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Incorporation of *Himanthalia elongata* seaweed to enhance the phytochemical content of breadsticks using *Response Surface Methodology* (RSM)

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23 **Abstract**

24 Optimization of incorporating seaweed into breadsticks was carried
25 out using response surface methodology (RSM). Ten formulations of
26 breadsticks were processed by varying concentrations of seaweed
27 (X_1 = 5 to 15% of overall flour concentration) and white flour (X_2 = 10
28 to 30% of overall flour concentration) using a central composite
29 design. The remaining flour concentrations were comprised of
30 wholemeal flour. Predicted models were found to be significant ($P <$
31 0.05) for total phenolic content (TPC), DPPH radical scavenging
32 activity, texture and color. Predicted values for each of the responses
33 were in good agreement with the experimental values. Seaweed
34 concentration had most significant effect on phytochemical
35 constituents of the breadsticks with TPC and DPPH activity
36 maximized when 17.07% *H. elongata* was incorporated into the flour
37 ($P < 0.05$). An acceptable edible texture and color of breadsticks was
38 also achieved at this concentration. Multiple response optimization
39 demonstrated that phytochemical content of *H. elongata* breadsticks
40 may be maximized with dried seaweed and white flour
41 concentrations of 17.07 and 21.89%, respectively, in the total flour.
42 Total dietary fiber increased from 4.65 to 7.95% in the optimized
43 sample, representing a 43.65% increase as compared to the control
44 ($P < 0.05$). A sensory panel evaluated the acceptability of the

seaweed breadsticks, as compared to the control, in terms of aroma, color, texture, taste and overall acceptability. There was no significant difference ($P > 0.05$) between the seaweed breadsticks and the control which shows that such fiber-rich seaweed bakery products are acceptable to consumers and have potential of increasing seaweed consumption among non-seaweed consumers.

Keywords: Functional foods; seaweeds; antioxidants; fiber; RSM.

1. Introduction

Marine food, due to its phenomenal biodiversity is a treasure house of many novel healthy food ingredients and biologically active compounds such as those found in seaweeds. Despite having so many health benefits, marine functional foods have been underexploited for food purposes. Bakery products are widely consumed throughout the world and are the best sources of incorporating marine functional ingredients and reaching the targeted population (Kadam and Prabhasankar, 2010). Bread is an excellent product in which incorporation of 'nutraceuticals' is attempted. One of the latest enrichments has been the addition of omega-3 PUFA to

65 improve essential fatty acid intake. In Europe, consumption of bread
66 enriched with omega-3 PUFA is steadily increasing because
67 Europeans recognise the healthy component of such products.
68 Therefore, the near future for nutrition could potentially include
69 extending the use of breads as vehicles for different micronutrients
70 (Kadam and Prabhasankar, 2010).

71 Seaweed contains a significant amount of soluble polysaccharides,
72 and has potential function as dietary fiber. The seaweed
73 polysaccharides possess a higher Water Holding Capacity (WHC)
74 than cellulosic fibers. There is an interest in seaweed hydrocolloids
75 for human nutrition as they can act as dietary fiber since their
76 physiological effects are closely related to their physicochemical
77 properties such as solubility, viscosity, hydration, and ion-exchange
78 capacities in the digestive tract (Lahaye and Kaeffer, 1997). Dietary
79 fiber (DF) is the edible portion of plants (or analogous carbohydrates)
80 which is resistant to digestion and adsorption in the human small
81 intestine with complete or partial fermentation in the large intestine
82 (Gelroth and Ranhotra, 2001). The term DF comprises
83 polysaccharides, oligosaccharides and associated plant compounds
84 (AACC, 2001).

85 Brown seaweeds are known to contain more bioactive components
86 than red or green seaweeds (Seafoodplus, 2008). Some of the

bioactive compounds identified in brown seaweeds include phylopheophylin, phlorotannins, fucoxanthin and various other metabolites (Hosakawa et al., 2006). Such antioxidants from natural sources can be added to products as an ingredient to increase the quality and shelf-life which also considerably enhances the consumer preference (Farag et al., 2003).

Development of functional foods is currently one of the most intensive areas of food product development worldwide. Product optimization is an effective strategy to accomplish successful development of the product with respect to a number of attributes. If a food product cannot be re-engineered or modified to fulfill consumer desires and demand for the product, it will not succeed (Robinson, 2000). The present study aimed to identify a food-based application for dried edible Irish seaweed in order to encourage consumption amongst non-seaweed eaters. The idea was to scientifically evaluate and improve the quality and nutritional content of a bakery product upon the incorporation of seaweeds. Wheat is the principal cereal used in the preparation of a variety of bakery products, however there is a current trend to move away from white breads towards whole grains such as whole meal flour. Therefore in the present study, white flour concentration was also varied and the overall flour consisted of varying levels of dried seaweed, white and

wholemeal flours. The main objective was to optimize the dried seaweed and white flour concentrations in the development of a new bakery based functional product and to investigate its effect on the phytochemical content of breadsticks.

2. Materials and methods

2.1 Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate (Na_2CO_3) and total dietary fiber kit were purchased from Sigma Aldrich Chemie (Steinheim, Germany).

2.2 Seaweed material

H. elongata was purchased from Quality Sea Veg., Co Donegal, Ireland. The seaweeds were collected in October 2011 and stored at 4 ℃ until further use.

2.3 Preparation of samples

H. elongata was washed thoroughly with tap water to remove epiphytes and salt, dried with absorbent paper and then cut into 3 cm long pieces before dehydration.

2.4 Dehydration procedure

Drying temperature and time was decided based on results of our previous kinetic experiments (Gupta et al., 2011). Seaweed samples (5 g) were placed on a drying tray in a single layer. Drying of seaweed was carried out in a drier (Innova 42, Mason Technology, Ireland) at 40 ℃ air drying temperature over a period of 24 hours. Air velocity was 2.0 ± 0.1 m s^{-1} measured with VWR Enviro-meter digital anemometer (VWR, Ireland). The dried seaweed was then ground into a fine powder using a blender (Rotor, Germany).

2.5 Experimental design

To investigate the effect of factors (seaweed and white flour concentration) on phytochemical constituents, color and texture of breadsticks, a central composite design with two factors was utilised. The central composite design was applied using STATGRAPHICS

148 Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA,
149 USA). The total number of experiments generated from the software
150 with two factors was 10 (= $2^k + 2k + 2$), where k is the number of
151 factors. Eight experiments were augmented with two duplicates at
152 the centre points. The level of codes for the independent variables
153 are presented in Table 1. The design matrix and variable
154 combinations of seaweed and white flour concentrations in
155 experimental runs are shown in Table 2. The independent variable
156 concentrations applied in the response surface methodology (RSM)
157 study (Seaweed 5 - 15% and white flour 10 - 30%) were percentage
158 of the of the overall flour concentration, with wholemeal flour making
159 up the remaining quantity up to 100%. Therefore as a percentage of
160 the overall mix of 411 g, these values consisted of 1.82 - 10.33 and
161 3.65 - 20.67% (seaweed and white flour, respectively).

162

163 Experimental data from the central composite design was analysed
164 and fitted to a polynomial regression model below:

165 $$Y = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_{11} \chi^2_1 + \beta_{22} \chi^2_2 + \beta_{12} \chi_1 \chi_2 \quad \textbf{Eq. 1}$$

166

Where; Y is 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 the lack of fit, coefficient of determination (R^2) and the Fisher's test value (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).

A multi-response analysis of the response surface design was performed using the desirability approach to optimize seaweed and white flour concentrations. The desirability function is an approach for solving the problem of optimization of several responses and is applied when various responses have to be considered at the same time and it is necessary to find optimal compromises between the total numbers of responses taken into account. This methodology is based on first constructing a desirability function for each individual response, and then it is possible to obtain the overall desirability.

2.6 Seaweed breadstick preparation

Seaweed and flour blends were prepared by the replacement method according to the RSM experiment. The percentages of seaweed and white flour from the RSM (Table 2) are based on percentages of overall flour in the mix (flour consisted of 60.79% of the mix), with wholemeal flour comprising the remaining component of the mix. The concentrations of ingredients for each of the experiments can be seen in Table 3. Firstly, the yeast was dissolved in the water and added to the dry ingredients (except seaweed). The ingredients were mixed at slow speed for 2 min, then at medium speed for 4 min (Hobard A120 mixer, Hobard MFG Co. Ltd, London, UK). Seaweed was then added and mixed again for a further 2 min. The dough was placed on trays and left to develop for 45 min then moulded into breadstick shapes by hand and proofed in a dough proofer (Sveba Dahlen, Sveba Dahlen, Fristan, Sweden) at 33 ℃, 78% RH for 40 min. The breadsticks were then baked in an oven (Sveba Dahlen, DC 44, Sveba Dahlen, Fristan, Sweden) at 210 ℃ for 20 min with 10 seconds of steam at the beginning.

2.7 Extraction of phytochemicals

Seaweed and breadstick samples (5 g) were powdered in liquid nitrogen using a mortar and pestle, then extracted with 50 ml of methanol (60%) under nitrogen atmosphere for 2 hours as described by Cox et al. (2010).

2.8 Total phenolic content

The total phenolic concentration was measured using the Folin-Ciocalteau method as outlined by Cox et al. (2012). The total phenolic contents were expressed as mg gallic acid equivalent per 100 gram dry basis (db) (mg GAE/100 g db).

2.9 DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Jaiswal et al. (2011).

2.10 Texture evaluation

Shear tests were performed using an Instron Universal Testing Machine (Model 4301, Canton MA, USA) supported with Bluehill 2 version 2.14 analysis software for materials testing. A Warner Bratzler cutter was used in the shear tests. An aluminium plate with dimensions of 10 x 6 cm^2, thickness of 1.3 cm and with an opening of 3 mm in the centre was supported in the Instron base. Breadstick samples (5 g) were sheared at a speed of 200 mm/min. The cutting implement was allowed to travel the depth of the seaweed, cutting through the sample and seaweed hardness was defined as the peak of force-deformation curve recorded in Newtons per mm (N/mm). Ten replications of each sample were carried out.

2.11 Color measurement

At specified experimental times (Table 2), breadsticks (original 5 g FW) underwent color analysis using a colorimeter (CIE Lab ColorQuest XE). The colorimeter was calibrated against a standard white reference tile (L* = 93.97; a* = -0.08 and b* = 1.21). The color values were represented on the CIE color scales in terms of L* (lightness/darkness), a* (redness/greenness) and b*

(yellowness/blueness). From these values, total color change from fresh (ΔE) was calculated according to the following equation:

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad \textbf{Eq. 2}$$

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.

2.12 Total Dietary Fiber

Total dietary fiber (TDF) was determined by Sigma analysis kit (Sigma-Aldrich, Inc., USA) based on AOAC method 991.43. Samples were cooked at 100 ºC with heat stable α-amylase to initiate gelatinization, hydrolysis and depolymerisation of starch. The samples were incubated at 60 ºC with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose). The samples were then treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerised protein and glucose. The residue was filtered, washed, dried and weighed. One duplicate was analysed for protein

and the other was incubated at 525 ºC to determine ash. The TDF was determined as the weight of the filtered and dried residue less the weight of the protein and ash.

2.13 Sensory characteristics

The sensory acceptance test was conducted in a standardized sensory test room (ISO 8589, 2007). Untrained panelists (n = 20) were recruited from staff and students of the Dublin Institute of Technology using a five-point hedonic scale. Samples (20 g) were served on white paper plates with random three-digit numbers and water at room temperature was provided for mouth-rinsing between samples. The panelists were asked to assign scores for aroma (maximum of 5), appearance (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall acceptability of the product (maximum of 5), where 5 was “like extremely” and 1 was “dislike extremely”. The overall quality (maximum of 25) was computed by combining scores of all five attributes.

288 **2.14 Statistical analysis**

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

297

298 **3. Results and Discussion**

299 **3.1 Statistical analysis of results obtained by experimental**
300 **design**

301 The effect of a range of drying temperatures on the drying kinetics
302 and phytochemical constituents of *H. elongata* was investigated and
303 results showed that drying was optimized at 40 ℃ and therefore
304 these drying conditions were applied in the current study (Gupta et
305 al., 2011). The rationale behind adding seaweed to breadsticks was
306 based on the fact that bakery products are widely consumed;
307 therefore addition of *H. elongata* would widen the consumer base

and would further improve the nutraceutical properties of this product. Dried seaweed is also a convenient and cost effective ingredient as drying reduces the volume thus lowering transport costs and therefore can be considered a viable ingredient to add value to existing products.

Preliminary experiments were carried out in order to determine the maximum levels of seaweed which could be added to the breadsticks with respect to texture and flavour. Higher seaweed concentrations (≥ 20%) led to unacceptable end products as the baked product was quite tough and difficult to chew. Once the maximum level of seaweed was established at 15%, RSM was applied. In this study, ten experiments were performed to determine the optimum concentrations of seaweed and flour blends required to maximize the phytochemical level in breadsticks. The effects of independent variables (seaweed and white flour concentrations) for each of the response variables (TPC, DPPH, texture and color) are presented in Table 4.

The models for each of the responses were analyzed separately before overall optimum seaweed and flour concentrations for the breadstick recipe were determined. Predicted and experimental values for each of the responses are presented in Table 5 and were in good agreement with the experimental values. Response surface

330 plots were generated to illustrate the effects of blanching time and
331 temperature on each of the responses (Fig. 1a-d).

332

333 **3.2 Effects of process variables on total phenolic content**

334 Experimental results for total phenolic content (TPC) were fitted to a
335 full quadratic second order polynomial equation and the model
336 obtained for TPC of the breadsticks was:

337 $Z = 3.77979 + 5.72532^* X_1 + 0.305353^* X_2 + 0.140273^* X_1^2 - 0.0129^*$
338 $X_1^* X_2 - 0.00315601^* X_2^2$ **Eq. 3**

339

340 (See Table 1 for definitions of X_1 and X_2). In order to determine the
341 significance of the model, ANOVA was carried out on the data. The
342 *F*-value for seaweed concentration (X_1) was high (762.40) indicating
343 that this factor was highly significant (Table 4). All other interaction
344 factors and white flour concentration (X_1) had low *F*-values which
345 suggest that TPC had mainly resulted from the addition of seaweed.
346 The model explained 99.48% (R^2 of 0.9948) of the variation in TPC
347 which is quite significant. This indicates that only 0.52% of the
348 variation in TPC was due to factors not included in the model.

349 The *P*-values were used to check the significance of each coefficient,
350 which also indicated the interaction strength of each parameter. The
351 smaller the *P*-value, the larger the significance of the corresponding
352 coefficient is. *P*-values indicated that, among the test variables and
353 their interactions, X_1 (seaweed concentration) was highly significant
354 ($P < 0.05$) but all other factors; X_2 (white flour concentration), X_1*X_1
355 (seaweed concentration × seaweed concentration), X_1*X_2 (seaweed
356 concetration × white flour concentration) and X_2*X_2 (white flour
357 concentration × white flour concentration) were insignificant model
358 terms with *P*-values > 0.05.

359 The polynomical response models were expressed as three-
360 dimensional (3D) surface plots to better visualise the relationship
361 between the seaweed and white flour concentrations as independent
362 variables and phytochemical properties as response variables. The
363 response plot (Fig. 1a) showed that TPC increased sharply with
364 increasing seaweed concentration ($P < 0.05$), while TPC remained
365 unchanged with increasing white flour concentration as observed in
366 Table 4.
367 The addition of seaweed to the breadsticks significantly increased
368 the TPC ($P < 0.05$). An 81.03% increase was seen when the overall
369 flour concentration was substituted with 17.07% seaweed. These
370 results are higher than those reported for other cereal based food

products which were incorporated with seaweed. Prabhasankar et al. (2009a) studied the influence of adding brown seaweed, *Sargassum marginatum*, to pasta. The TPC in cooked pasta increased from 9 to 13 mg GAE/100 g with 5% addition of the brown seaweed. Although the previous study showed that phenolics leached into processing water, these results are still significantly lower than those of the present study. Comparing with the same seaweed concentration, the results of 5% incorporation of seaweed in breadsticks increased the TPC from 27.67 to 38.99 mg GAE/100 g db which is also higher than that of Prabhasankar et al. (2009a).

The breadsticks containing maximum *H. elongata* concentration (17.07%) showed an increase in the TPC from 27.67 to 145.88 mg GAE/100 g db which is an increase of 81.03%, as compared to the control. Prabhasankar et al. (2009b) also reported that an addition of 30% *Undaria pinnatifida* seaweed increased the TPC of pasta from 9 - 27 mg GAE/100 g. Again, this is considerably less than obtained in the present study. TPC of bread samples with different percentages of ginger powder were studied by Balestra et al. (2011). TPC levels increased from 14.30 to 48.50 GAE/100 g db with 6% addition of ginger powder. This clearly shows that the seaweed breadsticks had higher levels of total phenols compared to that of other nutraceutical cereal based products such as bread and pasta.

393

394 **3.3 Effects of process variables on DPPH radical scavenging**
395 **activity**

396 The model obtained for the DPPH radical scavenging activity of the
397 breadsticks was:

398 $Z = 13.2787 + 4.76275^* X_1 + 0.92469^* X_2 - 0.1438^* X_1^2 + 0.0087^* X_1^*$
399 $X_2 - 0.0242^* X_2^2$ **Eq. 4**

400

401 There was a significant ($P < 0.05$) influence of the linear factor of X_1
402 (seaweed concentration) on the model. The linear factor of X_2 (white
403 flour concentration) and all quadratic factors and interactions $X_1^*X_1$
404 (seaweed concentration × seaweed concentration), $X_1^*X_2$ (seaweed
405 concetration × white flour concentration) and $X_2^*X_2$ (white flour
406 concentration × white flour concentration) were insignificant model
407 terms with *P*-values > 0.05 in terms of DPPH radical scavenging
408 activity. This showed that seaweed concentration had the greatest
409 impact on the DPPH radical scavenging activity of the breadsticks
410 which was expected as seaweed exhibit high levels of DPPH radical
411 scavenging activity. The fit of the model was further confirmed by a
412 high coefficient of determination, 0.9973 meaning that 99.73% of the

413 variation in DPPH activity was explained by the model. The response
414 surface plots generated showed that DPPH radical scavenging
415 activity increased with increasing seaweed concentration while the
416 activity remained more or less constant with respect to the effect of
417 white flour concentration (Fig. 1b). The lack of significance of the
418 white flour concentration on the DPPH activity of the breadsticks is
419 further confirmed by the circular shape of the contour plots which
420 indicates that the interactions are negligible.

421 The DPPH radical scavenging activity of the control breadsticks
422 (containing no seaweed) was 34.81%. Replacement of flour with
423 17.07% seaweed increased the DPPH activity to 65.24%,
424 representing a significant increase of 46.64% in DPPH activity ($P <$
425 0.05). Any level of seaweed above 5% significantly increased the
426 DPPH activity of the seaweed breadsticks ($P < 0.05$). Balestra et al.
427 (2011) also found a significant increase in DPPH activity with the
428 addition of 6% ginger powder to breads (86.75% increase). In
429 seaweed incorporated pasta, it was found that addition of 30% brown
430 seaweed increased the DPPH activity from 6.83 to 9.79%
431 (Prabhasankar et al., 2009a) which is significantly lower than the
432 activity in the present study. In our previous studies, it is reported that
433 dehydration can lead to slight decreases in DPPH activity but thermal
434 processing such as boiling, applied after drying can lead to significant

435 increases in the activity (Cox et al., 2011). It is possible that the
436 temperature upon baking of the breadsticks could also have
437 increased the DPPH radical scavenging activity of extracts from the
438 final product. This indicates that addition of *H. elongata* seaweed to
439 breadsticks would provide a good source of antioxidants.

440

441 **3.4 Effects of process variables on the texture**

442 For a novel food product, it is necessary to study the impact of added
443 ingredients on food quality attributes. Hardness or firmness is an
444 important factor in the quality of breadsticks. The texture of dried *H.*
445 *elongata* can be quite tough and processing is often required to make
446 it more palatable. Common food processing methods such as boiling
447 can lead to loss of phytochemicals (Cox et al., 2011). To overcome
448 the issues with the noticeable toughness of dried *H. elongata*, the
449 dried seaweed was ground into a powder and was then incorporated
450 into breadsticks. The model obtained for texture of the breadsticks
451 was:

452 $Z = 69.7308 - 0.0399788^* X_1 - 0.122297^* X_2 + 0.141849^* X_1^2 -$
453 $0.0002^* X_1^* X_2 + 0.0019626^* X_2^2$ **Eq. 5**

454

There was a significant ($P < 0.05$) influence of seaweed concentration, X_1, and the quadratic terms X_1*X_1 (seaweed concentration × seaweed concentration) on the model (Table 3). However, there was no significant influence of white flour concentration (X_2) or the quadratic term X_2*X_2 (seaweed concentration × seaweed concentration) or interaction term X_1*X_2 (seaweed concentration × white flour concentration) on the model. The fit of the model was confirmed by a satisfactory R^2 value of 0.9981 which is very high. The response surface plot (Fig. 1c) showed that the texture became harder with increasing seaweed concentration, but there were no major changes in hardness with increasing white flour concentration which was expected.

The hardness of the control breadsticks was calculated as 74.38 N/mm using an Instron texture analyser, and fortification of flour with seaweed at all levels (2.93 to 17.07%) significantly increased the hardness of the breadsticks ($P < 0.05$). Hardness was maximized in the present study, when flour was replaced with 17.07% seaweed (108.84 N/mm). Prabhasankar et al. (2009a and 2009b) also found that adding seaweed to pasta (1 - 5%) increased the firmness of the product. Chang and Wu (2008) added 4 - 8% green seaweed to noodles and also found that there was an increase in the hardness with increasing seaweed concentration.

3.5 Effects of process variables on the color

Commonly *H. elongata* is dried and during the dehydration process, color darkens from brown to almost black (Cox et al., 2012). Color is an important characteristic for baked products because together with texture and aroma, it contributes to consumer preference. It is dependant on physicochemical characteristic of the dough (water content, pH, reducing sugars and amino acid content) and on the operating conditions applied during baking (temperature, relative humidity, modes of heat transfer) (Esteller and Lannes, 2008). The consumer understanding of the expected color of baked goods is well known and this characteristic color would be expected with new baked products. The model obtained for color change of breadsticks with added seaweeds was:

$$Z = -0.562436 + 2.64694^{*} X_1 + 0.499152^{*} X_2 - 0.159474^{*} X_1^2 + 0.03885^{*} X_1^{*} X_2 - 0.0233189^{*} X_2^2 \quad \textbf{Eq. 6}$$

Color analysis of the breadsticks indicated that the linear factor of seaweed concentration (X_1) had an insignificant effect on the color of the breadsticks ($P > 0.05$) however the quadratic factors of seaweed concentration ($X_1^{*}X_1$) were significant ($P < 0.05$). X_2 (white flour concentration) also had a significant ($P < 0.05$) effect on the color of

498 the breadsticks. There was no significant interaction of the quadratic
499 term X_2*X_2 (white flour concentration × white flour concentration) or
500 interaction term X_1*X_2 (seaweed concentration × white flour
501 concentration) on the model ($P > 0.05$) and the R^2 value obtained
502 was 0.7780. This indicated that both seaweed and white flour
503 concentrations had some influence on the color of the breadsticks.
504 This was further confirmed by the response surface plot (Fig. 1d) as
505 it had a spherical response surface which indicated that color change
506 increased with increasing seaweed concentration but then gradually
507 decreased, while white flour concentration also affected color change
508 as it increased slightly with increasing flour concentration but then
509 also decreased slightly. The color change of all samples was
510 significantly different ($P < 0.05$) indicating that the different flour
511 blends with varying concentrations of seaweed, white and wholemeal
512 flour had a significant effect on the color of the breadsticks. This was
513 expected as the color of the seaweed is quite dark so varying the
514 seaweed concentrations in the flour from 2.93 to 17.07% would
515 obviously cause a difference in overall color of the baked
516 breadsticks.

517

518

519 **3.6 Optimization**

520 Optimum conditions of seaweed and flour concentrations in
521 breadsticks were determined to obtain maximum phytochemicals and
522 enhance dietary fiber as the rational was to develop a functional food
523 product. As the texture (hardness) and color of the breadsticks were
524 acceptable throughout the ten experiments, they were not included
525 as factors in the optimisation. These factors (texture and color) were
526 sensorially evaluated by a sensory panel to determine acceptability.
527 The second order polynomial models obtained in this study for TPC
528 and DPPH responses were utilised in order to determine the
529 specified optimum conditions. Optimum seaweed and white flour
530 concentrations for maximising phytochemical constituents are
531 depicted in Fig. 2.

532 By applying the desirability function method (an approach for solving
533 the problem of optimising several responses which have to be
534 considered at the same time) the concentrations were obtained for
535 the breadsticks with optimum phytochemical level. Multiple response
536 optimisation indicated that phytochemicals in breadsticks could be
537 maximized with 17.07% seaweed and 21.89% white flour
538 concentrations in the overall flour. The response values predicted
539 under these conditions by the multiple response optimisation were
540 142.75 mg GAE/100 g db for TPC and 64.58% for DPPH radical

scavenging activity. A validation experiment was carried out by preparing breadsticks with the optimized dried seaweed and white flour concentrations. The phytochemical constituent contents were 138.25 mg GAE/100 g db for TPC and 65.01% for DPPH radical scavenging activity.

8.3.7 Total dietary fiber

In view of the therapeutic potential of dietary fiber, more fiber incorporated food products are being developed. Fig. 3 shows the total dietary fiber (TDF) content of the breadsticks. Dried seaweed contained 39.56% TDF, control breadsticks had 4.65% TDF and the seaweed breadsticks as optimized using RSM (17.07% seaweed added) contained 7.95% TDF which represents a 43.65% increase in the total dietary fiber when compared to breadsticks with no added seaweeds. Addition of seaweed significantly increased the TDF of the breadsticks as compared to the control ($P > 0.05$). These results are higher than those reported in the literature for final products containing seaweed. Prabhasankar et al. (2008) developed a seaweed pasta which had 4% fiber, but the amount of seaweed added was considerably less (2.5%). Cofrades et al. (2008) found that the addition of 5% *H. elongata* to meat systems only contributed

562 2.52% TDF to the final product. The same authors also found that the
563 incorporation of *Porphyra umbilicalis* seaweeds at 5%, only fortified
564 meat products with 1.77% fiber. The effect of enrichment of bread
565 with rice bran fiber was studied by Hu et al. (2009) and addition of up
566 to 6% rice bran fiber resulted in 4.98% TDF in the final product.
567 Therefore, in the current study, the optimized breadsticks had a
568 higher TDF in the final product (7.95%), this higher level would also
569 be due to the fact that more seaweed could be added to the
570 breadsticks then to the products in the other studies outlined in
571 literature.

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573 **8.3.8 Sensory analysis**

574 Table 6 summarises the sensory scores for aroma, appearance,
575 texture, taste and overall acceptability of control and seaweed
576 breadsticks. When developing functional bakery products, it is
577 important to design a product with physiological effectiveness that
578 will be accepted by consumers in terms of appearance, taste and
579 texture (Siró et al., 2008). The samples tested by the sensory panel
580 in this study were the control (with no added seaweed), breadsticks
581 with 10% of the flour replaced with seaweed (6.08% concentration of
582 seaweed overall) and the optimized sample from the RSM study

583 which would have the maximum level of antioxidants (17.07%
584 seaweed in overall flour blend or 10.33% seaweed in the final
585 product).

586 Aroma, appearance, texture and taste were found to be significantly
587 different to the control breadsticks ($P > 0.05$). Although there was a
588 significant difference, the scores for each of the seaweed breadsticks
589 were only slightly lower than that of the control, and all three
590 breadsticks were at acceptable values suggesting potential
591 incorporation of seaweeds in bakery products.

592 The results of the present study are promising as some food
593 products with added fiber are often rated as unacceptable by sensory
594 panels once they exceed a certain concentration. For example, Hu et
595 al. (2008) found that the addition of rice bran fiber above 4% was
596 unacceptable by consumers. Also, Prabhasankar et al. (2009) found
597 that there was a significant difference in pasta with 10% replacement
598 of semolina with seaweed as compared to the control ($P > 0.05$).
599 This indicates that breadsticks are a good product for seaweed
600 incorporation at high levels without affecting the overall quality of the
601 product.

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4. Conclusion

Response surface methodology using central composite design was demonstrated to be an effective technique for optimizing *H. elongata* and white flour concentrations for enhancement of phytochemical constituents in seaweed breadsticks. From the response surface plots, seaweed concentration was found to have the most significant effect on phytochemical content of the breadsticks. The high coefficients of determination of the variables at a 95% confidence level indicated that second order polynomial models could be employed to predict critical phytochemical parameters of breadsticks containing *H. elongata* along with texture and color. These breadsticks would provide the consumer with higher levels of dietary fiber (7.95%) and phytochemicals (TPC: 138.25 mg GAE/100 g db; DPPH: 65.01%) and have an appealing color and texture. There was a significant difference found in the sensory scores for seaweed breadsticks as compared to the control ($P > 0.05$), however all scores were at acceptable levels which is promising.

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Table 1. Level of codes for independent variables used in the central composite design

Independent variables	Symbol	-2	-1	0	+1	+2
Seaweed concentration (%)*	X_1	2.93	5	10	15	17.07
White flour concentration (%)*	X_2	5.86	10	20	30	34.14

***Percentage of overall flour concentration (100%) with the remaining flour consisting of wholemeal**

Table 2. Design matrix and variable combinations in experimental runs

Experiment	Seaweed concentration (%)*	White flour concentration (%)*
1	15.00	10.00
2	10.00	20.00
3	5.00	30.00
4	10.00	20.00
5	17.07	20.00
6	10.00	5.86
7	5.00	10.00
8	2.93	20.00
9	10.00	34.14
10	15.00	30.00

***Percentage of overall flour concentration (100%) with the remaining flour consisting of wholemeal**

764 **Table 3. Design of experiments for seaweed breadsticks**

Experiment	Seaweed (%)	White flour (%)	Wholemeal flour (%)	Salt (%)	Butter (%)	Yeast (%)	Water (%)
1	9.12	6.08	45.59	1.21	1.21	2.13	34.65
2	6.08	12.16	42.55	1.21	1.21	2.13	34.65
3	3.04	18.24	39.51	1.21	1.21	2.13	34.65
4	6.08	12.16	42.55	1.21	1.21	2.13	34.65
5	10.33	12.16	38.30	1.21	1.21	2.13	34.65
6	6.08	3.65	51.06	1.21	1.21	2.13	34.65
7	3.04	6.08	51.67	1.21	1.21	2.13	34.65
8	1.82	12.16	46.81	1.21	1.21	2.13	34.65
9	6.08	20.67	34.04	1.21	1.21	2.13	34.65
10	9.12	18.24	33.43	1.21	1.21	2.13	34.65

Table 4. Two-way ANOVA for the independent variables on the response of total phenolic content, DPPH, texture and color of seaweed breadsticks

Source	Total phenolic content		DPPH		Texture		Color	
	F-Ratio	*P*-value	*F*-Ratio	*P*-value	*F*-Ratio	*P*-value	*F*-Ratio	*P*-value
X_1	762.40	0.0000	66.82	0.0012	2020.32	0.0000	1.50	0.2874
X_2	0.11	0.7548	0.12	0.7464	2.17	0.2145	0.22	0.0345
$X_1 * X_1$	3.13	0.1515	4.65	0.0973	74.44	0.0010	9.93	0.0345
$X_1 * X_2$	0.09	0.7760	0.06	0.8192	0.00	0.9829	2.06	0.2242
$X_2 * X_2$	0.03	0.8812	2.11	0.2203	0.23	0.6579	3.40	0.1390

R^2 values: 0.9948 (total phenolic content), 0.9973 (DPPH), 0.9981 (texture) and 0.7780 (color)

Table 5. Predicted (Pred.) and experimental (Exp.) values of total phenolic content, DPPH, texture and color of seaweed breadsticks

Experiment No.	Total phenolic content (mg GAE/100g db)		DPPH (%)		Texture (N/mm)		Color (∆E)	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	118.02	122.02	60.25	60.50	99.36	99.99	11.85	11.75
2	78.99	77.33	52.18	57.08	81.51	81.82	19.17	18.39
3	38.99	40.30	40.44	40.76	70.90	71.14	5.47	8.50
4	75.66	77.33	61.98	57.08	82.12	81.82	17.6	18.39
5	145.88	142.84	65.24	64.46	108.84	108.66	12.79	12.07
6	80.16	75.99	51.36	51.62	83.82	82.86	14.35	14.36

7	34.55	38.01	41.21	40.76	71.05	72.04	12.34	13.29
8	28.11	25.84	35.11	35.32	69.85	69.16	10.96	8.76
9	78.54	77.40	53.69	52.86	81.47	81.56	16.02	13.08
10	119.88	121.74	61.22	62.24	99.17	99.05	12.75	14.73

Values are presented as mean (n = 6).

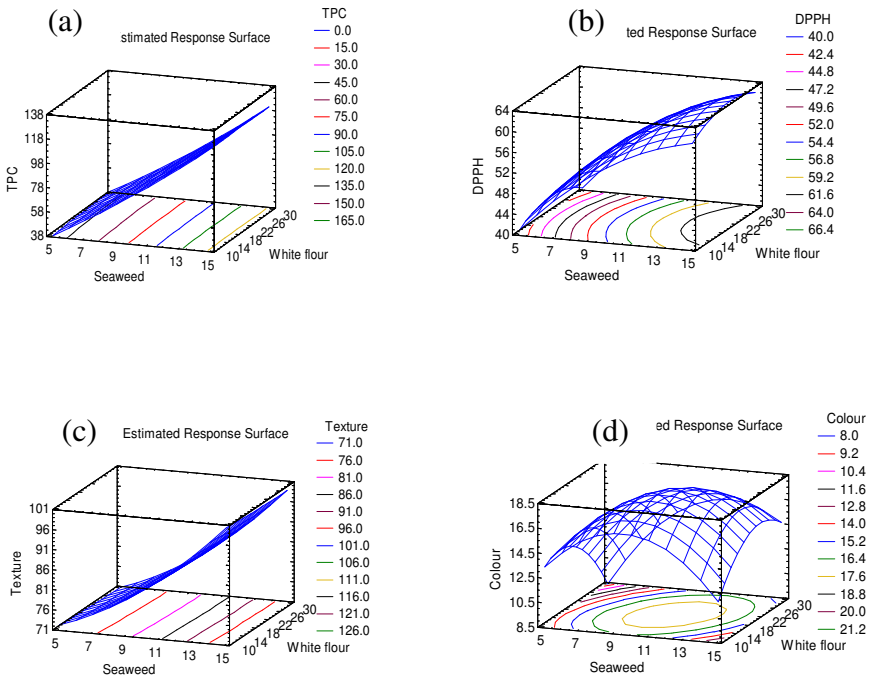
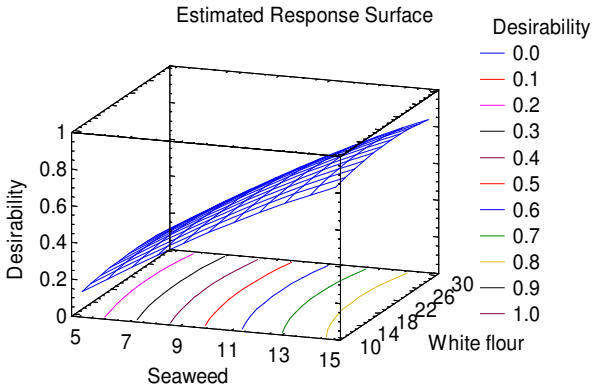


Fig. 1. Response surface plots showing effects of seaweed and white flour concentrations (%) on (a) the total phenolic content (GAE/100 g db), (b) DPPH radical scavenging activity (%), (c) texture (N/mm) and (d) color (ΔE) of seaweed breadsticks



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23 **Fig. 2. Response surface plot showing optimized effect of**
24 **seaweed and white flour concentrations (%) to maximize**
25 **phytochemical constituents of breadsticks**

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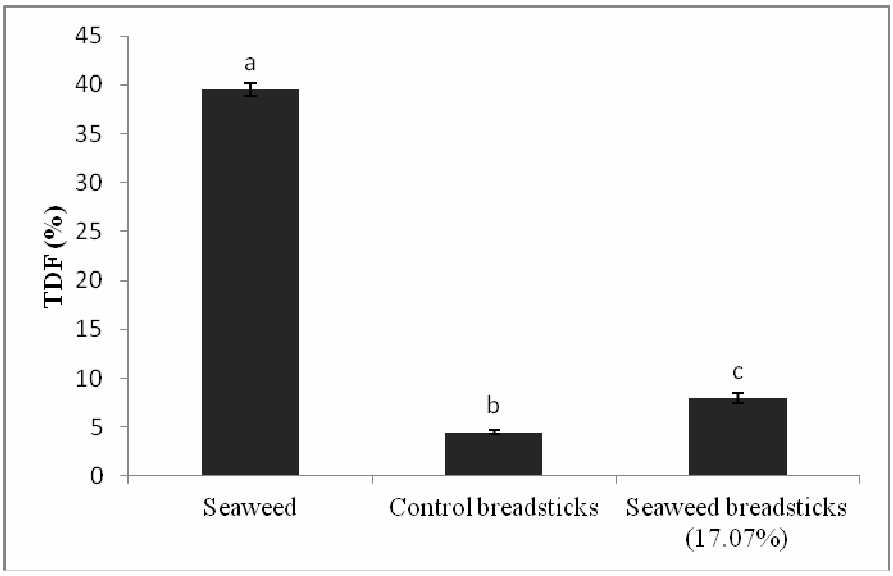

Fig. 3. Total dietary fiber content of seaweed, control and seaweed breadsticks

Each value is presented as mean ± SD (n = 3).
Means above each bar with different letters (a-c) differ significantly (*P* < 0.05).

Table 6. Mean scores for aroma, appearance, texture and taste of the control and seaweed breadsticks

	Sensory attributes				
Breadsticks	**Aroma**	**Appearance**	**Texture**	**Taste**	**Overall acceptability**
Control	4.35±0.81a	4.40±0.50a	3.95±0.75a	3.8±0.61a	3.75±0.71a
10% seaweed	3.80±0.61b	3.30±0.92b	3.40±0.94b	3.50±0.68b	3.55±0.68b
17.07% seaweed	3.25±1.06c	3.30±0.92c	3.55±0.94c	2.75±0.85c	2.80±0.76c

Each value is presented as mean ± SD (n = 20).
Means within each column with different letters differ significantly (*P* < 0.05).