

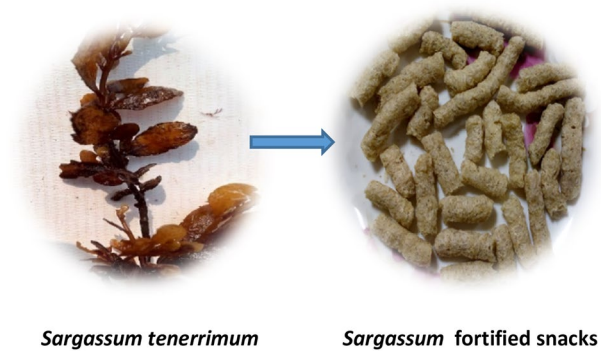
Fortification of Extruded Product with Brown Seaweed (*Sargassum tenerrimum*) and Its Process Optimization by Response Surface Methodology

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Abstract The aim of the present study was to develop an extruded product fortified with seaweed (*Sargassum tenerrimum*) using a co-rotating twin screw extruder and to optimize the process parameters using response surface methodology. The Box–Behnken design was employed with three independent variables i.e. feed moisture (X_1), *Sargassum* powder level (X_2) and barrel temperature (X_3) with three levels for designing the experiment. The expansion ratio and porosity were considered as response variables. Feed moisture had the most significant ($p < 0.05$) linear effect on both the response variables whereas *Sargassum* level and barrel temperature shown significant ($p < 0.05$) quadratic effect. The optimum level of independent variables i.e. feed moisture, *Sargassum* level and barrel temperature for maximum expansion ratio were 16.45%, 4.33% and 123.08 °C respectively whereas for maximum porosity were 16.06%, 4.51% and 124.04 °C respectively. The present investigation can be used to develop extruded products fortified with seaweeds as functional food using the optimized process condition as a means to increase the nutritional quality of the traditional extruded snacks which is considered as a junk food.

Graphical Abstract



Keywords Extruded product · *Sargassum tenerrimum* · Response surface methodology · Box–Behnken design · Expansion ratio · Porosity

Introduction

Seaweed refers to any large marine benthic algae that are multicellular, macrothallic and thus differentiated from most algae that are of microscopic size. It forms one of the most commercially important marine living renewable resources. It has been used as a staple diet from the immemorial time as they are nutritionally rich materials [1]. Edible seaweeds are rich in bioactive antioxidants, soluble dietary fibres, proteins, minerals, vitamins, phytochemicals and polyunsaturated fatty acids. Protein content of seaweed dry matter varies from 5 to 10% (brown algae) to 30–40% (red algae) where as it has low-fat content, ranging from 1 to 5% on dry weight basis [2]. However, seaweeds fats are reported to have a higher proportion of essential fatty acids compared to terrestrial plants.

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These are an excellent source of minerals which accounts upto 36% of its dry weight. It has also high fibre content, making upto 3–50% of dry weight. Seaweeds have been reported to have therapeutic properties for health and disease management such as antioxidant, anticoagulant, antihypertensive, antiobesity, anticancer, antidiabetic, antihyperlipidemic, anti-inflammatory immunomodulatory, anti-estrogenic, thyroid stimulating, neuro protective, antiviral, antifungal, antibacterial and tissue healing properties in-vivo condition [3, 4] attributed the antioxidant capacity mainly to phenolic compounds present in the seaweeds.

Extrusion is a method of continuous food processing in which raw food materials are thermo-mechanically cooked in a screw barrel assembly by a combination of moisture, pressure and temperature in order to be mechanically sheared and shaped [5]. Extrusion can be categorised as a high-temperature short time (HTST) processing. It is one of the most ideal food processing technologies which have been used since the mid-1930s for the production of different kind of products like breakfast cereals, ready-to-eat snack foods, other textured foods etc. [6]. Starch is the main constituent of the extruded products and responsible for most of their structural attributes. These products are rich in energy but are nutritionally poor and give a possibility to add some beneficial nutrients to them [7]. Therefore, incorporation of seaweed to the extruded products will give a great opportunity to fortify nutrient level of the extruded product. This promotes the consumption of seaweed as food. But very less information on the incorporation of seaweeds in extruded products is available. The results of this study will give benefits to the consumers who are looking for more functional and nutritional extruded products. The objective of present study was to develop an extruded product fortified with *Sargassum tenerrimum* as a functional food and to optimize the level of fortification by using RSM.

Materials and Methods

Raw Material

The seaweed (*S. tenerrimum*) was collected from Punta Mandavi beach, Ratnagiri district of Maharashtra, India. The seaweed was manually sorted, washed well with potable water and dried using mechanical drier until the moisture content reached below 10%. The dried seaweed is then pulverised using lab model pulverizer and sieved with 1 mm mesh to obtain fine powder. The commercially available corn flour and rice flour were used as base ingredients for the production of extruded product.

Preparation of Extruded Product Fortified with *Sargassum*

A blend of corn flour and rice flour in the ratio of 70:30 based on preliminary experiments was fortified with different levels i.e. 2.5, 5, 7.5 and 10% of *Sargassum* for selecting the levels for optimization. Extrusion process was carried out by using twin screw co-rotating extruder (Basic Technology Pvt Ltd, Kolkata, India) at a screw speed of 340 rpm, 115 °C barrel temperature, 105 °C die temperature and different feed moisture levels i.e. 13.5, 15, 17.5 and 20%. The feeding rate of the extruder was kept at 119 g/minute. An extrusion die with 5 mm diameter was used to produce extruded products.

Proximate Composition

The proximate composition was determined using the standard methods as described in AOAC [8]. Total nitrogen content was analysed using the Kjeldahl method with the help of Pelican, Kelplus-KES12L VAI/classic DX VATS apparatus. Crude protein was calculated by multiplying the nitrogen value with a conversion factor (6.25). Fat content was determined using Soxhlet method and ash content was determined by weight loss after 5 h of burning at temperature 600 °C in a microwave furnace (Phoenix, SEM, USA). Total carbohydrate content was estimated using Anthrone reagent method [9].

Total Phenolic Content (TPC)

Total phenolic content was determined by Folin–Ciocalteu method [10]. The standard curve was produced using Gallic acid as standard and the total phenol content was expressed in mg of Gallic acid equivalents (GAE/g).

Fatty Acid Profile

The extraction of fat from *Sargassum* was done using the method as described by the Folch [11] and preparation of fatty acid methyl esters by the AOAC method [12]. Fatty acids were determined by using GC-MS (QP2010, Shimadzu, U.S.A.) which was equipped with DB Wax (30 m × 0.25 mm internal diameter × 0.25 µm film thickness) capillary column (Cromlab S. A.). The temperature of injector and detector was set at 250 °C. The initial column temperature was maintained for 2 min at 50 °C. The injection was performed with an injection volume of 1 µL. Helium gas was used as carrier gas.

Physical and Textural Properties of Extruded Products

The sectional expansion was measured as the ratio of the diameter of extruded product to the die diameter [13]. The sectional expansion was used to express the expansion ratio of extruded product while the porosity was measured using the method as described by Pandey and Rizvi [14].

The textural characteristics of extruded products were measured using a texture analyser (Perten, TVT-300XP(H), Perten Instruments AB, Sweden) using three point bend rig with trigger force 50 mN. The initial speed and test speed were set at 1 and 3 mm/s respectively. The sample height and starting distance from the sample were set at 15 and 5 mm respectively. The peak force and the slope (N/mm) of the force-time and force-distance curve were measured and evaluated as hardness and crispness, respectively [15].

Colour and Sensory Characteristics of Extruded Products

The colour of the extruded products was measured using colourimeter (Labscan XE, Hunter colourlab) and the values were calculated using Easy Match QC (software inbuilt in the colourimeter). The sensory evaluation was conducted on randomly selected extrudate samples immediately after removing from the pouches. The sensory panel consists of 15 trained panellists. Parameters like puffing, colour, seaweediness, crispness, surface, taste and overall acceptability were recorded on a hedonic scale of 1–9. The mean panel score for the sample was calculated. A score of 4 was taken as border line of acceptability.

Experimental Design

The software Unscrambler (CAMO Software AS, Version 9.7, Norway) was used for experimental design, data analysis and model building following by RSM. The Box–Behnken design with three independent variables was employed to optimize the processing parameters in this study. The three independent variables used in this study were feed moisture (X_1), *Sargassum* level (X_2) and barrel temperature (X_3) with three levels. The values were based on the trials performed and sensory evaluation by expert panellists. The Box–Behnken experimental design consists of 15 run with three central points. The experimental data from different treatments was analysed using multiple regression analysis. Therefore, fitting was done to a second order model for the selected responses i.e. expansion ratio and porosity. This model can be expressed with the coded variables (X_1 , X_2 , and X_3) with the following equation:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_1^2X_1^2 + B_2^2X_2^2 + B_3^2X_3^2 + e \quad (1)$$

In this equation, Y represents the dependent variable (the estimated response) and X_i ($i=1-3$) represent the independent variables. Coefficients of the polynomial were represented by B_0 (constant term), B_1 , B_2 , and B_3 (linear coefficients), B_1^2 , B_2^2 , and B_3^2 (quadratic coefficients) B_{12} , B_{13} , and B_{23} (interactive coefficients) and e represented the random error.

Statistical Analysis

The experimental data from different treatments as previously mentioned in Table 7 was analyzed using multiple regression analysis using the Unscrambler software (CAMO Software AS, Version 9.7, Norway) and the data obtained from sampling were analyzed by Statistical Package for Social science (SPSS) software version 19. It was performed to examine the differences in the parameters analysed.

Results and Discussion

Proximate Composition

In the present study, the proximate composition of *S. tenerrimum* was found to have $11.78 \pm 0.68\%$ protein, $1.57 \pm 0.12\%$ fat, $30.58 \pm 0.71\%$ ash and 56.4 ± 1.71 carbohydrates (see Table 1). The crude protein content in the present study was in the range reported for brown seaweeds [16]. In an another study it was reported that the protein content of brown seaweeds was low i.e. 3–15% of the dry weight and also total carbohydrate and fat content of *S. variegatum* was 36 and 1% respectively [17]. The chemical composition of *S. naozhouense* was constituted

Table 1 Proximate composition of dried *Sargassum tenerrimum* powder

Parameter	Value (g/100 g sample)
Crude protein	11.78 ± 0.68
Ash	30.58 ± 0.71
Crude fat	1.57 ± 0.12
Carbohydrate	56.40 ± 1.71

Values expressed as mean \pm SD (n=3)

Table 2 Fatty acid composition of *Sargassum tenerrimum*

Sl. no.	Retention time	Short illustration	Fatty acid	Area (%)
1	14.945	C12:0	Lauric acid	0.10
2	16.050	C13:0	Tridecanoic acid	0.06
3	16.330	C14:0	Myristic acid	15.23
4	17.403	C15:0	Pentadecylic acid	2.79
5	18.312	C16:0	Palmitic acid	64.97
6	21.257	C17:0	Heptadecanoic acid	2.07
7	23.120	C18:0	Stearic acid	1.75
8	29.333	C20:0	Arachidic acid	0.13
9	32.811	C22:0	Behenic acid	0.68
10	36.813	C24:0	Lignoceric acid	0.39
11	23.699	C18:1(n-9)	Oleic acid	7.93
12	23.721	C18:1(n-7)	Cis-vaccenic acid	3.21
13	33.052	C22:1(n-9)	Erucic acid	0.15
14	29.509	C18:2(n-6)	Linoleic acid	0.22
15	31.434	C20:4(n-6)	Arachidonic acid	0.32

Total saturated fatty acid (Sl. no. 1–10)=88.17%; Total MUFA (Sl. no. 11–13)=11.29%; Total PUFA (Sl. no. 14–15)=0.54%

of 35.18% ash, 11.20% protein and 1.06% lipid as reported by Peng et al. [18]. Although the fat content was low, it contained polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) about 0.54 and 11.29% respectively (Table 2). Therefore, it can be considered as a good quality fat and higher protein content in *S. tenerrimum*, which can be categorised as a good source of protein for human consumption.

Fatty Acids Content

The fatty acids composition of dried *S. tenerrimum* was obtained as presented in Table 2. The fatty acids profile revealed 88.17% saturated fat and 11.83% unsaturated fat including MUFA and PUFA. Palmitic acid (64.97%) was the principal fatty acid among the saturated fatty acids followed by Myristic acid (15.23%). Tridecanoic acid (0.06%) was found to be the least among the saturated fatty acids. The similar fatty acid composition was reported by Peng et al. [18] for *S. naozhouense* in which saturated fatty acids, C14:0 and C16:0 were most abundant whereas C18:1 and C20:4 were dominant in MUFA and PUFA respectively. The studies related to fatty acids composition of four different of *Sargassum* i.e. *S. thumbergii*, *S. muticum*, *S. fusiforme* and *Sargassum* sp.

indicated that Palmitic acid C16:0 was the major fatty acid in all seaweed which accounts 20.60–36.66 of all fatty acids content and C18:1(n-9) among MUFA [4].

Total Phenolic Content (TPC)

The plant polyphenols and other food phenolics have beneficial effects on human health and disease. They have Cardio-Protective, Anti-Cancer, Anti-Diabetic and antioxidants properties [19]. In seaweeds, the majority of antioxidant capacity is attributed to their polyphenol content. Therefore, the TPC assay can be used as a method for assessment of antioxidant activity of seaweeds.

In the present study, the phenolic content of *S. tenerrimum* was found to be 3.53 ± 0.26 mg GAE/g of dry seaweed. The present result was significantly different from previous reports [20] where, it was 16.24 mg tannin/g of dry weight and the value of total phenolic content varies with a change in the amount of dry seaweed. Sadasivan and Manickan [21] also reported that the total phenolic content was 2.54 mg/g of dry weight in the same seaweed. The difference in the total phenolic content may be related to the seasonal and geographical variation of seaweed [22, 23]. This was not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations [24].

Physical and Textural Properties of Extruded Products

Physical and textural properties of an extruded product are dependent on the process conditions like extruder type, feed moisture, temperature, screw speed, feed rate and its ingredients [25]. Different studies have reported that the product characteristics such as crispness, puffiness, colour, texture, appearance etc. have an important bearing on the acceptability of the final product [25, 26]. The effects of fortification of *Sargassum* on different physical and textural parameters of extruded products in the present study were presented in Table 3. The study has shown that with the increased *Sargassum* level, expansion ratio and porosity decreased significantly ($p < 0.05$). Similarly, expansion ratio and porosity decreased with increased in feed moisture level ($p < 0.05$). Bisharat et al. [27] found that expansion ratio and porosity of the extruded product decreased with increasing initial moisture content and olive paste or broccoli concentration. This lower expansion ratio may be due to the presence of non-plasticizing substances, such as fibres that can cause rupture of cell walls and prevent air bubbles to expand upto the maximum level [28, 29]. The expansion also decreased with increase in protein or feed moisture content [30]. In the present study, the highest expansion ratio and porosity values were found at 13.5% moisture and 2.5% *Sargassum* i.e. 2.70 ± 0.09 and

Table 3 Effect of *Sargassum tenerrimum* incorporation on different parameters of extrudates

Sample (moisture/ <i>Sargassum</i>)	Expansion ratio	Porosity	Hardness (N)	Crispness (mm/N)
13.5%, 2.5%	2.70 ± 0.09 ^e	0.61 ± 0.06 ^e	2.02 ± 1.09 ^a	1.04 ± 0.61 ^e
13.5%, 5%	2.60 ± 0.11 ^{b,e}	0.54 ± 0.04 ^{c,d,e}	3.35 ± 0.43 ^{a,b}	0.56 ± 0.26 ^d
13.5%, 7.5%	2.49 ± 0.13 ^{c,d}	0.53 ± 0.03 ^{b,c,d}	5.94 ± 1.93 ^{b,c}	0.22 ± 0.07 ^{a,b,c}
15%, 2.5%	2.94 ± 0.11 ^g	0.57 ± 0.06 ^{d,e}	4.4 ± 1.90 ^{a,b,c}	0.44 ± 0.29 ^{c,d}
15%, 5%	2.83 ± 0.06 ^f	0.58 ± 0.06 ^{d,e}	7.01 ± 1.59 ^{c,d}	0.31 ± 0.04 ^{a,b,c,d}
15%, 7.5%	2.65 ± 0.14 ^e	0.52 ± 0.02 ^{b,c,d}	11.20 ± 1.09 ^e	0.189 ± 0.04 ^{a,b,c}
17.5%, 2.5%	2.95 ± 0.14 ^g	0.55 ± 0.03 ^{c,d,e}	4.91 ± 1.21 ^{b,c}	0.27 ± 0.05 ^{a,b,c,d}
17.5%, 5%	2.51 ± 0.11 ^{c,d}	0.55 ± 0.03 ^{c,d,e}	8.69 ± 3.68 ^{d,e}	0.37 ± 0.24 ^{b,c,d}
17.5%, 7.5%	2.47 ± 0.18 ^c	0.47 ± 0.03 ^b	18.18 ± 2.15 ^f	0.12 ± 0.06 ^{a,b}
20%, 2.5%	2.50 ± 0.08 ^{c,d}	0.54 ± 0.04 ^{c,d,e}	16.42 ± 1.32 ^f	0.22 ± 0.03 ^{a,b,c}
20%, 5%	2.54 ± 0.14 ^{c,d}	0.48 ± 0.02 ^{b,c}	18.81 ± 1.80 ^f	0.04 ± 0.01 ^a
20%, 7.5%	2.12 ± 0.11 ^b	0.36 ± 0.02 ^a	27.59 ± 2.78 ^g	0.03 ± 0.01 ^a
20%, 10%	1.84 ± 0.08 ^a	0.30 ± 0.01 ^a	42.97 ± 2.85 ^h	0.02 ± 0.01 ^a

Results were given as means ± standard deviation (n = 10 for expansion ratio, n = 3 for porosity and n = 5 for hardness and crispness); different superscripts in the same column indicate significant differences between samples (p < 0.05). Screw speed 340 rpm, 115 °C barrel temperature and 105 °C die temperature are kept constant

0.61 ± 0.06 respectively at 115 °C barrel temperature. The lowest values of expansion ratio and porosity were at 20% moisture and 10% *Sargassum* i.e. 1.84 ± 0.08 and 0.30 ± 0.01 respectively.

Hardness is expressed as the peak force required breaking the extruded product by a probe. It has an inverse relation with the crispness. In the present study, it was revealed that with an increase in *Sargassum* level and feed moisture level, the hardness of the extruded product increased (p < 0.05) continuously. However, the crispness decreased (p < 0.05) with increased in *Sargassum* level and moisture level. This may be related to the fact that during the extrusion process, fibre reduces the degree of cooking which results to decrease in the expansion ratio. Previous studies also reported that addition of fibre in extruded cereals increases density and hardness, and a decrease in crispness [31, 32]. Studies reported that the hardness of extruded product increases as the feed moisture content increase [33, 34]. The increase in hardness at higher feed moisture may be due to the reduction in expansion caused by the increase in moisture content. The highest value of hardness was 42.97 ± 2.85 N at 20% feed moisture and 10% *Sargassum*. Similarly, the highest value of crispness was 1.04 ± 0.61 mm/N at 13.5% feed moisture and 2.5% *Sargassum*.

Colour Analysis

Colour parameters (L*, a* and b*) of extruded products were shown in Table 4. The L* and b* values gradually decreased (p < 0.05) with increased in *Sargassum* level in extruded products indicating that expanded products

changed from light yellow to dark. The sample with 20% moisture and 10% *Sargassum* was having the lowest value of L*, a* and b* (p < 0.05). There was an inverse relationship between L*, a* or b* with *Sargassum* content in extruded products. These results give an idea of the colour changes with a positive change in *Sargassum* level of the developed extruded products. Similar results were also found in extruded products prepared from *Porphyra columbina* [35].

Sensory Characteristics of Extruded Products

The sensory evaluation of extruded samples developed with different *Sargassum* level and feed moisture levels at 115 °C barrel temperature and 340 rpm screw speed was done for the important parameters i.e. puffing, colour, sea weediness, crispness, surface, taste and overall acceptability by 15 trained panellists (Table 5). During the sensory evaluation, randomized samples were served in blind trials and were evaluated on a continuous intensity scale ranging from 1 (no intensity) to 9 (maximum intensity). All the sensory panellists were trained before sensory evaluation. The Quality Descriptive Analysis (QDA) was used for above purpose [36]. The sensory analysis of the different extruded products revealed that the overall acceptability score decreases with increased in *Sargassum* level (p < 0.05). When the *Sargassum* level was more than 7.5%, the overall acceptability score was below acceptable level. Similarly puffing, crispness and colour were decreasing with increased in *Sargassum* level (p < 0.05). When the moisture level increased, puffing and crispness were

Table 4 Effect of *Sargassum tenerrimum* fortification on colour parameters of extrudates

Sample (moisture/ <i>Sargassum</i>)	L*	a*	b*
13.5%, 2.5%	55.48 ± 5.46 ^{b,c}	0.61 ± 0.30 ^b	17.20 ± 2.37 ^{b,c}
13.5%, 5%	58.06 ± 4.97 ^{c,d}	1.01 ± 0.26 ^{c,d}	16.33 ± 2.35 ^{b,c}
13.5%, 7.5%	57.11 ± 4.32 ^{b,c,d}	1.26 ± 0.35 ^{d,e}	16.78 ± 1.88 ^{b,c}
15%, 2.5%	62.17 ± 4.56 ^d	0.12 ± 0.14 ^a	16.94 ± 2.56 ^{b,c}
15%, 5%	57.99 ± 7.47 ^{c,d}	0.91 ± 0.45 ^c	16.93 ± 2.25 ^{b,c}
15%, 7.5%	57.56 ± 3.07 ^{c,d}	1.57 ± 0.33 ^f	17.13 ± 0.94 ^{b,c}
17.5%, 2.5%	55.40 ± 10.14 ^{b,c}	0.57 ± 0.54 ^b	17.13 ± 1.66 ^{b,c}
17.5%, 5%	55.35 ± 4.47 ^{b,c}	1.16 ± 0.21 ^{c,d,e}	17.13 ± 1.95 ^{b,c}
17.5%, 7.5%	54.05 ± 4.42 ^{b,c}	1.34 ± 0.25 ^{e,f}	16.04 ± 1.21 ^{b,c}
20%, 2.5%	54.15 ± 3.89 ^{b,c}	0.56 ± 0.34 ^b	15.62 ± 2.01 ^b
20%, 5%	51.81 ± 4.77 ^{a,b}	1.59 ± 0.27 ^f	16.65 ± 0.89 ^{b,c}
20%, 7.5%	48.53 ± 3.61 ^a	2.17 ± 0.28 ^g	17.76 ± 1.37 ^c
20%, 10%	48.13 ± 2.07 ^a	2.14 ± 0.18 ^g	13.61 ± 0.75 ^a

Results were given as means ± standard deviation (n=10); different superscripts in the same column indicate significant differences between samples ($p < 0.05$). Screw speed 340 rpm, 115 °C barrel temperature and 105 °C die temperature are kept constant

Table 5 Effect of *Sargassum tenerrimum* fortification on sensory properties of extrudates

Sample (moisture/ <i>Sargassum</i>)	Puffing	Crispiness	Seaweed-iness	Colour	Surface	Taste	Overall acceptability
Control	8.4 ± 0.74 ^d	7.8 ± 1.15 ^d	8.6 ± 0.51 ^f	8.33 ± 0.65 ^g	7.78 ± 0.90 ^f	7.27 ± 1.05 ^d	7.81 ± 0.74 ^e
13.5%, 2.5%	7.87 ± 1.01 ^{c,d}	7.30 ± 0.88 ^d	7.17 ± 0.88 ^{d,e}	7.90 ± 0.85 ^{f,g}	7.67 ± 0.91 ^f	7.13 ± 1.01 ^d	7.66 ± 0.74 ^e
13.5%, 5%	7.20 ± 1.13 ^c	7.00 ± 1.18 ^{c,d}	6.63 ± 1.34 ^{d,e}	6.57 ± 0.92 ^{d,e}	6.50 ± 1.08 ^{c,d,e}	6.40 ± 1.00 ^{c,d}	6.73 ± 1.08 ^d
13.5%, 7.5%	7.23 ± 1.02 ^c	7.13 ± 0.90 ^{c,d}	6.64 ± 0.94 ^{d,e}	6.10 ± 1.39 ^{c,d}	6.23 ± 0.86 ^{c,d,e}	6.57 ± 1.05 ^{c,d}	6.60 ± 1.00 ^d
15%, 2.5%	7.87 ± 1.08 ^{c,d}	7.53 ± 1.14 ^d	7.47 ± 0.83 ^e	7.67 ± 0.84 ^{f,g}	7.43 ± 1.27 ^f	6.87 ± 0.92 ^d	7.63 ± 0.74 ^e
15%, 5%	7.50 ± 0.71 ^c	7.07 ± 0.92 ^{c,d}	6.63 ± 0.97 ^{d,e}	6.57 ± 1.07 ^{d,e}	6.53 ± 1.02 ^{d,e}	6.37 ± 1.11 ^{c,d}	6.70 ± 0.88 ^d
15%, 7.5%	7.00 ± 1.31 ^c	7.03 ± 1.54 ^{c,d}	5.73 ± 1.16 ^{b,c}	6.17 ± 0.75 ^{c,d}	6.00 ± 1.11 ^{c,d}	6.50 ± 1.18 ^{c,d}	6.50 ± 1.02 ^d
17.5%, 2.5%	7.57 ± 0.92 ^{c,d}	6.27 ± 1.27 ^c	7.53 ± 0.64 ^e	7.27 ± 0.88 ^{e,f}	7.03 ± 1.12 ^{e,f}	6.43 ± 1.12 ^{c,d}	6.80 ± 0.92 ^d
17.5%, 5%	6.03 ± 0.97 ^b	5.20 ± 1.28 ^b	6.70 ± 0.84 ^{d,e}	6.27 ± 0.88 ^{c,d}	6.07 ± 1.47 ^{c,d}	5.83 ± 1.46 ^{b,c}	5.83 ± 0.92 ^c
17.5%, 7.5%	5.70 ± 1.32 ^b	5.07 ± 1.35 ^b	6.47 ± 0.77 ^{c,d}	5.57 ± 1.10 ^c	5.60 ± 0.90 ^c	5.27 ± 1.28 ^b	5.50 ± 1.05 ^c
20%, 2.5%	5.77 ± 1.22 ^b	4.43 ± 1.36 ^b	6.83 ± 0.11 ^{d,e}	5.43 ± 1.25 ^c	5.97 ± 1.37 ^{c,d}	5.63 ± 1.37 ^{b,c}	4.67 ± 0.96 ^b
20%, 5%	3.80 ± 0.94 ^a	3.40 ± 1.24 ^a	5.13 ± 0.67 ^b	4.53 ± 1.27 ^b	4.37 ± 1.18 ^b	3.93 ± 1.01 ^a	3.75 ± 0.78 ^a
20%, 7.5%	3.13 ± 1.36 ^a	5.17 ± 1.46 ^b	3.90 ± 1.17 ^a	4.13 ± 1.41 ^b	3.6 ± 0.96 ^{a,b}	3.40 ± 1.06 ^a	3.40 ± 1.06 ^a
20%, 10%	3.00 ± 0.93 ^a	2.93 ± 1.34 ^a	4.93 ± 1.75 ^b	3.27 ± 1.28 ^a	3.27 ± 0.90 ^a	3.33 ± 1.59 ^a	3.13 ± 0.83 ^a

Results were given as means ± standard deviation (n=15); different superscripts in the same column indicate significant differences between samples ($p < 0.05$). Screw speed 340 rpm, 115 °C barrel temperature and 105 °C die temperature are kept constant

decreasing ($p < 0.05$). Overall acceptability was below an acceptable level when the moisture level exceeds 17.5%. Therefore, *Sargassum* level of 2.5–7.5% and feed moisture level of 13.5–17.5% were selected for optimization.

Optimization of Extrusion Process Variables by RSM

RSM is a mathematical modelling technique that establishes regression equation to describe interrelations between input parameters and product properties [37, 38]. It has been successfully demonstrated that RSM can be used in optimizing process variables [39–41]. Several

studies have used RSM for optimization of extrusion process [19, 26, 42, 43].

In the present study, optimization of extrusion was done using Box–Behnken Design of RSM by employing the quadratic model to study the individual, interactive and square effects of the independent variables on the dependent variable (sampling code was given as Table 6). The values of 15 samples for the experiment are presented in Table 7. The obtained data was analysed using multiple regression analysis using the Unscrambler (CAMO Software AS, version 9.7, Norway) to fit a full response surface model for expansion ratio and porosity including all linear (X_1, X_2, X_3), interaction

Table 6 Independent variables and their coded levels

Independent variable	Symbol	Coded levels		
		-1	0	1
Feed moisture (%)	X ₁	13.5	15.5	17.5
<i>Sargassum</i> level (%)	X ₂	2.5	5	7.5
Barrel temperature (°C)	X ₃	115	125	135

Table 7 Box–Behnken experimental design with the observed responses

Runs	Feed moisture (%)	<i>Sargas-sum</i> level (%)	Barrel temperature (°C)	Expansion ratio	Porosity
1	13.5	2.5	125	2.35	0.53
2	17.5	5	125	2.72	0.59
3	13.5	7.5	125	2.58	0.56
4	17.5	7.5	125	2.66	0.57
5	13.5	5	115	2.46	0.54
6	17.5	5	115	2.55	0.55
7	13.5	5	135	2.29	0.51
8	17.5	5	135	2.56	0.55
9	15.5	2.5	115	2.61	0.57
10	15.5	7.5	115	2.40	0.54
11	15.5	2.5	135	2.54	0.56
12	15.5	7.5	135	2.26	0.49
13	15.5	5	125	2.76	0.63
14	15.5	5	125	2.86	0.62
15	15.5	5	125	2.90	0.64

(X₁X₂, X₁X₃, X₂X₃), and quadratic terms (X₁², X₂², X₃²) and the model was developed. All the insignificant terms (P>0.05) were eliminated to develop the fitted response surface model equations. The significance of all terms in the polynomial functions was assessed statistically using F-value at a probability (p) of 0.001, 0.01 and 0.05. R² values were used to judge the adequacy of the models. The ANOVA of the responses were studied for finding the significance of variables and its interaction and square effects. The response

surface model equations for expansion ratio and porosity were revealed as:

$$\text{Expansion ratio} = 2.846 + 0.05065X_1 - 0.08048X_2^2 - 0.144X_3^2$$

$$\text{Porosity} = 0.632 + 0.008542X_1 - 0.01992X - 0.01992X_2 - 0.03373X_3^2$$

The response surface models developed for the degree of expansion ratio and porosity accounted for 86 and 91.9% of observed variations respectively. The models were significant at 95% (p<0.05) level (Table 8). It was found that for both responses, the individual effect of feed moisture was the most influencing factor (p<0.05) and it presented the positive effect. It was revealed that only square effect of temperature showed the significant effect at 95% (p<0.05) level of expansion ratio. In the case of porosity the square effects of all the variables shown the significant effect at 95% (p<0.05) level. All the interactive effects didn't have any significant effects on both the dependent variables.

Response surface plots for expansion ratio and porosity were generated as a function of two independent variables while kept other independent variable at their centre point. Figures 1, 2, 3 and 4 have shown the estimated response function and the effects of the independent variables (X₁, X₂, and X₃) on the dependent variables values. The convex surface plots indicated there was a maximum predicted value of response variables. Both expansion ratio and porosity values were increasing as the temperature and feed moisture (%) increased at a certain level and then decreased further. Same situation happens in all temperature-*Sargassum* level and moisture%-*Sargassum* level combinations. Expansion of extruded products increased with increasing in temperature but at low temperatures, there was a reduction in the expansion because starch was not completely molten [44]. Expansion ratio has proportional relation to the temperature up to a certain value but decreased at a higher temperature because of increasing dextrinization and weakening starch structure.

Table 8 ANOVA for the response surface model determined between response variables and independent variables

Source of variation	Degree of freedom	Sum of squares	Mean squares	F _{statistics}	P value
Expansion ratio					
Model	7	0.459	0.06561	6.124	0.0145
Residual	7	0.075	0.01071		
Total	14	0.534	0.03816		
Porosity					
Model	8	0.02503	0.003129	8.538	0.0087
Residual	6	0.002199	0.0003665		
Total	14	0.02723	0.001945		

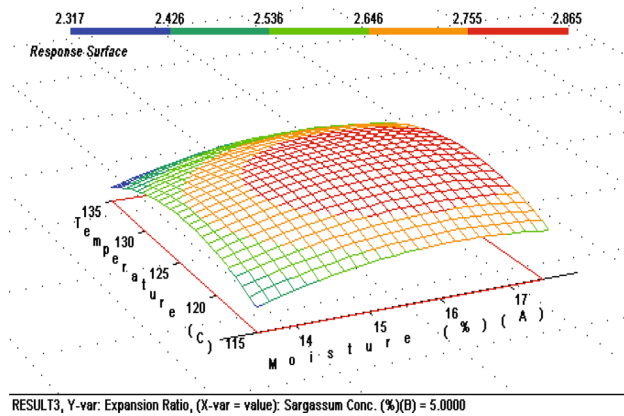


Fig. 1 Response surface plot showing the effect of temperature and moisture on expansion ratio

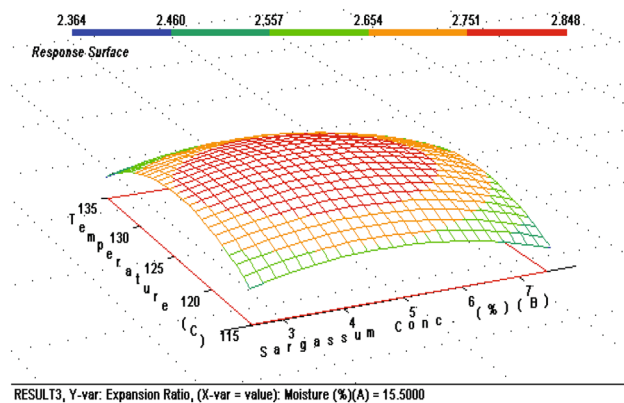


Fig. 2 Response surface plot showing the effect of temperature and *Sargassum* level on expansion ratio

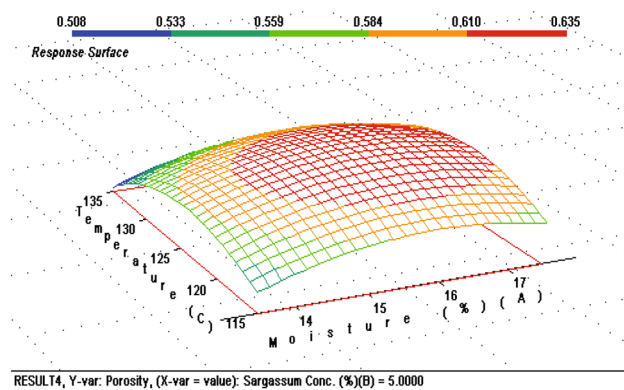


Fig. 3 Response surface plot showing the effect of temperature and moisture on porosity

The predicted maximum response value for expansion ratio and porosity were 2.87 and 0.64 respectively. The levels of independent variables for this maximum expansion ratio and porosity were found to be 16.45% and 16.06%

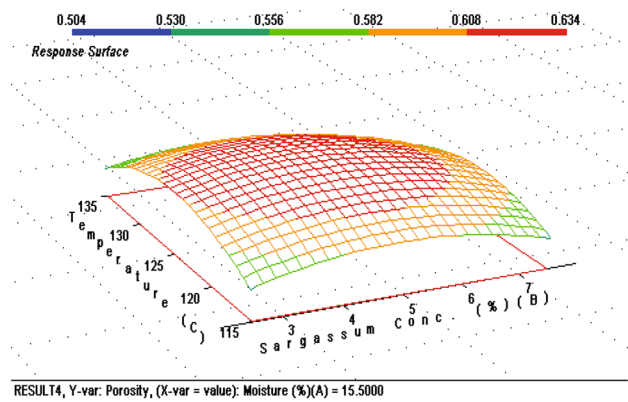


Fig. 4 Response surface plot showing the effect of temperature and *Sargassum* level on porosity

feed moisture, 4.33 and 4.51% *Sargassum* and 123.08 and 124.04 °C barrel temperature respectively.

Conclusion

The cereal based extruded products can be fortified with seaweeds to increase nutritional and functional qualities as seaweeds exemplified therapeutic properties. The present study demonstrates that the cereal based extruded products can be fortified with brown seaweed to increase their nutritional and functional qualities. Further, the seaweed fortified extruded products provide health benefits to the consumers because of the known therapeutic properties of seaweed. Response surface analysis revealed that the feed moisture had the most significant linear effect on expansion ratio and porosity parameters of extruded product whereas *Sargassum* level and temperature shown the significant quadratic effect on all the response variables. The optimum level of independent variables i.e. feed moisture, *Sargassum* level and barrel temperature for maximum expansion ratio were 16.45, 4.33% and 123.08 °C respectively whereas for maximum porosity were 16.06, 4.51% and 124.04 °C respectively.

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