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Statistics-based optimization of the extraction process of kelp polysaccharide and its activities

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ABSTRACT

Statistics-based response surface methodology (RSM) was used to optimize the extraction process of kelp polysaccharide and its activities were evaluated. Single factor experiment was first designed for determining the optimal range of each of four factors and these factors were further optimized using RSM with a CCD design. The optimal conditions were as follows: pH 3.4, temperature 83 °C, extraction time 3.95 h and ratio of water to kelp 1:23. Under the above conditions, the yield of kelp polysaccharide obtained was 1.26%. Scavenging percentages of free radicals •OH, $•O_2^-$ and DPPH by kelp polysaccharide were up to 90.8% (1.6 mg/mL), 85% (1 mg/mL) and 23.8% (1 mg/mL), respectively. An increase of 86% in the biosynthetic activity of collagen was obtained at a kelp polysaccharide concentration of 0.25%. All these results indicate that kelp polysaccharide may be a good candidate as an effective ingredient of cosmetics for future use.

1. Introduction

Brown seaweed kelp (*Laminaria japonica*) is one of the most important economic seaweeds cultivated in China, Japan and Korea, *etc*. It is consumed widely as a marine vegetable in these countries (Gao, Qin, & Zhang, 2006; Shao, Zhen, Jian, & Su, 2007; Suzuki, Fuyuya, & Takeuchi, 2006). Kelp contains a variety of bioactive substances, such as polysaccharides, agar, alginic acid and carrageenan (Chapman, 1970). Polysaccharide-containing extracts from plants, edible fungi, epiphytes and animals have been used widely for the treatment of some difficult diseases in the Traditional Chinese Medicine (TCM) (Mau, Chao, & Wu, 2001; Mau, Lin, & Chen, 2002; Mau, Tsai, Tseng, & Huang, 2005). The application of kelp as drugs in the TCM has also been documented well (Zhang et al., 2007). Moreover, a number of polysaccharides from plants and edible fungi have been studied in detail, some of which are used widely in the fields of foods, cosmetics, environmental protection, energy and pharmaceuticals, *etc*. (Mau et al., 2001, 2002; Tong et al., 2009; Yuan, Zhang, Fan, & Yang, 2008).

Currently, an increasing attention has been given to search for alternative natural antioxidants for replacing synthetic ones, such as BHA, BHT and TBHQ, and alternative natural components of cosmetics because of safety concerns (Wang, Zhao, Zhao, & Jiang, 2007). Many documents have reported that polysaccharides from some organisms have a strong antioxidative ability and a good promotion to the biosynthesis of collagen (Lu & Foo, 2000; Miliauskas, Venskutonis, & van Beek, 2004, Ray, 2006; Vinogradov, Brade, Brade, & Holst, 2003). However, the information pertinent to kelp polysaccharide has been still scarce so far. It is therefore interesting and attractive to investigate whether it has also above activities.

In the present study, the extraction process of kelp polysaccharide was optimized using the response surface methodology with a CCD design, in combination with a single factor design. *In vitro* antioxidative activity of the extracted kelp polysaccharide and its promotion to the biosynthesis of collagen were also investigated.

2. Materials and methods

2.1. Materials and chemicals

Kelp was purchased from the Cuiyuan commercial market, Hangzhou, Zhejiang Province, China. Absolute alcohol, β-hydroxyl acid, $FeSO_4$, H_2O_2 and methanol were purchased from Hangzhou Huipu Chemical Co. Ltd., Zhejiang Province, China. Pyrogallol, picric acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and DMEM were purchased from the Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Human dermal primary fibroblast P16, Fetal bovine serum (FBS), Sirius Red F3BA were purchased from the Sijiqing Bioengineering Materials Co. Ltd., Hangzhou, Zhejiang Province, China. The other reagents used in the experiments were analytical grade and used as the routine method.

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2.2. Kelp pretreatment

Kelp was rinsed carefully with the distilled water three times and dried at 105 °C for 15 min. It was then grounded in a high speed disintegrator (Model SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder. The powder was diluted with a 2% $CaCl_2$ solution by 5-fold (w/v) in a 100-mL conical flask. The extraction of kelp polysaccharide was carried out according to the following predesigned conditions.

2.3. Single factor experimental design

The effect of pH, temperature, extraction time and ratio of water to kelp on the yield of kelp polysaccharide was first studied by single factor design. One factor is changed, while the other factors keep constant in each experiment. The effect of each factor was evaluated by determining the yield of kelp polysaccharide.

2.4. CCD experimental design

After the optimal range of each factor was determined, a central composite design (CCD) with four independent factors at five levels was performed to further find the optimal value of each factor and the interaction among them. For the statistical calculation, factors are coded according to the following equation:

$$\chi_i = \frac{X_i - X_0}{\Delta X}, \quad i = 1, 2, 3, \ldots k \tag{1}$$

where χ_i is the coded value of an independent factor, X_i is the real value of an independent factor, X_0 is the real value of an independent factor on the center point and ΔX is the step change value. The coded value of each factor and its real value are presented in Table 1. The whole design consists of 30 experimental points in random order. Six replicates at the center of the design are used to allow for the estimation of a pure error sum of squares (Table 1). For predicting the optimal value of each factor, a second-order polynomial equation is fitted to correlate the relationship between factors and the response. The model equation used for the analysis is given below:

$$Y = \beta_0 + \sum \beta_i \chi_i + \sum \beta_i \chi_i \chi_j + \sum \beta_{ii} \chi_i^2 \tag{2}$$

where Y is the predicted response, β_0 is the intercept term, β_i is the linear coefficient, β_{ii} is the squared coefficient and β_{ij} is the interaction coefficient. χ_i and χ_j represent independent factors in the form of coded values. The accuracy and general ability of the polynomial model can be evaluated by a determination coefficient R^2.

2.5. Extraction of kelp polysaccharide and determination of the yield

The extracted slurry under each experimental condition was centrifuged at 5000 rpm for 20 min to collect the supernatant. The supernatant was then concentrated to one-fifth of the initial volume using a rotary evaporator (Senco Technology and Science Incorporation, Shanghai, China) at 55 °C under the vacuum condition. The resultant solution was mixed with four volumes of the absolute ethanol and kept at 4 °C overnight. The solution was centrifuged at 5000 rpm for 20 min to obtain the precipitate. The precipitate was washed six times with the absolute ethanol and collected as the extract of kelp polysaccharide. The extract was air-dried at 50 °C until its weight was constant, and then weighed with a balance (AY120, Shimadzu, Japan). The yield of kelp polysaccharide (%) was calculated as given below:

$$\text{polysaccharide yield } (\%) = \frac{A}{B} \times 100 \tag{3}$$

where A and B are the weight of kelp polysaccharide (g) and kelp powder (g), respectively.

Table 1
Experimental results of the response surface methodology.

Runs	Coded levels				Uncoded levels				Polysaccharide yield (%)
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	
1	−1	−1	1	−1	3	75	4.5	1:15	0.724
2	−1	−1	−1	1	3	75	4.5	1:25	0.649
3	0	0	0	0	3.5	80	4	1:20	1.05
4	0	0	0	0	3.5	80	4	1:20	1.19
5	−1	1	1	−1	3	85	4.5	1:15	0.969
6	1	−1	1	1	4	75	4.5	1:25	0.942
7	−1	1	−1	−1	3	85	3.5	1:15	0.888
8	0	0	0	−2	3.5	80	4	1:10	0.811
9	1	1	−1	1	4	85	3.5	1:25	0.754
10	1	1	−1	−1	3	85	3.5	1:15	0.565
11	−1	1	1	1	3.5	85	4.5	1:25	0.942
12	0	0	0	0	4	80	4	1:20	1.09
13	1	−1	1	−1	3.5	75	4.5	1:15	1.03
14	0	2	0	0	4.5	90	4	1:20	1.08
15	2	0	0	0	3.5	80	4	1:20	1.02
16	0	0	0	2	3.5	80	4	1:30	1.10
17	0	0	2	0	3.5	80	5	1:20	1.03
18	−1	−1	−1	−1	3	75	3.5	1:15	0.55
19	0	−2	0	0	3.5	70	4	1:20	0.666
20	0	0	0	0	3.5	80	4	1:20	1.19
21	−2	0	0	0	2.5	80	4	1:20	0.726
22	1	1	1	1	4	85	4.5	1:25	0.89
23	1	1	1	−1	4	85	4.5	1:15	0.633
24	1	−1	−1	−1	4	75	3.5	1:15	0.715
25	1	−1	−1	1	4	75	3.5	1:25	1.02
26	−1	−1	1	1	3	75	3.5	1:25	0.547
27	0	0	0	0	3.5	80	4	1:20	1.09
28	0	0	0	0	3.5	80	4	1:20	1.18
29	−1	1	−1	1	3	85	3.5	1:25	0.994
30	0	0	−2	0	3.5	80	3	1:20	0.446

2.6. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging experiments were carried out as described by Smirnoff and Cumbes (1989) with minor modifications: the reaction mixture, containing β-hydroxyl acid (6 mmol/L, 1 mL), $FeSO_4$ (2 mmol/L, 1 mL) and H_2O_2 (6 mmol/L, 1 mL), was prepared and incubated at 37 °C for 15 min, and then the absorbance at 510 nm (A_c) was detected. The kelp polysaccharide prepared was added to the above reaction mixture to scavenge the generated hydroxyl radical at 37 °C for 15 min, and then the absorbance value at 510 nm (A_i) was detected. H_2O_2 was used as the control and its absorbance value at 510 nm was A_j. The scavenging percentage of hydroxyl radical was calculated according to the following equation:

$$\text{scavenging percentage } (\%) = 1 - \frac{A_i - A_j}{A_c} \times 100 \quad (4)$$

2.7. Superoxide radical scavenging activity

Superoxide radical scavenging experiments were carried out as described by Nandi and Chatterjee (1987). The reaction mixture, including 4 mL Tris–HCl buffer (0.05 M, pH 8.2), pyrogallol (0.2 mM, 1 mL) and different concentrations of kelp polysaccharides (0.2–1 mg/mL), was incubated at 25 °C for 20 min. After the incubation, the reaction mixture was detected by determining the absorbance value at 420 nm (A_1). The polysaccharide sample was substituted with the distilled water as the control and the absorbance value at 420 nm was also detected (A_2). The scavenging percentage of superoxide radical was calculated using the following equation:

$$\text{scavenging percentage } (\%) = 1 - \frac{A_1}{A_2} \times 100 \quad (5)$$

2.8. DPPH radical scavenging activity

DPPH radical scavenging experiments were carried out as described by Katsube et al. (2004). Different concentrations of kelp polysaccharides (0.2–1 mg/mL) was added to 2 mL DPPH solution (6.5×10^{-5} mol/L). The mixture was shaken and allowed to stand at 25 °C for 30 min in dark. The absorbance value at 517 nm was measured against a blank (water and methanol replacing kelp polysaccharide and DPPH solution, respectively). The scavenging percentage was calculated by the following equation:

$$\text{scavenging percentage } (\%) = 1 - \frac{A_1 - A_2}{A_0} \times 100 \quad (6)$$

where A_0 is an absorbance value of the control sample (water replacing kelp polysaccharide of the mixture sample), A_1 is an absorbance value of the mixture sample and A_2 is an absorbance value of the control sample (methanol replacing DPPH solution of the mixture sample).

2.9. Biosynthetic activity of collagen

Human dermal primary fibroblast P16 was seeded in a 24-well plate at a density of 1×10^5 cells per well in DMEM containing 10% FBS. After the incubation at 37 °C for 24 h, the medium was replaced with 1 mL DMEM containing different concentrations of polysaccharides (0–0.25%, w/v). After the incubation at 37 °C for another 48 h, the cell culture supernatant and cell extract lyses were obtained by rapid freezing and thawing, and then dried onto the plates. Plates were incubated at 37 °C for overnight (humidified) and then kept at 37 °C for 24 h. The well was filled with 1 mL of 0.1% (w/v) Sirius Red F3BA in the saturated picric acid and samples were stained at 25 °C for 1 h. The plate was washed five times with 2 mL of 10 mM HCl for 10 s per wash. The collagen bound stain was then extracted with 2 mL of 0.1 M NaOH for 5 min. Absorbance values at 565 nm were detected (Wang et al., 2001).

3. Results and discussion

3.1. Single factor experimental analysis

The results of single factor experiments are presented in Fig. 1. Four factors were found to have a significant impact on the yield of kelp polysaccharide. Fig. 1(A) shows that the relationship between the yield of kelp polysaccharide and pH. It was very clear that the yield of kelp polysaccharide increased when pH increased from 2 to 3.5. The highest yield of it was 0.83% at pH 3.5. When pH was over 3.5, the yield of kelp polysaccharide decreased dramatically as pH increased. The extraction coefficient increases when the extraction temperature increases due to the increase of the polysaccharide solubility (Braga, Moreschi, & Meireles, 2006; Li, Cui, & Kakuda, 2006). To study the effect of different temperatures on the yield of kelp polysaccharide, the extraction process was carried out at 40, 50, 60, 70, 80 and 90 °C. The yield of kelp polysaccharide increased dramatically when extraction temperatures increased from 40 to 80 °C. As shown in Fig. 1(B), the maximal yield (0.88%) of kelp polysaccharide was obtained at 80 °C. This tendency is in good agreement with some reports of other authors (Ray, 2006; Vinogradov et al., 2003). The extraction time is another factor that affects the extraction efficiency and the selectivity of kelp polysaccharide. It has been reported that a longer extraction time favors the production of kelp polysaccharide (Liu, Wei, Guo, & Kennedy, 2006; Ros et al., 2004). The effect of different extraction times on the yield of kelp polysaccharide is shown in Fig. 1(C). A longer extraction time showed a positive effect on its yield. When the extraction time varied from 2 to 4 h, the increase of the extraction yield was relatively rapid. The extraction yield of kelp polysaccharide reached a maximal value of 0.9% at 4 h, and no longer changed as the extraction time prolonged. This indicates that the extraction time of 4 h is sufficient to obtain a higher yield of kelp polysaccharide. The effect of different ratios of water to kelp on the yield of kelp polysaccharide is shown in Fig. 1(D). The yield of kelp polysaccharide increased significantly from 0.9 to 1.05 when ratios of water to kelp varied from 1:10 to 1:20. This is probably due to the increase in the driving force for the mass transfer of kelp polysaccharide (Bendahou, Dufresne, Kaddami, & Habibi, 2007). However, when the ratios varied from 1:20 to 1:30, the yield of kelp polysaccharide decreased, and no longer changed when the ratio was over 1:30.

3.2. CCD experimental analysis

Based on the results of single factor experiments, a central composite design was used for determining the optimal value of each of four factors and the interaction among them. The central composite design of four factors in the coded level, along with the yield of kelp polysaccharide as the response, is presented in Table 1. The highest yield of kelp polysaccharide obtained was 1.19% at run 20. By applying a multiple regression analysis on the experimental data, a second-order polynomial model in the coded unit explained the role of each factor and their second-order interaction during the extraction of kelp polysaccharide:

$$\begin{aligned} Y = {} & 1.13 + 0.037A + 0.053B + 0.080C + 0.052D - 0.14AB \\ & + 0.008788AC + 0.041AD - 0.018BC + 0.024BD \\ & - 0.033CD - 0.075A^2 - 0.076B^2 - 0.11C^2 - 0.054D^2 \end{aligned} \quad (7)$$

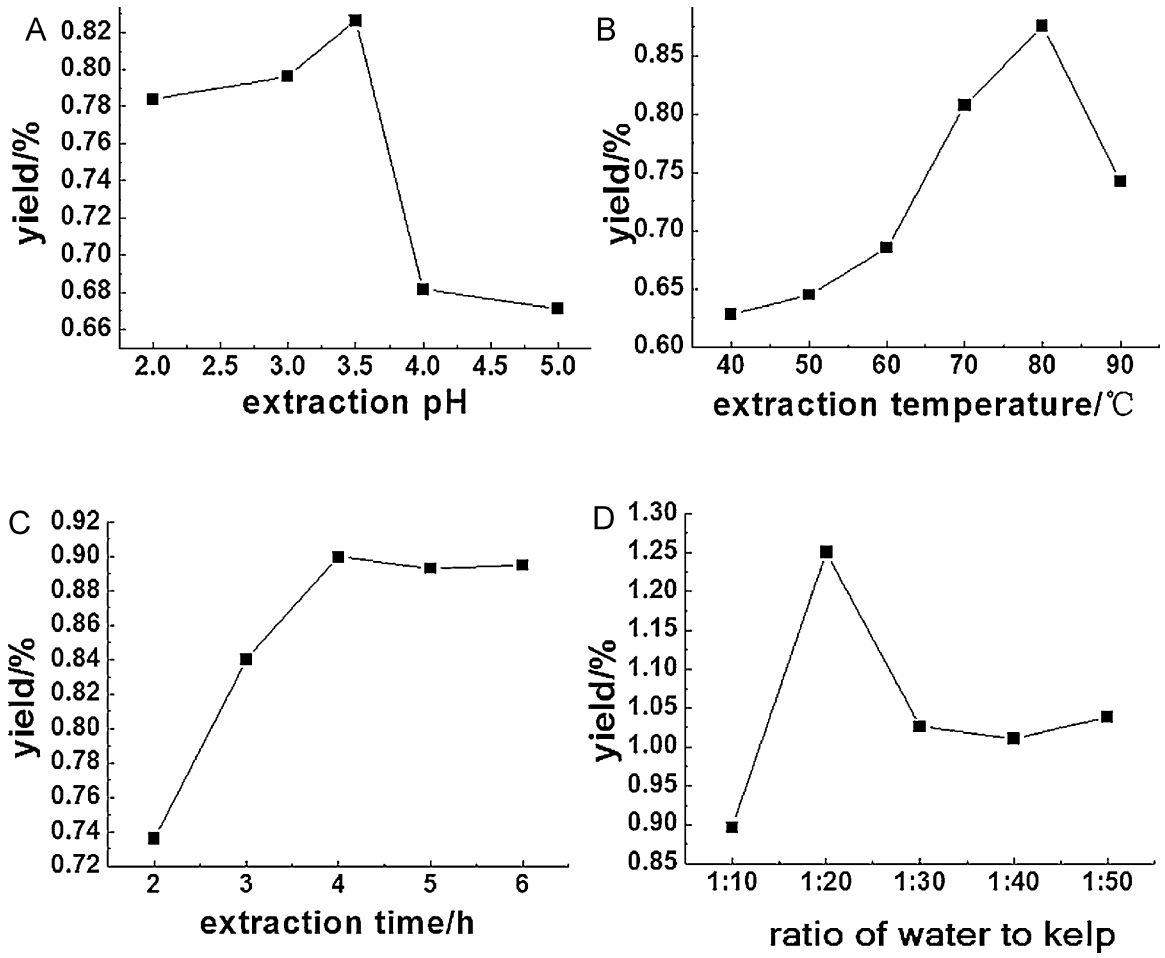

Fig. 1. Effect of pH, temperature, extraction time and ratio of water to kelp on the yield of kelp polysaccharide. (A) pH, (B) temperature, (C) extraction time and (D) ratio of water to kelp.

where Y is the predicted yield of kelp polysaccharide, A is the pH, B is the temperature, C is the extraction time and D is the ratio of water to kelp.

The statistical significance of Eq. (7) was checked by an F-test. The analysis of the variance (ANOVA) for the second-order polynomial model is shown in Table 2. It was evident that the model was highly significant, as suggested by the model F value and a low probability value ($p = 0.0002$). The analysis of factors (F-test) showed that the second-order polynomial model was adjusted well to the experimental data. The coefficient of the variation (CV) indicates the degree of the precision to which treatments are compared. The higher the value of CV is, the lower the reliability of the experiment is. Here, a lower value of CV (12.22) indicated a better precision and reliability of experiments (Box & Hunter, 1978). The precision of a model can be checked by determination coefficient (R^2) and correlation coefficient (R). The determination coefficient (R^2) was calculated to be 0.8729, indicating that 87.29% of the variability in the response could be explained by this model. The closer the value of R to 1 is, the better the correlation between the experimental and predicted values is. Here, the value of R (0.9119) for Eq. (7) indicated a close agreement between experimental results and theoretical values predicted by the model equation. Linear and quadratic terms were both significant at the 1% level. Therefore, the quadratic model was selected in this optimization study.

The significance of the regression coefficient was tested by a t-test. The regression coefficient and corresponding p-value for the model are also given in Table 2. The p-value is used as a tool to check the significance of each coefficient, which is necessary to understand the pattern of the mutual interaction among the best factors. The smaller the p-value is, the bigger the significance of the corresponding coefficient is (Li & Lu, 2005; Li et al., 2007; Liu, Weng, Zhang, Xu, & Ji, 2003). Results showed that among the independent

Table 2
Analysis of the variance (ANOVA) for the second-order polynomial model.

Source	Sum of square	Degree of freedom	Mean square	F-value	p-Value
Model	12	14	0.086	7.36	0.0002[a]
A	0.032	1	0.032	2.76	0.1175
B	0.068	1	0.068	5.87	0.0286[a]
C	0.15	1	0.15	13.28	0.0024[a]
D	0.065	1	0.065	5.56	0.0324[a]
AB	0.3	1	0.3	25.64	0.0001[a]
AC	1.236E−003	1	1.236E−003	0.11	0.7491
AD	0.027	1	0.027	2.33	0.1474
BC	5.027E−003	1	5.027E−003	0.43	0.5211
BD	9.506E−003	1	9.506E−003	0.82	0.3805
CD	0.017	1	0.017	1.5	0.2401
A^2	0.15	1	0.15	13.24	0.0024[a]
B^2	0.16	1	0.16	13.54	0.0022[a]
C^2	0.32	1	0.32	27.81	<0.0001[a]
D^2	0.081	1	0.081	6.92	0.0189[a]
Residual	0.17	5	0.012		
Lack of fit	0.15	10	0.015	3.87	0.074
Pure error	0.02	5	3.993E−003		
Cor total	1.37	29			
	$R^2 = 0.8729$		CV = 12.22		

Correlation coefficient (R) = 0.9119.
[a] Statistically significant at 95% of confidence level ($p < 0.05$).

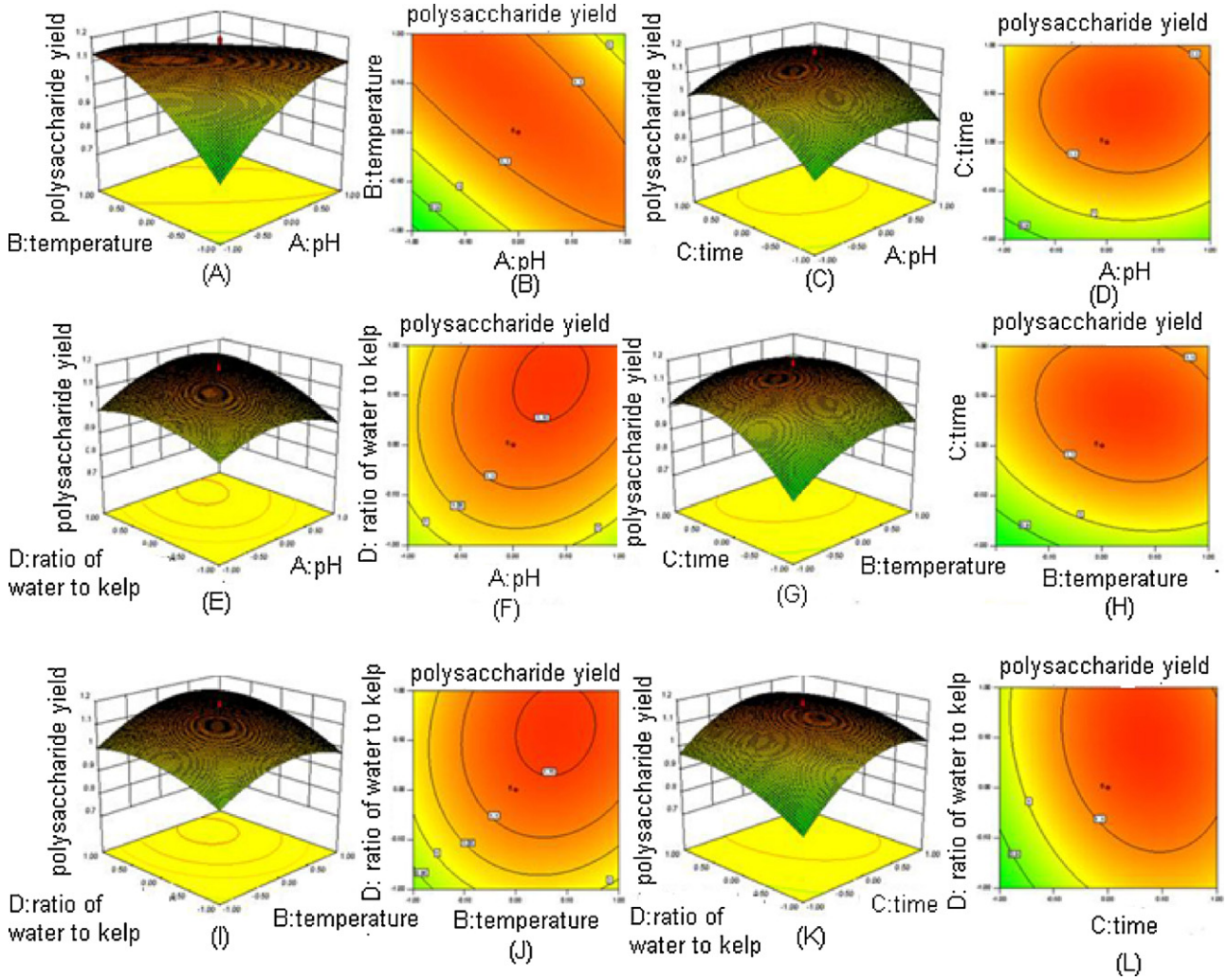

Fig. 2. 3D response surface plots and 2D contour plots showing the effect of pH, temperature, extraction time and ratio of water to kelp on the yield of kelp polysaccharide. (A) $Y = f(A,B)$, (C) $Y = f(A,C)$, (E) $Y = f(A,D)$, (G) $Y = f(B,C)$, (I) $Y = f(B,D)$ and (K) $Y = f(C,D)$. *A*: pH, *B*: temperature, *C*: extraction time and *D*: ratio of water to kelp.

factors, *B* (temperature), *C* (extraction time) and *D* (ratio of water to kelp) had a significant effect on the yield of kelp polysaccharide ($p < 0.05$). The positive coefficient of them showed a linear effect to increase the yield of kelp polysaccharide. Quadratic terms of four factors and the interaction between *A* and *B* had also a significant effect. Negative coefficients of AB, A^2, B^2, C^2 and D^2 showed a significant effect to decrease in the yield of kelp polysaccharide. However, no interaction between the other factors was found to contribute to the response at a significant level. In this case, B, C, D, AB, A^2, B^2, C^2 and D^2 are significant model terms.

The 3D response surface plot and 2D contour plot are graphical representations of the regression equation. As shown in Fig. 2(A) and (B), pH and temperature had a significant impact on the yield of kelp polysaccharide. The yield of kelp polysaccharide enhanced dramatically with increase in pHs from 3.0 to 4.0 and temperatures from 70 to 85 °C. With further increase in them, the yield of kelp polysaccharide tended to decrease slightly. The 2D contour plot shows that the interaction between pH and temperature is significant. Higher yield of kelp polysaccharide could be obtained by combining an appropriate pH with temperature. From Fig. 2(C) and (D), it could be seen that the maximal yield of kelp polysaccharide could be achieved when time and pH were 4 and 3.5, respectively. The circular 2D contour plot shows that the interaction between time and pH is not significant, which was also verified by the analysis of the variance in Table 2. Fig. 2(E) and (F) indicated that the yield of kelp polysaccharide increased with the increase in the ratio of water to kelp and pH. The yield of kelp polysaccharide was found to decrease with further increase in the two factors and no significant interaction was found between them. Fig. 2(G) and (H) showed that the yield of kelp polysaccharide increased with the increase in temperatures, and it was also found to increase rapidly with increase in temperatures from 70 to 85 °C. But when the temperature was over 85 °C, the yield of kelp polysaccharide decreased with increasing temperatures. Fig. 2(I) and (J) showed that the yield of kelp polysaccharide increased with increasing extraction times and ratios of water to kelp. Fig. 2(K) and (L) showed that the yield of kelp polysaccharide increased with increase in the ratio of water to kelp and extraction time, but it was also found to increase relatively slow. This implies that the interaction between extraction time and ratio of water to kelp is not significant. By employing the Design-Expert 7.0 software, the optimal values of the tested factors were pH 3.4, temperature 83 °C, extraction time 3.95 h and ratio of water to kelp 1:23. Under the optimal conditions, the maximal yield of kelp polysaccharide predicted was 1.27%. In order to validate the adequacy of the model equation, three rechecking experiments were performed and the yield of kelp polysaccharide obtained was 1.26%, which was in good agreement with the predicted yield. This also demonstrates the validation of the RSM model.

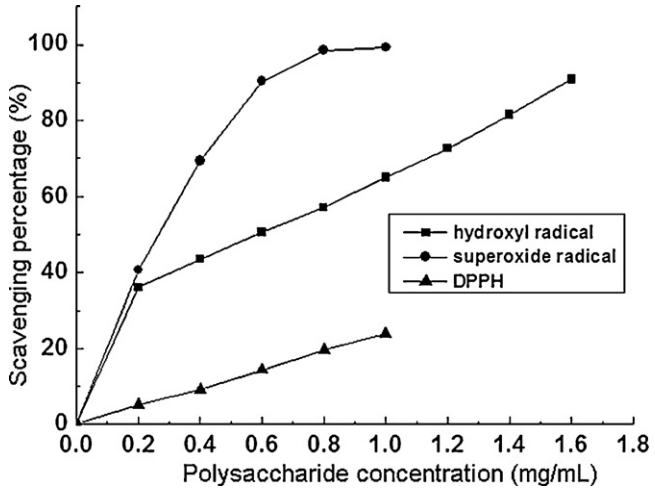

Fig. 3. Scavenging percentages of free radicals by kelp polysaccharide: hydroxyl radical, superoxide radical and DPPH. Scavenging percentages are expressed as the mean of three measures.

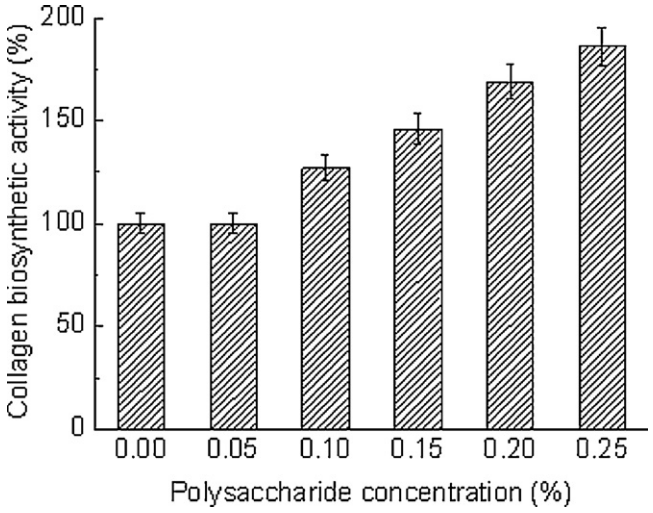

Fig. 4. Biosynthetic activities of collagen at different concentrations of kelp polysaccharide. The activity is significant ($p < 0.01$) and the values are mean ± S.E.

3.3. *In vitro* antioxidative ability of kelp polysaccharide

Hydroxyl radical (•OH) can cross cell membrane easily and react with the majority of biomolecules, including carbohydrates, proteins, lipids and DNA, which causes tissue damage or cell death. Removal of •OH is therefore important for the antioxidative defense (Mau et al., 2001, 2005). Fig. 3 shows the scavenging percentages of free radical •OH by polysaccharides at 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mg/mL. At the above concentrations, kelp polysaccharide exhibited an obvious antioxidative activity. The inhibiting percentage of •OH increased with increase in the concentration of kelp polysaccharide, and was 90.8% at 1.6 mg/mL. Although superoxide radical is a relatively weak oxidant, it can decompose to form stronger ROS, such as single oxygen and hydroxyl radical, which initiates the peroxidation of lipids. It is also known to initiate the lipid peroxidation indirectly as a result of the formation of H_2O_2, the precursor of •OH (Meyer & Isaksen, 1995). The scavenging of superoxide radical is therefore important for the antioxidative defense. As shown in Fig. 3, kelp polysaccharide had a better antioxidative activity. The scavenging activity increased with increase in the concentration of kelp polysaccharide. At 1 mg/mL of kelp polysaccharide, the scavenging activity of superoxide radical was 85%. DPPH free radical is accepted widely as a tool for evaluating the free radical scavenging capacity of antioxidants. The scavenging activity of DPPH by kelp polysaccharide is presented in Fig. 3. Kelp polysaccharide exhibited a strong scavenging ability to DPPH and the scavenging percentage was 23.8% at 1 mg/mL. These results indicate that kelp polysaccharide has a strong antioxidative ability and may be an alternative candidate for replacing synthetic antioxidants, such as BHA, BHT and TBHQ.

3.4. Collagen biosynthetic activity

Some polysaccharides have been suggested to play a possible role during the early stage of healing of a variety of connective tissues such as cell proliferation and synthesis of matrix components (Kaji et al., 2002; Wiig, Abrahamsson, & Lundborg, 1996). Hyaluronan has been regarded as a representative biomaterial for this use (Croce et al., 2001). The importance of hyaluronan has been described extensively for the homeostasis of connective tissues during embryogenesis and aging and its role in tissue repair. The effect of exogenous hyaluronan on the synthesis of total protein, collagen and hyaluronan was elucidated elaborately in HDF. Data strongly indicate that a relatively high concentration of hyaluronan in the extracellular space, such as during development and in the first phase of tissue repairs, will partially limit the deposition of the extracellular matrix, and of collagen in particular (Croce et al., 2001). Therefore, hyaluronan has been used widely for an important component of cosmetics. Recently, other polysaccharides have also been used as alternative ingredients for enhancing the collagen biosynthesis in skin cells (Katzman & Jeanloz, 1971; Kougias et al., 2001). The treatment of β-glucan produced from *Schizophyllum commune* increased the biosynthetic activity of collagen by 32% at a concentration of 0.04%, whereas yeast β-glucan gave a 10% increase. In this study, the treatment of fibroblasts with different concentrations of kelp polysaccharides increased the biosynthetic activity of collagen significantly and it was dose-dependent (Fig. 4). A maximal increase of 86% was obtained at a concentration of 0.25%. However, almost no increase was found when its concentration was lower than 0.05%.

4. Conclusions

The present study optimized the extraction process of kelp polysaccharide by response surface methodology with a CCD design and investigated its activities. The second-order polynomial model was established to depict the effect of four factors on the yield of kelp polysaccharide. The optimal conditions for the extraction of kelp polysaccharide were as follows: pH 3.4, temperature 83 °C, extraction time 3.95 h and ratio of water to kelp 1:23. The maximal yield of kelp polysaccharide was 1.26% under the above conditions. Kelp polysaccharide was demonstrated to have a strong antioxidative ability *in vitro* and a good promotion to the biosynthesis of collagen. These results indicate that kelp polysaccharide may have a potential application as an effective component of cosmetics in future.

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