Technical paper

Prediction of Chromaticity of Soy Sauce after Pasteurization Using Statistical Models

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A method to predict the chromaticity of many kinds of raw soy sauce after pasteurization from main chemical constituents was studied. The raw soy sauce pasteurization temperatures were set at three levels in the range of 65 to 90°C and at five time levels in the range of 0 to 60 min. A spectral absorption of the raw and pasteurized soy sauces in the wavelength range of 380 to 780 nm was used to obtain optical density at 450 nm (E_{450}), and chromaticity was measured. It was confirmed that relationship between browning rate constant calculated from change in E_{450} over the studied time and temperature range was applicable to the Arrhenius equation. A pioneer non-linear mathematical model to predict the chromaticity of soy sauce after pasteurization from the main chemical constituents was generated by the multiple regression and least square methods. Effectiveness of the proposed models was confirmed using actual data.

Keywords: soy sauce, chromaticity, pasteurization, mathematical model

Introduction

Soy sauce is heated to control its color and flavor as well as to kill microorganisms. Chromaticity is one of the most important indicators for controlling the quality of soy sauce because it is significantly related to the grade of a product by Japan Agriculture Standard (Hirose, 1994).

Some researchers have studied the relationship between the pasteurization methods and browning of soy sauce, which was monitored by measuring the optical density at 450 (E_{450}) or 500 nm (E_{500}). Okuhara *et al.* (1969) found a discontinuous point between 50 and 60°C by applying the change in E_{500} of soy sauce pasteurized at temperatures from 30 to 90°C to the Arrhenius equation. Onishi (1970) reported that E_{500} of raw soy sauce pasteurized at temperatures from 50 to 90°C linearly increased with time in the range of a few to 100 h. Motai *et al.* (1977) reported that E_{450} of raw soy sauce for dark color products pasteurized at temperatures from 70 to 100°C increased linearly with time in the range of 0 to 3 h, and that the data were applicable to the Arrhenius equation. They also studied the relationship between E_{450} and the chromaticity because quality of soy sauce products in many factories is controlled with reference to the chromaticity. The chromaticity is determined by comparing the color of soy sauce immediately after pasteurization with a standard liquid set using a visual sensory test designated by Japan Research Institute of soy sauce (1985) under a white light source. Motai *et al.* (1977) study

was found to be very important for practical application of the results of the browning by pasteurization. However, results from previously reported studies could be applied to very limited number of products because they examined only one type of raw soy sauce. Therefore, a study, which examines a variety of raw soy sauce, is required for application of the experimental results to a range of industrial products.

In this study, we attempted to create a mathematical model to predict the chromaticity after pasteurization from the main chemical constituents for seven kinds of raw soy sauce using statistical analysis. The effectiveness of the model was validated by experiments.

Materials and Methods

Examined raw soy sauce Seven kinds of raw soy sauce (normal dark color, special dark color, double fermented dark color, low sodium dark color, whole soy bean dark color, normal light color and special light color), provided by Shimajou Co. Ltd. (Kagawa prefecture, Japan), were used in this study.

Pasteurization of raw soy sauce Raw soy sauce samples (1 mL each) were vacuum encapsulated in a Vacuum Hydrolysis Tube (8 mm ϕ × 60 mm, PIERCE Biotechnology, Inc., Rockford, Illinois, USA). The samples were then heated in a water bath controlled at three temperature levels (65, 80 and 90°C) and four time levels (15, 30, 45 and 60 min). The heated samples were rapidly cooled in ice-cold water. Data for validation of mathematical models to predict the chromaticity after pasteurization were collected by pasteurization experiments at 85°C for 20 min

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using six kinds of raw soy sauce (normal dark color, normal light color, double fermented dark color, mixture of normal light color and special light color, mixture of normal dark color and normal light color, and mixture of special dark color and special light color). Equal amounts of two kinds of raw soy sauce were used in their mixtures.

Measurement of spectral transmittance and calculation of chromaticity A V-530iRM spectrophotometer (JASCO Corporation, Hachioji, Japan) was used to obtain spectral transmittance data for the samples in a wavelength range of 380 to 780 nm at 5 nm intervals using the method by Makino *et al.* (2004). According to JIS Z 8701 and 8729 (Japanese Industrial standards; Japanese Standards Association, 2001), the CIELAB ($L^*a^*b^*$) values under D_{65} illuminants recommended by the Commission Internationale de l'Eclairage (CIE) in 1976 were calculated from the spectral transmittance data. The chromaticity was calculated using Eq. 1 previously proposed by Makino *et al.* (2004). The chromaticity (C) of soy sauce is usually determined by a sensory test using a standard liquid set as described in the Introduction section. However, C below 2 cannot be evaluated by this method because the set does not contain any standard liquid for this chromaticity level. Therefore, Eq. 1, which can be applied to calculate C below 2, was used for determining C in the current study.

$$C = 0.855L^* - 0.001(b^*)^2 + 0.413 \quad (1)$$

Analysis of main chemical constituents in raw soy sauce Main chemical constituents in raw soy sauce were analyzed according to the methods proposed by Japan Research Institute for soy sauce (1985). The pH value, total Kjeldahl nitrogen, formol nitrogen, Brix scale, and sodium chloride were measured by the pH electrode method, semi-micro Kjeldahl method, titrimetric analysis, sugar refractometry and Mohr method, respectively. Reducing sugar and pentose concentrations were measured by 3,5-Dinitrosalicylic acid and orcin- Fe^{3+} -hydrochloric acid methods, respectively (Fukui, 1990).

Kinetic and statistical analysis of soy sauce browning Motai *et al.* (1977) confirmed that browning of dark color soy sauce linearly increased with time between 1 to 3 h in a temperature range of 70 to 90°C by monitoring E_{450} . The relationship between browning rate constant calculated from the time changes and pasteurization temperatures was found to be suitable for application to the Arrhenius equation. Therefore, the browning rate constants for the examined raw soy sauces were calculated using Eq. 2 from the change in E_{450} over time in the current study.

$$E_{450} = kt + M \quad (2)$$

In addition, the slopes and intercepts of the linear regression lines, generated by k values plotted according to Arrhenius equation at each temperature, were calculated by Eq. 3. The dependent variable in the current study was the Napierian logarithm, while Motai *et al.* (1977) adopted logarithm to the base 10.

$$\ln k = \frac{A}{T} + B \quad (3)$$

If the k value is calculated by connecting the constants (A and B in Eq. 3) with the constituents, the main chemical constituents in a raw soy sauce can determine the relationship between E_{450} and pasteurization method. A model to calculate the k value was constructed by stepwise multiple regression analysis with StatView® ver. 4.5 (Hulinks Inc., Tokyo, Japan). The dependent variables were the A and B values in Eq. 3. The independent variables were calculated as square, cube, reciprocal, reciprocal square, reciprocal cube, exponent, reciprocal exponent and cross product of the main chemical constituents. The mathematical models indicating the relationship between the dependent and the independent variables constructed by the multiple regression analysis are shown as Eqs. 5 and 6 in the Results and discussion section.

The E_{450} value for any pasteurization method can be predicted from the main chemical constituents by simultaneously solving Eqs. 2, 3, 5 and 6. However, the quality of soy sauce is usually controlled not by E_{450} but by chromaticity measured using a visual sensory test with the standard liquid set in factories. An equation to predict chromaticity from the main chemical constituents was generated from the experimental data for E_{450} and chromaticity determined by Eq. 1 using SigmaProt® ver. 9.01 (Hulinks Inc., Tokyo, Japan), a nonlinear least square fitting program that employs the Marquardt-Levenberg algorithm.

$$C = h(E_{450}) \quad (4)$$

Equations 2 to 6 can be used to predict the chromaticity of any pasteurization method from the main chemical constituents.

Results and Discussion

Relationship between E_{450} and kinds of raw soy sauce subjected to pasteurization under different conditions is presented in Table 1. It was confirmed that the browning of all soy sauce samples progressed over pasteurization time because E_{450} value increased. Moreover, the reaction accelerated with temperature. The results of the current study are in agreement with the data published by Motai *et al.* (1977). Then the slope (k value) was calculated by fixing the intercept at the data of raw soy sauce at zero min as an initial state. The Arrhenius plots of the slopes, calculated by the linear regression analysis using Eq. 2 and the experimental data in Table 1, are shown in Fig. 1. Main chemical constituents of raw soy sauce are presented in Table 2. The browning progresses with the Maillard reaction, which occurs between amino acids and reducing sugars under an anaerobic condition, was adopted in the current study. Although double fermented dark color soy sauce includes higher concentration of total Kjeldahl nitrogen and reducing sugar related to the Maillard reaction than the other soy sauces, its browning did not progress remarkably due to the initial depth of brown.

Table 1. Optical density of pasteurized soy sauce at 450 nm (E_{450}).

Type of soy sauce	Pasteurization temperature, K	Pasteurization time, min				
		0	15	30	45	60
Normal dark color	338	8.57	10.10	10.10	10.88	11.72
	353	8.57	11.06	11.06	11.02	11.83
	363	8.57	10.63	10.80	11.64	12.51
Special dark color	338	9.62	10.70	11.61	12.17	12.17
	353	9.62	10.42	12.24	12.32	12.09
	363	9.62	11.61	12.17	12.88	13.62
Double fermented dark color	338	21.77	22.43	23.11	23.62	23.88
	353	21.77	22.92	23.30	27.03	25.93
	363	21.77	26.29	28.03	31.73	28.19
Low sodium dark color	338	12.32	13.14	13.26	13.38	13.38
	353	12.32	12.82	13.10	13.26	14.58
	363	12.32	13.38	14.66	15.98	15.05
Whole soy bean dark color	338	12.90	14.75	14.92	13.99	15.36
	353	12.90	15.09	14.88	16.77	16.17
	363	12.90	15.05	15.98	16.81	16.63
Normal light color	338	5.54	7.24	6.58	7.19	7.06
	353	5.54	6.82	7.47	7.40	7.75
	363	5.54	7.90	8.73	8.45	9.21
Special light color	338	3.54	3.80	4.08	4.19	4.15
	353	3.54	4.19	4.41	4.58	5.00
	363	3.54	4.71	4.95	5.83	6.25

Fig. 1. The Arrhenius plot of reaction rate constant for change in optical density at 450 nm (E_{450}) of pasteurized soy sauce. The closed circle, triangle, square, diamond, X, open circle and triangle denote the observed data for normal dark color, special dark color, double fermented dark color, whole soy bean dark color, low sodium dark color, normal light color and special light color soy sauces, respectively. The full lines denote the linear regression lines.

Table 2. Main chemical constituents of raw soy sauce.

Type of soy sauce	pH	Total Kjeldahl nitrogen, g•100mL ⁻¹	Formol nitrogen, g•100mL ⁻¹	Brix scale	Sodium chloride, g•100mL ⁻¹	Reducing sugar, g•100mL ⁻¹	Pentose, g•100mL ⁻¹
Normal dark color	4.67	1.65	0.70	35.9	16.9	2.55	0.81
Special dark color	4.82	1.95	1.10	38.0	16.4	2.59	0.61
Double fermented dark color	4.76	2.01	1.05	40.4	15.5	4.53	0.74
Low sodium dark color	4.89	1.68	0.90	29.3	6.1	2.68	0.56
Whole soy bean dark color	4.72	1.86	1.08	37.9	16.8	2.35	0.52
Normal light color	4.80	1.62	1.00	35.7	16.7	2.46	0.59
Special light color	4.75	1.50	0.75	35.2	16.5	3.14	0.63

The results of the relationship between the constants A and B with the main chemical constituents of raw soy sauce by the multiple regression analysis were presented as follows:

$$A = 10^3 F^3 + 1.4 \times 10^5 D^{-1} - 2.4 \times 10^5 e^{-D} + 8.7 \times 10^3 NP - 5.0 \times 10^4 \quad (5)$$

$$B = -2.7 F^3 - 430 D^{-1} + 768 e^{-D} - 24 NP + 142 \quad (6)$$

The chemical constituents related to the Maillard reaction were selected as independent variables as expected. The constants A and B positively correlate with the apparent activation energy for browning and the reaction rate k pasteurized $K = \infty$, which are the indices expressing the difficult level of the browning. It was interesting that F^3 , D^{-1} , e^{-D} and NP selected as the independent variables among many variables to calculate the constants A and B were the same. A non-linear relationship between the browning reaction and main chemical constituents is suggested because the Eqs. 5 and 6 were synthesized by non-linear independent variables. The A value is equivalent to -1 time of the apparent activation energy of browning reaction divided by the universal gas constant ($8.31441 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$). This indicates that the absolute values of A positively correlate with the apparent activation energy. The apparent activation energy of browning reaction was dependent on the kind of soy sauce according to the results in Fig. 1 because the slopes were different for each soy sauce. Motai *et al.* (1977) described that the browning of soy sauce by heating is due to a Maillard reaction initiated by heating a mixture including amino acids and sugars. The brown pigment is the melanoidin produced by polymerization of the Amadori compounds, dicarbonyl compounds, furan compounds and a pyrrole compound as intermediates of the Maillard reaction (Hayase, 1993). The apparent activation energy in the current study relates to the reaction mentioned above, and the non-linearly correlates with the concentrations of the total Kjeldahl nitrogen, formol nitrogen, reducing sugar and pentose.

Correlation of E_{450} between the experimental data for the six kinds of soy sauce pasteurized at 85°C for 20 min

Fig. 2. Correlation between observed and predicted values of optical density of pasteurized soy sauce at 450 nm (E_{450}). The closed circle, open circle, closed square, open square, closed triangle and open triangle denote the actual data for dark color, light color, double fermented dark color, the mixture of light and especially light color, the mixture of dark and light color, and the mixture of concentrated dark and especially light color soy sauces, respectively. The mixtures contain two soy sauces in 1 to 1 ratios. The full line is the linear regression line.

for validation and the calculated values using Eqs. 2, 3, 5 and 6 is shown in Fig. 2. The correlation coefficient was found to be 0.996, which was statistically significant at 99.9% level. This finding supports that the equations to predict E_{450} after pasteurization from the main chemical constituents of raw soy sauce are effective.

Relationship between E_{450} and the chromaticity for all the soy sauce before and after pasteurization is shown in Fig. 3. The E_{450} value and chromaticity non-linearity increased and decreased with the brownness. The E_{450} value was converted to the chromaticity by a linear equation in the $E_{450} \cdot \text{mL}^{-1}$ range of 9 to 20 and in the chromaticity range of 9 to 21 in the report by Motai *et al.* (1977). The data range approximated by Motai *et al.*

Fig. 3. The relationship between optical density of pasteurized soy sauce at 450 nm (E_{450}) and chromaticity. The open circle and full line denote the actual data and nonlinear regression line, respectively.

(1977) included a plot similar to the Fig. 3 plot in this study. However, the relationship was not linear, similar to the results shown in Fig. 3 of the current study. In this study, this non-linear relationship was disclosed in many kind of soy sauce for the first time. The exponential function generated by the least square method using the data in Fig. 3 was as follows:

$$C = 74.1e^{-0.195E_{450}} \quad (7)$$

Correlation of the chromaticity between the experimental data for the six kinds of soy sauce pasteurized at 85°C for 20 min for validation and the calculated values using Eqs. 2, 3, 4, 5 and 6 is shown in Fig. 4. The correlation coefficient was found to be 1.00, which was statistically significant at 99.9%. This finding supports that the equations to predict the chromaticity after pasteurization from the main chemical constituents of raw soy sauce is effective. A method to directly connect the chromaticity with the main chemical constituents could be realized.

According to the results mentioned above, the chromaticity of soy sauce after pasteurization can be predicted from the concentrations of total Kjeldahl nitrogen, reducing sugar, formol nitrogen and pentose of raw soy sauce. A pasteurization plan to prepare a product with a desired chromaticity could be realized by the trial and error method using the mathematical models proposed in this study. A pasteurization method may be determined by back calculation method from a desired chromaticity. For the determination, a solution will be calculated by a numerical nonlinear analysis because a solution can not be calculated analytically using non linear equations. While the results in the current study were obtained under the anaerobic condition, they should be applicable to practical use in industry because the browning by oxidation does not have a significant effect and the browning by the Maillard reaction is dominant for the

Fig. 4. Correlation of chromaticity of soy sauce between observed and predicted values. The closed circle, open circle, closed square, open square, closed triangle and open triangle denote the actual data for dark color, light color, double fermented dark color, the mixture of light and especially light color, the mixture of dark and light color, and the mixture of concentrated dark and especially light color soy sauces, respectively. The mixtures contain two soy sauces in 1 to 1 ratios. The full line is the linear regression line.

pasteurization over 65°C (Motai *et al.*, 1977). There are several kind of pasteurization equipment such as jacket heating kettle, corrugated tube heating kettle, plate heater and many others (Shimura, 2001). The chromaticity control in diverse ways is possible by generating mathematical models using the methods proposed in the current study and the experimental pasteurization data obtained by any equipment. Conversely, changes in chromaticity during storage have to be predicted by different models because the pasteurized soy sauces were rapidly cooled in this study.

Notations

- a^* Redness axis of coordinates for Commission International de l'Eclairage 1976 L^* , a^* , b^*
- A Constant, $K \cdot \text{min}^{-1}$
- b^* Yellowness axis of coordinates for Commission International de l'Eclairage 1976 L^* , a^* , b^*
- B Constant, min^{-1}
- C Soy sauce chromaticity
- D Reducing sugar in raw soy sauce, $\text{g} \cdot 100\text{mL}^{-1}$
- E_{450} and E_{500} Optical density of soy sauce at 450 and 500 nm, respectively
- f Function to calculate A
- F Formol nitrogen in raw soy sauce, $\text{g} \cdot 100\text{mL}^{-1}$
- g Function to calculate B
- h Function to calculate C from E_{450}
- k Browning rate constant, min^{-1}
- L^* Lightness axis of coordinates for Commission International de l'Eclairage 1976 L^* , a^* , b^*

M Constant

N Total Kjeldahl nitrogen in raw soy sauce, $g \cdot 100mL^{-1}$

P Pentose in raw soy sauce, $g \cdot 100mL^{-1}$

t Pasteurization time, min

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