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Application of response surface methodology to study the influence of hydrothermal processing on phytochemical constituents of the Irish edible brown seaweed *Himanthalia elongata*

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Abstract

We used response surface methodology to investigate the effect of time and temperature of hydrothermal processing (blanching) on the phytochemical content, texture and colour of a semi-dried brown seaweed (*Himanthalia elongata*). A central composite design was employed with a hydrothermal processing time of 10–30 min and temperature of 60–90°C. Predicted models were found to be significant for total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, total flavonoids, total condensed tannins, texture and colour. The predicted values for each of the responses were in good agreement with experimental values. Processing time had the most significant effect on phytochemical constituents of *H. elongata*. An acceptable edible texture and colour of seaweed was also achieved during the blanching procedure. Thus, central composite design and response surface methodology can be used to model phytochemical content, texture and colour of *H. elongata* while minimising the number of experiments required. Multiple response optimisation demonstrated that the phytochemical content of *H. elongata* may be maximised by blanching for 20.4 min at 80.5°C.

Keywords: antioxidant; blanching; response surface methodology; seaweed; texture.

Introduction

Marine algae have been used as foods in Asia in the preparation of salads, soups and also as low-calorie foods (Jiménez-Escrig and Sanchez-Muniz 2000), and in Western countries mainly as a source of polysaccharides (agar, alginates and carrageenans). As in other photosynthetic organisms, seaweeds contain various kinds of inorganic and organic substances that may benefit human health. Seaweeds contain high levels of minerals, vitamins, essential amino acids, indigestible carbohydrates and dietary fibre (Jiménez-Escrig and Goni 1999).

Consumption of seaweeds increases the intake of dietary fibre and lowers the occurrence of some chronic diseases (diabetes, obesity, heart diseases and cancers), which are associated with low fibre diets of Western countries (Southgate 1990). The content of total dietary fibre ranges from 0.33 to 0.50 g g^{-1} dry weight (Lahaye 1991, Jiménez-Escrig and Goni 1999, Rupérez and Saura-Calixto 2001). Accordingly, the fibre contents of a range of seaweeds are higher than those found in most fruits and vegetables (Dawczynski et al. 2007).

Factors in the marine environment may promote the formation of free radicals and other strong oxidizing agents, but seaweeds seldom suffer any serious photodynamic damage during metabolism, implying that their cells have protective antioxidative mechanisms and compounds (Matsukawa et al. 1997). Reactive oxygen species, such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors, and result in extensive oxidative damage that can lead to age-related degenerative conditions, cancer and a wide range of other human diseases (Reaven and Witzum 1996, Aruoma 1999).

Phenolic compounds can act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie and Mohamed-Saiel 2006). These phenolic compounds are commonly found in photosynthetic organisms, including seaweeds (Duan et al. 2006). Polyphenols represent a diverse class of compounds, including flavonoids (i.e., flavones, flavonols, flavanones, flavononols, chalcones and flavan-3-ols), lignins, tocopherols, tannins and phenolic acids (Shukla et al. 1997). Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein, which induces human cardiovascular diseases (Amic et al. 2003). Therefore, the consumption of foods with high levels of these phytochemicals may protect the body and food sources against these events (Chandini et al. 2008).

Several seaweeds are perishable in their fresh state and deteriorate within a few days after harvest. The traditional way to preserve these plant products is by sun drying, to conserve their desirable qualities, reduce storage volume and to extend shelf life (Lim and Murtijaya 2007). Many dried seaweeds are rehydrated and cooked by blanching in hot water. Processing and preparation of vegetables, especially thermal treatment, which are applied before consumption, may affect phytochemical content. Blanching makes vegetables more palatable but can lead to leaching of nutrients (Oboh 2005). Phenolic compounds are sensitive to heat; hence, blanching and boiling of vegetables for a few minutes may

cause significant loss of phenolic content, which can leach into boiling water (Amin et al. 2006). Since seaweeds are consumed after processing, it is important to investigate the effects of hydrothermal processing on their phytochemical contents.

The "one-at-a-time approach" can be used to optimise processing time and temperature. However, this method is extremely time consuming and disregards the complex interactions among various physicochemical parameters. To optimise the processing parameters, we used response surface methodology (RSM). RSM is a suite of mathematical and statistical techniques used to search for optimum conditions of factors for desirable responses; it evaluates the relative significance of several treatment factors even in the presence of complex interactions. The design leads to the generation of contour plots by linear or quadratic effects of the key variables, and a model equation is derived that fits the experimental data to calculate the optimal response of the system (Parthirana and Shahidi 2005, Zhang et al. 2007, Prasad et al. 2011).

The main objective of our study was to investigate the effects of time and temperature of hydrothermal processing on phytochemical content, texture and colour of *Himanthalia elongata* (L.) S.F. Gray. We used RSM to optimise the time and temperature of the hydrothermal processing conditions for seaweeds that had previously been semi-dried (40°C, 2 h). In addition, we developed a model equation to predict and determine optimum processing conditions. Semi-dried seaweed was used as it has been optimised in previous drying studies (Gupta et al. 2011).

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate (Na_2CO_3), sodium benzoate, vanillin, hydrochloric acid (HCl), (+)-catechin, aluminium chloride ($AlCl_3$) and quercetin were purchased from Sigma Aldrich Chemie (Steinheim, Germany).

Seaweed material

Himanthalia elongata (Phaeophyceae) was purchased from Quality Sea Vegetables (Burton Port, Co Donegal, Ireland), and authentication was provided by the supplier. Samples were collected in January and February 2010, washed thoroughly with freshwater to remove epiphytes and salts, and stored at 4°C until analysis.

Preparation of samples

Thalli were washed thoroughly with tap water, dried with absorbent paper and then cut into 3-cm long pieces before processing. An overview of the steps involved in the preparation and treatment of the samples is presented in Figure 1.

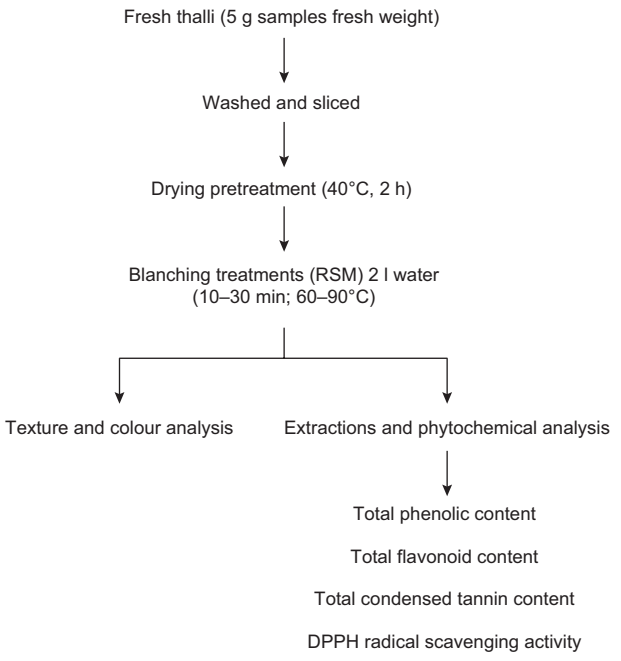

Figure 1 *Himanthalia elongata*: overview of steps in sample preparation, treatments and analysis.

Drying pretreatment

We applied a drying pretreatment to the seaweed before hydrothermal processing as optimised in previous studies. Drying temperature and time were selected based on optimised results (Gupta et al. 2011). Seaweed samples (5 g) were placed on a drying tray in a single layer. They were air-dried in a drier (Innova 42; New Brunswick, Edison, NJ, USA) at 40°C for 2 h. Air velocity was 2.0±0.1 m s^{-1} measured with VWR Enviro-meter digital anemometer (VWR, Dublin, Ireland).

Hydrothermal processing

Semi-dried seaweed samples were hydrothermally processed by immersion in 2 l of distilled water kept at the specified blanching temperatures in a water bath (Lauda, Aqualine AL5; Lauda-Brinkmann, Delran, NJ, USA). The processing treatments applied are presented in Table 1. After processing, the cooked seaweeds were drained on a wire mesh strainer and placed on ice to cool before the extraction procedure.

Experimental design

To investigate the effect of factors (blanching time and temperature) on phytochemical constituents of *Himanthalia elongata*, we used a central composite design with two factors. The central composite design was applied using STATGRAPHICS Centurion XV (StatPoint Technologies Inc., Warrenton, VA, USA). The total number of experiments generated from the software with two factors was 10 ($=2^k+2k+2$), where k is the number of factors. Eight experiments were augmented with

Table 1 *Himanthalia elongata*: design matrix and variable combinations in experimental runs.

Experiment no.	Blanching time (min)	Blanching temperature (°C)
1	20.00	75.00
2	20.00	96.21
3	20.00	53.78
4	5.85	75.00
5	20.00	75.00
6	30.00	60.00
7	10.00	90.00
8	10.00	60.00
9	34.14	75.00
10	30.00	90.00

two duplicates at the centre points. The variable combinations in experimental runs are shown in Tables 1 and 2. The independent variables for hydrothermal processing were blanching time (10–30 min) and blanching temperature (60–90°C), factors with the most significant effect on the seaweed during the blanching process. Experimental data from the central composite design were analysed and fitted to a polynomial regression model:

$$Y=\beta_0+(\beta_1\chi_1)+(\beta_2\chi_2)+(\beta_{11}\chi^2{}_1)+(\beta_{22}\chi^2{}_2)+(\beta_{12}\chi_1\chi_2) \quad (1)$$

where Y is the response calculated by the model, β_0 is a constant, and β_i, β_{ii} and β_{ij} are linear, squared and interaction coefficients, respectively.

The adequacy of the model was evaluated by lack of fit, coefficient of determination (R^2) and Fisher's test ratio (F-value) obtained from the analysis of variance (ANOVA) generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level (α=0.05). Three-dimensional response surface plots and contour plots were generated by keeping one response variable at its optimal level and plotting that against two factors (independent variables). The independent variables selected are presented in Table 2. We performed multi-response analysis of the response surface design using the desirability approach to optimise blanching time and temperature. The desirability function approach is one of the most widely used methods in industry for the optimisation of multiple response processes. It is based on the idea that the "quality" of a product or process that has multiple quality characteristics, with one of them outside of some "desired" limits, is completely unacceptable. The method finds operating conditions $\boldsymbol{x}$ that provide the "most desirable" response values. This methodology is based on first constructing a desirability function for each individual response, and then it is possible to obtain the overall desirability.

Table 2 *Himanthalia elongata*: level of codes for independent variables used in the central composite design.

Independent variables	Code	-2	-1	0	+1	+2
Blanching time (min)	X_1	5.85	10	20	30	34.14
Blanching temperature (°C)	X_2	53.78	60	75	90	96.21

Texture evaluation

At specified experimental times (Table 1), seaweed samples [original 5 g fresh weight (FW)] were removed for instrumental texture analysis. Shear tests were performed using an Instron Universal Testing Machine (Instron, Norwood, MA, USA) supported with Bluehill 2 version 2.14 software for materials testing. A Warner Bratzler cutter was used in the shear tests. An aluminium plate with dimensions of 10×6 cm, thickness of 1.3 cm and an opening of 3 mm in the centre was supported in the Instron base. Seaweed samples (5 g) were sheared at a speed of 200 mm min^{-1}. The cutting implement was allowed to travel the depth of the seaweed, cutting through the sample; seaweed hardness was defined as the peak of force-deformation curve recorded as N mm^{-1}. Ten replications of each sample were performed.

Colour measurement

At specified experimental times (Table 1), seaweed samples (original 5 g FW) were removed to undergo colour analysis by colorimetry (CIE Lab ColourQuest XE; Hunter Associates, Reston, VA, USA). The colorimeter was calibrated against a standard white reference tile (L*=93.97; a*=-0.08 and b*=1.21). The colour values were represented on the CIE colour scales in terms of L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). From these values, total colour change from fresh (DE) was calculated according to the following equation:

$$DE=\sqrt{(L^*-L^*_0)^2+(a^*-a^*_0)^2+(b^*-b^*_0)^2} \quad (2)$$

where, L^*_0, a^*_0 and b^*_0 are the readings at time zero and L*, a* and b* are the individual readings at each drying time.

Extraction of phytochemicals

Blanched seaweed samples (original 5 g FW) were powdered in liquid nitrogen using a mortar and pestle, then extracted in 50 ml of methanol (60% v/v), as optimised in previous studies, under a nitrogen atmosphere for 2 h. The extraction was carried out at 40°C and 100 rpm in a shaker incubator (Innova 42). Samples were filtered and centrifuged at 9168×*g* for 15 min (Sigma 2K15; Sigma Laborzentrifugen, Osterode am Harz, Germany). Resulting extracts were evaporated to dryness using a vacuum polyevaporator (Buchi Syncore Polyvap; Buchi Labortechnik, Flawil, Switzerland) at 60°C. A pressure gradient program was designed for evaporating the solvents under vacuum conditions of 33,700 and 7200 Pa for methanol and water, respectively.

Total phenolic content

Total phenolic concentration was measured using the Folin-Ciocalteau method (Taga et al. 1984). In this procedure, a

100-µl aliquot of stock sample (extract concentration 1000 µg ml^{-1} of water) was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then, 100 µl of 50% Folin-Ciocalteau's phenol reagent was added. After incubation for 30 min at room temperature in darkness, absorbance was read at 720 nm using a spectrophotometer (Milton Roy Spectronic 1201; Hewlett Packard, Bracknell, UK). Total phenolic contents of the whole seaweeds were expressed as mg gallic acid equivalent per 100 g FW (mg GAE 100 g^{-1} FW).

DPPH radical scavenging activity

Free radical scavenging activity was measured with DPPH following Yen and Chen (1995) with some modifications. Briefly, a 100-µl aliquot of test sample (50 µg ml^{-1}) was placed in a 96-well microtitre plate and 100 µl of 0.16 mM DPPH methanolic solution were added. The mixture was shaken and incubated for 30 min in darkness at 25°C. Changes in the absorbance of the samples were measured at 517 nm using a microplate reader (Powerwave; Biotek, Winooski, VT, USA).

The ability to scavenge the DPPH radical was calculated using the following equation (Duan et al. 2006):

$$\text{Scavenging effect } (\%)=\left[1-\left(\frac{A_{\text{sample}}-A_{\text{sample blank}}}{A_{\text{control}}}\right)\right]\times 100 \qquad (3)$$

where $A_{control}$ is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and $A_{sample\ blank}$ is the absorbance of the sample only (sample without any DPPH solution).

Total flavonoid content

Total flavonoid contents were determined according to the method of Zhishen et al. (1999). Briefly, a 250-µl aliquot of each extract was mixed with 1.25 ml of double distilled (dd) H_2O and 75 µl of 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3 \cdot H_2O$ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with dd H_2O. Following thorough mixing of the solution, the absorbance against a blank was determined at 510 nm. Quercetin was used to prepare the standard curve, and results were expressed as mg quercetin equivalents (QE) 100 g^{-1} FW (mg QE 100 g^{-1} FW) for whole seaweeds.

Total condensed tannin content

Total condensed tannin contents were determined according to the method of Julkunen-Titto (1985). Briefly, a 50-µl aliquot of each extract was mixed with 1.5 ml of 4% vanillin (prepared with methanol), and then 750 µl of concentrated HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. The absorbance against a blank was read at 500 nm. (+)-Catechin was used to prepare the standard curve, and results were expressed as mg catechin equivalents (CE) 100 g^{-1} FW (mg CE 100 g^{-1} FW) for whole seaweeds.

Statistical analysis

All experiments were carried out in triplicate and replicated at least twice. Data from the central composite design were subjected to a second-order multiple regression analysis using least-squares regression to obtain the parameter estimated for the mathematical model. The regression analysis and ANOVA were performed with STATGRAPHICS Centurion XV software (StatPoint Technologies Inc., Warrenton, VA, USA). Differences were considered statistically significant when $p<0.05$.

Results and discussion

Statistical analysis of results obtained by experimental design

Processing and preparation of vegetables, especially thermal treatment, applied before consumption may affect the phytochemical level of the food. Heat applications, such as blanching or boiling are common practices in the processing of food products to render them palatable and microbiologically safe. Since seaweed would need to undergo some heat treatment before consumption, it was relevant to assess the effects of hydrothermal heat treatment on the stability of seaweed antioxidant properties. Seaweeds are perishable in their fresh state and deteriorate within a few days of harvest, and are most commonly dried outdoors under atmospheric conditions as a means of preservation. Dried seaweeds are commonly blanched before consumption; therefore, the present study aimed to optimise the hydrothermal blanching procedure for the rehydration of semi-dried *Himanthalia elongata.* We previously investigated the effect of a range of drying temperatures on the drying kinetics and phytochemical constituents of *H. elongata* and found that drying at 40°C for 2 h increased phytochemical content as compared with fresh seaweeds (Gupta et al. 2011). Therefore, this optimised semi-dried seaweed was used in the present study.

Phenolic compounds are sensitive to heat; thus, blanching of vegetables for a few minutes may cause a significant loss of phenolic content, which can leach into boiling water (Amin et al. 2006). Therefore, in the present study, the time and temperature of hydrothermal processing were chosen as factors for the RSM study as they have major effects on phytochemical levels.

The effects of independent variables on each of the response variables are presented in Table 3. Statistical analysis indicated that models proposed were adequate with no significant lack of fit and high R^2 values for all of the responses. The models for each of the responses were analysed separately before an overall optimum processing condition for the hydrothermal processing procedure was determined. Predicted and experimental values for each of the responses are presented in Table 4. Response surface plots were generated to illustrate

Table 3 *Himanthalia elongata*: two-way ANOVA on the effects of independent variables on dependent variable responses: total phenolic content, DPPH, total flavonoids, total condensed tannins, texture and colour of blanched thalli.

Source	Total phenolic content		DPPH		Total flavonoids		Total condensed tannins		Texture		Colour	
	F-ratio	p-Value	F-ratio	p-Value	F-ratio	p-Value	F-ratio	p-Value	F-ratio	p-Value	F-ratio	p-Value
X_1	198.68	0.0001	77.22	0.0009	85.26	0.0008	57.42	0.0016	11.83	0.0263	8.42	0.0440
X_2	25.55	0.0072	1.24	0.3273	0.64	0.4675	11.15	0.0289	0.58	0.4898	45.27	0.0025
X_1*X_1	107.81	0.0005	52.36	0.0019	2.00	0.2303	3.60	0.1307	28.36	0.0060	4.17	0.1106
X_1*X_2	68.71	0.0012	25.07	0.0075	4.91	0.0911	0.27	0.6325	0.95	0.3847	3.03	0.1566
X_2*X_2	86.06	0.0008	123.94	0.0004	21.78	0.0095	3.31	0.1429	0.62	0.4767	5.41	0.0805

R^2 values: 0.9908 (total phenolic content), 0.9832 (DPPH), 0.9658 (total flavonoids), 0.9528 (total condensed tannins), 0.9174 (texture) and 0.9407 (colour).

Table 4 *Himanthalia elongata*: predicted (Pred.) and experimental (Exp.) values of total phenolic content, DPPH radical scavenging activity, total flavonoids, total condensed tannins, texture and colour of blanched thalli.

Experiment no.	Total phenolic content (mg GAE 100 g^{-1} FW)		DPPH (%)		Total flavonoids (mg QE 100 g^{-1} FW)		Total condensed tannins (mg CE 100 g^{-1} FW)		Texture (N mm^{-1})		Colour (ΔE)	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	64.19	64.24	99.56	99.13	11.69	12.00	11.65	11.90	31.93	31.80	10.37	10.07
2	47.63	48.24	91.21	91.27	8.67	8.76	8.82	8.30	34.70	33.31	13.67	13.68
3	54.13	55.45	92.22	92.07	8.66	9.31	10.63	11.86	31.41	32.28	9.92	9.30
4	59.27	60.43	90.54	91.14	13.61	14.23	17.78	17.84	40.49	40.86	10.36	10.37
5	64.28	64.24	98.71	99.13	12.31	12.00	12.15	11.90	31.68	31.80	9.78	10.07
6	53.55	52.46	96.72	97.28	9.18	8.79	11.80	10.61	33.09	33.00	10.34	11.09
7	62.43	61.59	92.76	92.26	13.19	12.84	13.34	13.82	36.39	37.01	12.99	12.85
8	56.21	54.86	89.59	89.24	12.47	11.72	16.56	15.79	38.59	37.60	8.31	8.62
9	39.53	40.31	98.11	97.43	7.85	7.97	9.10	9.75	37.10	36.21	12.88	12.26
10	36.10	35.52	92.72	93.13	6.90	6.91	7.48	7.54	33.53	35.04	12.75	13.05

Values are means (n=6).

the effects of blanching time and temperature on each of the responses (Figure 2A–F).

Effects of process variables on total phenolic content

Time and temperature combinations used for the processing of vegetables are crucial in optimising the concentration of bioactive compounds. RSM was applied to obtain conditions that would result in minimal loss of phytochemicals into blanching water. The 10 experiments proposed by the RSM [two factors with five levels (Table 2), including two replicates at the centre point] were used for fitting a second-order response surface model. The two centre point runs provided a measure of process stability and inherent variability. Experimental results for total phenolic content (TPC) were fitted to a full quadratic second-order polynomial equation and the model obtained for TPC of hydrothermally processed *Himanthalia elongata* was

$$Z=-150.541+(5.02044*X_1)+(4.74852*X_2) -(0.0693325*X_1^2)-(0.0394485*X_1*X_2) -(0.0275309*X_2^2) \quad (4)$$

(see Table 2 for definitions of X_1 and X_2). To determine the significance of the model, we performed ANOVA on the data. For each experimental factor, the variance was partitioned into linear, quadratic and interaction components to assess the adequacy of the second-order polynomial function and the relative importance or significance of the terms. The coefficients of the model were estimated with multiple regression analysis. The quality of fit for the second-order equation was expressed by the coefficient of regression, R^2. The R^2 values provided a measure of how much variability in the observed response values may be explained by variability in the experimental factors and their interactions. The goodness of fit of the model was examined with an F-test. The F-test is a statistically valid measure of how well the factors describe the variation in the data about the mean. The greater the deviation of the F-value from unity, the more certain it is that the factors explain adequately the variation in the data around the mean, and the estimated factor effects are real. The F-values for each of the factors of TPC were very high, up to 198.68 (time X_1), indicating that these factors were highly significant (Table 3). The model explained 99.08% (R^2 of 0.9908) of the variation in TPC.

The p-values were used to check the significance of each coefficient and also to estimate the interaction strength of each parameter. The smaller the p-value, the larger the significance of the corresponding coefficient. Corresponding

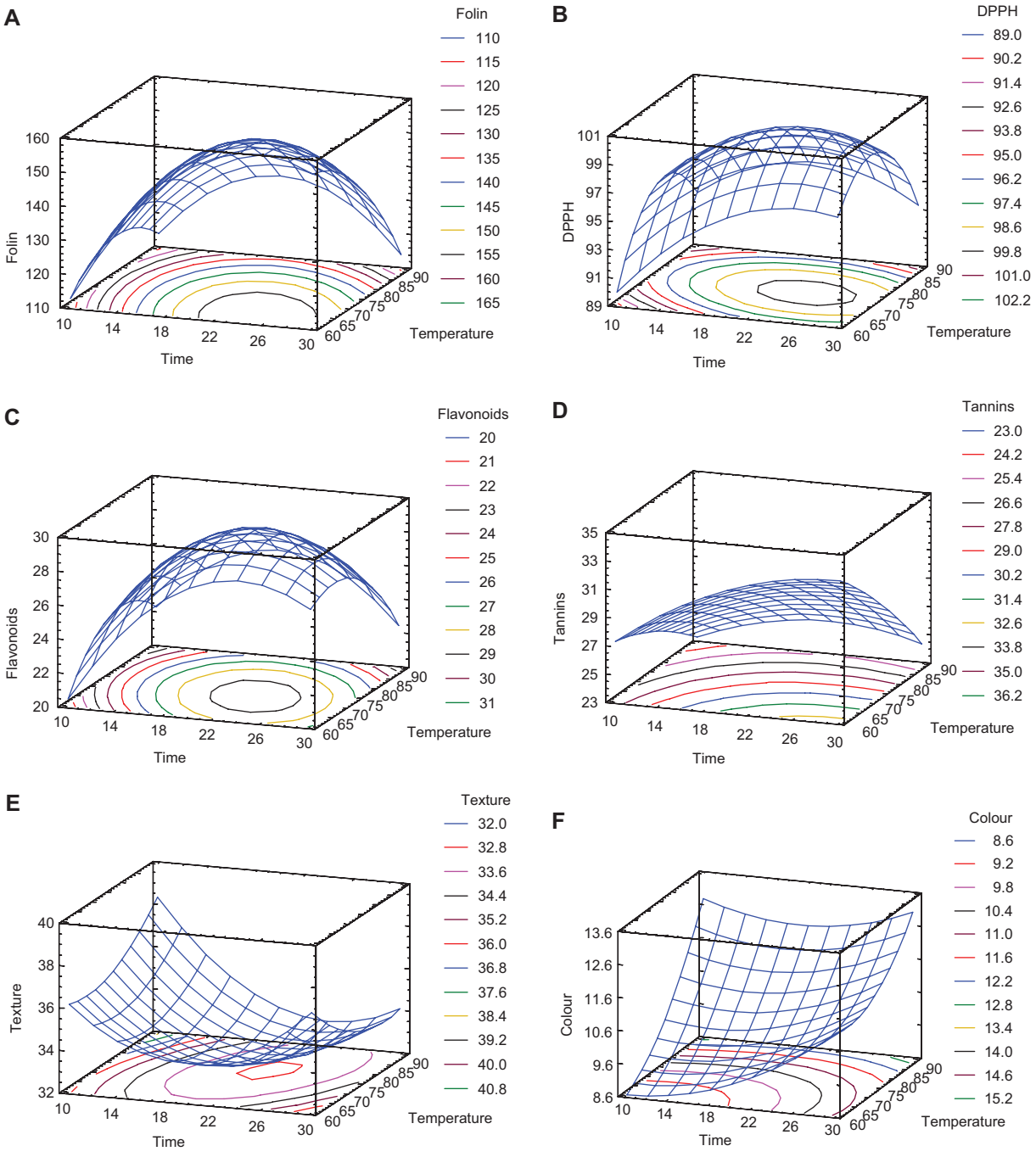

Figure 2 *Himanthalia elongata*: response surface plots showing effects of blanching time (min) and temperature (°C) on (A) total phenolic content (GAE 100 g^{-1} FW), (B) DPPH radical scavenging activity (%), (C) total flavonoids (mg QE 100 g^{-1} FW), (D) total condensed tannins (mg CE 100 g^{-1} FW), (E) texture (N mm^{-1}) and (F) colour (ΔE).

p-values indicated that among the test variables and their interactions, X_1 (blanching time), X_2 (blanching temperature), $X_1{*}X_1$ (time×time), $X_1{*}X_2$ (time×temperature) and $X_2{*}X_2$ (temperature×temperature) were significant model terms with $p<0.05$. Therefore, it is important to find optimum blanching time and temperature at which the TPC loss is minimised while at the same time making the product edible.

The polynomic response models were expressed as three-dimensional surface plots to better visualise the relationship between blanching time and temperature as independent variables and phytochemical properties as response variables. The fitted response surface plots were generated by statistically significant models using Statgraphics software to illustrate the interactions of parameters required for optimum responses. The response plot (Figure 2A) shows that blanching time and

temperature both had significant effects on the TPC, with significant interaction between the factors. The shape of the contour plots (circular or elliptical) indicates whether the mutual interactions between variables are significant or not. A circular contour plot indicates that the interactions are negligible. An elliptical contour plot indicates that the interactions between related variables are significant (Muralidhar et al. 2001). The response surface plot showed that increasing blanching time and temperature resulted in decreases in TPC.

Treatment of vegetables for consumption exposes the phytochemicals present to detrimental factors that may lead to alterations in concentrations and health-related quality. Processing, such as blanching can result in reduction of constituents through leaching or thermal destruction (Rungapamestry et al. 2007). Phenolic compounds are sensitive to heat; boiling of vegetables for a few minutes may cause a significant loss of phenolic content, which can leach into blanching water (Amin et al. 2006). Xu and Chang (2008) reported a 40–50% loss in TPC of legumes due to leaching of phenolics in boiling water. Bunea et al. (2008) also reported a 50% loss of TPC in spinach blanched for 10 min. We observed a reduction in TPC from 142.6 to 64.2 mg GAE 100 g^{-1} FW when the seaweed was blanched at 75°C for 20 min. This is a good result considering the long blanching time. The texture of *H. elongata* is relatively hard, and long cooking times are required to make it edible; such levels of phytochemical losses are therefore expected and are in line with other results in literature (Amin et al. 2006, Bunea et al. 2008, Xu and Chang 2008).

Effects of process variables on DPPH radical scavenging activity

The model obtained for the DPPH radical scavenging activity of hydrothermally processed thallus extract was

$$Z=-24.7231+(2.08795*X_1)+(2.7056*X_2) -(0.0242313*X_1^2)-(0.01195*X_1*X_2) -(0.0165694*X_2^2) \quad (5)$$

(see Table 2 for definitions of X_1 and X_2). There were significant ($p<0.05$) influences of the linear factor X_1 (blanching time), and all interactions X_1*X_1 (time×time), X_1*X_2 (time×temperature) and X_2*X_2 (temperature×temperature) on the DPPH radical scavenging activity. X_2 (blanching temperature) had no significant linear effect in the model ($p>0.05$) (Table 3). The fit of the model was further confirmed by a high coefficient of determination ($R^2=0.983$). The response surface plots generated showed slight increases in DPPH radical scavenging activity as blanching time increased (Figure 2B). The DPPH scavenging results indicate better performance than in some previous reports (Turkmen et al. 2005, Chandini et al. 2008). While thermal processing often increases DPPH radical scavenging activity per unit mass of extract from vegetables, the potency of the extract in the current study was very high. Turkmen et al. (2005) found that boiled greenbeans and peas had 70.8% and 17.9% scavenging activity at 6 mg ml^{-1} extract concentration, respectively. *Himathalia elongata* blanched for 20 min at 75°C achieved 99.5% scavenging at a much lower concentration of extract (50 µg ml^{-1}).

Effects of process variables on total flavonoid content

Flavonoids are another important phytochemical found in vegetables; they may be lost during processing. The model obtained for total flavonoid content in blanched thalli was

$$Z=-28.9533+(0.33379*X_1)+(1.07504*X_2) -(0.00448804*X_1^2)-(0.00501146*X_1*X_2) -(0.00658426*X_2^2) \quad (6)$$

(see Table 2 for definitions of X_1 and X_2). The linear factor X_1 (blanching time) had a significant influence on the model ($p<0.05$) while X_2 (blanching temperature) and the quadratic interaction factors, X_1*X_1 (time×time) and X_1*X_2 (time×temperature), did not ($p>0.05$). The quadratic effect of temperature, X_2*X_2, was significant ($p<0.05$). The fit of the model was further confirmed by a satisfactory R^2 value of 0.9658. The response surface plots showed the total flavonoid content was maximised with increasing time. There was little effect as temperature was increased (Figure 2C). The circular contour plot confirmed a negligible interaction between the variables. Flavonoids commonly accumulate in epidermal cells of plant organs, where they are found as glycosides and in non-glycosidic forms (aglycones) (Sakihama et al. 2002). Release of flavonoids and increased chemical extraction of these compounds is inducible by blanching (Olivera et al. 2008). This release of flavonoids coupled with contact and leaching into water may strongly reduce flavonoid content in samples blanched at high temperatures for long durations. The results of the present study are similar to Olivera et al. (2008), who found that blanching decreased TFC in Brussels sprouts; however, these authors found less leaching of flavonoids than the present study (their blanching times were significantly shorter).

Effects of process variables on total condensed tannin content

Phlorotannins are a group of phenolic compounds restricted to polymers of phloroglucinol and have been identified in several brown algae. Many studies have shown that phlorotannins are the only phenolic group in brown algae (Jormalainen and Honkanen 2004, Koivikko et al. 2007). The model obtained for the total condensed tannin content of hydrothermally processed thalli was

$$Z=2.2226-(0.527452*X_1)+(0.559293*X_2) +(0.00948114*X_1^2)-(0.00184167*X_1*X_2) -(0.00404377*X_2^2) \quad (7)$$

(see Table 2 for definitions of X_1 and X_2). There were significant ($p<0.05$) influences of X_1 and X_2 in the model. All interaction terms were non-significant ($p>0.05$). The fit of the model was further confirmed by a satisfactory R^2 value of 0.9528. Tannin levels were as low as 7.48 mg CE 100 g^{-1} FW, which represented an almost 90% reduction from fresh samples. As components of food, tannins reduce the biological value of dietary proteins (Bressani 1993). Therefore, such losses of these anti-nutritional components can be beneficial. Somsub et al. (2008) also found decreases in tannin levels of cooked

legumes and vegetables. From the response surface plots generated for blanched *Himanthalia elongata* (Figure 2D), it can be seen that varying time and temperature caused little enhancement or reduction in total condensed tannins as all blanching time/temperature combinations had a significant effect in the reduction of tannins as compared with fresh seaweed.

Effects of process variables on the texture

Cooking time and cooked texture, appearance and flavour are important cooking quality characteristics (Xu and Chang 2008). The texture of dried *Himanthalia elongata* is quite tough, and cooking such as blanching is required to make it palatable. Cooking times (10–30 min) were chosen on the basis of the results of preliminary experiments in which an edible texture was first determined by the tactile method. To overcome the subjectivity of the tactile method, a combination of tactile and instrumental textural methods were used to decide the edible texture of seaweed. From these methods, it was found that a softness of 30–32 N mm^{-1} was an acceptable edible texture. RSM was used to study texture to take into account the required combination of an edible texture and optimised phytochemical composition.

The model obtained for texture of hydrothermally processed thalli was

$$Z=65.7186-(1.83977*X_1)-(0.394061*X_2)+(0.0336521*X_1^2)+(0.00439167*X_1*X_2)+(0.00220277*X_2^2) \quad (8)$$

(see Table 2 for definitions of X_1 and X_2). There was a significant ($p<0.05$) influence of blanching time, X_1; the interaction term X_1*X_1 (time×time) was also significant (Table 3). There was no significant influence of X_2 in the model. The fit of the model was confirmed by a satisfactory R^2 value of 0.9174. The response surface plot (Figure 2E) showed that the texture softened as the blanching time increased, but there were no major changes in texture with increasing temperature.

Effects of process variables on the colour of thalli

Colour is as an important element in food choice, often reflecting our expectations of flavours (Lavin and Lawless 1998, Leon et al. 1999, Hutchings 2003). Commonly, *Himanthalia elongata* is dried and during the process, colour darkens from brown to almost black. The seaweed becomes bright green upon blanching under appropriate conditions, which is an important colour change. The model obtained for colour of hydrothermally processed *H. elongata* was

$$Z=15.5294+(0.101367*X_1)-(0.293434*X_2)+(0.00621854*X_1^2)-(0.00377769*X_1*X_2)+(0.00314807*X_2^2) \quad (9)$$

(see Table 2 for definitions of X_1 and X_2). X_1 and X_2 were significant model terms $p<0.05$. There was no significant interaction term in the model (Table 3). The R^2 value was 0.9407. The response surface plot (Figure 2F) indicated that overall colour of seaweed increased (became brighter) as time and temperature increased; this is an important colour change as it makes the seaweed more visually attractive.

Optimisation

Optimum conditions of blanching for enhancing each of the bioactive compounds were slightly different. A number of combinations of variables produced maximum total phenolic content, DPPH radical scavenging activity and total flavonoids while still achieving good colour and texture. It was also important to minimise total condensed tannins. As a result, emphasis was placed on optimising the phytochemical constituents (total phenolic content, DPPH activity and total flavonoids). Optimum blanching conditions for maximising phytochemical constituents are depicted in Figure 3. The multi-response analysis of response surface design using the desirability approach was used to optimise blanching time and temperature. The desirability function is an approach for solving the problem of optimising several responses and is applied when various responses have to be considered at the same time. A desirability function is first constructed for each individual response, and then it is possible to obtain the overall desirability. Multiple response optimisation indicated that phytochemicals in thalli could be maximised by blanching for 20.4 min at 80.5°C. This is a promising finding as the time is short and the temperature is lower than boiling. The response values predicted under these conditions by the multiple response optimisation were 63.5 mg GAE 100 g^{-1} FW for TPC, 99.5% for DPPH radical scavenging activity, 13.2 mg QE 100 g^{-1} FW for TFC and 12.8 mg CE 100 g^{-1} FW for TTC. A validation experiment was carried out by blanching the seaweed at this optimised time and temperature combination. The phytochemical constituent contents were 61.5 mg GAE 100 g^{-1} FW for TPC, DPPH radical scavenging activity was 98.9%, TFC was 11.5 mg CE 100 g^{-1} FW and TTC was 10.2 mg QE 100 g^{-1} FW. Texture was suitable at 30.3 N mm^{-1}

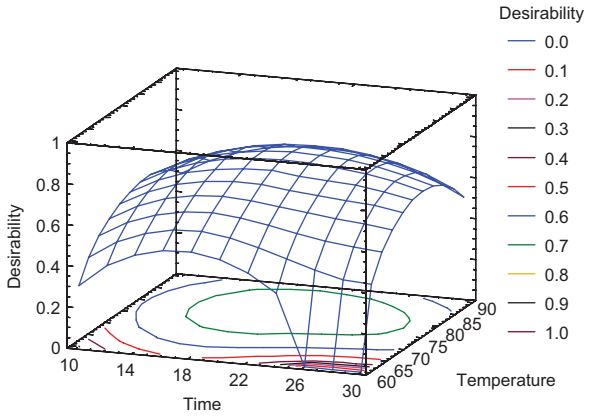

Figure 3 *Himanthalia elongata*: response surface plot showing optimised effect of blanching time (min) and temperature (°C) to maximise phytochemical constituents.

and total colour was 10.5, which indicated a significant and important colour change.

Conclusion

RSM using a central composite design was demonstrated to be an effective technique for optimising blanching conditions for enhancement of phytochemical constituents in semi-dried *Himanthalia elongata*. From the response surface plots, blanching time was found to have the most significant effect on phytochemical content of the seaweed. The high coefficients of determination of the variables at a 95% confidence level for the six mathematical models indicated that second-order polynomial models may be employed to predict critical phytochemical parameters of *H. elongata* along with texture and colour. These findings may be applied in the development of new functional foods from *H. elongata*.

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