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Effects of Strawberry Puree and Red Pepper Powder Contents on Physicochemical Properties of *Kochujang* Analyzed Using Response Surface Methodology

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Abstract

Response surface methodology (RSM) was used for the analysis and optimization of the production process of strawberry *Kochujang*. Experiments were carried out according to a central composite design, selecting strawberry puree content and red pepper powder content as independent variables and soluble solids content, moisture content, water activity, color characteristics (*L-, *a**-, and *b**-values) as response variables. The polynomial models developed by RSM were highly effective for describing the relationships between the study factors and the responses. *Kochujang* containing a higher amount of red pepper powder had a higher soluble solids content; on the contrary, soluble solids content decreased with the increase in the strawberry puree content in the sample. Moisture content increased with increased strawberry puree content but decreased with increased red pepper powder content. Water activity increased with the increase in strawberry puree content in the sample but was less affected by the amount of red pepper powder content. Decreases in *L**-values with increasing amount of red pepper powder were noted. *a**-values decreased with the increases in red pepper powder content but increased with the increase in strawberry puree content in the *Kochujang* formulation. *b**-values decreased with the increases in red pepper powder content but was less affected by the strawberry puree content. Overall optimization, conducted by overlaying the contour plots under investigation, was able to point out an optimal range of the independent variables within which the six responses were simultaneously optimized. The point chosen as representative of this optimal area corresponded to strawberry puree content=14.36% and red pepper powder content=11.33%, conditions under which the model predicted soluble solids content=59.31°Brix, moisture content=45.30% (w.b.), water activity=0.758, *L**-value=24.81, *a**-value=7.250, and *b**-value=10.19.**

Key words: *Kochujang*, strawberry puree, physicochemical, RSM, optimization

INTRODUCTION

Kochujang is a fermented traditional Korean hot pepper paste, long used to provide hot, sweet, and savory tastes along with other fermented traditional Korean condiments. *Kochujang* is made of hot red pepper, soybean, starch paste and its hot, sweet, and savory tastes originate from digestion of these ingredients (1-4). *Kochujang* contains more vitamin B_1, B_2, C, and folic acid than *doenjang* (soybean paste) or *ganjang* (soy sauce) and known to have health benefits such as weight and blood-pressure reductions (3).

The quality of *Kochujang* usually depends on the microorganisms which control the fermentation process, mixing ratio of raw materials, fermentation time, and so on. In order to improve the functionality of *Kochujang*, several attempts were made to increase the bioactive components by using kiwifruit (5), *Lycium chinense* fruit (6), sea tangle chitosan (7), and apple and persimmon (8).

Fruits and vegetables contain large amount of antioxidants which strongly contribute to reduced risks of diseases such as cancer, heart attack, and stroke (9). Strawberry fruit contain high concentrations of ascorbic acid which have protective roles against reactive oxygen compounds (10). Anthocyanins in the strawberry also have potent antioxidant properties (11) and reduce oxidative stress-induces neurotoxicity (12). Strawberry is also reported to serve as one of our most important dietary sources of phenolic compounds (13,14).

Attempts were made to perform a systematic investigation on how different amounts of strawberry puree and red pepper powder in the formulation of *Kochujang* influence their physicochemical properties using response surface methodology (RSM), as well as to determine optimal conditions for producing strawberry *Kochujang*, and to provide reliable experimental data for the *Kochujang* making process in developing new varieties of fermented foods.

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MATERIALS AND METHODS

Materials

Kochujang pre-mixture was obtained from Poorun Foods Co., Ltd. (Yeoungcheon, Gyeongbuk, Korea), which was prepared by blending 22% wheat powder (Imported from USA by Dong Ah Flour Mills Co., Ltd., Seoul, Korea), 20% wheat grain (Imported from China), 10.5% salt (Sea salt flakes, Salinity higher than 99%, Imported from Mexico), and 47.5% purified water. Wheat flour was first steamed with pressure after spraying with the warm water and was then blended with ground wheat grain (inoculated with 0.05% spore suspension of *Aspergillus oryzae* starter and incubated at 35～40°C for 48～52 hr) in uniform sizes and salt, then stored in a fermentation tank (Capacity 20 kL, inside coating with fiberglass reinforced plastic) for 1 month. Corn syrup (100% corn starch, TS Co., Ltd., Incheon, Korea), red pepper powder (Imported from China), mixed condiments (contained 38% red pepper powder, 15% salt, 7% garlic, and 4% onion; Korea-China Trade Co., Daejeon, Korea), and spirits (Haitai & Company, Seoul, Korea) were also obtained from Poorun Foods Co., Ltd. (Yeoungcheon, Gyeongbuk, Korea). Fresh strawberries were procured from a local market, which were cultivated at Koryeong (Gyeongbuk, Korea), washed with tap water, and drained. Strawberries were pureed using a hand blender (MR 5550 M CA, Braun Española S.A., Barcelona, Spain) and simmered at 80°C for 15 min. pH, moisture content, and soluble solids content of the strawberry puree were 3.85, 90.25%, and 9.2°Brix, respectively.

Kochujang preparation

Kochujang was prepared using the commercially manufacturing practice by Poorun Foods Co., Ltd. Aged *Kochujang* pre-mixture and 30% corn syrup were pasteurized at 70°C while blending 8% mixed condiments, 3% spirits, and different amounts of strawberry puree (12.929%～27.071%) and red pepper powder (10.7574%～19.2426%) according to the central composite design. The mixtures were then cooled to 40～45°C, placed in a pot, and aged for 30 days at room temperature (23～24°C).

Experimental design

The experimental design used was a central composite design with two factors and five levels. In this experimental design, there were five coded factor levels: $-\sqrt{2}$, -1, 0, 1, and $\sqrt{2}$. The factor and respective coded and uncoded levels are given in Table 1. The effect of two independent responses (*Y*) was modeled using a polynomial response surface. Table 2 shows the actual design of experiments which contain 12 randomized experimental runs, including four replicates at the center point for evaluating the experimental error and the suitability of the mathematical model. The second-order response function for the experiments was predicted by the following equation:

$$Y = \beta_0 + \sum_{i=1}^{2}\beta_i x_i + \sum_{i=1}^{2}\beta_{ii}x_i^2 + \sum_{i=1}^{1}\sum_{i \leq j}^{2}\beta_{ij}x_i x_j$$

where β_0 is the value of the fixed response at the central

Table 1. Independent variables and their coded and actual values used for analysis

Independent variable	Unit	Symbol	Coded levels				
			-1.4142	-1	0	1	1.4142
Strawberry puree	%	x_1	12.929	15	20	25	27.071
Red pepper powder	%	x_2	10.7574	12	15	18	19.2426

Table 2. The central composite experimental design with the observed responses

Exp.	Independent variables		Physicochemical properties					
	Strawberry puree (x_1)	Red pepper powder (x_2)	Soluble solids (°Brix)	Moisture content (%, w.b.)	Water activity	Color		
						*L**-value	*a**-value	*b**-value
1	1.4142	0	57.83	47.13	0.79	24.58	6.56	9.09
2	0	0	60.30	44.91	0.77	24.41	6.37	9.22
3	0	0	60.30	44.91	0.77	24.41	6.37	9.22
4	0	-1.4142	58.03	46.77	0.77	24.87	7.25	10.28
5	-1	1	62.57	42.79	0.75	24.12	6.14	8.81
6	0	1.4142	62.13	43.39	0.76	24.04	5.52	8.06
7	1	-1	57.23	48.01	0.78	24.82	7.08	10.11
8	0	0	60.30	44.91	0.77	24.41	6.37	9.22
9	-1	-1	59.43	45.34	0.76	24.68	7.14	10.11
10	1	1	59.73	44.44	0.78	24.51	6.18	8.65
11	-1.4142	0	62.07	43.68	0.75	24.35	6.37	9.14
12	0	0	60.30	44.91	0.77	24.41	6.37	9.22

point of the experiment which is the point (0,0); β_1 and β_2 are linear; β_{11} and β_{22} are quadratic, and β_{12} is interaction coefficient, respectively.

Soluble solids content measurement

Soluble solids content was determined at room temperature with a refractometer (PR-301, Atago Co., Tokyo, Japan). Measurements were performed in triplicate and mean values were reported.

Moisture content and water activity measurements

The moisture content was determined using a convection oven (FOL-2, Jeio Tech Co., Incheon, Korea) at 105°C for 24 hrs. Water activity of each sample was measured using a water activity meter (TH-500, Novasina, Swiss). Measurements were performed in triplicate and mean values were reported.

Color analysis

CIELAB color characteristics (L^*, a^*, and b^*) of the *Kochujang* samples were determined using a Chromameter (model CR-200, Minolta Co., Osaka, Japan) calibrated with a calibration plate using Y=94.2, x=0.3131, and y=0.3201. The Chromameter used xenon pulse-diffused illumination (D65 illuminant) with three response detectors set at 0° viewing angle. In addition, the machine was preset to use the 2° observer. Measurements were performed in triplicate and mean values were reported.

Statistical analysis

Statistical analysis of variance (ANOVA) and multiple regression were performed using the Design-Expert v.7.0 software (15) to fit the equation. The results included the significance of the model and of each of its terms, the estimated model coefficients, the coefficient of determination, and the lack of fit test.

RESULTS AND DISCUSSION

Diagnostic checking of fitted models

The independent and dependent variables were fitted to the quadratic model equation and examined for the goodness of fit. Regression analysis and ANOVA were conducted for the model to examine the statistical significance of the model terms. ANOVA of the effect of strawberry puree and red pepper powder for preparing *Kochujang* as linear, quadratic, and interaction terms on the response variables are shown in Table 3. The results indicated that the fitted quadratic models accounted for more than 94.7% of the variation in the experimental data, which were highly significant (R^2>0.94). It has been suggested that a model with R^2 greater than 0.8 indicates a good fit (16).

Effects on soluble solids content

Minimum soluble solids content of 57.23°Brix was found with a strawberry puree content (x_1) of 25% and a red pepper powder content (x_2) of 12%, whereas maximum soluble solids content of 62.57°Brix was recorded for a strawberry puree content of 15% and a red pepper powder content of 18% (Table 2). It can be observed from ANOVA that linear and square terms in the model were significant and the regression model explained 99.21% of the total variability in soluble solids content of strawberry *Kochujang* (Table 3). From Table 4, it may also be observed that x_2 term had a linear positive effect on soluble solids content ($p<0.01$). *Kochujang* containing a higher amount of red pepper powder had a higher soluble solids content. On the contrary, soluble solids content decreased with the increased strawberry puree content in the sample (Fig. 1) as is also indicated by the significant quadratic term of x_1 ($p<0.05$) in Table 4. This is probably due to the increase of relative moisture content with higher amount of strawberry puree in the sample. A similar decrease in the soluble solids content with the increase in the amount of strawberry puree in *Kochujang* formulation was also reported (17).

Effects on moisture content and water activity

Moisture content was significantly affected by the variables from which the *Kochujang* was formulated.

Table 3. Analysis of variance on the independent variables as linear, quadratic and interaction terms on the response variables

Source	DF	Soluble solids (°Brix)	Moisture content (%, w.b.)	Water activity	Color		
					L^*-value	a^*-value	b^*-value
Regression	5	32.1745***	26.0896***	0.0016***	0.6491**	2.4465**	4.3767**
Linear	2	31.5792***	25.5063***	0.0015***	0.6136***	2.3693	4.3572***
Square	2	0.4929*	0.3232	4.16E-5*	0.0199	0.0747	0.0130
Interaction	1	0.1024	0.2601	2.50E-5*	0.0156	0.0025	0.064
Residual error	6	0.2687	0.2712	7.53E-6	0.0273	0.1360	0.1730
Lack-of-fit	3	0.2687	0.2712	7.53E-6	0.0273	0.1360	0.1730
Pure Error	3	2.01E-12	5.45E-13	0	4.83E-13	7.60E-14	9.13E-14
Total	11	32.4432	26.3608	0.0016075	0.6764	2.5825	4.5497
R^2		0.9917	0.9897	0.9952	0.9597	0.9474	0.9620

***Significant at $p\leq 0.001$, **Significant at $p\leq 0.01$, *Significant at $p\leq 0.05$.

Table 4. Regression coefficients of the second-order polynomial for the response variables

Coefficients	Soluble solids (°Brix)	Moisture content (%, w.b.)	Water activity	Color		
				L*-value	a*-value	b*-value
β_0	47.1005***	47.0526***	0.7193***	28.2554***	12.5623	13.6020**
Linear						
β_1	0.2741	0.1260	0.0002	-0.1141	-0.1788	0.0072
β_2	1.2858**	-0.3049	0.0040	-0.3039*	-0.4144	-0.3404
Quadratic						
β_{11}	-0.0098*	0.0090*	0.0000	0.0018	0.0040	0.0007
β_{22}	-0.0199	0.0063	-0.0003*	0.0045	0.0067	0.0049
Crossproduct						
β_{12}	-0.0107	-0.0170	0.0002*	0.0041	0.0017	-0.0027

***Significant at p≤0.001, **Significant at p≤0.01, *Significant at p≤0.05.

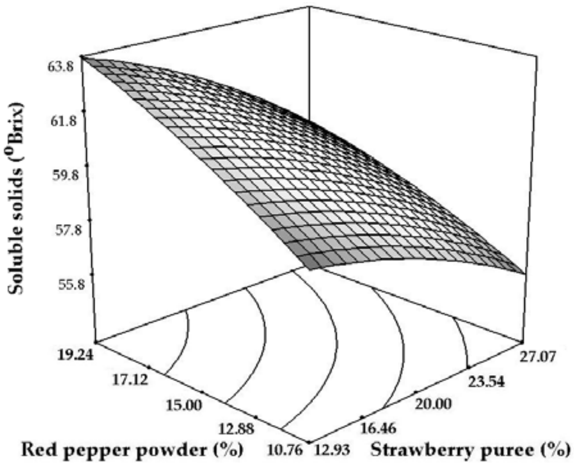

Fig. 1. Effects of strawberry puree and red pepper powder contents on soluble solids content of strawberry *Kochujang*.

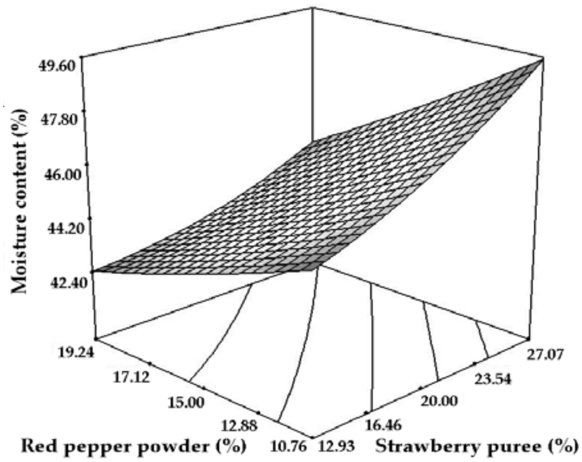

Fig. 2. Effects of strawberry puree and red pepper powder contents on moisture content of strawberry *Kochujang*.

Minimum moisture content (42.79%, w.b.) occurred with a strawberry puree content of 15% and a red pepper powder content of 18% while maximum moisture content (48.01%, w.b.) was recorded at a strawberry puree content of 25% and a red pepper powder content of 12% (Table 2). ANOVA revealed that strawberry puree content and red pepper powder content are significant parameters linearly affecting moisture content ($p<0.001$). The positive coefficient of the first order term of x_1 in Table 4 indicated that moisture content increased with increases in the strawberry puree content. Similar results were reported previously (17). In addition, the negative coefficient of the first order term of x_2 indicated that moisture content decreased with increases in the red pepper powder content. The regression model explained 99.0% of the total variability ($p<0.001$) in moisture content of strawberry *Kochujang*. Fig. 2 also confirms the above findings.

Water activity ranged from 0.75～0.79 (Table 2). The regression model explained 99.5% of the total variability in water activity of strawberry *Kochujang* and linear, quadratic, and interaction terms in the model were significant at $p<0.001$, $p<0.05$, and $p<0.05$, respectively (Table 3). Water activity increased with the increase in strawberry puree content in the sample but less affected by the amount of red pepper powder content (Fig. 3). A similar effect of strawberry puree content on the water activity of *Kochujang* was reported by others (17).

Effect on color

The effects of strawberry puree content and red pepper powder content on *Kochujang* color are also given in Table 2. The values of L*, a*, and b* were in the ranges of 24.04～24.87, 5.52～7.25, and 8.06～10.28, respectively. It is interesting to note that the highest values of L*, a*, and b* were obtained with the *Kochujang* made with 20% strawberry puree and 10.76% red pepper powder while the lowest values with 20% strawberry puree and 19.24% red pepper powder.

As shown in Table 4, the L*-values of strawberry *Kochujang* were significantly affected by the first-order (linear) term of x_2 ($p<0.05$). The contour plot in Fig. 4a indicated decreases in L*-values with increasing amounts of red pepper powder. Significant effect of

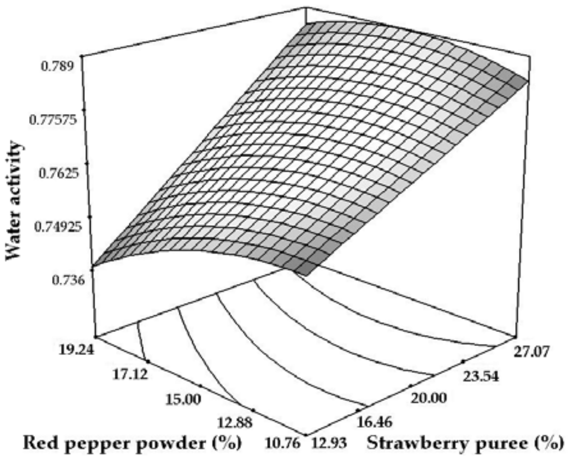

Fig. 3. Effects of strawberry puree and red pepper powder contents on water activity of strawberry *Kochujang.*

strawberry concentration on the *L**-values were reported by others (18).

The *a**-value, which measures the reddish color of *Kochujang,* usually comes from capsanthin of red pepper and has a big impact on the quality and marketability of *Kochujang* (18). The regression model explained 94.7% of the total variability in *a**-value of strawberry *Kochujang*. The *a**-values decreased with the increases in red pepper powder content but increased with the increase in strawberry puree content in the *Kochujang* formulation (Fig. 4b). Seog et al. (18) reported similar results that increases in strawberry puree concentration from 10 to 30% led to a significant increase in *a**-values of strawberry *Kochujang*, from 12.51 to 13.83 among samples fermented for 30 days ($p<0.05$).

The *b**-values of strawberry *Kochujang* were significantly ($p<0.001$) affected by the linear term and the regression model explained 96.2% of the total variability in *b**-value of strawberry *Kochujang*. The *b**-values decreased with the increases in red pepper powder content but were less affected by the strawberry puree content (Fig. 4c). Insignificant changes in *b**-values with the substitution of strawberry puree in the *Kochujang* formulation up to 30% were noted by Seog et al. (18).

Optimization

Numerical and graphical optimizations were carried out for the process parameters for production of strawberry *Kochujang.* The desired goals for each variable and response were chosen as summarized in Table 5. Table 5 also shows software-generated optimum condition for independent variables with the predicted values of responses, in the ranges strawberry puree content

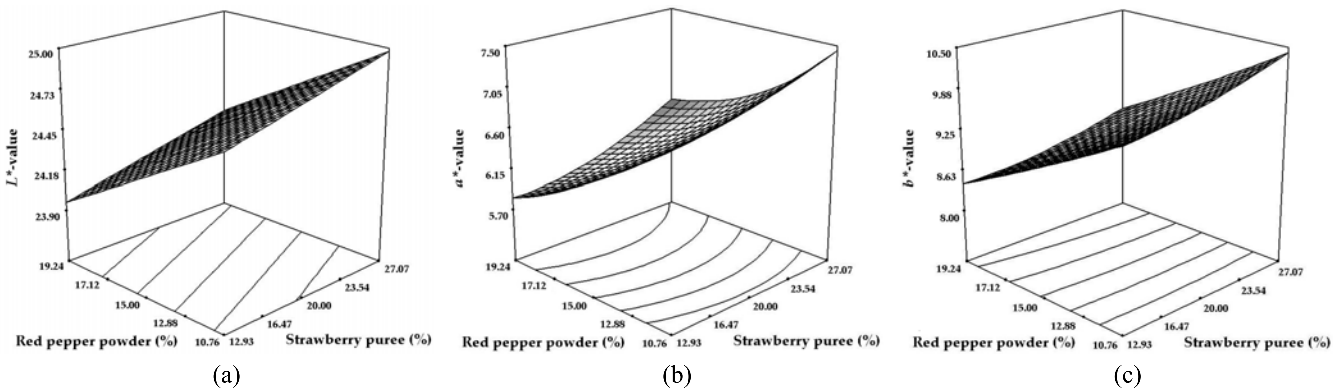

Fig. 4. Effects of strawberry puree and red pepper powder contents on color characteristics of strawberry *Kochujang.* (a) *L**-value, (b) *a**-value, and (c) *b**-value.

Table 5. Criteria and outputs of the numerical optimization of the all the responses affected by strawberry puree and red pepper powder contents

Criteria	Goal	Limit	Outputs		
			1	2	3
x_1: Strawberry puree (%)	Minimize	12.93～27.07	14.36	14.43	14.52
x_2: Red pepper powder (%)	Minimize	10.76～19.24	11.33	11.32	11.30
Y_1: Soluble solids (°Brix)	In the range	58.63～59.78	59.31	59.29	59.27
Y_2: Moisture content (%, w.b.)	In the range	44.97～45.58	45.30	45.32	45.35
Y_3: Water activity	In the range	0.754～0.760	0.758	0.759	0.759
Y_4: *L**-value	In the range	24.68～24.84	24.81	24.81	24.81
Y_5: *a**-value	In the range	7.051～7.287	7.250	7.250	7.250
Y_6: *b**-value	In the range	10.00～10.21	10.19	10.19	10.19
Desirablity			0.915	0.914	0.911

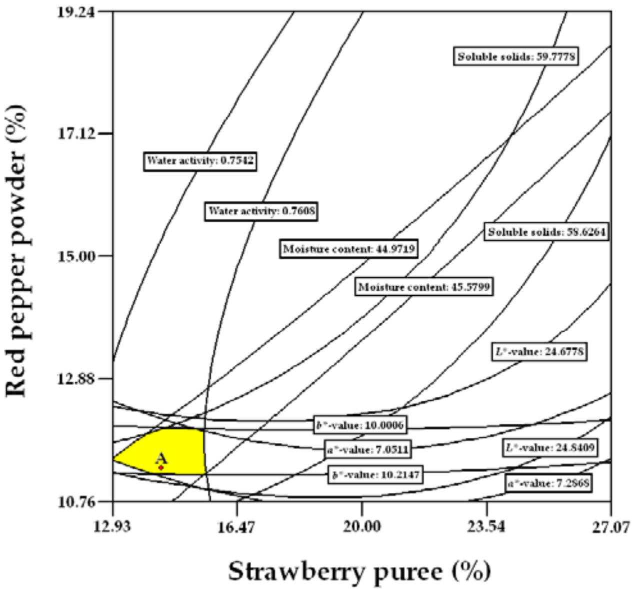

Fig. 5. Superimposed contour plots for significant responses as affected by strawberry puree and red pepper powder contents.

=14.35～14.52% and red pepper powder content=11.30～11.33% for achieving the highest values of soluble solids content=59.27～59.31°Brix, moisture content=45.30～45.35% (w.b.), water activity=0.758～0.759, *L**-value=24.81, *a**-value=7.250, and *b**-value=10.19.

From the set of constraints and outputs given in Table 5, contour plots of relevant and statistically significant responses were generated and the overlaying plots are displayed in Fig. 5. The shaded area in Fig. 5 represents the x_1-x_2 domain satisfying the imposed criteria. Thus, optimum production conditions can be drawn from this delimited area to achieve a specific goal. For example, point A in Fig. 5 determines the following criteria and goals: strawberry puree content=14.36%, red pepper powder content=11.33%, soluble solids content=59.31 °Brix, moisture content=45.30% (w.b.), water activity=0.758, *L**-value=24.81, *a**-value=7.250, and *b**-value=10.19.

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