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Optimization of seaweed and water content for the production of seaweed paste using response surface methodology

***Xiren Guli Keyimu , Aminah Abdullah**

School of Chemical Sciences and Food Technology, Faculty Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan , Malaysia

Abstract : The effects of seaweed concentration on the quality characteristics of seaweed paste [seaweed 30-70% (w/v), water 30-70% (w/v)] of *Kappaphycus alverazii* species were evaluated by response surface methodology (RSM) to determine the optimum amount of ingredients. The interactive effect of fresh seaweed and water percentage on the hardness and gel strength (g) of the paste was determined. Results showed that the experimental data could be adequately fitted into a second-order polynomial model with multiple regression coefficients (R^2) of 0.9805 and 0.7931 for the hardness and gel strength respectively. The hardness and gel strength of seaweed paste were dependent on the ratio of fresh seaweed mixing with water. The proposed optimum amount of ingredients for the production of seaweed paste is at percentage of 44.97 % fresh seaweed and 55.02 % water content. Based on the result obtained, the RSM demonstrated a suitable approach for the adding ingredients optimization of *Kappaphycus alverazii* paste.
Keywords: seaweed paste, kappaphycus alverazii, response surface methodology.

Introduction

Seaweeds have been commonly used for human consumption in many parts of the world, due to they contain vital components of food nutrients for human nutrition. They are recognized as valuable sources of protein, elements, fiber and essential amino acids[1]. Seaweeds are contain sticky components and it has various functions, for example, action of improving bowel movement, antiallergic action, anticoagulant action, antiviral action, anticancer action, action of reducing cholesterol, decreasing blood pressure and therapeutic effect on indefinite complaint[2]. However, fresh seaweeds are perishable in effect on indefinite complaint[2]. However, fresh seaweeds are perishable in nature and require immediate processing or preservation[3], and not utilized extensively for their low processing suitability. As seaweed paste having small seaweed particle can be taken in a short treatment time through a simple method, low production costs and high working efficiency. Seaweed paste can be used for various foods, cosmetics, formulations, daily necessities, materials for building and the seaweed paste can also be used for films, sheets[2].

For a novel food product, it is necessary to study the impact of added ingredients on food quality attributes. The present study was conducted for development and optimization of value added fresh seaweed based paste, using response surface methodology. Furthermore, these interactions between the seaweed and water will be linked to the rheological characteristics of paste aimed at successful product development.

Material and Methods

Seaweed Preparation

The sample *Kappaphycus alvarezii* seaweeds were collected from the coastal area of Sabah in Malaysia. The samples were thoroughly rinsed and soaked in water for 117 minutes[3], and then soaked in 5% lemon juice overnight to eliminate a fishy odour.

Preparation of seaweed paste

Water and odour eliminated wet seaweed were weighed according to the experimental design (Table 2). Mixed in a tank, and stirring the resulting composition at 20℃.

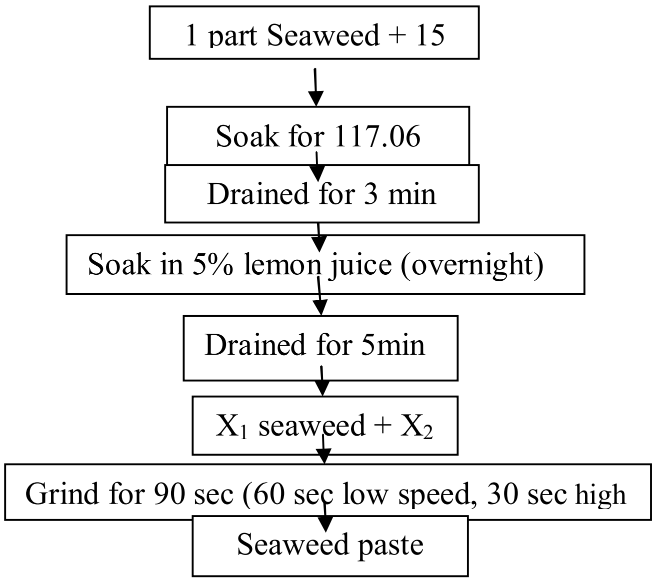

Figure 1. Steps for the preparation of seaweed paste

Hardness determination

The hardness of the seaweed paste was measured using a Stable Micro Systems (SMS) Texture Analyzer (Texture Technologies Corp. Scarsdale, Godalming, UK) using cone probe (No. P/45℃). A paste was placed horizontally on the plate and double compression was applied using a cylindrical probe (75.0 mm in diameter) at a test speed of 1.0 mm/s and deformation of 1.5 mm. The test was performed in triplicate. The maximum peak of the first compression (N) in the force-time curves indicates the hardness value [4, 5].

Measurements of gel strength

The gel strength of seaweed paste was determined according to the method by Wainewright[6] with some modification. 50 g of seaweed paste was mixed with 50 ml of distilled water and heated on the hot plate with continuous stirring with magnetic stirrer until all the seaweed paste is dissolved and boiled. The gel solution was then poured into 3 different containers with 30 g each and allowed to set at room temperature before being kept at 7 ^{o}C for 18 hours. After cool maturation, the gel strength, expressed in g, was measured while the samples were still at 7oC. Gel strength of the samples were determined by using TA.XT2 Texture Analyser (Stable Micro System Ltd. Surrey, England) with 5 kg load cell and 20 mm cylindrical probe with a speed of 1 mm/sec. Maximum force (g) used for the probe to penetrate into the gel 4mm in depth was recorded as gel strength.

Experimental design

Experiments were conducted according to Central Composite Design (CCD), with two variables at five levels each, to optimize the process variables for development of seaweed based paste. The CCD design predicts uniformly at all constant distances from their center points. The independent variables for development of seaweed paste were amount of water (ratio) and seaweed (%) (Table1). The dependent variables chosen were hardness and % moisture. Experimental design in uncoded form of process variables, along with results for optimization of process variables for seaweed paste is given in table 3.

Table1. Experimental design range and values of the independent variables in the central composite design for the production of seaweed paste

Independent variable	Symbol	Level				
			Coded value			
		-α (-1.414)	-1	0	+1	+α (+1.414)
			Actual value			
seaweed	X1	21.72	30	50	70	78.28
water	X2	21.72	30	50	70	78.28

Table 2. Experimental design of seaweed paste (*Central points of experimental design)

Ran order	Coded independent variable		Actual independent variable	
	X1 water (%)		X2	seaweed (%)
1	+α	0	78.28	50
2	0	0	50	50
3	0	-α	50	21.72
4	0	0	50	50
5	-1	+1	30	70
6	-1	-1	30	30
7	+1	+1	70	70
8	0	+α	50	78.28
9	+1	-1	70	30
10	0	0	50	50
11	0	0	50	50
12	0	0	50	50
13	-1	0	21.72	50

A second-order polynomial equation was used to express the hardness (Y1) and gel strength (Y2) of the seaweed paste as a function of the independent variables as follows:

$$Y\,1= \beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_{11}X_1^2+ \beta_{22}X^2_2 + \beta_{12}X_1X_2 + \beta_{12}X_1^2X_2 + \beta_{12}X_1X_2^2$$

$$Y\,2= \beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_{11}X_1^2+ \beta_{22}X^2_2 + \beta_{12}X_1X_2$$

where Y is the predicted response factor, and β_0, β_1, β_2, β_{11}, β_{22} and β_{12} are constant regression coefficients of the model, in which β_0 is the intercept term, β_1 and β_2 are linear coefficients, β_{11} and β_{22} are quadratic coefficients and β_{12} is the interactive coefficient. $X_1$and X_2 are independent factors and combination of factors X_1X_2 represent an interaction between the individual factors in that term.

Data analysis

The experimental design and statistical analysis were performed using response surface methodology (RSM) with Design Expert Version 7.0 software. Data was also analysed using SPSS for ANOVA and Duncan tests. All experiments were done using three replication.

Result and Discussion

The hardness and gel strength of the seaweed paste obtained from all the experiments are given in Table 3. Regression analysis was employed to fit a full response surface model for every response investigated including all linear (X_1 and X_2), interaction (X_1X_2) and quadratic terms (X_1^2 and X_2^2).

Table 3. Actual levels of independent variables along with the observed values for the response variables, hardness and gel strength of seaweed pastes

Run #	X1	X2	Hardness (N)	gel strength (g)
1	50	50	1.37	163.13
2	21.72	50	0.39	145.97
3	30	70	0.24	148.11
4	78.28	50	2.16	140.51
5	70	70	1.28	160.67
6	50	78.28	0.56	143.3
7	50	50	1.23	174.63
8	50	50	1.52	166
9	50	50	1.32	168.98
10	50	50	1.8	175.47
11	30	30	0.91	158.06
12	70	30	3.48	153.72
13	50	21.72	2.76	155.14

X_1=seaweed; X_2=water

Table 4. Analysis of coefficients for coded models used to fit hardness, gel strength and moisture experimental date for seaweed pastes

		hardness			Gel strength	
	Coefficient	F	Prob<F	Coefficient	F	Prob<F
Independent variables						
seaweed, X_1	0.63	39.3	0.0015	0.062	6.604	0.9802
water, X_2	-0.78	60.72	0.0006	-2.47	1.04	0.3425
Interactions						
X_1X_2	-0.38	14.68	0.0122	4.22	1.52	0.2576
X_1^2	-0.084	1.23	0.3176	-10.97	17.82	0.0039
X_2^2	0.11	2.05	0.2112	-7.98	9.43	0.0180
$X_1^2X_2$	0.06	0.18	0.6870	-	-	-
$X_1X_1^2$	0.28	3.84	0.1073	-	-	-

X_1=seaweed; X_2=water

Table 5. Model equation fitted for hardness and gel strength experimental date for seaweed pastes

Responses	Model Equation	Model	Lack of Fit	R^2
hardness	Actual Equation	<0.0006	0.9525	0.9805
	$-5.6749+0.22427X_1+0.17359X_2-5.1691X_1X_2-5.86984X_1^2-1.45819X_2^2+7.53968X_1^2X_2+3.4588X_1X_2^2$	(Significance)	(Not Significant)	
	Coded Equation			
	$+1.45+0.63X_1-0.78X_2-0.38X_1X_2-0.084X_1^2$			

	$+0.11X_2^2+0.06X_1^2X_2+0.28X_1X_2^2$			
Gel strength	Actual Equation	<0.024	0.2001	0.7931
	$83.58134+2.21836X_1+1.34435X_2$ $+0.010562\ X_1X_2-0.027434X_1^2-0.019959X_2^2$	(Significance)	(Not Significant)	
	Coded Equation			
	$169.64+0.062X_1-2.47X_2+4.22X_1X_2-10.97X_1^2-7.98X_2^2$			

X_1=seaweed; X_2=water

Table 6. Analysis of variance (ANOVA) for the 2nd order response surface model of Hardness and Gel strength

	Hardness					**Geln Strengh**				
	ss	**df**	**ms**	**f**	**p-value**	**ss**	**df**	**ms**	**f**	**p-value**
Model	10.04	7	1.43	35.98	0.0006	1261.67	5	252.33	5.37	0.024
A	1.57	1	1.57	39.3	0.0015	0.031	1	0.031	6.604	0.9802
B	2.42	1	2.42	60.72	0.0006	48.73	1	48.73	1.04	0.3425
AB	0.59	1	0.59	14.68	0.0122	71.4	1	71.4	1.52	0.2576
A2	0.049	1	0.049	1.23	0.3176	837.69	1	837.69	17.82	0.0039
B2	0.082	1	0.082	2.05	0.2112	443.38	1	443.38	9.43	0.018
A2B	7.276	1	7.276	0.18	0.687					
AB2	0.15	1	0.15	3.84	0.1073					
Residual	0.2	5	0.04			329.11	7	47.02		
Lack of Fit	2.000	1	2.000	4.018	0.9525	214.16	3	71.39	2.48	0.2001
Pure Error	0.2	4	0.05			114.95	4	28.74		
Cor Total	10.24	12				1590.78	12			

The regression coefficients for the 2nd order response surface model in terms of coded units are shown in Table 4. The examination of the fitted model was necessary to ensure that it provided an adequate approximation to the true system[7]. To develop the fitted response surface model equations, all insignificant terms ($p > 0.05$) were eliminated and the fitted models are shown in Table 5. Analysis of variance (ANOVA) showed that the resultant quadratic polynomial models adequately represented the experimental data with the coefficients of multiple determinations (R2) for the responses, Y1 and Y2 were 0.9805 and 0.7931, respectively. This indicated that the quadratic polynomial models obtained were adequate to describe the influence of the independent variables studied on all the responses. A summary of the analysis of variance (ANOVA) for the predictive model is shown in Tables 6. For any of the term in the models, a large F-value and a small P-value would indicate a more significant effect on the respective response variables [8].

Hardness of seaweed paste

The hardness of seaweed paste varied between 0.24 N to 3.48 N (Table 3 and Figure 1). The analysis of variance of the linear regression model demonstrated that the model is significant with F value of 35.98 (Table 6). The value of multiple correlation coefficients (R^2) of 0.9805 (Table 5), indicates a good agreement between the experimental and predicted values. The results revealed that seaweed and water content influenced the hardness of seaweed paste. The estimated parameter and corresponding P values suggests that the independent variable, water content (X_2) had a more significant effect on the hardness compared to seaweed content (X_1) (Table 6), and also water content (X_2) had a negative coefficient value.

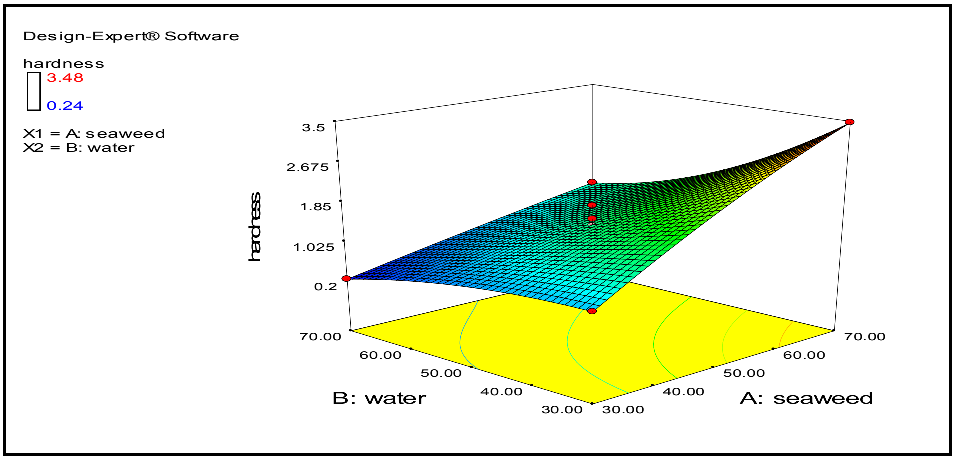

Figure 1. Response surface graph for hardness as a function of seaweed pastes

The hardness of seaweed paste was at the maximum value of 3.48 N when 70% fresh seaweed was mixed in 30% distilled water. The lowest hardness was obtained when 21.72% of fresh seaweed was mixed with 50% of distilled water. From this, it was observed that an increase of water content resulted in a reduction of hardness, but increase of seaweed content resulted in an increase of hardness. This finding is concurrent with Cox and Abu-Ghannam[9] who observed the hardness of the breadsticks and fortification of flour with seaweed at all levels (2.93 to 17.07%) significantly increased the hardness of the breadsticks ($P < 0.05$). Prabhasankar et al. [10] also found that adding seaweed to pasta (1 - 5%) increased the firmness of the product.

Gel strength of seaweed paste

The gel strength of seaweed paste is shown in Table 3 and Figure 2. The results indicated that the maximum level of gel strength (175.47 g) was achieved when the fresh seaweed was mixed with distilled water at the same percentage of 50% each. The lowest gel strength was obtained when 78.28 % of fresh seaweed was mixed with 50 % of distilled water. The analysis of variance of the linear regression model demonstrated that the model is significant with F value of 5.37 (Table 6). The value of multiple correlation coefficients (R^2) of 0.7931 (Table 6), indicates agreement between the experimental and the predicted values.

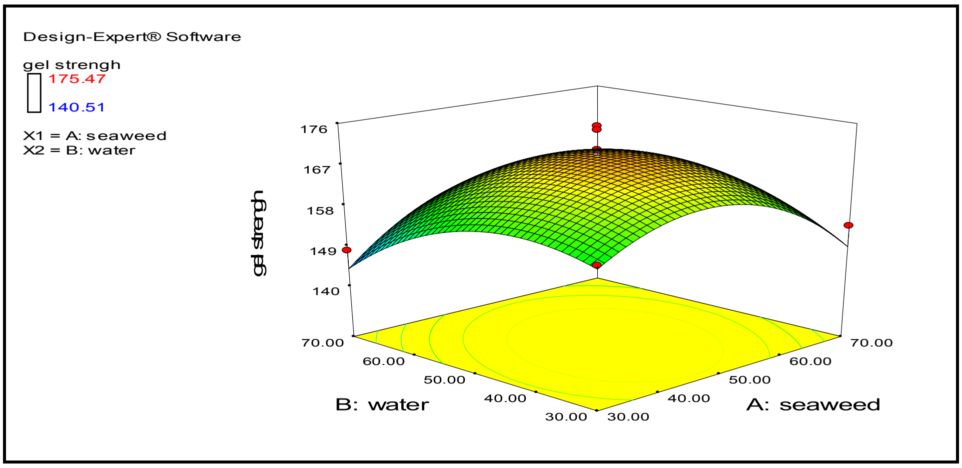

Figure 2. Response surface graph for gel strength as a function of seaweed pastes

The results showed that the seaweed and water content have an effect on the gel strength of the seaweed paste. The variable having the largest effect on the response was the quadratic term of the seaweed content (X_1^2) and water content (X_2^2) ($P < 0.05$). However, the effect of the linear term of seaweed and water content (X_1 and X_2) was insignificant ($P > 0.05$). Likewise, there was no significant effect for the interactive terms (X_1X_2) ($P > 0.05$) on all the measured responses. This may be due to the leaching of water soluble protein which is responsible for the formation of gel[3]. This finding was supported by Shitole et al.[11] who discovered that extract from seaweed can be used as a natural gel enhancer for lesser sardine surimi industry.

Optimization of added ingredients for seaweed paste

For determination of the optimum levels of independent variables, multiple response optimizations were used. A numerical optimizer was applied to determine the exact value of multiple response optimizations that led to the overall optimum condition. In this experiment, the seaweed and water contents were set in a range. Hardness was set at minimize while gel strength was set as maximize. There is 1 suggested solution for the production of seaweed paste. Results obtained show that, the region was predicted to be 44.97 % fresh seaweed and 55.02 % water content. For optimized condition, hardness and gel strength were predicted to be 1.12% and 167.54 g respectively (Table 7), with the desirability 0.751. The % error for hardness and gel strength were 9.14 and 2.66 respectively.

Table 7. Predicted and experimental data for the responses at optimum point for seaweed paste

Responses	Predicted value	Experimental value	Residual	Residual error* (%)
Hardness (N)	1.1176	1.024	-0.094	9.14
Gel Strength (G)	167.543	163.208	-4.335	2.66

*The residual error (%) has been computed as [(Experimental value – Predicted value)/Actual value] x 100

Conclusion

This study has demonstrated the feasibility of using the response surface methodology (RSM) in optimizing the seaweed and water content for the production of seaweed paste. The hardness and gel strength of seaweed paste were dependent on the percentage of seaweed and water. The proposed optimum amount of ingredients for the production of seaweed paste is at percentage of 44.97 % fresh seaweed and 55.02 % water content. For optimized condition, hardness and gel strength were predicted to be 1.12% and 167.54 g respectively. The optimum formulation obtained in this study can be used to determine the effects of seaweed paste on changes in textural characteristics.

Acknowledgement

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