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Optimization of Maillard Reaction in Model System of Glucosamine and Cysteine Using Response Surface Methodology

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ABSTRACT: Sulfur-containing amino acids play important roles in good flavor generation in Maillard reaction of non-enzymatic browning, so aqueous model systems of glucosamine and cysteine were studied to investigate the effects of reaction temperature, initial pH, reaction time, and concentration ratio of glucosamine and cysteine. Response surface methodology was applied to optimize the independent reaction parameters of cysteine and glucosamine in Maillard reaction. Box-Behnken factorial design was used with 30 runs of 16 factorial levels, 8 axial levels and 6 central levels. The degree of Maillard reaction was determined by reading absorption at 425 nm in a spectrophotometer and Hunter's L, a, and b values. ΔE was consequently set as the fifth response factor. In the statistical analyses, determination coefficients (R^2) for their absorbance, Hunter's L, a, b values, and ΔE were 0.94, 0.79, 0.73, 0.96, and 0.79, respectively, showing that the absorbance and Hunter's b value were good dependent variables for this model system. The optimum processing parameters were determined to yield glucosamine-cysteine Maillard reaction product with higher absorbance and higher colour change. The optimum estimated absorbance was achieved at the condition of initial pH 8.0, 111°C reaction temperature, 2.47 h reaction time, and 1.30 concentration ratio. The optimum condition for colour change measured by Hunter's b value was 2.41 h reaction time, 114°C reaction temperature, initial pH 8.3, and 1.26 concentration ratio. These results can provide the basic information for Maillard reaction of aqueous model system between glucosamine and cysteine.

Keywords: glucosamine, cysteine, sulfur-containing amino acids, Maillard reaction, response surface methodology

INTRODUCTION

Maillard reaction is one of most important and complex processes in food processing due to participation of large numbers of complex mixtures into products through different pathways. The basis of Maillard reaction is non-enzymatic browning reaction between amino acids and reducing sugars, which takes place in thermally processed (1). Maillard reaction plays a major role in food stability, flavour development, nutrition, and health (2-5). It produces formation of complex mixtures of coloured and colourless range of flavor volatile to melanoidins, a series of brown pigments with high molecular weight. Generally, alkyl pyrazine has been reported as important product of Maillard reaction for unique sensory properties in food industries (6) and formation of colour directly related with these products (7).

At the early stage of Maillard reaction, the free amino group of amino acid reacts with the carbonyl group of reducing sugar forms reversible Schiff base and rearranges to Amadori rearrangement products or Heyns rearrangement product (8,9). The presence of open chain of sugar molecules in aqueous solution of aldehyde or ketone is less than 1% of total sugar in solution. Therefore, ring opening reaction is initiated by nucleophilic attack of nitrogen atom of the amino group of amino acid to electrophilic carbonyl group of reducing sugar (10). At the intermediate stage, highly UV absorbing and colourless compounds are formed and in the advance phase of reaction, Amadori products undergo further transformation to fluorescent, coloured substances and cross linked polymers (11,12). The final result is the formation of reductions, furfurals, pyrazines, and other cyclic substances.

Maillard reaction properties give desirable characters to some kind of food processing (baking, cocoa and coffee roasting, and cooking of meat) and undesirable charac-

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ters to other processing (milk and fruit juice). Although the Maillard reaction products have negative effect on sensory characteristics of the food (colour changes and volatile compound formation) and nutrition metabolism in human body (amino acid unavailability for metabolism), (13) it has beneficial effects, not only in food processing but also in food preservation. Maillard reaction products have antioxidant ability through scavenging oxygen radicals or chelating metals according to the findings of Bersuder et al. (14), Lingnert et al. (15), Wijewickrama et al. (16), and Monti et al. (17). Maillard reaction is a complex reaction and it is influenced by many factors such as temperature, pH, water activity, heating time, buffer concentration, reactant concentration and reactant source, and sugar involved to the reaction (11,16). Changing of any these products will alter reaction rate, reaction pathways and reaction end product.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for the improvement and optimization of complex processes. The main advantage of RSM is its ability to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions to provide sufficient information for statistical acceptable results (18). It has been successfully demonstrated that this technique can be used in optimizing process variables (19-22). Although flavour scientists have done lots of experiments on Maillard reactions between different types of reducing sugars and amino acids, little information is available on chemical structures of hundreds of unknown products and their effects on the sensory character of foods. Especially the optimum conditions for the reaction have not discovered. This study involved two chemical compounds which are namely glucosamine and cysteine. It is very important to study Maillard reaction between these two compounds rather than to study the reaction between reducing sugar and amino acids because glucosamine contains both an aldehyde moiety with electrophilic carbonyl group and a nucleophilic amino group and cysteine contains another nucleophilic amino group and sulfur atom. The overall objective of this work is to investigate the effect of heating time, temperature, initial pH, and concentration ratio of glucosamine and cysteine in Maillard reaction to optimize the processing conditions using response surface method.

MATERIALS AND METHODS

Materials

D-Glucosamine, L-cysteine, sodium hydroxide, and hydrochloric acids were used. Sodium hydroxide, hydrochloric acid, and L-cysteine were parched from Junsei Chemicals Co., Ltd. (Tokyo, Japan). D-glucosamine was purchased from Jiangsu Jiushoutang Organisms Manufacture Co., Ltd. (Xinghua, China). All other chemicals used were of analytical grade.

Experimental design for reaction

According to prior experimental findings, temperature (X_1), initial pH (X_2), heating time (X_3), and concentration ratio (X_4) are the most influential factors on the Maillard reaction between glucosamine and cysteine. In order to evaluate the effects and interactions of these four factors, RSM was used in designing this experiment. The Box-Behnken factorial design with four independent variables (temperature, initial PH, heating time, and concentration ratio) and absorbance, Hunter's Lab colour scale of L, a, and b values were used as a response (dependent) variable. This design was constructed based on a 33 factorial design, three replications of the central run, leading to 15 sets of experiments, allowing each experimental response to be optimized.

Temperatures were achieved by using a dry oven (Dongwon Scientific System, Busan, Korea). The pH levels were achieved by using 0.1 M NaOH and 0.1 M HCl accordingly and verified by the using of pH meter (827 pH Lab, Metrohm AG, Herisau, Switzerland). The ranges of the concentration ratios were achieved by mixing 0.1 M glucosamine and 0.1 M cysteine according to Table 1. The reactions were conducted in triplicate and each replicate were adjusted the total volume of 20 mL.

The ranges of independent variables, temperature (X_1), pH (X_2), heating time (X_3), and concentration ratios (X_4) were set in five levels of code values of −2, −1, 0, +1, and +2 according to Table 1.

Table 1. Coded levels of independent variables in the response surface design of Maillard reaction between glucosamine and cysteine

Independent variable	Coded unit				
	−2	−1	0	+1	+2
Temperature (°C, X_1)	80	90	100	110	120
pH (X_2)	6	7	8	9	10
Heating time (h, X_3)	2/3	1 1/3	2	2 2/3	3 1/3
Concentration ratio (X_4) (glucosamine : cysteine)	0.6 (0.03:0.07)	0.8 (0.04:0.06)	1 (0.05:0.05)	1.2 (0.06:0.04)	1.4 (0.07:0.03)

Determination of degree of Maillard reaction

The degree of Maillard reaction was determined by absorption at the wavelength of 425 nm by using a spectrophotometer (Ultospec 2000, Amersham Pharmacia Biotech, Buckinghamshire, UK). Distilled water was used as the standard for reference.

Determination of degree of colour changes

The evaluation of colour of the heated Maillard reaction product of glucosamine and cysteine mixtures was carried out using a colourimeter (Colour JC 801, Stable Micro Systems, Godalming, UK) according to the CIE Lab scale. The system provides the values of three colour components; L* (black-white component, luminosity) and the chromaticness coordinates, a* (+ red to − green component) and b* (+ yellow to − blue component). The colour changes in the media were determined by reading the Hunter's L, a, and b values using a colour reader. Distilled water was used as the standard for reference.

Statistical analyses

Analysis of variance (ANOVA) was performed using statistical analysis software (Version 9.2, SAS Institute Inc., Cary, NC, USA). ANOVA tables were generated and the effect and regression coefficients of individual linear, quadratic, and interaction terms were determined. The statistical significance of the regression coefficients was determined by using the F-test and the applicability of the model was checked with significance coefficients of determination (R^2) and the coefficient of variation (CV) values. The optimum processing conditions were obtained by using graphical and numerical analysis based on the criterion of desirability. Data was subjected to multiple regression analysis using SAS (Version 9.2) to fit the following second order polynomial equation:

$$Y=b_0+\sum_{i=1}^{4}b_iX_i+\sum_{i=1}^{4}b_{ii}X_i^2+\sum_{i<j=2}^{4}b_{ij}X_iX_j$$

where b_o is the intercept, b_i, b_{ii}, and b_{ij} are the linear, quadratic, and interaction coefficients, respectively.

The response surface plots were then plotted to present the individual, interactive, and quadratic effects of the independent variables on the responses.

RESULTS AND DISCUSSION

Response surface modelling for absorbance

The details of the real values of absorbance and Hunter's colour values of 30 experimental runs of Box-Behnken design for response surface methodology were summarized in Table 2 and 3. The ANOVA confirms adequacy of the statistical models since their probability>F values are less than 0.05 and statistically significant at the 95% confidence level. The results show that the quadratic model equation is significant ($P<0.05$) on the account. The models present high R^2 and low CV. These values are obtained as follows: $R^2=0.94$ and CV=18.98. This result indicates that a good precision and reliability of the experiments were carried out. The significance of each coefficient is determined by F value and probability>F value. The smaller the magnitude of the probability>F value, the more significant is the corresponding coefficient. Table 4 shows the good matching model for the RSM with a linear and quadratic regression model, due to its high R^2 of 0.91. The fitted model equations are as follows: The multiple polynomial regression analysis result was used to fit the data into the following second order equation model:

$$Y_1=-10.975810+0.337375\times X_1-1.261243\times X_2+0.514981\times X_3-5.050096\times X_4-0.001994\times X_1^2+0.014374\times X_2^2-0.170158\times X_3^2+0.609391\times X_4^2+0.006625\times X_1\times X_2-0.003375\times X_1\times X_3+0.004376\times X_1\times X_4+0.095626\times X_2\times X_3+0.600003\times X_2\times X_4+0.140625\times X_3\times X_4$$

where independent variables of X_1, X_2, X_3, and X_4 denote temperature (°C), pH, heating time (h), and concentration ratio, respectively.

The ANOVA results for the quadratic model for absorbance shows that the linear coefficients of pH and concentration ratio were significant ($P<0.05$). This signifies that the linear effects of initial pH ($P<0.05$) and concentration ratio ($P<0.05$) were dominant over the quadratic and interaction terms. The interaction effects between heating time and concentration ratio was significant. The interaction effects between heating time, temperature and initial pH were not significant, but they slightly influenced the absorbance. Only the quadratic effect of pH was significant to absorbance, while others were not significant. The quadratic effects of heating time, temperature, and concentration ratio were negative to absorbance.

Fig. 1A presents the variation of the absorbance with temperature and initial pH at a given heating time (2 h) and given concentration ratio (1). According to the graph of Fig. 1A, the absorbance increased rapidly with increasing temperature. The same results were also observed in the reaction between reducing sugar and amino group of works of Cerami (5) and O'Brien et al. (23). Temperature and duration of heating were studied by Maillard, reporting that the rate of the reaction increases with temperature (1). Reaction variation of glucosamine and cysteine according to the temperature behaves as the same as the reaction variation of sugar and amino group, but

Table 2. The Box-Behnken matrix and response dependent variables of absorbance and Hunter's L, a, and b for the response surface analysis of Maillard reaction between glucosamine and cysteine

	Independent variables (Coded)[1]				Response variables				
					Absorbance	Hunter's Lab colour scale L, a, and b values			
Runs	X_1	X_2	X_3	X_4	Y_1	Y_2 (L)	Y_2 (a)	Y_2 (b)	Y_2 (ΔE)[2]
1	1	1	−1	−1	1.37±0.03	85.57±2.54	0.79±0.23	59.84±2.32	104.43
2	1	−1	−1	−1	0.71±0.10	96.19±0.53	−10.18±0.46	36.24±3.79	103.30
3	−1	1	−1	−1	0.18±0.01	98.11±0.46	−2.52±0.02	12.08±0.42	98.88
4	1	1	1	−1	0.58±0.05	95.15±1.41	−5.12±0.39	31.84±2.94	100.47
5	−1	−1	1	−1	0.73±0.07	95.92±0.55	−7.41±0.37	31.81±2.34	101.33
6	1	−1	1	1	1.95±0.02	88.23±0.96	−6.24±0.21	61.83±0.71	107.92
7	−1	1	1	1	1.04±0.01	90.94±0.23	−2.22±0.03	51.70±0.36	104.63
8	−1	−1	−1	1	0.76±0.03	96.12±0.73	−6.70±0.09	31.88±0.34	101.49
9	0	0	0	0	1.09±0.05	90.55±1.02	−3.80±0.12	50.97±0.95	103.98
10	0	0	0	0	1.27±0.18	89.20±1.37	−2.78±0.42	53.20±4.15	103.90
11	0	0	0	0	1.36±0.08	88.98±1.06	−2.63±0.38	56.32±2.09	105.34
12	1	1	−1	−1	0.37±0.03	97.16±0.80	−4.50±0.18	22.02±2.00	99.73
13	1	−1	1	−1	1.35±0.02	94.02±0.56	−10.66±0.04	51.81±0.88	107.88
14	−1	1	−1	1	0.45±0.00	95.60±0.36	−3.78±0.15	26.76±0.26	99.34
15	−1	−1	−1	−1	0.35±0.01	98.20±0.09	−4.39±0.07	17.80±0.20	99.90
16	−1	−1	1	1	1.38±0.01	92.58±0.92	−8.49±0.12	45.92±0.49	103.69
17	1	−1	−1	1	1.48±0.02	93.74±0.18	−9.93±0.36	48.63±0.45	106.07
18	−1	1	1	−1	0.37±0.02	96.83±0.43	−4.34±0.09	21.82±1.28	99.35
19	1	1	1	1	2.13±0.15	80.20±2.34	8.41±2.52	78.65±3.38	112.64
20	0	0	0	0	1.09±0.02	90.36±0.56	−3.24±0.21	50.46±0.08	103.54
21	0	0	0	0	1.30±0.00	88.70±0.16	−2.90±0.09	53.64±1.41	103.70
22	0	0	0	0	1.31±0.08	91.11±1.21	−3.49±1.25	54.80±3.12	106.37
23	−2	0	0	0	0.58±0.03	84.91±2.31	−0.43±1.24	33.91±0.93	91.44
24	2	0	0	0	2.29±0.18	80.74±1.08	6.03±0.19	79.40±0.80	113.40
25	0	−2	0	0	0.63±0.04	95.15±0.18	−7.45±0.05	24.98±0.61	98.65
26	0	2	0	0	0.45±0.03	96.35±1.29	−5.59±0.22	24.81±1.46	99.65
27	0	0	−2	0	0.39±0.04	89.99±2.36	−1.52±0.43	23.73±4.40	93.08
28	0	0	2	0	1.68±0.07	87.07±0.59	−1.25±1.09	64.29±2.20	108.24
29	0	0	0	−2	0.34±0.01	93.87±4.58	−4.70±0.27	18.95±0.87	95.88
30	0	0	0	2	2.45±0.03	77.61±0.93	9.05±0.14	78.60±0.68	110.83
		R^2			0.94	0.79	0.73	0.96	0.79

The data represent means±standard deviation of three replicate.
[1]Independent variables are described in Table 1.
[2]$\Delta E = \sqrt{L^2 + a^2 + b^2}$.

the kinetic of the reaction may be different due to attachment of amino group to carbonyl group. Further studies should be done on the kinetics of the reaction between glucosamine and cysteine. It may also be seen that the absorbance increased with the increasing of initial pH and after initial pH is around 8.0, the absorbance decreased with increasing initial pH.

The variation of observance is curvilinear in nature with the increment of initial pH. Lerici et al. (13) described that intermediate stages of the nonenzymatic browning reaction produce more UV-absorbing compound and cause to higher absorbance. This observation is similar to the observation of Maillard reaction between fructose and lysine studied by Ajandouz et al. (24). Higher the starting pH value causes higher absorbance if fructose presents with lysine. The UV-absorbance quickly reached the maximum value at higher pH values and decrease thereafter because of almost complete degradation of sugar during the first stage in the heating period. The decrease of UV absorbance may result the transformation of some intermediate products into brown polymers.

Fig. 1B shows the effect of temperature and heating time on absorbance at a fixed initial pH of 8.0 and concentration ratio of 1. Fig. 1B shows that the absorbance increases rapidly and linearly with the increasing temperature. The absorbance increased rapidly with time at the first stage, while decreased slowly after 2.3 h.

Fig. 1C shows the effect of heating time and concentration ratio on absorbance at a fixed initial pH of 8.0 and temperature of 100°C. The absorbance increased linearly with increasing of concentration ratios within the range of 0.6 to 1.4. Similar observations were explained by Benjakul et al. (25) as the fluorescence intensity of

Table 3. Results of the estimated coefficients of independent variables for model equations in absorbance and Hunter's b values in the response surface analysis of Maillard reaction between glucosamine and cysteine

Parameter	DF	Absorbance		Hunter's b value	
		Estimate	Pr>|t|[1)]	Estimate	Pr>|t|
Intercept	1	−10.975810	0.1918	−483.335833	0.0316
Temp	1	0.337375	0.0034	11.139333	0.0004
pH	1	−1.261243	0.1803	−22.524167	0.3388
Time	1	0.514981	0.6632	19.607500	0.5156
Ratio	1	−5.050096	0.2321	−66.125000	0.5306
Temp×Temp	1	−0.001994	0.0002	−0.077791	<0.0001
pH×pH	1	0.014374	0.7287	−1.809062	0.1003
Time×Time	1	−0.170158	0.0827	−6.750703	0.0109
Ratio×Ratio	1	0.609391	0.5579	4.023438	0.8783
Temp×pH	1	0.006625	0.2326	0.491188	0.0025
Temp×Time	1	−0.003375	0.6787	0.060844	0.7684
Temp×Ratio	1	0.004376	0.8717	0.277812	0.6870
pH×Time	1	0.095626	0.2498	2.048437	0.3287
pH×Ratio	1	0.600003	0.0396	10.715625	0.1339
Time×Ratio	1	0.140625	0.7297	−2.498437	0.8088

DF, degree of freedom; Pr, probability; Temp, temperature; Time, heating time; Ratio, concentration ratio.
[1)]An absolute value of the t value in Student's *t*-test.

the fructose-glycine model system increased with increasing reactant concentrations. According to his explanation, "This might be due to transformation of intermediates to polymer compounds in the presence of reactants at high concentration". Benjakul et al. (25) have found that fluorescence intensity increases when the concentration of sugar increases in Maillard reaction products from a porcine plasma protein-sugar model system. Moreover, Baisier and Labuza (26) and Lerici et al. (13) have discovered that concentrations and ratios of reactants have significant impact on the reaction. In general, the variation of absorbance in Maillard reaction between glucosamine and cysteine can be explained as "Fluorescent compounds formed prior to the generation of brown pigments are possibly the intermediate precursors of brown pigments" (26,27). The variation of absorbance with concentration ratio and initial pH at a constant temperature and constant time is presented in Fig. 1D. It is evident that at a fixed temperature and heating time, the absorbance increased rapidly with initial pH at the first stage, while decreased afterwards. At a fixed temper-

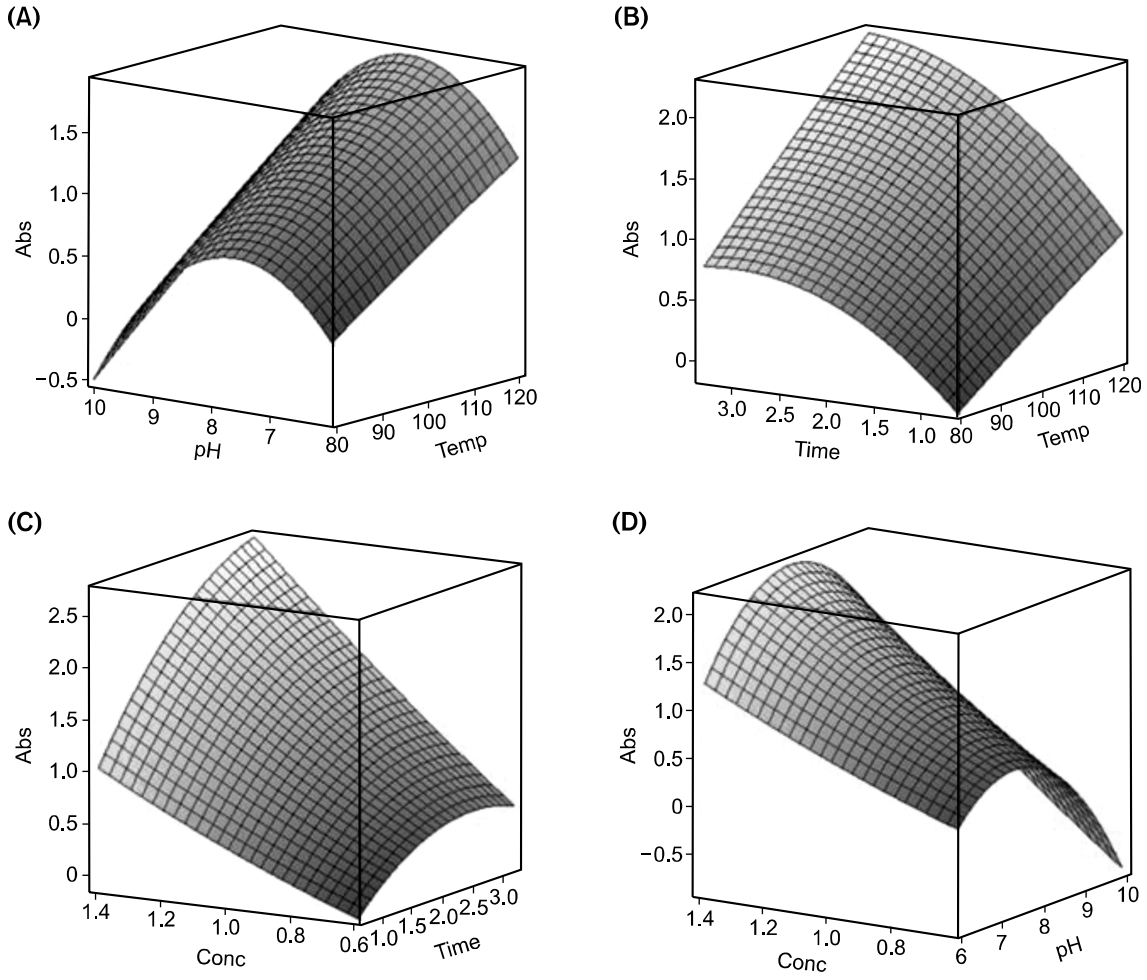

Fig. 1. Three dimensional response surface plots showing the interactive effects of (A) temperature and pH, (B) temperature and heating time, (C) heating time and concentration ratio, and (D) concentration ratio and pH on absorbance at 425 nm. In each plot, the other two components are set at their central values (pH 8.00, temperature 100°C, concentration ratio 1.00, and heating time 2 h). Abs, absorbance; Temp, temperature; Time, heating time; Conc, concentration ratio.

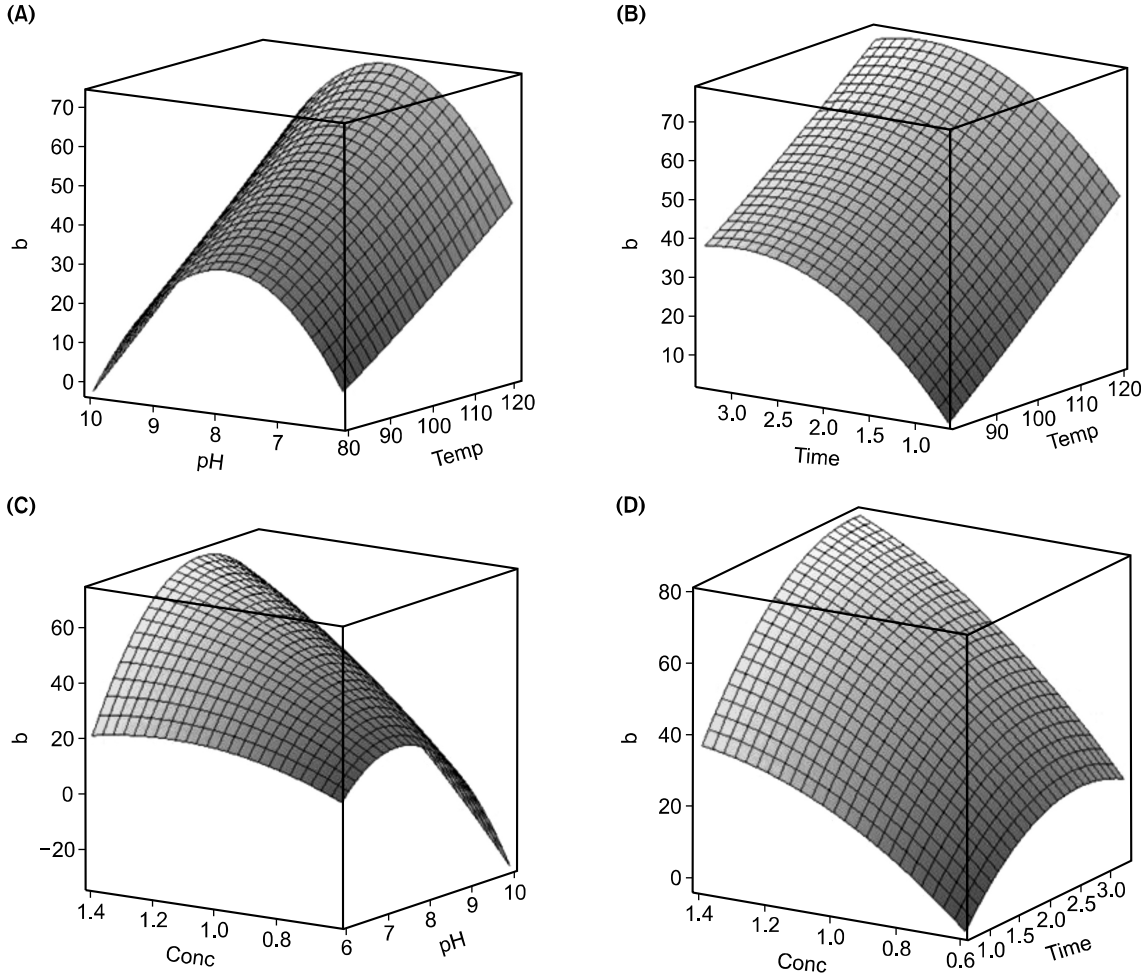

Fig. 2. Three dimensional response surface plots showing the interactive effects of (A) temperature and pH, (B) temperature and heating time, (C) concentration ratio and heating time, and (D) concentration ratio and pH on the Hunter's Lab colour scale b value. In each plot, the other two components are set at their central values (pH 8.00, temperature 100°C, concentration ratio 1.00, and heating time 2 h).

ature, initial pH, and heating time, concentration ratios affect to increase absorbance. The increasement is observed as linear relationship. The central composite design matrix showed that the optimum absorbance can be achieved with the independent variable set at concentration ratio 1.30, initial pH 8.01, temperature 111°C, and reaction time of 2.47 h.

Response surface modelling for Hunter's b value.

Table 2 gives the details of the actual Hunter's b (Y_2) values obtained from each of the 30 experimental runs. Among the response variables of L, a, b, and ΔE, variable b (Y_2) has a higher R^2 value according to the ANOVA results in Table 2. The ANOVA result of Hunter's Lab colour scale b value shows that the quadratic model equation is significant ($P<0.05$) on the account. The models present high R^2 and CV. These values are obtained as follows: $R^2=0.96$ and CV=13.43. This result indicates that a good precision and reliability of the experiments carried out. The significance of each coefficient is determined by F value and probability>F value. The fitted model equations are as follows. The multiple polynomial regression analysis result was used to fit the data into the following second-order equation model:

$$Y_4=-483.335833+11.139333\times X_1-22.524167\times X_2+19.607500\times X_3-66.125000\times X_4-0.077791\times X_1^2-1.809062\times X_2^2-6.750703\times X_3^2+4.023438\times X_4^2+0.491188\times X_1\times X_2+0.060844\times X_1\times X_3+0.277812\times X_1\times X_4+2.048437\times X_2\times X_3+10.715625\times X_2\times X_4-2.498437\times X_3\times X_4$$

where independent variables X_1, X_2, X_3, and X_4 denote temperature (°C), pH, heating time (h), and concentration ratio, respectively.

Hunter's b value has strong relationship with degree of colour change of Maillard reaction according to R^2 (Table 2). Table 4 shows the good matching model for the RSM with a linear and quadratic regression model, due to its high R^2 of 0.91. The ANOVA results for the quadratic model for Hunter's b value have a positive linear effect with the initial pH ($P<0.05$). The interaction effects between pH and concentration ratio were sig-

Table 4. Statistical results of model equations for absorbance and Hunter's b value in the response surface analysis of Maillard reaction between glucosamine and cysteine

Regression	DF	Absorbance		Hunter's b value	
		R^2	Pr>F[1)]	R^2	Pr>F
Linear	4	0.7925	<.0001	0.7396	<.0001
Quadratic	4	0.1143	0.0019	0.1724	<.0001
Crossproduct	6	0.0334	0.2784	0.0468	0.0473
Total model	14	0.9402	<.0001	0.9588	<.0001

DF, degree of freedom; Pr, probability.
[1)]A value of F-distribution in analysis of variance (ANOVA).

nificant while others were not significant, but they slightly influenced the Hunter's b value. Only the quadratic effect of pH was significant to Hunter's b value, while others were not significant.

Fig. 2A presents the variation of the Hunter's b value with temperature and initial pH at a given heating time and a given concentration ratio. It can be seen from the figure that Hunter's b value rapidly increased with increasing temperature. It may also be seen from in Fig. 2A that Hunter's Lab colour scale b value increased with the increasing of initial pH. When initial pH is higher than 8.0, the Hunter's b value decreased. The variation of 'b' value for pH was curvilinear in nature. Fig. 2A shows the same pattern at the absorbance result of Fig. 1A and the b value was increased with the increasing temperature.

Cerami (5) and O'Brien et al. (23) described that the increasing temperature leads to an increase of reactivity of Maillard reaction. In casein and sugar model system, Morales and van Boekel (27) have observed that lightness (L*) of samples and the yellow and blue component (b*) have significant effect with severely heated samples. Lightness decreased significantly in severely heated samples, which was indicated the increased darkness. The reduction of lightness is the result of formation of brown pigments in the sugar and casein mixtures in the advanced stage of the Maillard reaction. Results of yellow and blue component (b*) of glucosamine and cysteine can be explained as the same one as Morales and van Boekel's (27) findings. The blue component (b*) varied same as absorbance with the changing of initial pH of the reaction. The absorbance quickly reached the maximum value at higher pH values and decreased thereafter because of almost complete degradation of sugar during the first stage in the heating period. The decrease of absorbance may result the transformation of some intermediate products into brown polymers (24).

Fig. 2B shows the effect of temperature and heating time on Hunter's b value at a fixed initial pH and fixed concentration ratio. Hunter's b value increased rapidly and linearly with the increased temperature. Hunter's b value increased rapidly with the reaction time at the first stage, while decreased slowly after 2.3 h.

Fig. 2C shows the effect of reaction time and concentration ratio on Hunter's b value at a fixed initial pH and temperature. The b value increased with the increased concentration ratios within the range of 0.6 to 1.4. The variation of b value with concentration ratio and initial pH at a constant temperature and constant reaction time is presented in Fig. 2D. It is evident that at a fixed temperature and heating time, the b value increased rapidly with an initial pH at the first stage, while decreasing afterwards. The central composite design matrix showed that the optimum colour change in absorbance measurement can be achieved by the independent variable set at 1.30 concentration ratio, initial pH 8.01, 111°C temperature, and 2.47 h reaction time.

CONCLUSION

The response surface methodology is a useful tool to investigate the optimum conditions of heating time, temperature, initial pH, and concentration ratio for targeting measurement of degree of Maillard reaction and measurement of degree of colour changes in glucosamine and cysteine Maillard reaction. The R^2 values of the all parameters show a good fit of the models with the experimental data at the 95% confidence level. The different conditions (heating time, temperature, initial pH, and concentration ratio) for Maillard reaction revealed that initial pH and concentration ratio had the significant effect on degree of Maillard reaction, while other two variables had an optimum zone for degree of Maillard reaction. The different conditions (heating time, temperature, initial pH, and concentration ratio) for colour change in Maillard reaction revealed that only the initial pH had significant effect. These results were well fitted with experimental data and obtained models can be used to the maximum values of the variables.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Maillard LC. 1912. Action des acides aminés sur les sucres; formation des mélanoïdines par voie méthodique. *CR Acad Sci* 154: 66-68.
2. George RW, Milton SF. 1983. *The Maillard reaction in foods and nutrition*. Waller GR, Feather MS, eds. American Chemical Society, Washington, DC, USA. p 14-23.
3. Fujimaki M. 1986. *Amino-carbonyl reactions in food and biological systems*. Namiki M, Kato H, eds. Kodansha, Tokyo, Japan. p 125-131.
4. Ledl F. 1990. *The Maillard reaction in food processing, human nutrition and physiology*. Finto P, Aeschbacher H, Hurrell R, Liardon R, eds. Birkhäuser Verlage, Basel, Switzerland. p 210-236.
5. Cerami A. 1994. *Maillard reactions in chemistry, food and health*. Labuza TP, Reineccius GA, Monnier VM, O'Brien J, Baynes JW, eds. The Royal Society of Chemistry, Cambridge, UK. p 103-115.
6. Davidek J, Velisek J. 1990. *Chemical changes during food processing (development in food science)*. Pokorný J, ed. Elsevier Science Ltd., New York, NY, USA. p 117-152.
7. Wong J, Shibamoto T. 1996. Genotoxicity of Maillard reaction products. In *The Maillard reaction: consequences for the chemical and life sciences*. Ikan R, ed. Wiley, Chichester, UK. p 129-160.
8. Jing H, Kitts DD. 2002. Chemical and biochemical properties of casein-sugar Maillard reaction products. *Food Chem Toxicol* 40: 1007-1015.
9. Rizzi G. 1994. Maillard reaction in foods. In *Maillard reactions in chemistry, food and health*. Labuza TP, Reineccius GA, Monnier VM, O'Brien J, Baynes JW, eds. The Royal Society of Chemistry, Cambridge, UK. p 11-19.
10. Yaylayan VA, Huyghues-Despointes A. 1994. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit Rev Food Sci Nutr* 34: 321-369.
11. Ames JM. 1990. Control of the Maillard reaction in food systems. *Trends Food Sci Technol* 1: 150-154.
12. van Boekel MAJS. 1998. Effect of heating on Maillard reactions in milk. *Food Chem* 62: 403-414.
13. Lerici CR, Barbanti D, Manzano M, Cherubin S. 1990. Early indicators of chemical changes in foods due to enzymic or non-enzymic browning reactions 1. Study on heat treated model systems. *Lebensmittel-Wissenschaft und Technologie* 23: 289-294.
14. Bersuder P, Hole M, Smith G. 2001. Antioxidants from a heated histidine-glucose model system. Investigation of the copper(II) binding ability. *J Am Oil Chem Soc* 78: 1079-1082.
15. Lingnert H, Erikssion C, Waller G. 1983. Characterization of antioxidative Maillard reaction product from histidine and glucose. In *The Maillard reaction in foods and nutrition*. Waller GR, Feather MS, eds. American Chemical Society, Washington, DC, USA. p 335-345.
16. Wijewickreme AN, Krejpcio Z, Kitts DD. 1999. Hydroxyl scavenging activity of glucose, fructose, and ribose-lysine model Maillard products. *J Food Sci* 64: 457-461.
17. Monti SM, Ritieni A, Graziani G, Randazzo G, Mannina L, Segre AL, Fogliano V. 1999. LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *J Agric Food Chem* 47: 1506-1513.
18. Lee J, Lin Y, Landen WO Jr, Eitenmiller RR. 2000. Optimization of an extraction procedure for the quantification of vitamin E in tomato and broccoli using response surface methodology. *J Food Compos Anal* 13: 45-57.
19. Lee GD, Kwon JH. 1998. The use of response surface methodology to optimize the Maillard reaction to produce melanoidins with high antioxidative and antimutagenic activities. *Int J Food Sci Technol* 33: 375-383.
20. Gallagher E, O'Brien CM, Scannell AGM, Arendt EK. 2003. Use of response surface methodology to produce functional short dough biscuits. *J Food Eng* 56: 269-271.
21. Shyu SL, Hwang LS. 2001. Effects of processing conditions on the quality of vacuum fried apple chips. *Food Res Int* 34: 133-142.
22. Zhang H, Wang Z, Xu SY. 2007. Optimization of processing parameters for cloudy ginkgo (*Ginkgo biloba* Linn.) juice. *J Food Eng* 80: 1226-1232.
23. O'Brien J. 1998. *The Maillard reaction in foods and medicine*. O'Brien J, Nursten HE, Crabbe MJC, Ames JM, eds. The Royal Society of Chemistry, Cambridge, UK. p 318-329.
24. Ajandouz EH, Tchiakpe LS, Dalle Ore F, Benajiba A, Puigserver A. 2001. Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *J Food Sci* 66: 926-931.
25. Benjakul S, Lertittikul W, Bauer F. 2005. Antioxidant activity of Maillard reaction products from a porcine plasma protein-sugar model system. *Food Chem* 93: 189-196.
26. Baisier WM, Labuza TP. 1992. Maillard browning kinetics in a liquid model system. *J Agric Food Chem* 40: 707-713.
27. Morales FJ, van Boekel MAJS. 1998. A study on advanced Maillard reaction in heated casein/sugar solutions: colour formation. *Int Dairy J* 8: 907-915.
28. Moreaux V, Birlouez-Aragon I. 1997. Degradation of tryptophan in heated β-lactoglobulin-lactose mixtures is associated with intense Maillard reaction. *J Agric Food Chem* 45: 1905-1910.