Oxidative Browning Validity of the Equation for a Relationship between Color Intensity and Molecular Weight of Melanoidin

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To demonstrate the validity of an equation, $E=k_1 M^{\alpha_1}$ (E : $\frac{1\%}{1\text{cm}}$ E_{450} , absorbance of 1% solution in 1 cm path at 450 nm, M : molecular weight, k_1 , α_1 : constant), intrinsic viscosity of the color components of melanoidin from a glycine-, diglycine- or triglycine-xylose system and of the color components of shoyu, separated selectively by utilizing their change of elution position on DEAE-cellulose chromatography during oxidative browning, has been investigated in connection with their color intensity or molecular weight.

The intrinsic viscosity increased with an increase of molecular weight of the color components from each of melanoidins. The Staudinger's equation, $[\eta]=k_2 M^{\alpha_2}$, was observed to fit the relationship between the intrinsic viscosity ($[\eta]$) and molecular weight of color component from each of melanoidins. On the other hand, an experimental equation, $E=k_3[\eta]^{\alpha_3}$ was obtained from a linear relationship between logarithmic intrinsic viscosity and $\log E$ of color components from each of melanoidins. The last equation can also be derived from the first two equations. Therefore, the equation, $E=k_1 M^{\alpha_1}$ was judged by this experiment to be valid for these melanoidins. Moreover, these melanoidins including the color components of shoyu was suggested to consist of a homologous series of symmetrically flexible chain polymer with different degrees of polymerization and color intensity, since the equation, $[\eta]=k_2 M^{\alpha_2}$ was applicable for the melanoidins solutions and none of color components from melanoidins showed optical rotation in visible wavelength region.

Mechanism of the representation of color from the chromophore of melanoidin has been also discussed.

Melanoidin pigments prepared from a model system¹⁾ and also from the color body of shoyu²⁾ were fractionated into eight color components and designated as P1 to P8 in the order of elution position of DEAE-cellulose column chromatography by stepwise elution with sodium chloride solution of varying concentrations. Fast eluting components were found to convert to slow eluting components with a concomitant increase in their molecular weights, probably as the result of polymerization of a structural unit of melanoidin during oxidative browning.³⁾ By utilizing the change in elution position on the DEAE-cellulose column under an oxidative environment, the color components of melanoidins from the model system or shoyu were selectively separated. Molecular weight (M)

and absorbance of 1% solution in 1 cm path at 450 nm, $\frac{1\%}{1\text{cm}}$ E_{450} (E), of the color components increased in the order of elution position from the DEAE-cellulose column and a linear relationship between $\log E$ and $\log M$ was unified as the following equation (1).

$$E=k_1 M^{\alpha_1} \quad (1)$$

This equation was also applicable to a number of melanoidins from model systems⁴⁾ and to the color of shoyu⁵⁾ (soy sauce) and miso⁶⁾ (soybean paste). We concluded that the oxidative browning (darkening) of melanoidin was attributable to an increase in its molecular weight by the polymerization of a structural unit of melanoidin and the amount of color increased according to the equation (1). The oxidative browning of shoyu and miso which

accompanied with the deterioration of flavor and taste could also be explained in terms of the equation (1).^{5,6)}

In order to demonstrate the validity of the equation (1), this communication will describe a relationship among the intrinsic viscosity, molecular weight and color intensity of the melanoidin from a glycine-, diglycine- or triglycine-xylose system and the color of shoyu.

EXPERIMENTAL

Preparation of melanoidin. Melanoidin was prepared by heating a mixture of 0.2 mole xylose and 0.2 mole amino compound in 150 ml of 0.2 M acetate buffer (pH 5.0) at 100°C for 2 hr. Shoyu was used after pasteurization.

Separation of color components. Selective separation of color components by utilizing the change in the elution position of color components of DEAE-cellulose column chromatography under an oxidative environment was performed by almost the same method as previously described.³⁾ Since a large amount of the color components was required for the determination of viscosity, every color components of P3 to P8 fractions which were formed from the original color components (P2~P7) under the oxidative environment were used. However, the viscosity of P3 fraction of melanoidin from diglycine- and triglycine-xylose systems and the color of shoyu could not be determined because of a minute yield of the color component.

Estimation of molecular weight. A method for the estimation of molecular weight of color components was the same as previously described.⁴⁾

Optical rotatory dispersion. Measurements of optical rotatory dispersion were made with a Jasco ORD/UV-5 recording spectro-polarimeter at room temperature. For the measurement in the region of 300 to 600 nm, 0.05% melanoidin solution was used with a cell of 50 mm path length.

Determination of intrinsic viscosity. Viscosity was measured with a Ostwald viscometer requiring 2.5 ml solution with a water flow time of 192.5 sec at 20° ± 0.01°C. Each sample was dissolved in distilled water.

RESULTS

1) Viscosity of melanoidins from model systems

Figures 1~3 show the independence of the

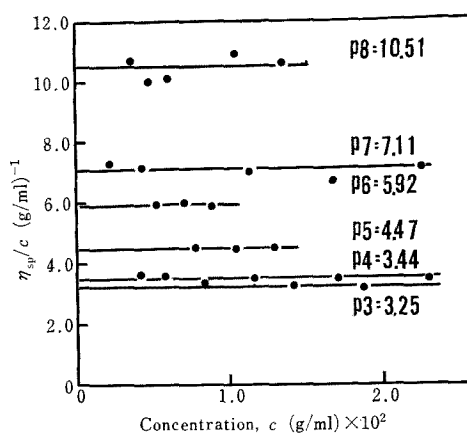


FIG. 1. Determination of Intrinsic Viscosity of the Color Components of Melanoidin from the Glycine-xylose System.

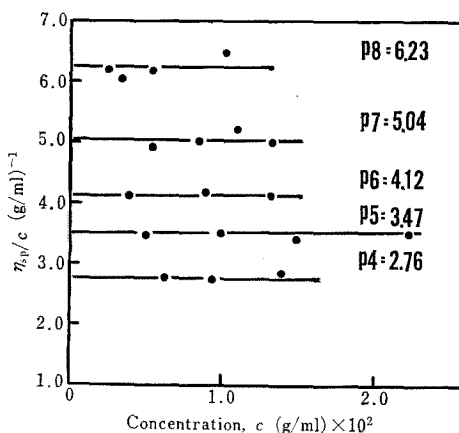


FIG. 2. Determination of Intrinsic Viscosity of the Color Components of Melanoidin from the Diglycine-xylose System.

reduced viscosity, η_{sp}/c , from the concentration of the color components of melanoidins from model systems. The intrinsic viscosity was determined at zero concentration of each melanoidin solution. The intrinsic viscosity increased with an increase of the molecular weight of the color component.

The authors previously reported that a series of the color components of melanoidin from the glycine-xylose system formed during oxidative browning appeared to be very similar in the chemical structure on the basis of spectral measurement, elemental analysis and amino acid analysis and different in molecular

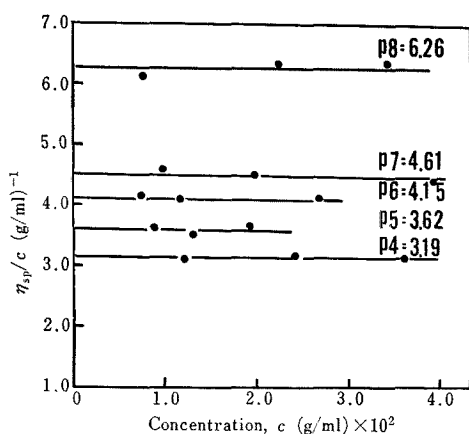


FIG. 3. Determination of Intrinsic Viscosity of the Color Components of Melanoidin from the Triglycine-xylose System.

weight or degree of polymerization and color intensity.³⁾ And moreover, melanoidin was generally observed to leak out endlessly from the cellophane tube by successive dialysis. Assuming by the above facts that melanoidin is a mixture of a homologous series of chain polymers consisting of a similar structural unit with the different degree of polymerization, Staudinger's equation (2),^{7~9)}

$$[\eta] = k_2 M^{\alpha_2} \quad (2)$$

is applicable for the relationship between the

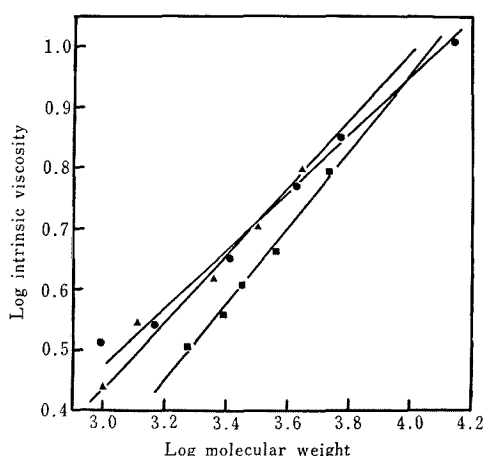


FIG. 4. Plot of Log Intrinsic Viscosity ($[\eta]$) against Log Molecular Weight (M) of the Color Components of Melanoidins from the Model Systems.

Empirical equation: glycine-xylose (●), $[\eta] = 0.096 M^{0.48}$, diglycine-xylose (▲), $[\eta] = 0.062 M^{0.55}$, triglycine-xylose (■), $[\eta] = 0.021 M^{0.66}$.

molecular weight and intrinsic viscosity ($[\eta]$) of the color components of melanoidin. Furthermore, the equation (3),

$$E = k_3 [\eta]^{\alpha_3} \quad (3)$$

can be derived from the equations (1) and (2). Conversely, the validity of the equation (1) will be supported if the equation (3) also fits to melanoidin molecules. Therefore, the relationship to satisfy the equation (3) is expected to exist between the intrinsic viscosity and color intensity of melanoidin.

As shown in Fig. 4, plot for $\log [\eta]$ vs. $\log M$ fairly formed defined straight lines. Apparently, Staudinger's equation (2) was found to be applicable for the color components of melanoidin obtained from the glycine-, diglycine- and triglycine-xylose systems. The data of Fig. 4 give the equations (4) to (6).

$$[\eta] = 0.096 M^{0.48} \quad (\text{glycine-xylose system}) \quad (4)$$

$$[\eta] = 0.062 M^{0.55} \quad (\text{diglycine-xylose system}) \quad (5)$$

$$[\eta] = 0.021 M^{0.66} \quad (\text{triglycine-xylose system}) \quad (6)$$

On the other hand, from the data of Fig. 5, the following equations are formulated.

$$E = 2.90 M^{0.29} \quad (\text{glycine-xylose system}) \quad (7)$$

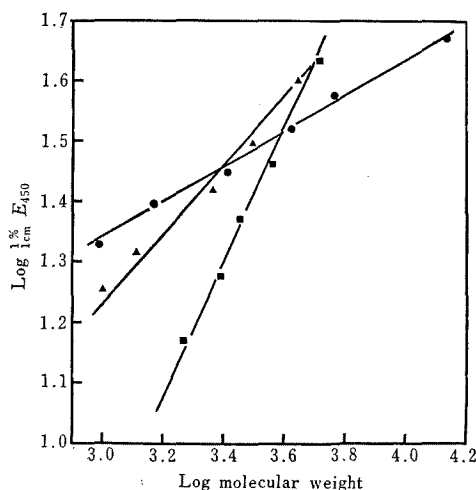


FIG. 5. Plot of $\log \frac{1}{100} E_{450}$ (E) against Log Molecular Weight (M) of the Color Components of Melanoidins from the Model Systems.

Empirical equation: glycine-xylose (●), $E = 2.90 M^{0.29}$, diglycine-xylose (▲), $E = 0.26 M^{0.60}$, triglycine-xylose (■), $E = 2.7 \times 10^{-3} M^{1.14}$.

$$E = 0.26 M^{0.60} \text{ (diglycine-xylose system)} \quad (8)$$

$$E = 2.7 \times 10^{-3} M^{1.14} \text{ (triglycine-xylose system)} \quad (9)$$

The equations (7) and (8) obtained here were fairly close to those previously reported,⁴⁾ although the separation method for color components was slightly different and the values of color intensity and molecular weight were a little high compared with those of previous experiments.⁴⁾ However, the equa-

tion (9) differed slightly from that previously calculated.⁴⁾

As expected by the equation (3), a relationship between $\log E$ and $\log [\eta]$ of each melanoidin was observed to be linear (Fig. 6) and was expressed in the following empirical equations.

$$E = 11.5 [\eta]^{0.61} \text{ (glycine-xylose system)} \quad (10)$$

$$E = 5.88 [\eta]^{1.05} \text{ (diglycine-xylose system)} \quad (11)$$

$$E = 2.19 [\eta]^{1.67} \text{ (triglycine-xylose system)} \quad (12)$$

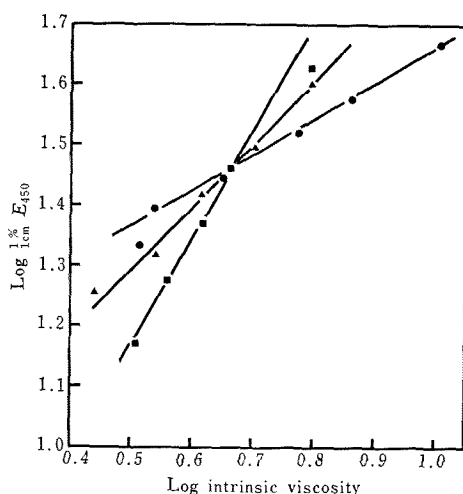


FIG. 6. Plot of $\log \frac{1\%}{1\text{cm}} E_{450} (E)$ against $\log$ Intrinsic Viscosity ($[\eta]$) of the Color Components of Melanoids from the Model Systems.

Empirical equation: glycine-xylose ($\bullet$), $E = 11.5 [\eta]^{0.61}$, diglycine-xylose ($\blacktriangle$), $E = 5.88 [\eta]^{1.05}$, triglycine-xylose ($\blacksquare$), $E = 2.19 [\eta]^{1.67}$.

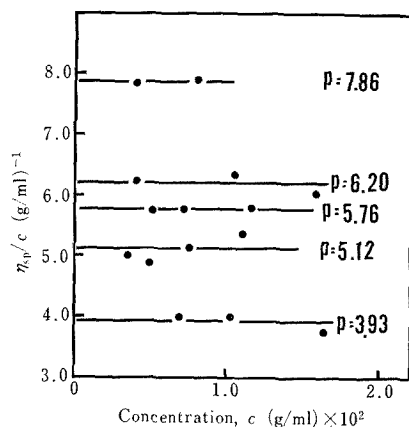


FIG. 7. Determination of Intrinsic Viscosity of the Color Components of Shoyu.

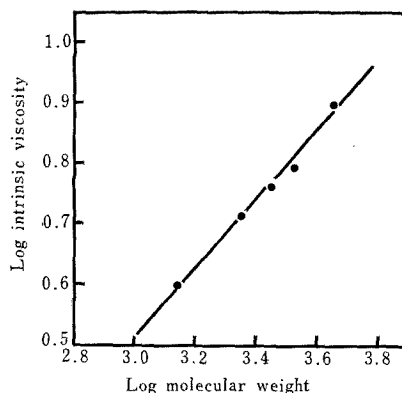


FIG. 8. Plot of $\log$ Intrinsic Viscosity ($[\eta]$) against $\log$ Molecular Weight (M) of the Color Components of Shoyu.

Empirical equation: $([\eta]) = 0.087 M^{0.58}$.

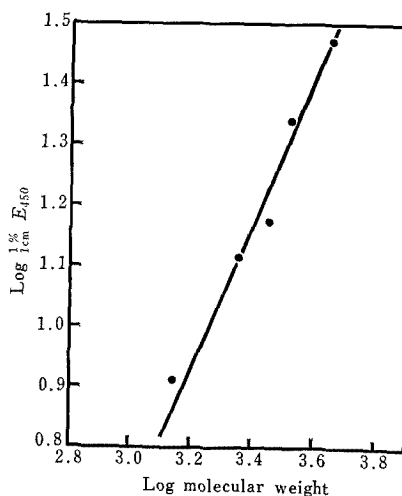


FIG. 9. Plot of $\log \frac{1\%}{1\text{cm}} E_{450} (E)$ against $\log$ Molecular Weight (M) of the Color Components of Shoyu.

Empirical equation: $E = 9.12 \times 10^{-4} M^{1.24}$.

The equations (10) to (12) are respectively in good agreement with those equations ($E=11.8[\eta]^{0.60}$, $E=5.38[\eta]^{1.09}$, $E=2.0[\eta]^{1.72}$) calculated from the equations (4) to (6) and the equations (7) to (9), and this is evidence for the validity of the equation (1).

2) Viscosity of the color components from shoyu

As shown in Fig. 7, the reduced viscosity of each color component of shoyu was independent of its concentration. The intrinsic viscosity was determined by extrapolation of the reduced viscosity to zero melanoidin concentration.

Figure 8 shows a linear relationship between $\log [\eta]$ and $\log M$ and gives the equation (13).

$$[\eta]=0.087 M^{0.58} \quad (13)$$

On the other hand, the following equation was formulated by a linear relationship between $\log E$ and $\log M$ (Fig. 9).

$$E=9.12 \times 10^{-4} M^{1.24} \quad (14)$$

The equation (14) was very close to the one previously reported.⁵⁾

Figure 10 also shows a linear relationship

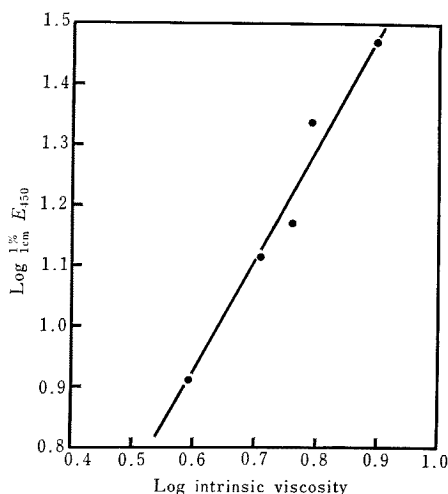


FIG. 10. Plot of $\log \frac{1}{1\text{cm}} E_{450} (E)$ against $\log$ Intrinsic Viscosity ($[\eta]$) of the Color Components of Shoyu.

Empirical equation: $E=0.69 [\eta]^{1.82}$.

between $\log [\eta]$ and $\log E$ of the color components of shoyu gives the following equation.

$$E=0.69 [\eta]^{1.82} \quad (15)$$

In this case, the equation (15) is not consistent with the equation ($E=0.17 [\eta]^{2.14}$) calculated from the equations (13) and (14). However, the equation (1) is valid for the color components of shoyu.

3) Optical rotation of melanoidins from model systems and the color of shoyu

In optical rotatory dispersion analysis, none of the color components of melanoidins from model systems and shoyu showed optical rotation throughout the visible wavelength region.

DISCUSSION

The authors previously reported³⁾ that melanoidin from the glycine-xylose system consisted of a series of color components (melanoidin) which were very similar to each other in their chemical structures but differing in the degree of polymerization and color intensity. Therefore, it can be assumed that melanoidins from model systems are composed of a series of homologous polymers. This assumption was justified by the results in this paper, since the equation (2), which is uniquely applicable to only homologous chain polymers, was applicable for the melanoidins from the glycine-xylose, diglycine-xylose and triglycine-xylose systems or from the color of shoyu. These melanoidins including the color components of shoyu exhibited no optical rotation in the region of visible wavelength. This finding suggests that the chemical structure of melanoidin is symmetric. It is assumed from these data that melanoidins from the glycine-, diglycine- or triglycine-xylose systems and the color of shoyu are made up of a homologous series of symmetrically flexible chain polymers. Most of melanoidins are also thought to have the same molecular shape, since the color of shoyu which is generally consisted of a mixture of various melanoidins formed by the Maillard reaction fol-

lowed Staudinger's equation (2). By this assumption, it can be explained that melanoidin pigments endlessly leak out from cellophane tubes in successive dialysis.

The α_2 value in the equation (2) is a factor representing linear expansion of a chain polymer of the random coil structure^{10,11)} and probably indicates the degree of flexibility of the chain polymer. The α_2 value is generally found to be 0.5 to 1.0. The flexibility of chain polymers increases with decreasing the α_2 value. When the α_2 value equals to 1.0, the molecule is a linear chain polymer. In the present paper, the α_2 value of melanoidin increased with the increase in the molecular size of the amino compounds participating in the Maillard reaction. Accordingly, the flexibility of a melanoidin molecule decreased with the increase in the molecular size of the amino compounds. From the α_2 values estimated in the present investigation, the increasing order of flexibility is assumed to be melanoidin in the triglycine-xylose, shoyu, diglycine-xylose and glycine-xylose systems.

On the contrary, the α_1 value of the equation (1) exhibits the appearance of color by the polymerization of melanoidin. In other words, it exhibits the degree of representation of color from the chromophore in the melanoidin molecule.

As shown in Fig. 11, a linear relationship between α_1 and α_2 values was found in melanoidins from the glycine-, diglycine- and triglycine-xylose systems. The data in Fig. 11 show that the degree of representation of color from the chromophore in melanoidin is suggested to be influenced by the flexibility of the molecule. Thus, in other words, the lower was the degree of flexibility, the more effective was the representation of color from the chromophore. This speculation can be well explained if most of chromophore was buried in the melanoidin molecule. In Fig. 12, a probable shape of the melanoidin molecules with different flexibility are proposed based on the assumption that melanoidin is a homologous series of symmetrically flexible chain polymers. On the other hand, a double

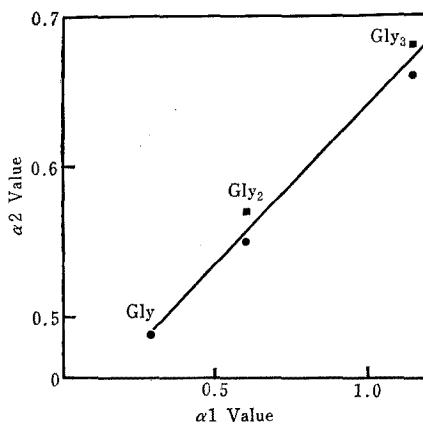


FIG. 11. Plot of α_2 against α_1 .

●, determined; ■, calculated from α_1 and α_3 : $\alpha_2 = \alpha_1/\alpha_3$. α_1 : $E = k_1 M^{\alpha_1}$, α_2 : $[\eta] = k_2 M^{\alpha_2}$, α_3 : $E = k_3 [\eta]^{\alpha_3}$.

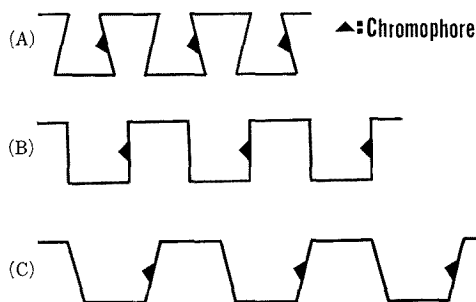


FIG. 12. Probable Shapes of Melanoidin Molecules.

- (A) Melanoidin from glycine-xylose system
 $\alpha_1 = 0.29$, $\alpha_2 = 0.48$.
- (B) Melanoidin from diglycine-xylose system
 $\alpha_1 = 0.60$, $\alpha_2 = 0.55$.
- (C) Melanoidin from triglycine-xylose system
 $\alpha_1 = 1.14$, $\alpha_2 = 0.66$.

bond in a melanoidin molecule was found as one of the chromophore by infrared analysis.³⁾ The representation of color from the chromophore seems to be more hindered by a chain molecule of the symmetrically flexible random structure accompanied with an increase in its flexibility.

As previously reported,^{3,4)} the amount of color increased, and the color tone of melanoidin became darker by increasing the degree of the polymerization of melanoidin due to oxidative browning. The investigation of viscosity of melanoidins revealed that the chain length of a melanoidin molecule increases

with the progress of the polymerization of melanoidin due to oxidative browning and that the increase in the amount of color and the darkening of color tone is due to the increase in the chain length of a melanoidin molecule.

The validity of the equation (1) is strongly supported by the fact that the equations (2) and (3) are also applicable to the melanoidins from the glycine-, diglycine- or triglycine-xylose system and to the color of shoyu.

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