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ORIGINAL PAPER

Optimization of whey protein concentrate and psyllium husk for the development of protein-fiber rich orange fleshed sweet potato (*Ipomoea batatas* L.) bread by using response surface methodology

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Abstract

The present study was concerned with to optimize the formulation of whey protein concentrate (WPC) and psyllium husk for development of protein-fiber rich orange fleshed sweet potato (OFSP) bread using response surface methodology. The variables considered for the study were WPC (03–09%) and psyllium husk (02–06%) while the responses were protein content (%), fiber content (%), overall acceptability, loaf weight (g), loaf volume (cm^3), specific volume (cm^3/g) and oven spring (cm). Bread was prepared using 30% OFSP flour and 70% wheat flour. WPC and psyllium husk were used as source of protein and fiber respectively. The level of alone WPC significantly affects ($p < 0.05$) on protein content whereas psyllium husk on fiber content of OFSP bread. There was significant effect of psyllium husk and non significant effect of WPC on overall acceptability. Moreover, the loaf volume and specific volume of bread was significantly affected by both the factors. The WPC was significantly and psyllium fiber non significantly affects on oven spring of OFSP bread. The optimization was carried out on WPC and psyllium husk in order to know which of the combination will give best protein-fiber content and overall acceptability with quality physical properties. The optimized bread sample was also evaluated for biofunctional (total carotenoids, total phenols, total flavonoids and antioxidant activity) components, and estimated glycemic index (EGI). The optimized OFSP bread containing 09% WPC and 06% psyllium husk was found most acceptable by consumers and it provides 17.72% protein and 8.02% fiber. The estimated glycemic index was found lower 52.58. The total carotenoid, total phenols, total flavonoids and DPPH inhibition of OFSP bread was found as 3.78 (mg/100 g), 51.32 (mg GAE/100 g), 26.80 (mg QE/100 g) and 43.53% respectively. The optimized sample of bread was found superior in carotene, protein and fiber content.

Keywords Bread · Orange fleshed sweet potato · Whey protein concentrate · Psyllium husk · Response surface methodology

Introduction

Bread is generally made from refined wheat flour and consumed widely in developed and developing countries as snack food or breakfast food. Now it's become the part of daily diet with over 9 millon kg of bread produced annually [1]. It is a fermented bakery product involved series of operations such as mixing of ingredients, fermentation, kneading, proofing, shaping and baking at 205 °C for 15–20 min [2]. Consumers are health conscious and demands for healthy and nutritious bread which made from refined wheat flour which is non nutritious, there is need to replace wheat flour with non wheat flour which provides nutritional benefits. Use of non wheat flours to replace wheat flour for preparation of bread also helps to reduce loads on production and cultivation of wheat especially in countries where climate and soil doesn't feasible for wheat production [3, 4]. Indigenous non wheat flours were utilized in replacing portions of wheat flour in bread making [5].

Sweet potato flour is best substitute to wheat flour in context to calorie content and micronutrient content. These are different varieties of sweet potato such as cream fleshed, orange fleshed and purple fleshed. It was reported that,

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orange fleshed sweet potato (OFSP) tubers rich in carotenoids content which resulted to high provitamin A activity, produces more edible energy per hector, per day than wheat and cassava [6]. The beta-carotene content in OFSP makes concentrate source of antioxidant and it was becomes the part of diet particularly pregnant women and children in developing countries to reduce the deficiency of vitamin A [7]. Addition of various proportion of sweet potato flour in wheat flour can increase the nutritive values in terms of fiber and carotenoids. This also helps in lowering the gluten level and prevent from celiac disease [8]. It was reported that, 30% level substitution of OFSP to wheat flour in bread preparation would increased the significant amount of vitamin A and also provide the chance to earn income for rural farmers [9, 10].

The replacement of refined wheat flour with orange fleshed sweet potato flour made the convenient way of fortification of nutrients but it lacks in protein and dietary fiber content. Many researchers developed bread using sweet potato flour but found low in protein [11, 12]. This protein deficiency in product could be fulfilled by adding whey protein concentrate (WPC). It is a dairy based protein rich ingredients widely used in development of functional food to upgrade the protein value, without adding many calories from fat [13]. Fortification of WPC in different food products helps to combat protein deficiency and obesity [14–17]. Moreover, incorporation of psyllium fiber into food increases the accountability of fiber especially soluble (70%) and insoluble (17%) fiber. This fiber derived from the plants of the *Plantago* genus that present different species [18]. Consumption of this fiber enriched food leads to regulation of large bowel function [19], to lower blood cholesterol levels [20], reducing the risk for heart diseases [21] and serum total cholesterol and LDL-cholesterol [20–23].

In the present investigation an attempt was carried out to optimize the level of WPC and psyllium fiber in OFSP bread using response surface methodology (RSM) as consumers are demanding protein and fiber enriched food products with additional health benefits. According to Khan [24] composite flour technology has many advantages among which are; it plays a vital role to complement the deficiency of essential nutrients.

In view of the above literature there is an urgent need to develop protein and fiber enriched orange fleshed sweet potato bread which is convenient and nutritious. Hence in the present investigation, sincere efforts have been made to formulate and evaluate the quality of bread prepared with OFSP, WPC, psyllium fiber and wheat flour increasing their carotene, protein and fiber content in the optimized bread.

RSM is one of the statistical technique which is effectively use for optimizing complex process and successfully applied to determine the optimum formulation [25, 26]. RSM helps to reduce number of unnecessary experiments and gives effective and selective experiments. Therefore the main objective of this study was to optimize the level of WPC and psyllium fiber for preparation of OFSP bread using RSM with the purpose of achieving maximum acceptability of produced bread.

Materials and methods

The study was conducted in the laboratory of the University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra. Raw materials such as wheat flour, yeast, sugar, salt and oil were procured from local market of Aurangabad and required chemicals were used from the laboratory stock. WPC, having a protein content of 70% (Procon 3700) was supplied by Ms. Mahaan Proteins Ltd., Uttar Pradesh, India. Psyllium husk powder, having dietary fiber 85.17% and soluble fiber 60.45% was supplied by Organic India Pvt. Ltd. Lucknow, India. Orange fleshed sweet potato tubers were received from ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala.

Materials

Orange fleshed Sweet potato flour, wheat flour, whey protein concentrate (WPC), psyllium husk, sugar, yeast, calcium propionate, salt, oil and water.

Preparation of orange fleshed sweet potato flour

Orange fleshed sweet potato harvested at three months maturity were washed free of dirt and manually peeled and sliced to round discs of approximately 5 mm thickness. The slices were sundried for 36 h till the moisture content was brought down to $< 10\%$ and powdered in a hammer mill into fine flour and pass through 85 mesh sieve of BSS standard (particle size 0.177 mm). The dry flour was packed in air tight containers and stored at room temperature (30 ± 1 °C) for further use [27].

Design generation and OFSP bread preparation

RSM was used to optimize the level of WPC and psyllium husk for OFSP bread. Wheat flour was substituted by 30% orange fleshed sweet potato flour to produce OFSP bread [9, 10]. After preliminary tests, the upper and lower limits for these variables were established. A central composite design was prepared and the levels of WPC and psyllium husk were considered as 03–09% and 02–06% respectively. Analysis of 13 combinations of these variables were performed. The experiment plans represented in Table 1.

Table 1 Experimental design matrix by central composite design with values of independent and dependent variables of OFSP bread

Run	Coded levels		Actual levels		Responses						
	x_1	x_2	X_1	X_2	Protein content (%)	Fiber content (%)	Overall acceptability	Loaf weight (g)	Loaf volume (cm^3)	Specific volume (cm^3/g)	Oven spring (cm)
1	0	0	6	4	15.23	6.43	8.3	152.2	330	2.17	0.6
2	+10.24	0	10.24	4	18.67	6.46	8	160.3	365	2.28	1.4
3	0	0	6	4	15.24	6.43	8.3	152.2	330	2.17	0.6
4	−1	−1	3	2	11.91	4.05	8	162.2	360	2.22	1.2
5	1	−1	9	2	17.28	4.08	8	164.24	370	2.25	1.3
6	1	1	9	6	17.3	8.1	9.5	165.1	340	2.06	0.8
7	−1.75	0	1.75	4	7.03	6.45	7.5	157.1	275	1.75	0.3
8	−1	1	3	6	11.93	8.07	8.5	163.5	270	1.65	0.6
9	0	−1.17	6	1.17	15.21	3.1	7.5	162.8	375	2.3	0.7
10	0	+6.82	6	6.82	15.24	8.31	9	160.6	280	1.74	0.5
11	0	0	6	4	15.23	6.44	8.3	152.21	335	2.2	0.6
12	0	0	6	4	15.23	6.43	8.3	152.22	330	2.17	0.6
13	0	0	6	4	15.24	6.43	8.3	152.2	330	2.17	0.6

X_1 represents whey protein concentrate (%) and X_2 represents psyllium husk (%)

Table 2 Ingredients required for preparation of protein and fiber enriched orange fleshed sweet potato bread

Ingredients	Quantity (%)
Orange fleshed sweet potato flour	30
Wheat flour	70
Sugar	15
Salt	02
Yeast	05
Oil	02
Calcium propionate	0.2
Water	70–100 ml
Whey protein concentrate (WPC)	Variable[a]
Psyllium husk powder	Variable[a]

[a]Amounts varied according to the experimental design (Table 1)

The responses used for optimization presented in Table 3 were protein content (maximize = 5), fiber content (maximize = 5), overall acceptability (maximize = 5), loaf weight (is in range = 5), loaf volume (is in range = 3), specific volume (is in range = 3) and oven spring (is in range = 3). Models were confirmed by comparing the average response to the prediction interval at 95% confidence level.

Preparation of OFSP bread

Total 13 runs of bread preparation were carried out (Table 1). For each experiments, all ingredients mentioned in Table 2 were weighed and levels of WPC and psyllium husk powder were added as per quantity mentioned in Table 1.

The WPC and psyllium husk powder levels and the amount of each ingredients added per 100 g of OFSP (30 g) and wheat flour (70 g) blend. For the preparation of bread, a straight dough method was used. Preparation method used for bread is presented in Fig. 1. The dry ingredients were placed in spiral mixer and mixed uniformly. Yeast was dissolved in warm water along with sugar under anaerobic condition for 10 min to activate it and mixed into dry ingredients. Finally water and oil were incorporated and mixtures were blended to form dough. It was knead properly and placed in aluminium baking pans for fermentation about 1 h. Baking of each sample was conducted in a laboratory baking oven (IMAYAM Engg. Works, Coimbatore, Model-AGNI E1) at 200 °C for 30 min. Bread loaves were removed and cooled at room temperature and packed in polyethylene bag and stored for further quality analysis.

Physical characteristics

Physical characteristics of bread samples such as loaf weight, loaf volume, specific loaf volume and oven spring were evaluated.

Loaf weight

Loaf weight was measured 30 min after the loaves were removed from the oven using a laboratory scale (CE-410I, Camry Emperors, China) and the readings recorded in grams.

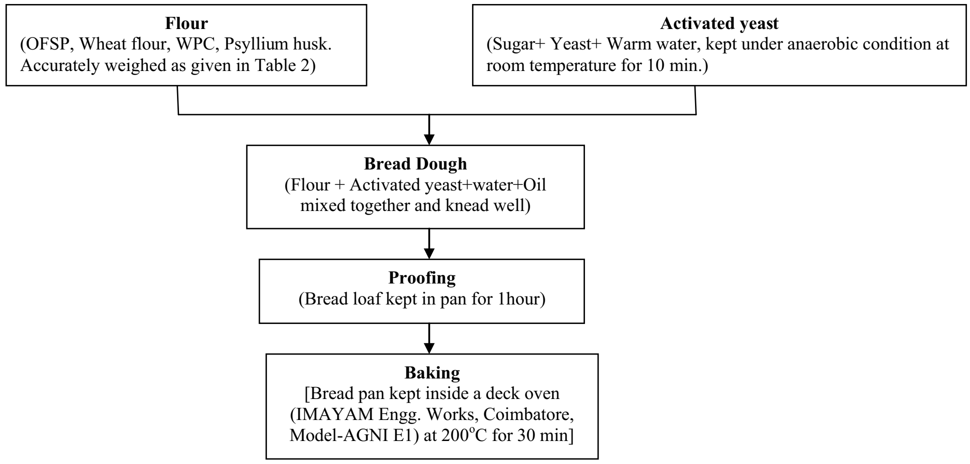

Fig. 1 Preparation process of orange fleshed sweet potato bread [28]

Table 3 Level of responses fixed for optimization of OFSP bread

Name	Goal	Lower limit	Upper limit	Importance
A: WPC	Is in range	3	9	3
B: Psyllium husk	Is in range	2	6	3
Protein content (%)	Maximize	7.03	18.67	5
Fiber content (%)	Maximize	3.1	8.31	5
Overall acceptability	Maximize	7.5	9.5	5
Loaf weight (g)	Is in range	152.2	165.1	5
Loaf volume (cm^3)	Is in range	270	375	3
Specific volume (cm^3/g)	Is in range	1.65	2.3	3
Oven spring (cm)	Is in range	0.3	1.4	3

WPC whey protein concentrate

Loaf volume

Loaf volume was measured using the rapeseed displacement method as modified by Giami et al. [29] as follows: A box of fixed dimensions (23.00 × 14.30 × 17.00 cm) of internal volume 5591.30 cm^3 was put in a tray, half filled with sorghum grains, shaken vigorously 4 times, then filled till slightly overfilled so that overspill fell into the tray. The box was shaken again twice, and then a straight edge was used to press across the top of the box once to give a level surface. The seeds were decanted from the box into a receptacle and weighed. The procedure was repeated three times and the mean value for seed weight was noted (C g).

A weighed loaf was placed in the box and weighed seeds (3500 g) were used to fill the box and leveled off as before. The overspill was weighed and from the weight obtained the weight of seeds around the loaf and volume of seed displaced by the loaf were calculated using the following equations:

$$\text{Seeds displaced by loaf (L)} = \text{C g} + \text{overspill weight} - 3500\,\text{g}.$$

$$\text{Volume of loaf (V)} = \frac{\text{L} \times 5591.30\,\text{cm}^3}{c}.$$

Specific volume

The specific loaf volume was determined by dividing the loaf volume by its corresponding loaf weight (cm^3/g) as described by Araki et al. [30].

Oven spring

Oven spring was estimated from the difference in height of dough before and after baking.

Nutritional composition

The protein and fiber content of bread were determined by standard method of AOAC [31]. The minerals in the sample were quantified by the method of flame photometry for K, P, Ca, Mg, Cu, Zn and Fe were determined by atomic absorption spectrophotometer (Elico, SL 243, Double beam) [32].

Total carotenoids content

Total carotenoids (TC) in sample were determined by taking the 5 g of sample and sample is homogenized using homogenizer with the extraction solvent (hexane:acetone:methanol = 2:1:1). Centrifugation step was repeated until the supernatant became colorless at 10,000 rpm for 10 min and collected the supernatant. Supernatant was pooled together and transferred them into a separating funnel. Carotene is extracted the using the solvent Hexane. The absorbance was measured at 450 nm with hexane as blank. Scanned the absorbance between 200–700 nm and also read the peak absorbance at 450 nm. The total carotenoids calculated using the following equation

$$\text{Total carotenoids (mg/100 g)} = \frac{A_{450} \times 0.386 \times \text{made up final volume in 'ml'}}{\text{Fresh weight of the sample taken in 'g'}}.$$

Antioxidant activity

Determination of antioxidant activity of sample was done by 2,2-diphenyl-2-picryl-hydrazyl (DPPH) inhibition method. Sample (1 g) was taken in 10 ml ethanol and was kept overnight for extraction. This eluted extract was taken (0.2 ml) and to it 1 ml of DPPH solution (80 µg/ml ethanol) was added. The sample sets were centrifuged at 3000 rpm for 15 min (Sigma laboratory centrifuge 3K 18, Germany). In cuvette, 0.5 ml of centrifuged solution was taken and to it 1 ml of ethanol was added. Absorbance was taken at 517 nm separately for blank and samples with pure ethanol as reference using Perkin Elmer UV/VIS spectrometer Lambda 25, Germany.

$$\%\ \text{DPPH inhibition} = \left(A_B - A_S/A_B\right) \times 100$$

where A_B = OD for blank; A_S = OD for sample.

Total phenols

The total phenolic content was determined using Folin–Ciocalteu (FC) reagent, as given by Singleton and Rossi [33] with some modifications. One gram of sample was kept overnight for extraction with 10 ml of 50% aqueous methanol. The mixture was centrifuged at 10,000 rpm for 15 min. 0.5 ml of centrifuged supernatant was added to test tube containing 5 ml FC reagent (10% aqueous solution) and 4 ml aq. sodium carbonate. The tubes were held for 15 min and were then analyzed by spectrometer for absorbance at 765 nm. Results were expressed as mg gallic acid equivalents/100 g sample.

Total flavonoids

Flavonoids content in the methanolic extract was determined by aluminum chloride calorimetric method [34]. Briefly 0.50 ml of methanolic extract of sample was diluted with 1.50 ml of distilled water and 0.50 ml of aluminum chloride added along with 0.10 ml 0f 1 M potassium acetate and 2.80 ml of distilled water. This mixture was incubated at room temperature for 30 min. The absorbance of resulting reaction mixture was measured at 415 nm UV–Vis spectrophotometer. Quantification of flavonoids was done on the basis of standard curve of quercitin prepared in methanol and results were expressed in mg quercetin equivalent (QE) per 100 g of sample.

Evaluation of estimated glycemic index (EGI)

In vitro starch digestibility

Total starch content in the samples was determined by the titrimetric method of Moorthy and Padmaja [35]. In vitro starch digestibility (IVSD) of samples was determined as per the procedures of Englyst et al. [36], McCleary and Monaghan [37] and Kim et al. [38] with slight modifications. Five grams (3 replicates) sample were mixed with HCl–KCl buffer (pH 1.5, 10.0 ml) and equilibrated at 37 °C for 10 min. Pepsin (SIGMA, USA) was added to initiate proteolysis (0.4 ml from 10.0 ml HCl-KCl buffer containing 1.0 g pepsin). Samples were incubated at 37 °C for 1 h, after which 40.0 ml sodium phosphate buffer (0.02 M; pH 6.9 containing 0.12 M sodium chloride) was added. After equilibrating for 10 min at 37 °C, 1.0 ml Panzynorm N (one tablet dissolved in 5.0 ml 0.02 M sodium phosphate buffer pH 6.9) was added and incubation continued for 20 min. One milliliter of the supernatant was withdrawn and heat inactivated at 100 °C. The aliquot was added to 3.0 ml sodium acetate buffer (0.2 M; pH 4.8) and incubated at 60 °C for a further 10 min with 0.25 ml Dextrozyme GA (M/s Novo Industries, Denmark). Incubation of the Panzynorm assay system was continued up to 120 min and aliquots of 1.0 ml were withdrawn at every 20 min interval. Samples were treated identically with 0.25 ml Dextrozyme GA. Glucose content in each aliquot was quantified using Glucose oxidase (EC 1.1.3.4)–peroxidase (EC 1.11.1.7) reagent (M/s Beacon Diagnostics Pvt. Ltd. Gujarat, India). Starch measured at 20 min [Glucose expressed as (g/100 g) × 0.9] was taken as the rapidly digested starch (RDS) and that measured at 120 min. was taken as RDS + slowly digested starch (SDS). Resistant starch (RS; starch remaining undigested after 120 min.) was computed as the difference between total starch content in the sample (g/100 g dwb) and (RDS + SDS) 120 min. Separate enzyme and substrate blanks were maintained for each sample.

The hydrolysis index (HI) is calculated as:

$$\text{HI} = \frac{\text{Total glucose released from 100 g cooked sample (on dry basis) at 120 min}}{\text{Total glucose released from 100 g white bread (on dry basis) at 120 min}} \times 100$$

Estimated glycemic index (EGI) could be computed using the formula of Goni et al. [39].

$$EGI = 39.71 + 0.549 \times HI.$$

Sensory characteristics of bread

The sensory evaluations of sample were carried out by trained panel comprised of scientist and senior research fellow of the institute who had some previous experience in sensory evaluation. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. The trained panellists evaluated all samples and also served with a glass of water to neutralize the taste before analyzing the next sample. Judgment were made through rating products on a nine points Hedonic Scale with corresponding descriptive terms ranging from 9 'like extremely to 1 'dislike extremely'. Hedonic scale was in the following sequence: like extremely—9, like very much—8, like moderately—7, like slightly—6, neither like nor dislike—5, dislike slightly—4, dislike moderately—3, dislike very much—2, dislike extremely—1.

Statistical analysis

The data obtained in the experiments were analyzed using RSM, so as to fit the quadratic polynomial equation generated by the Design-Expert software version 8.0.3.1 (Stat-Ease Inc., Minneapolis, USA). In order to correlate the response variable to the independent variables, multiple regression was used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using analysis of variance (ANOVA).

Results and discussion

Fitting the models

The observed values of all dependent variables (protein content, fiber content, overall acceptability, loaf weight, loaf volume, specific volume and oven spring) with the level of two independent variables (whey protein concentrate and psyllium husk) for all the samples of OFSP bread are given in Table 1. The observed values of protein and fiber content for all different samples ranges from 7.03 to 18.67% and 3.1 to 8.31% respectively. Whereas, overall acceptability varied from 7.5 to 9.5. The value for physical properties of bread such as loaf weight (152.2 to 165.1 g), loaf volume (270 to 375 cm^3), specific volume (1.65 to 2.3 cm^3/g) and oven spring (0.3 to 1.4 cm). The levels of responses were fixed for optimization of OFSP bread (Table 3). The lower limit and upper limit for WPC was 03% and 09% whereas for psyllium husk was 02% and 06% which were is in range. The protein content, fiber content and overall acceptability of OFSP bread were in maximum range. The optimized lower limit and upper limit for protein content, fiber content and overall acceptability were 7.03 to 18.67%, 3.1 to 8.31% and 7.5 to 9.5 respectively. Rest of the responses of OFSP bread was in the range.

Effect of independent factors level on protein content

Analysis of variance for specific volume of OFSP bread using Response Surface Quadratic Model is given in Table 4. The Model F-value of 29.77 and p value of 0.0001 represents that the quadratic model was significant for protein content. Based on the results, it was proved that there was significant effect of whey protein concentrate on the protein content of OFSP bread. But there was non-significant effect of psyllium husk powder on protein content because it is

Table 4 Analysis of variance for protein content of OFSP bread using response surface quadratic model

Source of variation	SS	df	MS	F value	p value
Model	100.69	5	20.14	29.77	0.0001*
A (whey protein concentrate)	92.49	1	92.49	136.70	< 0.0001*
B (psyllium husk powder)	0.0008	1	0.0008	0.0013	0.9727^{ns}
AB	0.0000	1	0.0000	0.0000	1.0000^{ns}
A^2	7.67	1	7.67	11.34	0.0120*
B^2	0.1313	1	0.1313	0.1940	0.6729^{ns}
Residual	4.74	7	0.6766		
Lack of fit	4.74	3	1.58	52,622.01	< 0.0001*
Pure error	0.0001	4	0.0000		
Correlation total	105.43	12			

SS sum of squares, *df* degree of freedom, *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk powder, *ns* non significant

*p < 0.05

rich source of fiber, whereas level of WPC (0.0001) more significantly effected on protein content.

The response surface graph for the combined effect of levels of WPC and psyllium husk powder on protein content of bread is given in Fig. 2a. It is evident from the graph that as the level of WPC increased in samples; protein content linearly increased because WPC was added to food to incline the protein content. Similar results were observed by Munaza et al. [40] in WPC enriched biscuits.

A quadratic model was obtained from the ANOVA which had a final equation shown in Eq. 1:

$$\text{Protein} = +4.76 + 2.53 * \text{W} - 0.26 * \text{P} + 9.84\text{E} - 16 * \text{WP} - 0.11 * \text{W}^2 + 0.03 * \text{P}^2 \ldots \quad (1)$$

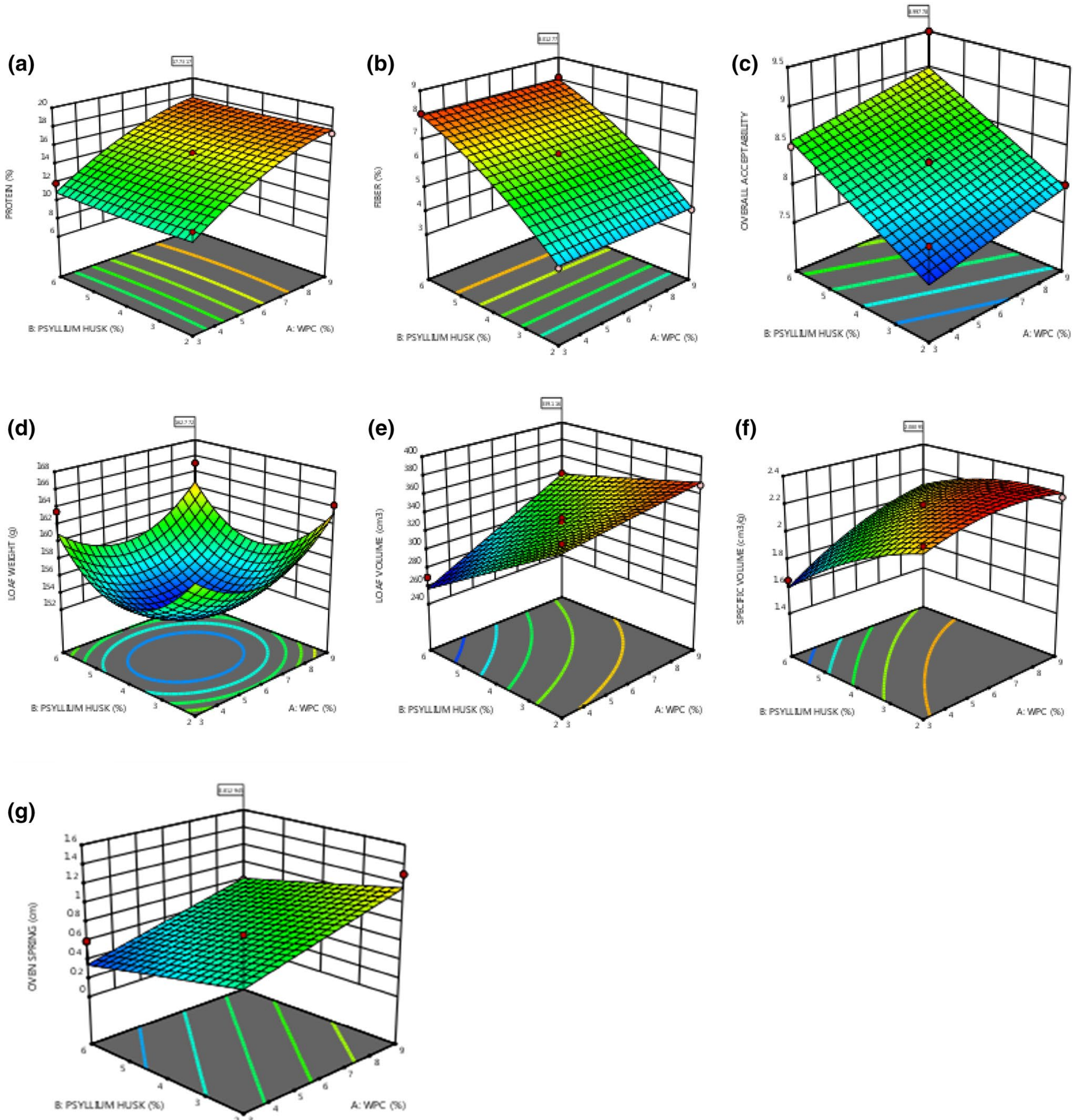

Fig. 2 Response surface graph for combined effect of WPC and psyllium husk on **a** protein content, **b** fiber content, **c** overall acceptability, **d** loaf weight, **e** loaf volume, **f** specific volume and **g** oven spring of OFSP bread

where W: whey protein concentrate, P: psyllium husk powder.

Effect of independent factors level on fiber content

The results of analysis of variance for fiber content of OFSP bread using response surface quadratic model are given in Table 5. The F-value of 754.44 and p value of 0.0001 indicate that the quadratic model for fiber content was significant. p value less than 0.0500 indicate model terms were significant. It is evident from the result that there is significant effect of only one factor psyllium husk powder on fiber content of bread. The level of WPC and interaction of both the factors exerted non-significant effect on fiber content of bread.

The response surface 3D graph for the combined effect of level of WPC and Psyllium husk powder on fiber content of bread is given in Fig. 2b. From the graph it is observed that as the level of psyllium husk increased, the level of fiber content increased. Dimitrios et al. [41] optimized recipe for bread preparation which contained 6.5% maize fiber and 102.5% water and yielded good quality bread with a total dietary fiber content 40% higher than that of ordinary wheat bread.

A quadratic model was obtained from the ANOVA which had a final equation shown in Eq. 2:

$$\begin{aligned} \text{Fiber} = &+1.14 - 0.01 * W + 1.69 * P + 1.30E \\ &- 16 * WP + 0.01 * W^2 - 0.09 * P^2 \ldots \end{aligned} \tag{2}$$

where W: whey protein concentrate, P: psyllium husk powder.

Table 5 Analysis of variance for fiber content of OFSP bread using response surface quadratic model

Source of variation	SS	df	MS	F value	p value
Model	30.63	5	6.13	754.44	< 0.0001*
A (whey protein concentrate)	0.0007	1	0.0007	0.0846	0.7795^{ns}
B (psyllium husk powder)	29.68	1	29.68	3655.19	< 0.0001*
AB	0.0000	1	0.0000	0.0000	1.0000^{ns}
A^2	0.0007	1	0.0007	0.0900	0.7729^{ns}
B^2	0.9255	1	0.9255	114.00	< 0.0001*
Residual	0.0568	7	0.0081		
Lack of fit	0.0568	3	0.0189	945.87	< 0.0001*
Pure error	0.0001	4	0.0000		
Correlation total	30.68	12			

SS sum of squares, *df* degree of freedom, *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk, *ns* non significant

*p < 0.05

Effect of independent factors level on overall acceptability

Response surface linear model with F value of 12.19 and p value of 0.0021 was found significant (p < 0.0001) for overall acceptability score (Table 6). The score of overall acceptability of bread samples was significantly (p < 0.0001) mainly affected by the level of psyllium husk and this may be due to the adverse effect on quality parameters of bread which was liked by panel members. As per Eq. 3 it was found that, there is positive effect of increasing level of WPC and psyllium husk on overall acceptability of bread.

The graph for combined effect of both the ingredients (WPC and psyllium husk powder) on overall acceptability score is given in Fig. 2c. From the graph it was estimated that the as the level of psyllium husk increases the overall acceptability score for bread samples were increases and this may be due to the increase in moisturizing ability of bread and had a very fine core and the elasticity and the porosity were within the permissible. Bread supplemented with 15% psyllium husk was found acceptable without affecting quality adversely [42].

A linear model was obtained from the ANOVA which had a final equation shown in Eq. 3:

$$\text{Overall acceptability} = +6.81 + 0.07 * W + 0.257 * P \ldots \tag{3}$$

where W: whey protein concentrate, P: psyllium husk powder.

Effect of independent factors level on loaf weight

Response surface quadratic model with F value of 15.08 and p value of 0.0013 was found significant (p < 0.0001) for loaf weight (Table 7). It was shown that, based on quadratic

Table 6 Analysis of variance for overall acceptability of OFSP bread using response surface linear model

Source of variation	SS	df	MS	F value	p value
Model	2.49	2	1.24	12.19	0.0021*
A (whey protein concentrate)	0.3643	1	0.3643	3.57	0.0881^{ns}
B (psyllium husk powder)	2.12	1	2.12	20.81	0.0010*
AB	1.02	10	0.1020		
Residual	1.02	6	0.1700		
Lack of fit	0.0000	4	0.0000		
Pure error	3.51	12			
Correlation total					

SS sum of squares, *df* degree of freedom; *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk, *ns* non significant

*p < 0.05

model of loaf weight, both the factors were non significantly affected on loaf weight of bread.

The graph for combined effect of both the ingredients (WPC and psyllium husk powder) on loaf weight score is given in Fig. 2d. From the graph it is estimated that the as the level of both the factors increased, there was increase in loaf weight but not statistically significant. This may be due to the reduction in level of wheat flour and addition of protein and fiber rich ingredients which cause reduction in carbon dioxide retention resulting in high loaf weight of the bread [28].

A quadratic model was obtained from the ANOVA which had a final equation shown in Eq. 4:

$$\text{Loaf weight} = +189.05 - 5.10 * W - 11.22 * P - 0.01 * WP + 0.45 * W^2 + 1.40 * P^2 \ldots \quad (4)$$

where W: whey protein concentrate, P: psyllium husk powder.

Effect of independent factors level on loaf volume

Response surface 2FI model with F value of 67.02 and p value of < 0.0001 was found significant ($p < 0.0001$) for loaf volume (Table 8). The score of loaf volume of the bread samples was significantly ($p < 0.0001$) affected by the different level of both factors WPC and psyllium husk powder.

The graph for combined effect of both the ingredients (WPC and psyllium husk) on loaf volume is given in Fig. 2e. It is estimated that the as the level of both factors increases the loaf volume of bread samples were decreases and this may be attributed to the dilution effect on the gluten network thereby reducing the gluten strength with a ripple effect of poor carbon dioxide gas formation and retention in the baked dough [43]. Similar types of results were reported that increasing level of fluted pumpkin flour and mushroom powder, decrease in loaf volume of bread [44, 45].

Table 7 Analysis of variance for loaf weight of OFSP bread using response surface quadratic model

Source of variation	SS	df	MS	F value	p value
Model	311.44	5	62.29	15.08	0.0013*
A (whey protein concentrate)	8.33	1	8.33	2.02	0.1985^{ns}
B (psyllium husk powder)	0.1131	1	0.1131	0.0274	0.8733^{ns}
AB	0.0484	1	0.0484	0.0117	0.9168^{ns}
A^2	119.06	1	119.06	28.82	0.0010*
B^2	221.05	1	221.05	53.51	0.0002*
Residual	28.92	7	4.13		
Lack of fit	28.92	3	9.64	1.205E+05	< 0.0001*
Pure error	0.0003	4	0.0001		
Correlation total	340.36	12			

SS sum of squares, *df* degree of freedom, *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk, *ns* non significant

*p < 0.05

Table 8 Analysis of variance for loaf volume of OFSP bread using response surface 2FI model

Source of variation	SS	df	MS	F value	p value
Model	14,357.34	3	4785.78	67.02	< 0.0001*
A (whey protein concentrate)	5370.58	1	5370.58	75.21	< 0.0001*
B (psyllium husk powder)	8086.76	1	8086.76	113.25	< 0.0001*
AB	900.00	1	900.00	12.60	0.0062*
Residual	642.66	9	71.41		
Lack of fit	622.66	5	124.53	24.91	0.0041*
Pure error	20.00	4	5.00		
Correlation total	15,000.00	12			

SS sum of squares, *df* degree of freedom, *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk, *ns* non significant

*p < 0.05

A 2FI model was obtained from the ANOVA which had a final equation shown in Eq. 5:

$$\text{Loaf volume} = +401.7 - 1.36 * W - 30.89 * P + 2.5 * WP \ldots \quad (5)$$

where W: whey protein concentrate, P: psyllium husk powder.

Effect of independent factors level on specific volume

Response surface quadratic model with F value of 56.50 and p value of < 0.0001 was found significant (p < 0.0001) for specific volume (Table 9). Specific volume of bread was found significantly affected by individual both factors as well as their interaction. Specific volume is the ratio of loaf volume to loaf weight. In present investigation, loaf volume and weight were found decreasing and increasing respectively so it was obvious to found decrease in specific volume. These results were found in agreement with the results reported by Bhise and Kaur [46] who also reported decreasing trend in specific volume of bread incorporated with oat, psyllium and barley fibers.

The graph for combined effect of WPC and psyllium husk on specific volume of bread sample is given in Fig. 2f. From the equation it is estimated that there was negative effect of level of psyllium husk powder on specific volume.

A quadratic model was obtained from the ANOVA which had a final equation shown in Eq. 6:

$$\text{Specific volume} = +2.06 + 0.08 * W - 0.04 * P + 0.01 * WP - 0.008 * W^2 - 0.017 * P^2 \ldots \quad (6)$$

Table 9 Analysis of variance for specific volume of OFSP bread using response surface quadratic model

Source of variation	SS	df	MS	F value	p value
Model	0.5785	5	0.1157	56.50	< 0.0001*
A (whey protein concentrate)	0.1769	1	0.1769	86.36	< 0.0001*
B (psyllium husk powder)	0.3011	1	0.3011	147.00	< 0.0001*
AB	0.0361	1	0.0361	17.63	0.0040*
A^2	0.0377	1	0.0377	18.41	0.0036*
B^2	0.0352	1	0.0352	17.18	0.0043*
Residual	0.0143	7	0.0020		
Lack of fit	0.0136	3	0.0045	25.22	0.0046*
Pure error	0.0007	4	0.0002		
Correlation total	0.5929	12			

SS sum of squares, *df* degree of freedom, *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk, *ns* non significant

*p < 0.05

where W: whey protein concentrate, P: psyllium husk powder.

Effect of independent factors level on oven spring

Response surface linear model with F value of 5.05 and p value of 0.0305 was found significant (p < 0.0001) for oven spring (Table 10). There was significant effect of WPC on oven spring of bread whereas; psyllium husk powder had negative effect on the oven spring. As per equation of linear model showed that, increase in level of psyllium husk, linear decrease in value of oven spring of bread sample. It showed similar trends as loaf volume. Researchers reported that, incorporation of fiber in bread, reduced oven spring. These research findings are in accordance with Wang et al. [47] who used many types of fibers in the composite flour bread formulations. They found that bread with a high percentage of fiber resulted in a lower oven spring value. This phenomenon is due to the reduction in dough viscosity and the increase in resistance to expansion [48].

The graph for combined effect of both the factors on oven spring value is given in Fig. 2g. A linear model was obtained from the ANOVA which had a final equation shown in Eq. 7:

$$\text{Oven spring} = +0.63 + 0.77 * W - 0.08 * P \ldots \quad (7)$$

where W: whey protein concentrate, P: psyllium husk powder.

Validation based upon desirability

The mean values for protein content (14.67%), fiber content (6.21%), overall acceptability (8.27), loaf weight (158.22 g), loaf volume (330 cm^3), specific volume

Table 10 Analysis of variance for oven spring of OFSP bread using response surface linear model

Source of variation	SS	df	MS	F value	p value
Model	0.6695	2	0.3347	5.05	0.0305*
A (whey protein concentrate)	0.4304	1	0.4304	6.49	0.0290*
B (psyllium husk powder)	0.2390	1	0.2390	3.61	0.0868^{ns}
AB	0.6629	10	0.0663		
Residual	0.6629	6	0.1105		
Lack of fit	0.0000	4	0.0000		
Pure error	1.33	12			
Correlation total					

SS sum of squares, *df* degree of freedom, *MS* mean sum of square, *A* whey protein concentrate, *B* psyllium husk, *ns* non significant

*p < 0.05

Table 11 Results of response surface models for OFSP bread

Statistical Parameters	Protein content (%)	Fiber content (%)	Overall acceptability	Loaf weight (g)	Loaf volume (cm^3)	Specific volume (cm^3/g)	Oven spring (cm)
Mean	14.67	6.21	8.27	158.22	330.00	2.09	0.7538
CV (%)	5.61	1.45	3.86	1.28	2.56	2.17	34.15
R^2 (%)	0.9551	0.9981	0.7091	0.9150	0.9572	0.9758	0.5025
Adjusted R^2 (%)	0.9230	0.9968	0.6510	0.8543	0.9429	0.9585	0.4030

CV coefficient of variations

Table 12 Optimized solutions with predicted and actual experimental values for OFSP bread

Sol. no	Level of ingredients (%)			Protein content (%)		Fiber content (%)		Overall acceptability		Loaf weight (g)		Loaf volume (cm^3)		Specific volume (cm^3/g)		Oven spring (cm)	
	WPC	PH	Desirability	Pre	Exp*	Pre	Exp*	Pre	Exp*	Pre	Exp*	Pre	Exp*	Pre	Exp*	Pre	Exp*
1	9.0	6.0	0.94	17.73	17.72	8.01	8.02	8.99	9.0	162.77	162.78	339.11	339.10	2.08	2.09	0.81	0.81
2	8.74	6.0	0.93	17.61	17.57	8.01	8.01	8.98	8.97	162.01	162.0	335.63	335.64	2.07	2.07	0.79	0.78
3	8.65	6.0	0.92	17.56	17.52	8.00	8.0	8.97	8.95	161.75	161.73	334.35	334.34	2.06	2.05	0.78	0.77
4	9.0	5.65	0.90	17.68	17.7	7.79	7.77	8.90	8.91	161.02	161.05	342.02	342.0	2.12	2.13	0.84	0.85

WPC whey protein concentrate, *PH* psyllium husk, *Pre* predicted, *Exp* experimental

(2.09 cm^3/g) and oven spring (0.75 cm) were analyzed through Design Expert Software (Table 11). The selected formulations (with desirability 0.94) of protein and fiber enriched orange fleshed sweet potato bread were prepared and further evaluated for validating predicted values. Overall acceptability was evaluated by same panel of judges. Based upon the validation experiments, the formulation with optimized levels for whey protein concentrate and psyllium husk powder as 09% and 06% respectively was found most suitable for preparation of protein and fiber enriched orange fleshed sweet potato bread (Table 12). The optimized sample with protein content (17.72%), fiber content (8.02%), overall acceptability (9.0), loaf weight (162.78 g), loaf volume (339.10 cm^3), specific volume (2.09 cm^3/g) and oven spring (0.81 cm) was also further evaluated for biofunctional components such as carotenoide, total phenols, total flavonoids content and antioxidant activity. Moreover, estimated glycemic index (EGI) and Mineral content were also estimated and compared with refined wheat flour bread. Shelf life of optimized bread sample was evaluated by microbial assessment.

Nutritional composition of optimized OFSP bread and refined wheat flour bread

The nutritional composition of the optimized bread prepared using 30% orange fleshed sweet potato, 70% wheat flour, 9% WPC and 6% psyllium husk was determined and the results are presented in Table 13 and compared with refined wheat flour bread. Results revealed that, optimized sample contained higher protein and fiber as compare to refined wheat flour bread which was increased from 7.28 to 17.72% and 3.72 to 8.02% respectively and it was due to addition of WPC and psyllium husk powder. However, optimized bread found rich in mineral content (K, P, Fe, Zn and Ca) because of substitution of wheat flour by 30% orange fleshed sweet potato flour. Incorporation of OFSP puree into bread baking

Table 13 Nutritional composition of optimized OFSP bread and refined wheat flour bread

Parameters	Optimized OFSP bread	Refined wheat flour bread
Protein (%)	17.72	7.28
Fiber (%)	8.02	3.72
Total carotenoid content (mg/100 g)	3.78	0.32
Total phenols (mg GAE/100 g sample)	51.32	33.8
Total flavonoids (mg QE/100 g sample)	26.80	12.41
% DPPH inhibition (antioxidant activity)	43.53	5.03
Estimated glycemic index (EGI)	52.58	92.5
Potassium (mg/100 g)	520.33	312.13
Phosphorus	25.63	23.09
Iron (mg/100 g)	10.06	4.01
Zinc (mg/100 g)	0.79	0.65
Calcium (mg/100 g)	38.54	27.51

can serve to enrich the bread energy and nutrients such as vitamins (pro-vitamin A) and minerals (Ca, K, Fe, Zn and P) and also add natural sweetness, color, flavor and dietary fiber [49, 50]. Similarly, carotenoid, phenols, flavonoide and % DPPH inhibition were found more on bread sample incorporated with OFSP as compare to refined wheat flour bread. OFSP is rich in β-carotene, total phenols, ascorbic acid, folic acid and minerals [51, 52]. Estimated glycemic index of wheat flour bread was found high and which was decreased from 92.5 to 52.58 in optimized protein and fiber enriched orange fleshed sweet potato bread. It was reported that, high protein and fat contributes to the lower glycemic response [53] and diet rich in fiber helps to reduce insulin resistance which found beneficial for diabetics [54]. Scientific finding revealed that orange fleshed sweet potato could be used as alternative to wheat flour for individuals diagnosed with celiac disease and incorporated in low glycemic index foods for diabetics [55].

Conclusion

Optimization of the levels of whey protein concentrate and psyllium husk powder for the processing of high protein and fiber bread with orange fleshed sweet potato is predicted based on nutritional composition, overall acceptability score and physical characteristics using RSM package. The formulation with 09% WPC, 06% psyllium husk powder, 30% OFSP and 70% wheat flour was considered to be the most appropriate for development of bread rich in protein, fiber, minerals and high content of carotenoids, total phenol, total flavonoids and antioxidant activity. Optimized OFSP bread was found low in glycemic index which would be suitable for diabetics. Developed bread had 17.72% protein and 8.02% fiber and meets nutritional requirement and provide health benefits

Compliance with ethical standards

Conflict of interest Authors declare that there is no conflict of interest.

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