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

4

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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

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

51

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

53

54 **1. Introduction**

55 Marine food, due to its phenomenal biodiversity is a treasure house
56 of many novel healthy food ingredients and biologically active
57 compounds such as those found in seaweeds. Despite having so
58 many health benefits, marine functional foods have been
59 underexploited for food purposes. Bakery products are widely
60 consumed throughout the world and are the best sources of
61 incorporating marine functional ingredients and reaching the targeted
62 population (Kadam and Prabhasankar, 2010). Bread is an excellent
63 product in which incorporation of 'nutraceuticals' is attempted. One of
64 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

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

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

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

113

114 **2. Materials and methods**

115 **2.1 Chemicals**

116 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol
117 reagent, gallic acid, sodium carbonate (Na_2CO_3) and total dietary
118 fiber kit were purchased from Sigma Aldrich Chemie (Steinheim,
119 Germany).

120

121 **2.2 Seaweed material**

122 *H. elongata* was purchased from Quality Sea Veg., Co Donegal,
123 Ireland. The seaweeds were collected in October 2011 and stored at
124 4 °C until further use.

125

126

127

128 **2.3 Preparation of samples**

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

132

133 **2.4 Dehydration procedure**

134 Drying temperature and time was decided based on results of our
135 previous kinetic experiments (Gupta et al., 2011). Seaweed samples
136 (5 g) were placed on a drying tray in a single layer. Drying of
137 seaweed was carried out in a drier (Innova 42, Mason Technology,
138 Ireland) at 40 °C air drying temperature over a period of 24 hours. Air
139 velocity was $2.0 \pm 0.1 \text{ m s}^{-1}$ measured with VWR Enviro-meter digital
140 anemometer (VWR, Ireland). The dried seaweed was then ground
141 into a fine powder using a blender (Rotor, Germany).

142

143 **2.5 Experimental design**

144 To investigate the effect of factors (seaweed and white flour
145 concentration) on phytochemical constituents, color and texture of
146 breadsticks, a central composite design with two factors was utilised.
147 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:

$$165 \quad Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad \text{Eq. 1}$$

166

167 Where; Y is response calculated by the model: β_0 is a constant and
168 β_i , β_{ii} and β_{ij} are linear, squared and interaction coefficients,
169 respectively.

170

171 The adequacy of the model was evaluated by the lack of fit,
172 coefficient of determination (R^2) and the Fisher's test value (F -value)
173 obtained from the analysis of variance (ANOVA) generated by the
174 software. Statistical significance of the model and model parameters
175 were determined at the 5% probability level ($\alpha = 0.05$). Three-
176 dimensional response surface plots and contour plots were
177 generated by keeping one response variable at its optimal level and
178 plotting that against two factors (independent variables).

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

188

189 **2.6 Seaweed breadstick preparation**

190 Seaweed and flour blends were prepared by the replacement method
191 according to the RSM experiment. The percentages of seaweed and
192 white flour from the RSM (Table 2) are based on percentages of
193 overall flour in the mix (flour consisted of 60.79% of the mix), with
194 wholemeal flour comprising the remaining component of the mix. The
195 concentrations of ingredients for each of the experiments can be
196 seen in Table 3. Firstly, the yeast was dissolved in the water and
197 added to the dry ingredients (except seaweed). The ingredients were
198 mixed at slow speed for 2 min, then at medium speed for 4 min
199 (Hobard A120 mixer, Hobard MFG Co. Ltd, London, UK). Seaweed
200 was then added and mixed again for a further 2 min. The dough was
201 placed on trays and left to develop for 45 min then moulded into
202 breadstick shapes by hand and proofed in a dough proofer (Sveba
203 Dahlen, Sveba Dahlen, Fristan, Sweden) at 33 °C, 78% RH for 40
204 min. The breadsticks were then baked in an oven (Sveba Dahlen, DC
205 44, Sveba Dahlen, Fristan, Sweden) at 210 °C for 20 min with 10
206 seconds of steam at the beginning.

207

208

209

210 **2.7 Extraction of phytochemicals**

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

215

216 **2.8 Total phenolic content**

217 The total phenolic concentration was measured using the Folin-
218 Ciocalteu method as outlined by Cox et al. (2012). The total
219 phenolic contents were expressed as mg gallic acid equivalent per
220 100 gram dry basis (db) (mg GAE/100 g db).

221

222 **2.9 DPPH radical scavenging activity**

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

226

227

228

229 **2.10 Texture evaluation**

230 Shear tests were performed using an Instron Universal Testing
231 Machine (Model 4301, Canton MA, USA) supported with Bluehill 2
232 version 2.14 analysis software for materials testing. A Warner
233 Bratzler cutter was used in the shear tests. An aluminium plate with
234 dimensions of 10 x 6 cm², thickness of 1.3 cm and with an opening of
235 3 mm in the centre was supported in the Instron base. Breadstick
236 samples (5 g) were sheared at a speed of 200 mm/min. The cutting
237 implement was allowed to travel the depth of the seaweed, cutting
238 through the sample and seaweed hardness was defined as the peak
239 of force-deformation curve recorded in Newtons per mm (N/mm). Ten
240 replications of each sample were carried out.

241

242 **2.11 Color measurement**

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

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

251

$$252 \quad \Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad \text{Eq. 2}$$

253

254 Where; L^*_0 , a^*_0 and b^*_0 are the readings at time zero and L^* , a^* and
255 b^* are the individual readings at each drying time.

256

257 **2.12 Total Dietary Fiber**

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

268 and the other was incubated at 525 °C to determine ash. The TDF
269 was determined as the weight of the filtered and dried residue less
270 the weight of the protein and ash.

271

272 **2.13 Sensory characteristics**

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

285

286

287

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 °C 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

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

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

325 The models for each of the responses were analyzed separately
326 before overall optimum seaweed and flour concentrations for the
327 breadstick recipe were determined. Predicted and experimental
328 values for each of the responses are presented in Table 5 and were
329 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. 1 a-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:

$$\begin{aligned} 337 \quad Z = & 3.77979 + 5.72532 * X_1 + 0.305353 * X_2 + 0.140273 * X_1^2 - 0.0129 * \\ 338 \quad & X_1 * X_2 - 0.00315601 * X_2^2 \end{aligned} \quad \text{Eq. 3}$$

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 \times seaweed concentration), $X_1 * X_2$ (seaweed
356 concentration \times white flour concentration) and $X_2 * X_2$ (white flour
357 concentration \times white flour concentration) were insignificant model
358 terms with P -values > 0.05 .

359 The polynomial 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

371 products which were incorporated with seaweed. Prabhasankar et al.
372 (2009a) studied the influence of adding brown seaweed, *Sargassum*
373 *marginatum*, to pasta. The TPC in cooked pasta increased from 9 to
374 13 mg GAE/100 g with 5% addition of the brown seaweed. Although
375 the previous study showed that phenolics leached into processing
376 water, these results are still significantly lower than those of the
377 present study. Comparing with the same seaweed concentration, the
378 results of 5% incorporation of seaweed in breadsticks increased the
379 TPC from 27.67 to 38.99 mg GAE/100 g db which is also higher than
380 that of Prabhasankar et al. (2009a).

381 The breadsticks containing maximum *H. elongata* concentration
382 (17.07%) showed an increase in the TPC from 27.67 to 145.88 mg
383 GAE/100 g db which is an increase of 81.03%, as compared to the
384 control. Prabhasankar et al. (2009b) also reported that an addition of
385 30% *Undaria pinnatifida* seaweed increased the TPC of pasta from 9
386 - 27 mg GAE/100 g. Again, this is considerably less than obtained in
387 the present study. TPC of bread samples with different percentages
388 of ginger powder were studied by Balestra et al. (2011). TPC levels
389 increased from 14.30 to 48.50 GAE/100 g db with 6% addition of
390 ginger powder. This clearly shows that the seaweed breadsticks had
391 higher levels of total phenols compared to that of other nutraceutical
392 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 $Z = 13.2787 + 4.76275 * X_1 + 0.92469 * X_2 - 0.1438 * X_1^2 + 0.0087 * X_1 * X_2 - 0.0242 * X_2^2$
399 **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 \times seaweed concentration), $X_1 * X_2$ (seaweed
405 concentration \times white flour concentration) and $X_2 * X_2$ (white flour
406 concentration \times 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:

$$452 \quad Z = 69.7308 - 0.0399788 * X_1 - 0.122297 * X_2 + 0.141849 * X_1^2 -$$
$$453 \quad 0.0002 * X_1 * X_2 + 0.0019626 * X_2^2 \qquad \qquad \qquad \text{Eq. 5}$$

454

455 There was a significant ($P < 0.05$) influence of seaweed
456 concentration, X_1 , and the quadratic terms X_1^2 (seaweed
457 concentration \times seaweed concentration) on the model (Table 3).
458 However, there was no significant influence of white flour
459 concentration (X_2) or the quadratic term X_2^2 (seaweed
460 concentration \times seaweed concentration) or interaction term $X_1^2 X_2$
461 (seaweed concentration \times white flour concentration) on the model.
462 The fit of the model was confirmed by a satisfactory R^2 value of
463 0.9981 which is very high. The response surface plot (Fig. 1c)
464 showed that the texture became harder with increasing seaweed
465 concentration, but there were no major changes in hardness with
466 increasing white flour concentration which was expected.

467 The hardness of the control breadsticks was calculated as 74.38
468 N/mm using an Instron texture analyser, and fortification of flour with
469 seaweed at all levels (2.93 to 17.07%) significantly increased the
470 hardness of the breadsticks ($P < 0.05$). Hardness was maximized in
471 the present study, when flour was replaced with 17.07% seaweed
472 (108.84 N/mm). Prabhasankar et al. (2009a and 2009b) also found
473 that adding seaweed to pasta (1 - 5%) increased the firmness of the
474 product. Chang and Wu (2008) added 4 - 8% green seaweed to
475 noodles and also found that there was an increase in the hardness
476 with increasing seaweed concentration.

477 **3.5 Effects of process variables on the color**

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

490 $Z = -0.562436 + 2.64694 * X_1 + 0.499152 * X_2 - 0.159474 * X_1^2 +$
491 $0.03885 * X_1 * X_2 - 0.0233189 * X_2^2$ **Eq. 6**

492

493 Color analysis of the breadsticks indicated that the linear factor of
494 seaweed concentration (X_1) had an insignificant effect on the color of
495 the breadsticks ($P > 0.05$) however the quadratic factors of seaweed
496 concentration ($X_1 * X_1$) were significant ($P < 0.05$). X_2 (white flour
497 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_2^2 (white flour concentration \times white flour concentration) or
500 interaction term $X_1 \times X_2$ (seaweed concentration \times 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.

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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

541 scavenging activity. A validation experiment was carried out by
542 preparing breadsticks with the optimized dried seaweed and white
543 flour concentrations. The phytochemical constituent contents were
544 138.25 mg GAE/100 g db for TPC and 65.01% for DPPH radical
545 scavenging activity.

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547 **8.3.7 Total dietary fiber**

548 In view of the therapeutic potential of dietary fiber, more fiber
549 incorporated food products are being developed. Fig. 3 shows the
550 total dietary fiber (TDF) content of the breadsticks. Dried seaweed
551 contained 39.56% TDF, control breadsticks had 4.65% TDF and the
552 seaweed breadsticks as optimized using RSM (17.07% seaweed
553 added) contained 7.95% TDF which represents a 43.65% increase in
554 the total dietary fiber when compared to breadsticks with no added
555 seaweeds. Addition of seaweed significantly increased the TDF of
556 the breadsticks as compared to the control ($P > 0.05$). These results
557 are higher than those reported in the literature for final products
558 containing seaweed. Prabhasankar et al. (2008) developed a
559 seaweed pasta which had 4% fiber, but the amount of seaweed
560 added was considerably less (2.5%). Cofrades et al. (2008) found
561 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 than to the products in the other studies outlined in
571 literature.

572

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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604 **4. Conclusion**

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

621

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

Independent variables	Symb ol	-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

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

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754 **Table 2. Design matrix and variable combinations in**
 755 **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

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

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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				
	<i>F</i> -Ratio	<i>P</i> -value	<i>F</i> -Ratio	<i>P</i> -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)

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

Experiment No.	Total phenolic content (mg GAE/100g db)		DPPH (%)		Texture (N/mm)		Color (ΔE)	
	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

3 **Values are presented as mean (n = 6).**

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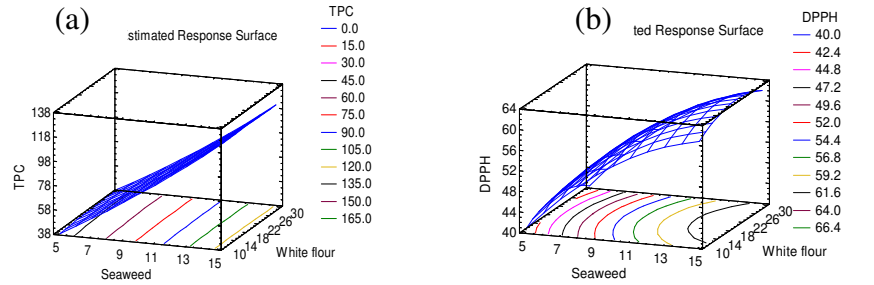
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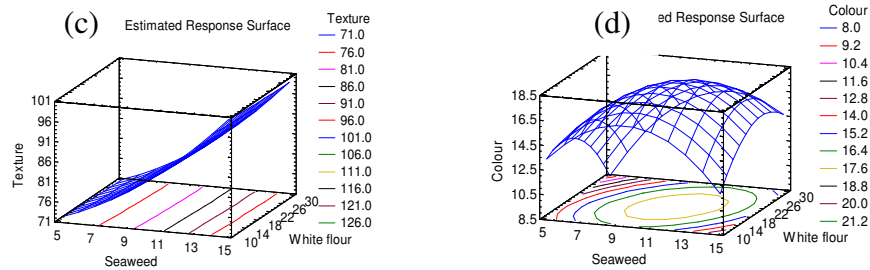
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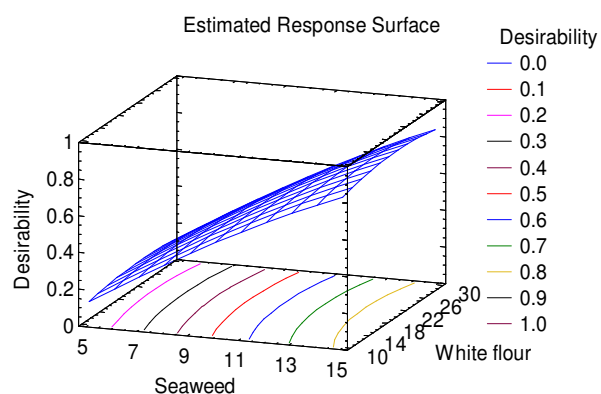
13 **Fig. 1. Response surface plots showing effects of seaweed and**
14 **white flour concentrations (%) on (a) the total phenolic content**
15 **(GAE/100 g db), (b) DPPH radical scavenging activity (%), (c)**
16 **texture (N/mm) and (d) color (ΔE) of seaweed breadsticks**

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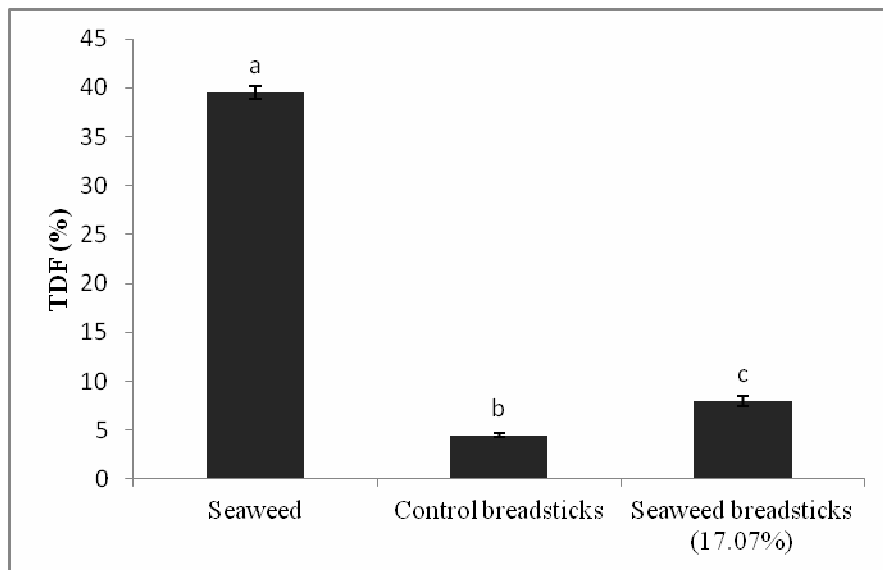
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35 **Fig. 3. Total dietary fiber content of seaweed, control and**
 36 **seaweed breadsticks**

37 **Each value is presented as mean \pm SD (n = 3).**

38 **Means above each bar with different letters (a-c) differ**
 39 **significantly ($P < 0.05$).**

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49 **Table 6. Mean scores for aroma, appearance, texture and taste**
 50 **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

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

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