International Food Research Journal 21(1): 471-477 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Optimization of soaking conditions for the production of seaweed (*Kappaphycus alverazii*) paste using response surface methodology

[1*]Siah, W. M., [2]Aminah, A. and [2]Ishak, A.

[1]*Food Technology Research Centre, Malaysian Agricultural Research and Development Institute, P.O. Box 12301, General Post Office, 50774 Kuala Lumpur, Malaysia*
[2]*School of Chemical Sciences and Food Technology, Faculty Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia*

Article history

Received: 14 October 2013
Received in revised form: 15 October 2013
Accepted: 21 November 2013

Keywords

Soaking conditions
Seaweed paste
Kappaphycus alverazii
Response surface methodology

Abstract

The effects of soaking conditions on the quality characteristics of seaweed paste of *Kappaphycus alverazii* species were studied. Response Surface Methodology (RSM) with a 2-factor, 5-level central composite design (CCD) was conducted to determine the optimum soaking conditions. The interactive effect of dry seaweed: soaking water ratio (X_1 = 1: 15-50) and soaking duration (X_2 = 30-120 min) on the gel strength (g), whiteness, expansion (%), moisture content (%) and protein content (g/100 g) of the paste were determined. Results showed that the experimental data could be adequately fitted into a second-order polynomial model with multiple regression coefficients (R^2) of 0.8141, 0.9245, 0.9118, 0.9113 and 0.9271 for the gel strength, whiteness, expansion, moisture content and protein content, respectively. The gel strength, whiteness, expansion, moisture content and protein content of seaweed paste were dependent on the ratio of dry seaweed to soaking water and also soaking duration. The proposed optimum soaking conditions for the production of seaweed paste is at a ratio of 1:15 (dry seaweed : soaking water) and soaking duration of 117.06 min. Based on the result obtained, the RSM demonstrated a suitable approach for the processing optimization of *Kappaphycus alverazii* paste.

Introduction

Seaweeds are renewable resources for many industries like food, pharmaceutical, cosmetics, textile, paper, paint and varnish. They are rich in protein, vitamins, minerals, trace elements and bioactive substances (Dhargalkar and Pereira, 2005; Cox *et al.*, 2010; Gupta and Abu-Ghannam, 2011). They are also the only source of phytochemicals such as agar, agarose, carrageenan and alginate which are widely employed as gelling, stabilizing and thickening agents (Mabeau and Fleurence, 1993; Jimenez-Escrig and Sanchez-Miniz, 2000). People in the Far East and Asian Pacific have a long tradition of consuming seaweeds as part of their diet, while in the Western countries, the principal uses of seaweeds are as sources of phycocolloids, thickening and gelling agents for various industrial applications and recently as components of functional foods (Shahidi, 2009).

Seaweeds are becoming an important commercial aquaculture product in Malaysia with significant increase in yields through the adoption of advanced cultivation approach. From a recorded volume of 1,000 MT in 1991, the production significantly increased to about 14,000 MT in 2009, accounting for about 4 – 10% of the total world production (Suhaimi, 2011). Sabah is the main seaweed producer in Malaysia and most of the production is farmed off the coast of Semporna. Currently, the two main species cultured widely are scientifically known as *Kappaphycus alvarezii* and *Euchema denticulatum* (Ahemad *et al.*, 2006). *K. alvarezii* is the major source of raw materials in the world for the production of kappa-carrageenan, which is an important ingredient in the industrial sector, especially in the production of foods and medicines (Bixler and Porse, 2010).

Fresh seaweeds are perishable in nature and require immediate processing or preservation and drying is the major processing technology practiced for preservation of seaweeds. Before they can be used in industrial processing, this hard, tough and dry seaweed need to be soften by soaking in water. During soaking, the dry seaweed absorbs water, expands and turns soft, and at the same time, some important water soluble nutrients like minerals and protein will leach out. The extent of leaching depends on the soaking conditions particularly on the quantity of soaking water and duration of soaking. From economic points of view, soaking in less water for the shortest period will save cost. However, this will produce seaweed with harder texture which is difficult to grind into soft and smooth paste. In order to produce seaweed paste with the desirable characteristics and minimum loss of nutrients, it is important to consider the soaking

*Corresponding author.
Email: *wmsiah@mardi.gov.my*, *caryen@gmail.com*
Tel: 03 8941 6896; Fax: 03 8942 2906

conditions. Therefore, this study was conducted in order to determine the optimum soaking conditions for producing seaweed paste with the optimum quality characteristics by using response surface methodology.

Materials and Methods

Sample preparation

Dried seaweed (*Kappaphycus alvarezii*) was obtained from Semporna, Sabah. The seaweed was washed under running water to remove debris and salt before being soaked. The seaweed paste was processed according to steps outlined in Figure 1. One part of washed seaweed was soaked in distilled water with predetermined ratio and duration following the experimental runs designed by RSM. The soaked seaweed was drained for 3 min before being weighed to determine percentage expansion. The seaweed was then ground using Warring Blender at low speed for 40 sec and subsequently followed by high speed for 20 sec. To ease the grinding process, 1.2 parts (based on weight of soaked seaweed) of distilled water was added to the soaked seaweed. The paste obtained was immediately analysed for gel strength, whiteness, moisture content and protein content.

Measurements of gel strength

The gel strength of seaweed paste was determined according to the method by Wainewright (1977) 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°C for 18 hours. After cool maturation, the gel strength, expressed in g, was measured while the samples were still at 7°C. 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.

Measurements of colour (whiteness)

Determination of colour was carried out on the seaweed paste which was placed in a petri dish to a height of 1.0 cm. Colour was measured using a Chroma meter CR10 (Minolta Camera Co.) based on the CIE 1976 $L^*a^*b^*$ colour system. The equipment was calibrated using a white tile for the Y, x, y values of 92.4, 0.3136 and 0.3193 respectively. Five colour readings were obtained and the results averaged. Whiteness was calculated based on the following equation:

$$\text{Whiteness} = L^* - 3b$$

Measurements of expansion

The expansion (%) of seaweed after soaked were calculated using the following equation:

$$\text{Expansion (\%)} = \frac{(\text{weight of soaked seaweed} - \text{weight of dry seaweed})}{\text{Weight of dry seaweed}} \times 100$$

Measurements of moisture content

Moisture content of seaweed paste was determined using an oven drying method (AOAC, 2000). Five gram samples were weighed in triplicate in pre-labeled, pre-dried and pre-weighted glass dishes and allowed to dry for 16 – 18 h at 105°C in an oven (Memmert, Germany). Following drying, samples were removed, placed in desiccators and weighed. Moisture content was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Measurements of protein content

Protein content of seaweed paste was determined according to standard AOAC method (AOAC 2000) by using Kjeltec Systems (FOSS, Denmark). Crude protein of the seaweed paste was calculated based on nitrogen factor of 6.25 (6.25 x nitrogen content).

Experimental design

Response surface methodology (RSM) (Khuari and Cornell, 1987) was used to optimize the processing parameters for the production of seaweed paste. Ratio of dry seaweed: soaking water (X_1) and duration (X_2) of soaking were considered as independent factors whereas the gel strength (Y_1), whiteness (Y_2), % expansion (Y_3), moisture content (Y_4) and protein content (Y_5) were considered as responses.

A central composite design (CCD) was employed in the present study. The total number of experimental combinations in the CCD is equal to $2k + 2k + \eta 0$, where k is the number of independent factors and $\eta 0$ is the number of repetitions of the experiments at the centre point. In this study, the CCD with two factors and five levels, including five replicates at the centre point, was used to fit the second-order-response surface. The range and centre point values of the independent variables are based on the results of the preliminary experiments. Table 1 and 2 show the factors, their values and the experimental design. Experimental runs were randomized to minimize the effect of unexpected variability in the observed responses.

Table 1. 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				
Soaking water ratio	X_1	15	20.13	32.5	44.87	50
Soaking duration (min)	X_2	30	43.18	75.0	106.82	120

Table 2. Experimental design for the processing conditions of seaweed paste (*Central points of experimental design)

Run order	Coded independent variable		Actual independent variable	
	X_1	X_2	Ratio	Duration (min)
1	-1	-1	20.13	43.18
2*	0	0	32.50	75.00
3	+1	+1	44.87	106.82
4*	0	0	32.50	75.00
5*	0	0	32.50	75.00
6	-α	0	15.00	75.00
7*	0	0	32.50	75.00
8*	0	0	32.50	75.00
9	0	-α	32.50	30.00
10	0	+α	32.50	120.00
11	+α	0	50.00	75.00
12	-1	+1	20.13	106.82
13	+1	-1	44.87	43.18

A second-order polynomial equation was used to express the gel strength (Y1), whiteness (Y2), expansion (Y3), moisture content (Y4) and protein content (Y5) of the seaweed paste as a function of the independent variables as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

where Y is the predicted response factor, and β_0, β_1, b_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.

Statistical analysis

The Design Expert (version 8) Statistical Programme (Stat-Ease, Inc. 2010) was used to develop the experimental plan for RSM. This software was also used for regression analysis of the data obtained, to estimate the coefficients of the regression equation and to perform the analysis of variance (ANOVA).

Results and Discussion

Model fitting and statistical analysis

The gel strength, whiteness, expansion, moisture content and protein content values 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_{12} and X_{22}). 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 (Zhou and Regenstein, 2004). 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. The gel strength, whiteness, expansion, moisture content and protein content were as predicted by the final models along with the corresponding observed experimental values which are given in Table 3. Comparison of these values indicated that there is an excellent agreement between the predicted and experimental data. Analysis of variance (ANOVA) showed that the resultant quadratic polynomial models adequately represented the experimental data with the coefficients of multiple determinations (R^2) for the responses, Y1, Y2, Y3, Y4 and Y5 were 0.8141, 0.9245, 0.9118, 0.9113 and 0.9271, 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 - 10. 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 (Yuan *et al*., 2008).

Table 3. Experimental and predicted values of responses obtained from the central composite experimental design

Run order	Coded value		Gel strength (g)		Whiteness		Expansion (%)		Moisture content (%)		Protein content (g/100 g)	
	X_1	X_2	Exp. response	Predicted response	Exp. Response	Predicted response	Exp. response	Predicted response	Exp. response	Predicted response	Exp. response	Predicted response
1	-1	-1	1833.46	2263.20	19.03	19.19	377.09	348.95	92.07	91.65	0.52	0.48
2	0	0	1272.97	1379.30	24.23	23.35	450.77	458.73	93.38	93.65	0.25	0.28
3	+1	+1	826.11	550.23	26.33	26.08	537.64	564.18	94.60	95.02	0.28	0.28
4	0	0	1274.09	1379.30	23.47	23.35	447.59	458.73	93.46	93.65	0.30	0.28
5	0	0	1261.41	1379.30	22.37	23.35	486.55	458.73	94.17	93.65	0.31	0.28
6	-α	0	2468.66	2190.09	21.30	21.76	358.74	376.13	92.02	92.24	0.46	0.50
7	0	0	1313.81	1379.30	23.27	23.35	469.32	458.73	93.63	93.65	0.26	0.28
8	0	0	1774.20	1379.30	23.43	23.35	439.40	458.73	93.62	93.65	0.29	0.28
9	0	-α	2653.24	2279.00	19.50	18.84	356.22	379.09	91.66	92.04	0.36	0.38
10	0	+α	833.82	1054.20	23.97	24.71	558.05	536.78	94.96	94.58	0.19	0.20
11	+α	0	867.67	992.38	26.00	25.63	538.60	522.81	94.68	94.46	0.41	0.40
12	-1	+1	1832.26	1841.54	24.37	23.54	451.65	454.73	93.32	93.44	0.36	0.33
13	+1	-1	1716.12	1860.70	21.37	22.12	451.62	446.94	93.33	93.21	0.39	0.39

Table 4. Regression coefficients for response surface models in terms of gel strength, whiteness, expansion, moisture content and protein content

Parameter	Term	Gel strength		Whiteness		Expansion		Moisture content		Protein content	
		Co-efficient	P-value	Co-efficient	P-value	Co-efficient	P-value	Co-efficient	P-value	Co-efficient	P-value
β_0	Intercept	1379.30	0.0170	23.35	0.0008	458.73	0.0014	93.65	0.0015	0.28	0.0007
β_1	X_1	-423.45	0.0083	1.37	0.0018	51.86	0.0007	0.79	0.0009	-0.035	0.0181
β_2	X_2	-433.03	0.0075	2.08	0.0002	55.75	0.0004	0.90	0.0004	-0.064	0.0008
β_{12}	X_1X_2	-222.20	0.2194	-0.095	0.8183	2.87	0.8277	0.005	0.9810	0.013	0.4650
β_{11}	X_1^2	105.97	0.4244	0.17	0.5941	-4.63	0.6449	-0.15	0.3573	0.085	0.0002
β_{22}	X_2^2	143.65	0.2880	-0.79	0.0348	-0.40	0.9683	-0.17	0.3013	0.0046	0.7173

Table 5. Response surface model for seaweed paste processing

Response	Quadratic polynomial model	R^2	F value
Gel strength	$Y1 = 1379.3 - 423.45X_1 - 433.03X_2$	0.8141	6.13
Whiteness	$Y2 = 23.35 + 1.37X_1 + 2.08X_2 - 0.79X_2^2$	0.9245	17.14
Expansion	$Y3 = 458.73 + 51.86X_1 + 55.75X_2$	0.9118	14.48
Moisture Content	$Y4 = 93.65 + 0.79X_1 + 0.90X_2$	0.9113	14.38
Protein content	$Y5 = 0.28 - 0.035X_1 - 0.064X_2 + 0.085X_1^2$	0.9271	17.80

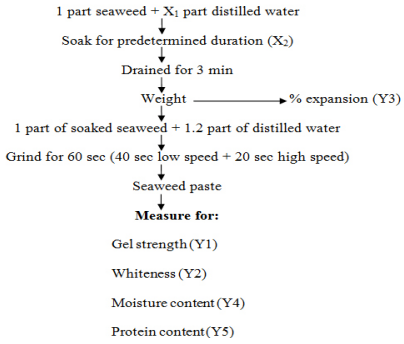

Figure 1. Steps for the preparation of seaweed paste

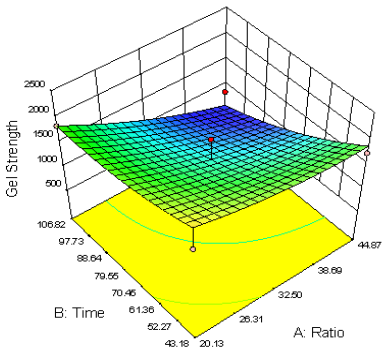

Figure 2. Response surface plot of gel strength as a function of soaking water ratio and duration

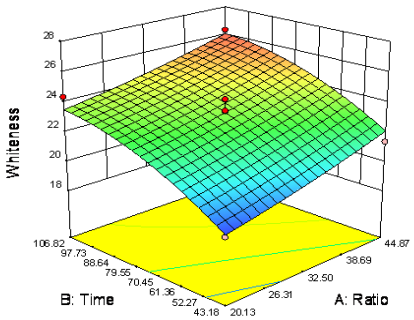

Figure 3. Response surface plot of whiteness as a function of soaking water ratio and duration

Effect of variables on the properties of produced seaweed paste

The three-dimensional response curve plots were generated using the equations in Table 5 to assess the effect of dry seaweed to soaking water ratio and soaking duration on the properties of seaweed paste. The significance of each variable and its interaction were assessed by evaluating the three-dimensional response curve plots (Figure 2-6) and also corresponding prob>F values (Tables 6-10). Both individual variables were found to be significant for the production of seaweed paste.

The gel strength is the most important physical property that determines the quality of seaweed paste. The gel strength of seaweed paste varied between 826.11 to 2653.24 g (Table 3 and Figure 2). The analysis of variance of the linear regression model demonstrated that the model is significant

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

Responses	Source	SS	DF	MS	F	P-value
Gel strength	Model	3.330E+006	5	6.659E+005	6.13	0.0170
	A	1.435E+006	1	1.435E+006	13.21	0.0083
	B	1.500E+006	1	1.500E+006	13.82	0.0075
	AB	1.975E+005	1	1.975E+005	1.82	0.2194
	A2	78118.50	1	78118.50	0.72	0.4244
	B2	1.436E+005	1	1.436E+005	1.32	0.2880
	Residual	7.601E+005	7	1.086E+005	3.82	0.1139
	Lack of fit	5.636E+005	3	1.879E+005		
	Pure error	1.965E+005	4	49127.05		
	Cor total	4.090E+006	12			

Table 7. Analysis of variance (ANOVA) for the 2nd order response surface model of whiteness

Responses	Source	SS	DF	MS	F	P-value
Whiteness	Model	54.40	5	10.88	17.14	0.0008
	A	14.98	1	14.98	23.60	0.0018
	B	34.53	1	34.53	54.42	0.0002
	AB	0.036	1	0.036	0.057	0.8183
	A2	0.20	1	0.20	0.31	0.5941
	B2	4.33	1	4.33	6.82	0.0348
	Residual	4.44	7	0.63	2.03	0.2525
	Lack of fit	2.68	3	0.89		
	Pure error	1.76	4	0.44		
	Cor total	58.84	12			

with F value of 6.13 (Table 5). The value of multiple correlation coefficients (R^2) of 0.8141, indicates a good agreement between the experimental and the predicted values. The results revealed that dry seaweed to soaking water ratio and soaking duration influenced the gel strength of seaweed paste. The estimated parameter and corresponding p values suggests that the independent variable, soaking duration (X_2) had a more significant effect on the gel strength compared to dry seaweed to soaking water ratio (X_1) (Table 6). The gel strength of seaweed paste was at the maximum value of 2653.24 g when the dry seaweed was soaked in distilled water at the ratio of 1:32.50 for the duration of 30 min. The lowest gel strength was obtained when 1 part of dry seaweed was soaked in 44.87 part of distilled water for the duration of 106.82 min. From this, it was observed that an increase in dry seaweed to water ratio and soaking duration resulted in a reduction of gel strength. This may be due to the leaching of water soluble protein which is responsible for the formation of gel. This suggestion is supported by the results which show that the seaweed paste with the lowest gel strength also had the lowest protein content.

The colour of the seaweed paste played an important role in determining the quality of the paste produced. Whiter coloured seaweed paste is considered as higher grade product. The whiteness values of seaweed paste ranged from 19.03 to 26.33 (Table 3 and Figure 3). The analysis of variance of the linear regression model demonstrated that the model is significant with F value of 17.14 (Table 5). The value of multiple correlation coefficients (R^2) of 0.9245, indicates a good agreement between the experimental and the predicted values. The results showed that dry seaweed to soaking water ratio and

Table 8. Analysis of variance (ANOVA) for the 2nd order response surface model of expansion

Responses	Source	SS	DF	MS	F	P-value
Expansion	Model	46563.59	5	9312.72	14.48	0.0014
	A	21515.72	1	21515.72	33.46	0.0007
	B	24865.70	1	24865.70	38.67	0.0004
	AB	32.83	1	32.83	0.051	0.8277
	A2	149.04	1	149.09	0.23	0.6449
	B2	1.09	1	1.09	1.697E-003	0.9683
	Residual	4501.53	7	643.08	2.81	0.1716
	Lack of fit	3054.32	3	1018.11		
	Pure error	1447.21	4	361.80		
	Cor total	51065.13	12			

Table 9. Analysis of variance (ANOVA) for the 2nd order response surface model of moisture content

Responses	Source	SS	DF	MS	F	P-value
Moisture content	Model	11.74	5	2.35	14.38	0.0015
	A	4.96	1	4.96	30.39	0.0009
	B	6.46	1	6.46	39.53	0.0004
	AB	1.000E-004	1	1.000E-004	6.122E-004	0.9810
	A2	0.16	1	0.16	0.97	0.3573
	B2	0.20	1	0.20	1.25	0.3013
	Residual	1.14	7	0.16	2.67	0.1831
	Lack of fit	0.76	3	0.25		
	Pure error	0.38	4	0.095		
	Cor total	12.88	12			

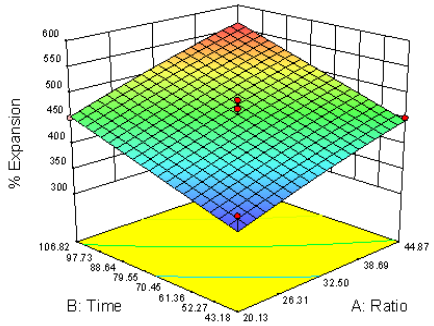

Figure 4. Response surface plot of expansion as a function of soaking water ratio and duration

soaking duration affect the whiteness of the seaweed paste. The estimated parameter and corresponding p values suggest that soaking duration (X_2) had a more significant effect on the whiteness of the paste produced compared to dry seaweed to soaking water ratio (X_1) (Table 7). The paste obtained had the whitest colour when the dry seaweed was soaked in distilled water at the ratio of 1:44.87 for the duration of 106.82 min. The lowest whiteness value was obtained when 1 part of dry seaweed was soaked in 20.13 part of distilled water for the duration of 43.18 min. Results indicated that, an increase in dry seaweed to soaking water ratio and soaking duration produced whiter-coloured paste.

In order to ease the grinding process after soaking and produce smoother paste, seaweed that absorbs water and expands to a certain extent is desired. Results for the expansion and moisture content of seaweed paste are shown in Table 3, Figures 4 and 5. For both of the responses, the maximum values were obtained when the dry seaweed were soaked in distilled water at the ratio of 1:32.50 for the duration of 120 min, while the minimum values were recorded

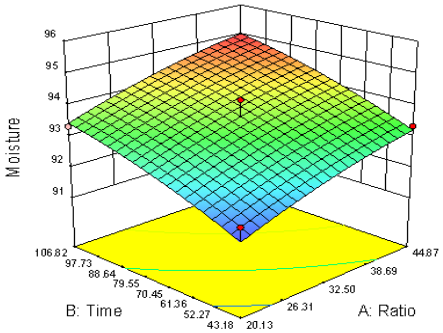

Figure 5. Response surface plot of moisture content as a function of soaking water ratio and duration

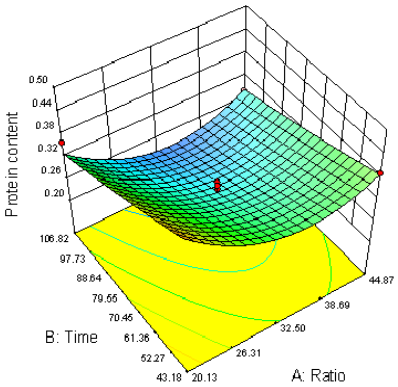

Figure 6. Response surface plot of protein content as a function of soaking water ratio and duration

when the seaweed were soaked in distilled water at the ratio of 1:32.50 for 30 min. Seaweed for both experimental runs were soaked in the same ratio of water but for different lengths of time. Results showed that the dry seaweed expanded more when soaked for a longer time. The absorption of water during soaking treatment contributes to the expansion of seaweed and it was observed that the moisture content had increased accordingly. This conclusion is supported by the p values shown in Table 9. The p values are used as a tool to check the significance of each of the coefficients, which in turn, are necessary to understand the pattern of the mutual interactions between the variables. The smaller the magnitude of the p, the more significant is the corresponding coefficient (Khuari and Cornell, 1987). According to this probability values, the duration of soaking was observed as the most significant factor compared to dry seaweed to soaking water ratio for both responses.

The protein content of seaweed paste which underwent different soaking conditions is shown in Table 3 and Figure 6. The results indicated that the maximum level of protein (0.52 g/100 g) was achieved when 1 part of dry seaweed was soaked in 20.13 part of distilled water for the duration of 43.18 min, while the minimum (0.19 g/100 g) was achieved when soaked in the ratio of 1: 32.5 for 120

Table 10. Analysis of variance (ANOVA) for the 2nd order response surface model of protein content

Responses	Source	SS	DF	MS	F	P-value
Protein content	Model	0.093	5	0.019	17.80	0.0007
	A	9.850E-003	1	9.850E-003	9.41	0.0181
	B	0.033	1	0.033	31.11	0.0008
	AB	6.250E-004	1	6.250E-004	0.60	0.4650
	A2	0.050	1	0.050	47.59	0.0002
	B2	1.488E-0.004	1	1.488E-0.004	0.14	0.7173
	Residual	7.327E-003	7	1.047E-003	2.31	0.2178
	Lack of fit	4.647E-003	3	1.549E-003		
	Pure error	2.680E-003	4	6.700E-004		
	Cor total	0.10	12			

Table 11. Possible optimal solution for production of seaweed paste

Number	Ratio	Duration (min)	Gel strength (g)	Whiteness	Expansion (%)	Moisture content (%)	Protein content (g/100 g)	Desirability	
1	15.00	117.06	2284.06	23.3017	443.774	93.1153	0.401251	0.748	Selected
2	15.00	117.88	2290.89	23.3044	445.081	93.1266	0.399466	0.747	
3	15.00	116.09	2276.22	23.2971	442.226	93.1018	0.403371	0.747	
4	15.00	71.93	2202.90	21.5351	371.132	92.1483	0.508783	0.713	
5	15.00	62.88	2256.15	20.7994	356.383	91.8718	0.53257	0.676	
6	15.54	61.10	2242.46	20.6801	356.22	91.8663	0.525377	0.662	

Table 12. Predicted and actual values of responses for confirmation run

Responses	Actual value	Predicted value	Residual	Residual error* (%)
Gel strength (g)	1996.32	2284.06	-287.74	14.41
Whiteness	24.06	23.30	0.76	3.16
Expansion (%)	452.11	443.77	8.34	1.84
Moisture content (%)	93.24	93.12	0.12	0.13
Protein content (g/100 g)	0.38	0.40	-0.02	5.26

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

min. The analysis of variance of the linear regression model demonstrated that the model is significant with F value of 17.80 (Table 5). The value of multiple correlation coefficients (R^2) of 0.9271, indicates a good agreement between the experimental and the predicted values. The results showed that dry seaweed to soaking water ratio and soaking duration have an effect on the protein content of the seaweed paste. The variable having the largest effect on the response was the quadratic term of dry seaweed to soaking water ratio (X_1^2), followed by linear term of soaking duration (X_2)($p < 0.001$); the linear term of dry seaweed to soaking water ratio also had a significant effect ($p < 0.05$) on the protein content of seaweed paste. However, the effect of the quadratic term of soaking duration 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. More water soluble protein in the seaweed leached out when the seaweed was soaked in higher dry seaweed to soaking water ratios of distilled water over an extended period.

Optimization process and confirmation run

The multiple response optimizations were performed with the numerical tools provided by the software. By using the numerical optimization function, the desired goals for the factors and responses are set. These goals are combined into an overall desirability function and the search for optimal solutions involves the maximization of the said function. In this experiment, the ratio of dry seaweed to soaking water was set at minimize while the soaking duration was set in a range. It is desirable to have maximum value for gel strength, whiteness and protein content, therefore, the goals for these responses under investigation were set as maximize. The expansion and moisture content were set in range. There are 6 possible optimal solutions for the production of seaweed paste. Out of the six possible optimal solutions, the suggested conditions to obtain the optimum value for all the responses is, 1 part of dry seaweed soaked in 15 parts of distilled water for the duration of 117.06 min with the desirability of 0.748 (Table 11). The production of seaweed paste soaked under optimal conditions as predicted by RSM was carried out for verification and the results are shown in Table 12. The actual readings for all the measured responses fitted well to the predicted data by the model particularly for the whiteness, expansion and moisture content which were within 95% prediction interval. The % error for gel strength and protein content were 14.41 and 5.26 respectively.

Conclusion

This study has demonstrated the feasibility of using the response surface methodology (RSM) in optimizing the soaking conditions for the production of better quality seaweed paste. The gel strength, whiteness, expansion, moisture content and protein content of seaweed paste were dependent on the ratio of dry seaweed to soaking water and also on the soaking duration. The proposed optimum condition for the production of seaweed paste is soaking 1 part of dry seaweed in 15 parts of distilled water for the duration of 117.06 min. The gel strength, whiteness, expansion, moisture content and protein content of seaweed paste produced using this optimized conditions were 1996.32, 24.06, 452.11, 93.24 and 0.38, respectively. The final gel strength (1996.32), whiteness (24.06), expansion (452.11), moisture content (93.24) and protein content (0.38) of seaweed paste produced using optimized conditions developed by RSM were respectively, 2.42, 1.26, 1.27, 1.02 and 2.0 times higher than those obtained in non-optimized conditions.

Acknowledgements

This study was supported by the Science Fund Grant (06-03-08-SF0342) from the Ministry of Science, Technology and Innovation, Malaysia (MOSTI), and Seaweed Downstream Research Centre (SDRC) (K-FS010410-1001). The authors would like to thank Ms. Nazarifah Ibrahim from

Food Technology Research Centre, MARDI, for her technical assistance.

References

Ahemad, S., Ismail, A. and Mohammad Raduan, M. A. 2006. The seaweed industry in Sabah, East Malaysia. Jati 11: 97-107.

AOAC. 2000. In. Horwitz, W. (Ed.). Official methods of analysis of the Association of Official Analytical Chemists (17th ed.). Washington, DC: Association of Official Analytical Chemists.

Bixler, H. J. and Porse, H. 2010. A decade of change in the seaweed hydrocolloids industry. Journal of Applied Phycology. Published online: 22 May 2010.

Cox, S., Abu-Ghannam, N. and Gupta, S. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal 17: 205-220.

Dhargalkar, V. K. and Pereira, N. 2005. Seaweed promising plant of millennium. Science and Culture 71: 60-66.

Gupta, S. and Abu-Ghannam, N. 2011. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innovative Food Science and Emerging Technologies 12: 600-609.

Jimenez-Escrig, A. and Sanchez-Muniz, F.J. 2000. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. Nutrition Research 20(4): 585-598.

Khuari, A. I. and Cornell, J. A. 1987. Response surface design and analysis. New York: Marcel Dekker.

Mabeau, S. and Fleurence, J. 1993. Seaweed in food products: Biochemical and nutritional aspects. Trends in Food Science and Technology 4(4): 103-107.

Shahidi, F. 2009. Nutraceuticals and functional foods: Whole versus processed foods. Trends in Food Science and Technology 20(9): 376-387.

Suhaimi, M. Y. 2011. Sustainable management of *Kappaphycus* and *Eucheuma* cultivation in Malaysia. Prosiding Seminar Rumpai Laut kebangsaan 2011, 16.

Wainewright, F. W. 1977. Physical test for gelatine and gelatine products. In. A. G. Ward & A. Courts (Eds.), The science and technology of gelatine, London, UK: Academic Press, Inc., 507-531.

Yuan, Y., Gao, Y., Mao, L. and Zhao, J. 2008. Optimization of conditions for the preparation of β-carotene nanoemulsions using response surface methodology. Food Chemistry 107: 1300-1306.

Zhou, P. and Regenstein, J. M. 2004. Optimization of extraction condition for Pollock skin gelatin. Journal of Food Science 69: 393-398.