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Optimisation of the production process of bread fortified with soya protein ısolate and fonio flour blend

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Abstract
In this study, the optimization of the production process of bread from composite flours was investigated, from $31.75 \leq$ wheat ≤ 49.21, $2.38 \leq$ soy protein isolate ≤ 15.87 and $1.32 \leq$ fonio flour ≤ 7.94 at 187.5 °C for 30min at constant temperature and time. Bread loaves were produced from the composite flour using the modified straight dough procedure. The proximate analysis of the bread samples was carried out. Analysis of variance indicated that the linear, quadratic and interactive effects of A (wheat flour), B (Fonio flour) and C (soy protein isolate) significantly ($p \leq 0.05$) affected all the composite bread properties evaluated. The emperical models fitted to the experimental data showed the model was significant with 13.03 (protein), 5.50 (fat content), 3.52 (crude fibre), 49.62 (moisture content), 6.13 (ash content) and 17.31% (carbohydrate) for the bread samples. The bread with optimal quality indexes with overall desirability index of (0.568), was obtained from 39.42% wheat, 6.28% SPI and 13.76% Fonio. The properties of this optimal bread were: protein 17.25%, fat 8.83%, crude fibre 2.81%, moisture content 28.47%, ash content 2.80%, carbohydrate 41.35%, colour 4.07, taste 4.40, odour l 3.89, texture 3.89 and acceptability 3.56.

Keywords: Proximate composition, composite bread, wheat flour, protein, optimization

Introduction

Food products made from cereals are significant sources of dietary energy that can supply the nutrient needs of people and boost food security. Bread, a widely available product in Nigeria, is obtained from wheat flour. According to Kent and Evers (1994) [13], wheat flour is the major component of most breads because of the distinctive visco-elastic dough it forms when mixed with water. Nevertheless, non-wheat flours are also being utilized in the production of good quality bread. The major challenge being faced by the bakery industry is the scarcity and or high cost of wheat flour thus the need to investigate other cheaper and more sustainable cereal flours as substitutes (Thompson *et al*., 2008) [28].

According to Dewettinck *et al*., (2008) [7] bread can be described as a fermented confectionary produced majorly from a combination of wheat flour, yeast, water and salt through a process involving mixing, kneading, proofing, shaping and baking. Though the consumption of baked products from wheat flour like breads, biscuits, doughnuts and cakes is very popular these days, the low protein content of wheat flour has continued to be a limiting factor in its usage (Young and Pellet, 2001) [29]. Many authors have reported the use of non-wheat flour in the production of bread, (Edema *et al*., 2005; Shittu *et al*., 2008; Mesfin and Shimelis, 2013) [9, 27, 17].

According to Muller and Krawinkel, (2005), Protein Energy Deficiency (PED) poses a significant nutritional problem as a result of undernutrition affecting people in the developing world.

Policies that have been used to solve protein deficiencies include food diversification, fortification of food with indispensable amino acids, supplementation with good quality protein (FAO, 1997) [10].

The fortification of bread and other cereal-based products with legume flours especially in areas where protein utilization is inadequate has long been recognized because of the high protein content of legumes. According to Okoye and Okaka (2009) [20], legumes can be used to complement other cereals when blended at optimum ratio.

Soybean, of the *leguminosae* family and *Papillionnideae* subfamily; is a rich source of essential nutrients containing various chemical compounds that have potent bioavailability. According to Kure *et al*. (1998) [14], soybean flour has about 35 - 45% protein and is considered an excellent source of high-quality plant protein containing all essential amino acids needed for proper growth and maintenance of body. Many authors have reported success with adding soy flour to bakery products towards increasing the daily soy intake in people's diet (Kure *et al*., 1998; Murphy *et al*., 1999; Dhingra and Jood, 2002) [14, 18].

Fonio is classified among grasses (*poaceae*) and ranked in the same subfamily as millet. The species can be white (*Digitaria exilis*) and black (*Digitaria iburu*) with local names such as *acha* and *iburu* respectively. *Fonio* grains are very tiny with 1000 weighing between 0.5-0.6g (Adoukonou-sagbadja *et al*., 2007). Polysaccharides are the main constituent (75%) followed by protein (8-10%) and fat (1%). It is also rich in calcium (20%) and phosphorus (25%) (Jideani, 1994) [12]. Delicacies prepared from fonio include porridges, fonio couscous or *injera* (spongy fermented flat wholegrain bread). Olapade and Oluwole (2013) [21] also reported that fonio flour can be used as a component in multi-composite flour blends like wheat-fonio-cowpea flour.

Nigerians face the challenges of deficiency in protein and shortage of calcium in their daily menu. Most of the bread produced are rich in carbohydrate but with less value in protein. It is therefore important to fortify the wheat flour with legumes (like soybeans and fonio flour) in order to produce nutritionally balanced products.

Although much work has been done on fortification of bread, the major thrusts of previous research have been on improving the nutritional properties without recourse to how these inclusions affect the end product in different proportions.

Soybean (rich in protein) and fonio (rich in both protein and calcium) crops will therefore improve the nutritional contents of bread if added during production. This study will therefore help to bring to light, effects of the different levels of fortification on the overall quality and properties of the end product. Thus, apart from improving the quality and nutritional composition of the bread, data on the effect of the different inclusion levels will be obtained.

Materials and Methods

Materials

The following were used in the study: Wheat flour (Dangote brand), Soybean, Fonio seeds, Sugar (Golden penny), Margarine (Simas brand, Assar-cozinhar industries Indonesia), baking improver (Batta Baker brand), Dry yeast (SKT- Royal instant yeast) and Salt (Dangote brand).

Methods

Preparation of Soybean Flour

For the preparation of the soybean flour, the method of Otegbayo *et al*., (2018) [23] with slight modification was used. Fig. 1 shows the production of soybean flour.

Soybean
↓
Sorting
↓
Steeping
↓
Drying
↓
Dehulling
↓
Milling
↓
Sieving
↓
Flour

Fig 1: Flow chart for the production of soybean flour

Preparation of Fonio seed Flour

The method of Coda *et al*., (2010) [6] was used. The *fonio* seeds were cleaned. The seeds were weighed, milled and sieved with a 250 µm mesh to get the fonio flour. The flour samples were then stored in polyethylene films until needed. The production process is presented in Fig. 2.

Sorting
↓
Drying
↓
Milling
↓
Sieving
↓
Flour

Fig 2: Flow chart for the production of fonio flour

Preparation of Soy Protein Isolate (SPI)

The isoelectric precipitation method as described by Petruccelli and Anon, (1995) [24] was used in the preparation of the soy protein isolate. The laboratory work was carried out at STEP B of Federal University of Technology Minna, Niger state. The production process for the soy protein isolate is presented in Fig. 3.

Soy flour [(10:1 water/soyflour, 60 °C, 30min, pH 8.5]
Centrifuge
↓
[(14, 000 xg, 15 °C, 30 min)] → Spent flour
Supernatant
↓
Precipitate [(2 min, 20 °C, pH 4.5)] Refrigerate (4 °C, 1hr)
↓
Centrifuge [(14, 000 xg, 4 °C, 30 min)] → Whey
Isolate curd Neutralize
↓
(P^H7) Freeze dry (-20 °C, 7days) Isolate

Fig 3: Flow chart for the production of soy protein isolate

Production of the wheat, soy protein isolate and fonio flour composite bread

The major ingredient (Wheat, SPI and Fonio) gives 52.91% in the formulation while the other ingredients include Water (26.46), Yeast (1.06), Salt (1.06), Granulated sugar (3.18), Margarine (2.12) and baking improver (13.23).

3.4 Composite bread baking

The modified straight dough method of Onuegbu *et al*. (2013) [22] was used in baking the bread. This was carried out at Agricultural and Bioresources Engineering laboratory, FUT, Minna.

Proximate Analysis

The Proximate composition (crude protein, crude fibre, crude fat, ash, moisture content and carbohydrate) of the bread samples was determined based on the method described by the Association of Official Analytical Chemists, A.O.A.C (2012) [4].

Experimental design

Optimization of the process parameters was carried out using process mixture design. The experimental plan for the mixture consists of three independent parameters – wheat flour, fonio flour and soya protein isolate. The experimental plan for the process consists of two independent parameters – baking temperature and baking time. The dependent variables are texture, taste, moisture content and colour variables in terms of L*, a* and b* of bread. The independent variables varied using a 2^3 factorial design matrix. The levels of the factorial design are illustrated in Table 1. The experimental layout for the experimental runs is presented in Table 2.

Table 1: Formulation constraints

Variables	Unit	Low	Levels	High
Wheat	%	31.746		49.207
Fonio	%	1.322		7.936
SPI	%	2.381		15.873

Table 2: Design matrix for fortified composite bread production

Run	Wheat flour (%)	Fonio flour (%)	Soy protein isolate (%)	Baking temperature (°C)	Baking time (min)
1	36.69	3.38	12.83	187.5	30
2	38.47	6.95	7.49	187.5	30
3	35.72	1.32	15.87	187.5	30
4	49.21	1.32	2.38	187.5	30
5	44.91	2.91	5.09	187.5	30
6	31.75	7.94	13.22	187.5	30
7	49.21	1.32	2.38	187.5	30
8	45.11	5.42	2.38	187.5	30
9	42.38	1.32	9.20	187.5	30
10	34.92	7.94	10.05	187.5	30
11	31.75	5.29	15.87	187.5	30
12	40.22	7.94	4.75	187.5	30
13	39.31	1.32	12.28	187.5	30
14	31.75	5.29	15.87	187.5	30
15	41.67	4.48	6.76	187.5	30
16	42.60	7.94	2.38	187.5	30
Control	52.91			187.5	30

Statistical analysis

The Design Expert Software (version 11) was used to carry out the statistical analysis; this provides test matrices for screening of all the factors in determining the best lines or points of fit, for the purpose of final optimization using a Response Surface Methodology (RSM).

Results

The results of the study are shown in Tables 3, 4, 5 and 6 respectively.

Proximate composition of composite bread samples

The result of the proximate analysis of the bread samples is as shown in Table 3.

Table 3: Mean proximate composition of composite bread samples

Run	A:Wheat Flour (%)	B:Fonio (%)	C:Soy protein isolate (%)	Protein (%)	Fat (%)	Crude fibre(%)	Moisture (%)	Ash content (%)	Carbohydrate (%)
1	36.69	3.38	12.83	16.52	4.57	2.69	29.00	2.50	44.72
2	38.47	6.95	7.49	14.12	4.18	2.76	25.50	3.50	49.94
3	35.71	1.32	15.87	18.11	3.82	2.51	30.00	2.50	43.06
4	49.20	1.32	2.38	12.15	6.66	2.82	27.00	2.00	49.37
5	44.91	2.91	5.09	12.73	4.85	2.79	25.50	2.50	51.63
6	31.75	7.94	13.23	16.75	3.05	2.65	24.00	3.00	50.55
7	49.21	1.32	2.38	12.25	5.38	2.83	26.50	3.00	50.04
8	45.11	5.42	2.38	12.45	3.25	2.08	23.00	3.90	55.32
9	42.38	1.32	9.20	14.25	6.63	2.75	31.00	2.50	42.87
10	34.92	7.94	10.05	14.32	6.50	2.73	31.50	2.40	42.55
11	31.75	5.29	15.87	18.12	3.48	2.23	22.50	3.80	49.87
12	40.22	7.94	4.75	12.75	4.33	2.78	26.10	3.00	51.04
13	39.31	1.32	12.28	16.48	4.23	2.92	29.50	2.80	44.07
14	31.75	5.29	15.87	18.09	7.47	2.3	26.50	3.60	42.04
15	41.67	4.48	6.76	13.03	6.07	2.5	28.00	3.30	47.10
16	42.59	7.94	2.38	12.47	3.38	2.84	23.00	3.80	54.51
Control	52.91			10.41	2.70	1.70	33.20	1.50	50.49

Optimum condition of composite bread

Table 4 shows the properties of the bread with overall desirability of 0.57.

Table 4: Optimum condition of the composite bread samples

Parameters	Unit	Optimal condition
Protein	%	17.25
Fat	%	5.83
Crude fibre	%	2.81
Moisture content	%	28.47
Ash content	%	2.80
Carbohydrate	%	41.35
Desirability		0.57

Discussion

Proximate analysis of the bread samples

Protein

Protein content ranged between 10.41 and 18.12 % (Table 3) and sample 11 was found to have the highest value of 18.12% at 187.5ºC and 30min respectively, while Sample 17 (control) was found to have lowest value of 12.15% at constant temperature and time. The protein content in this study agrees with the report of Mashayekh *et al.,* (2008) [16], Ndife *et al.,* (2011) [19] and Otegbayo *et al.,* (2018) [23]; they all reported an increase in protein content of bread with increase in soya flour. According to Alabi and Anuonye, (2007) and Aly *et al.,* (2012) [2], legumes having high protein content utilized in composite flour gave an improved quality in the protein content of cereal-based products. The rise in the protein content is expected as a result of the partial replacement of wheat flour with soy protein isolate and fonio flour both of which have high in protein content.

The result of the ANOVA (Table 6) showed that the linear and quadratic terms of the model significantly ($p< 0.05$) influenced the protein content. The mixture of wheat, soy protein isolate and fonio was positive for the protein content, which revealed that increase in the amount of soy protein isolate and fonio led to an increase the amount of protein content of the composite bread. The model F-value of 13.03 implies that the model is significant. The effect of the variables on the protein content are shown in Figures 4 and 5.

Table 6: ANOVA for the Quadratic Model for Protein Content

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	47.55	5	9.51	13.03	0.0004	Significant
(1) Linear Mixture	37.58	2	18.79	25.74	0.0001	
AB	4.34	1	4.34	5.94	0.0350	
AC	3.61	1	3.61	4.95	0.0503	
BC	6.66	1	6.66	9.12	0.0129	
Residual	7.30	10	0.7302			
Lack of Fit	2.59	5	0.5181	0.5499	0.7362	not significant
Pure Error	4.71	5	0.9422			
Cor Total	54.86	15				

Equation in terms of coded values

Protein = 0.0376A – 4.954B – 1.1642C + 0.1189AB + 0.0382AC + 0.1372BC

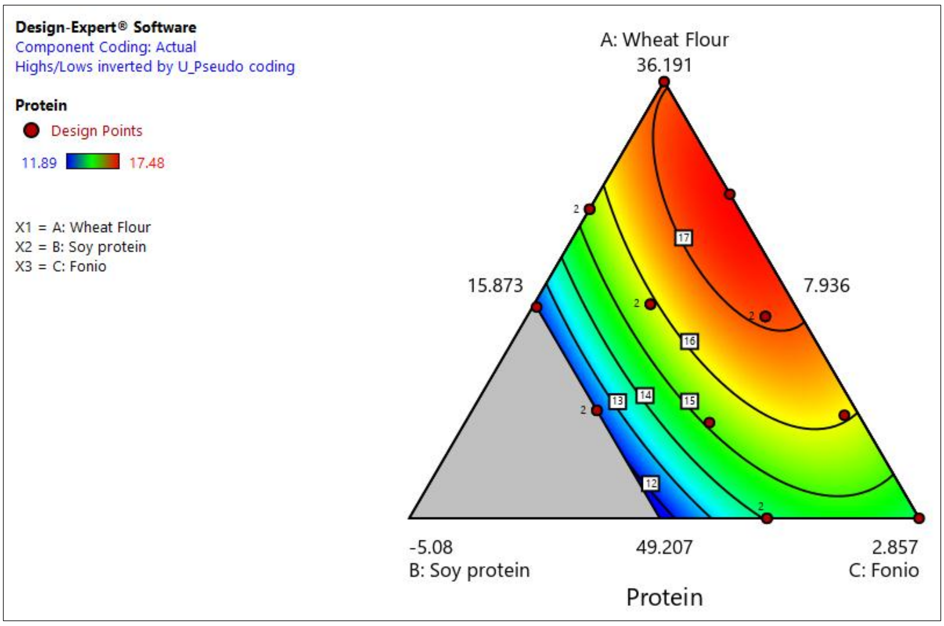

Fig 4: Mixture of wheat, soy protein isolate and fonio on protein content of bread

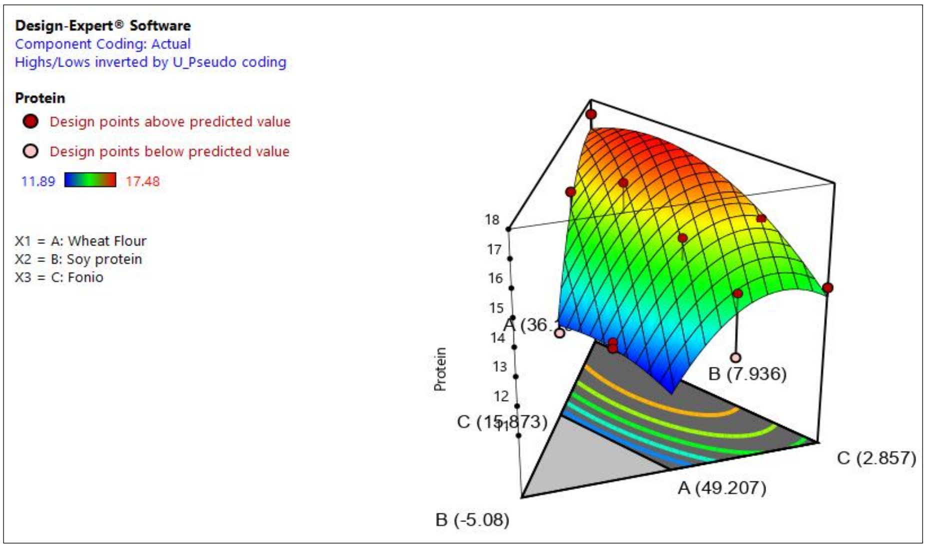

Fig 5: Three-dimensional surface plot of protein content model

Fat Content

The fat content ranged from 2.7 to 7.47% with sample 14 having the highest value of 7.47%, while the sample 17 (control) had the lowest value of 2.7% at constant temperature and time of 187.5ºC and 30min respectively (Table 3). There was an increase in the fat content with increase in fonio flour substitution. Ndife *et al.*, (2011) [19] reported an increase in fat content of bread made with composite flour containing soy flour. The result obtained was lower than the one reported by Otegbayo *et al*., (2018) [23].

Table 7 (ANOVA) shows that the linear and quadratic terms of the model significantly (p< 0.05) influenced the fat content. The mixture of wheat, soy protein isolate and fonio was positive for fat content, which revealed that increase in the quantity of fonio flour led to a rise in the fat content of the composite bread. The model F-value of 5.50 implies that the model is significant. The effect of variables on the fat content are shown in Figures. 6 and 7.

Table 7: ANOVA for the Quadratic Model of Fat Content

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	39.19	9	4.35	5.50	0.0252	Significant
(1) Linear Mixture	19.13	2	9.57	12.08	0.0079	
AB	0.0716	1	0.0716	0.0905	0.7737	
AC	0.4387	1	0.4387	0.5541	0.4848	
BC	0.0029	1	0.0029	0.0037	0.9538	
ABC	0.0124	1	0.0124	0.0157	0.9044	
AB(A-B)	0.4014	1	0.4014	0.5071	0.5032	
AC(A-C)	0.8590	1	0.8590	1.09	0.3377	
BC(B-C)	0.1509	1	0.1509	0.1906	0.6777	

Residual	4.75	6	0.7916			
Lack of Fit	0.0027	1	0.0027	0.0029	0.9593	not significant
Pure Error	4.75	5	0.9494			
Cor Total	43.94	15				

Equation in terms of coded values

Fat = 0.8978A – 103.582B – 15.387C + 3.156AB + 0.4056AC + 1.652BC – 0.0105ABC – 0.0287AB*(A-B) – 0.0038AB*(A-B) – 0.0176BC*(B-C)

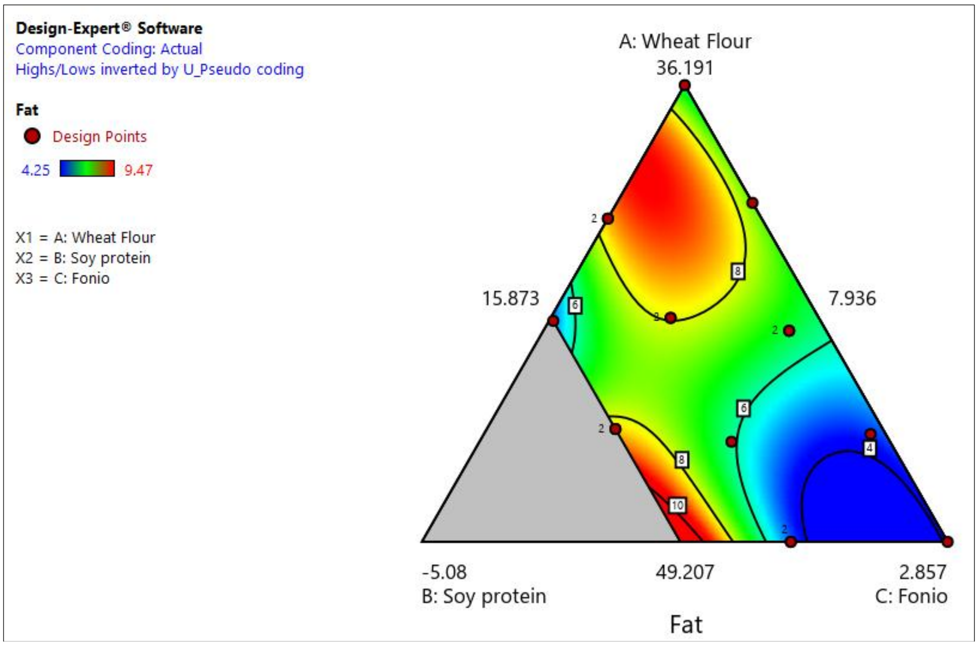

Fig 6: Mixture of wheat, soy protein isolate and fonio on fat content of bread

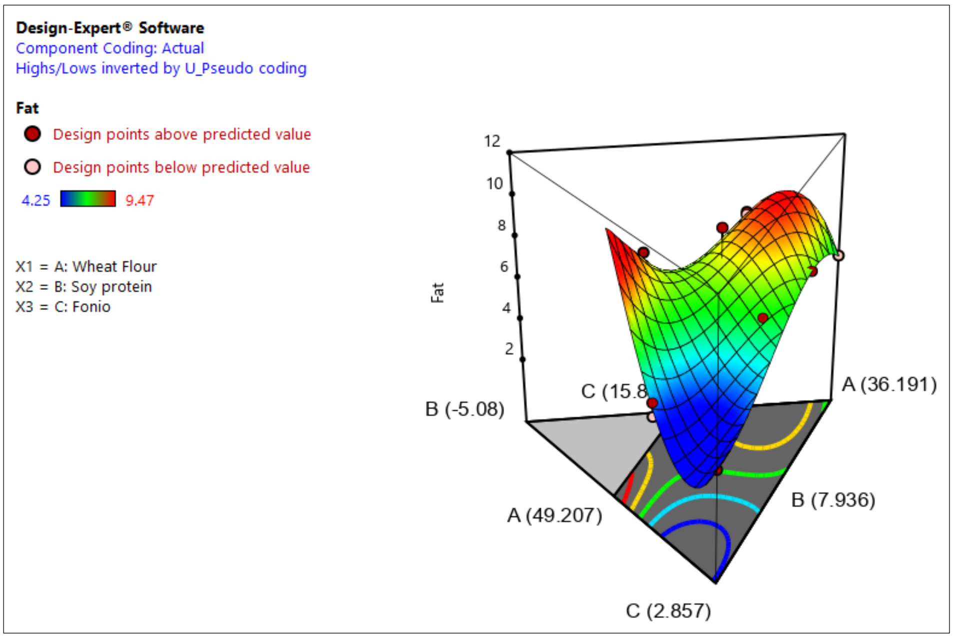

Fig. 7: Three-dimensional surface plot of fat content model

Crude fibre

There was a significant decrease ($p<0.05$) in the crude fibre content as the level of substitution of soy protein isolate increased. Sample 13 had the highest crude fibre content (2.92) with sample 17 (control) having the lowest (1.7). Mesfin and Shimelis, (2013) [17] however reported an increase in fibre content of composite flour comprising of maize and soybean with a comparable increase in the amount of soybean flour. Ndife *et al.*, (2011) [19] also reported a rise in the crude fibre content of composite bread made from whole wheat and soy flour. According to Marer and Martin (2003) [15], fibre helps in digestion. The effect of variables on the crude fibre content is shown in Figures 8 and 9.

Table 8: ANOVA for Response surface Mean Model for Crude Fibre

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5736	5	0.1147	3.52	0.0429	Significant
(1) Linear Mixture	0.1921	2	0.0961	2.94	0.0987	
AB	0.0036	1	0.0036	0.1109	0.7460	
AC	0.0045	1	0.0045	0.1394	0.7166	
BC	0.0946	1	0.0946	2.90	0.1194	
Residual	0.3262	10	0.0326			
Lack of Fit	0.1807	5	0.0361	1.24	0.4092	not significant
Pure Error	0.1455	5	0.0291			
Cor Total	0.8998	15				

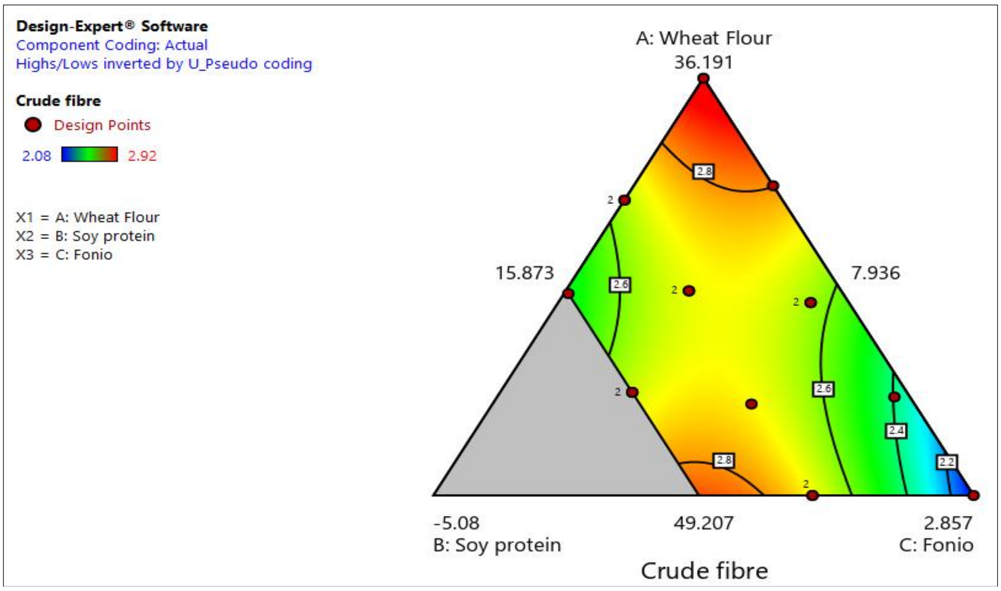

Fig. 8: Mixture of wheat, soy protein isolate and fonio on crude fibre of bread

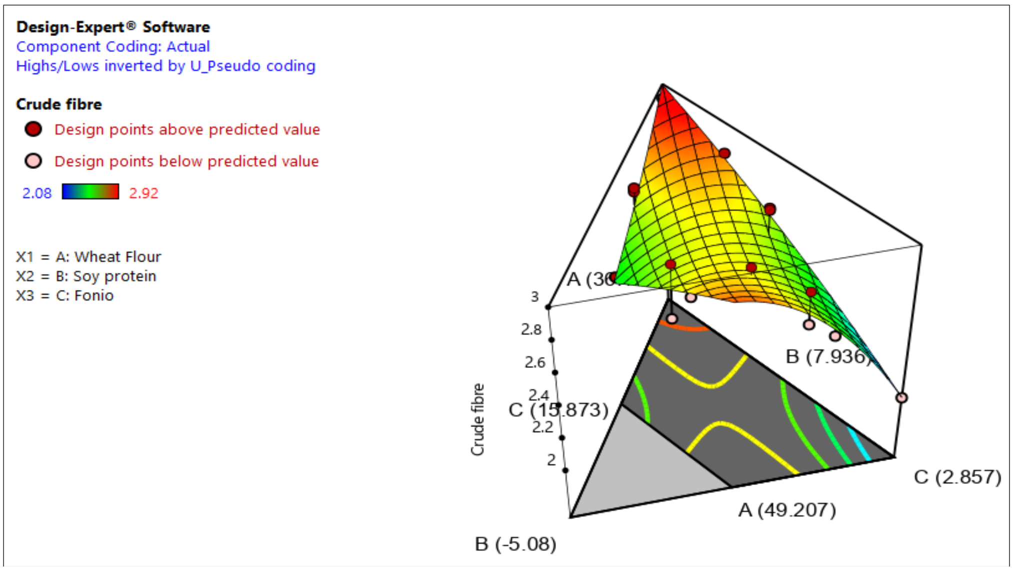

Fig. 9: Three-dimensional surface plot on crude fibre model

Moisture Content

The moisture content ranged between 22.5% and 33.2% (Table 3), with sample 17 (control) having the highest value, while sample 11 had the lowest value. The values obtained for moisture content of composite bread in this study were compared with the values of 12.94% reported by Gbadamosi and Oladeji, (2013) [11] for cassava; 11.08% reported for taro by Amandikwa, (2012) [3] and the value of 4.40% obtained for water yam by Saputri and Joni Kusnadi, (2013) [25]. The results showed that at higher amounts of soy protein isolate and low amounts of fonio flour, the moisture content was lower, while at lower level of protein isolate and higher level of fonio, the moisture content was higher; this shows that the higher level of fonio substitution has significant effect on the moisture content of composite bread produced. According to Bugusu *et al*. (2001) [5], the moisture content and water activity of a product majorly dictates the shelf life of the foods.

The fitted linear moisture content model with $p<0.05$ was significant as indicated in Table 8. The model F-value implies that the model was significant (p<0.05), the table also shows that model terms are significant. This reveals that the goodness of the model for moisture content based on the mixture design makes the model for predicting moisture content as a response valid. Adequate precision quantifies the signal to noise ratio. The effect of variables on the moisture content is shown in Figure 10.

Table 8: ANOVA for Moisture content

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	108.61	2	54.31	49.62	< 0.0001	significant
(1) Linear Mixture	108.61	2	54.31	49.62	<0.0001	
Residual	14.23	13	1.09			
Lack of Fit	9.80	8	1.22	1.38	0.3754	not significant
Pure Error	4.43	5	0.8860			
Cor Total	122.84	15				

Final Equation in terms Actual components
Moisture = 0.3231A + 0.3024B + 0.9936C

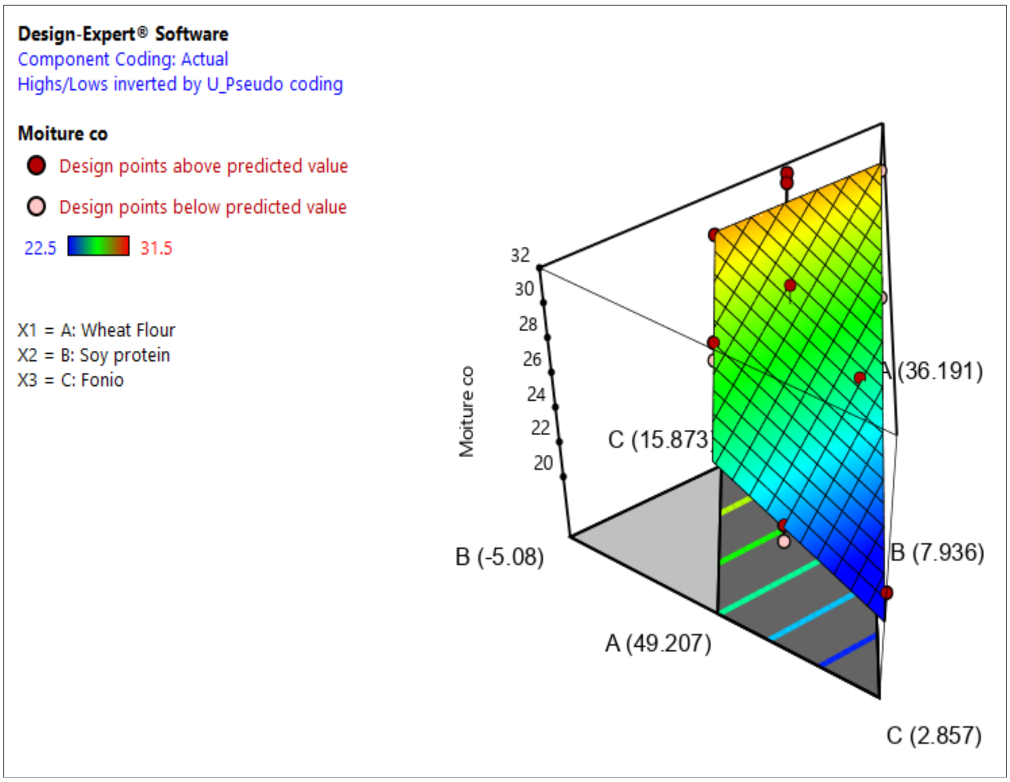

Fig 10: Three-dimensional surface plot of Moisture content model

Ash content

The ash content in the samples were between 1.5 and 3.9% (Table 3), with the control having the lowest value and sample 8 having the highest value. A rise in soy protein isolate substitution greatly increased the ash content of the product. Otegbayo *et al*., (2018) [23] reported a similar trend. The model's linear and quadratic terms of the ash content are significant as shown in Table 9 (ANOVA). The effect of variables on the ash content are shown in Figures 11 and 12.

Table 9: ANOVA for Ash content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.52	2	1.26	6.13	0.0134	Significant
(1) Linear Mixture	2.52	2	1.26	6.13	0.0134	
Residual	2.67	13	0.2055			
Lack of Fit	0.7716	8	0.0965	0.2538	0.9576	not significant
Pure Error	1.90	5	0.3800			
Cor Total	5.19	15				

Final equation in terms of Actual values:
Ash content = 0.0663A + 0.089B – 0.0306C

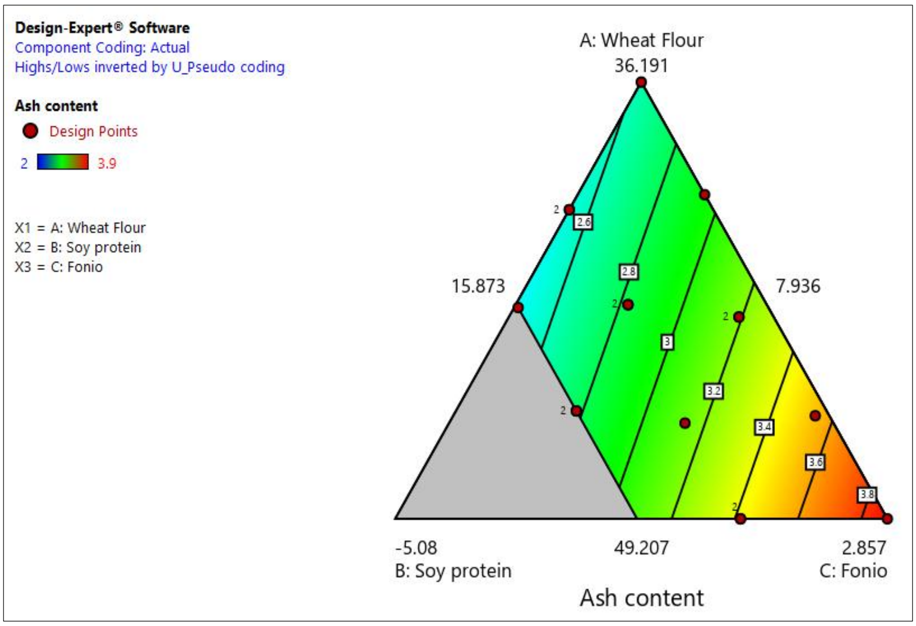

Fig. 11: Mixture of wheat, soy protein isolate and fonio on Ash content of bread

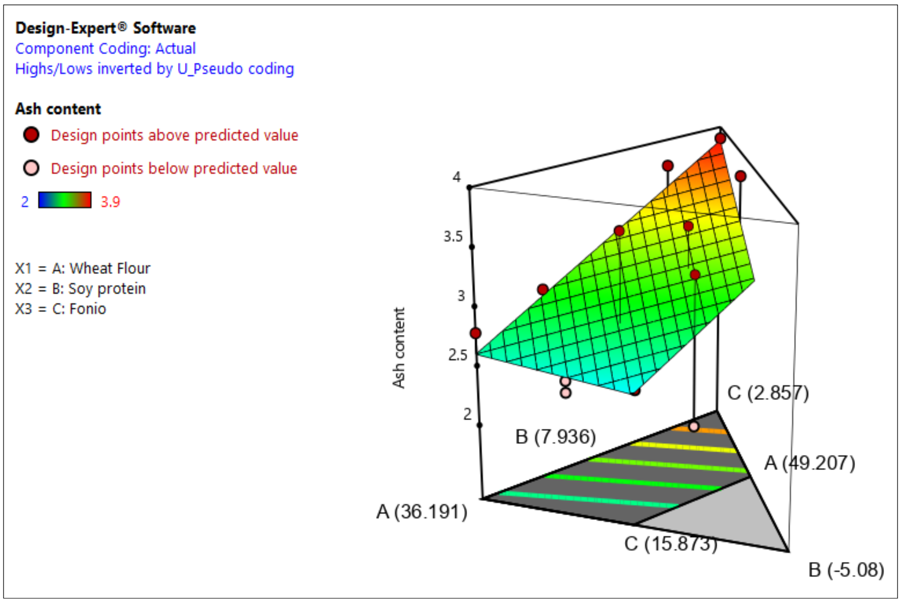

Fig. 12: Three-dimensional surface plot of Ash content model

Carbohydrate content

The carbohydrate content ranged between 42.04% and 55.32 (Table 3), with sample 8 having the highest value; while sample 14 had the lowest value. There was a decrease in the carbohydrate content as the amount of soy flour increased. This result agrees with the findings of Sanful and Darko, (2010) [26] and Aly *et al.*, (2012) [2], who reported a decrease in the carbohydrate content of composite flour obtained by substituting wheat flour with soy flour or chickpea flour at different levels.

Table 10 (ANOVA) shows that the linear mixture of soy protein isolate had a significant ($p<0.05$) influence on the carbohydrate content. The 3D effect of variables on the carbohydrate content are shown in Figures 13 and 14.

Table 10: ANOVA for Carbohydrate content

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	262.15	6	43.69	17.31	0.0002	significant
(1) Linear Mixture	223.52	2	111.76	44.29	< 0.0001	
AB	18.26	1	18.26	7.24	0.0248	
AC	1.15	1	1.15	0.4549	0.5170	
BC	13.44	1	13.44	5.33	0.0464	
ABC	14.70	1	14.70	5.82	0.0390	
Residual	22.71	9	2.52			
Lack of Fit	13.98	4	3.50	2.00	0.2325	not significant
Pure Error	8.73	5	1.75			
Cor Total	284.86	15				

Final Equation in terms of Actual components

Carbohydrate = 2.1779A + 44.1243B + 12.2851C – 0.99005AB – 0.3442AC – 2.5088BC + 0.04698ABC

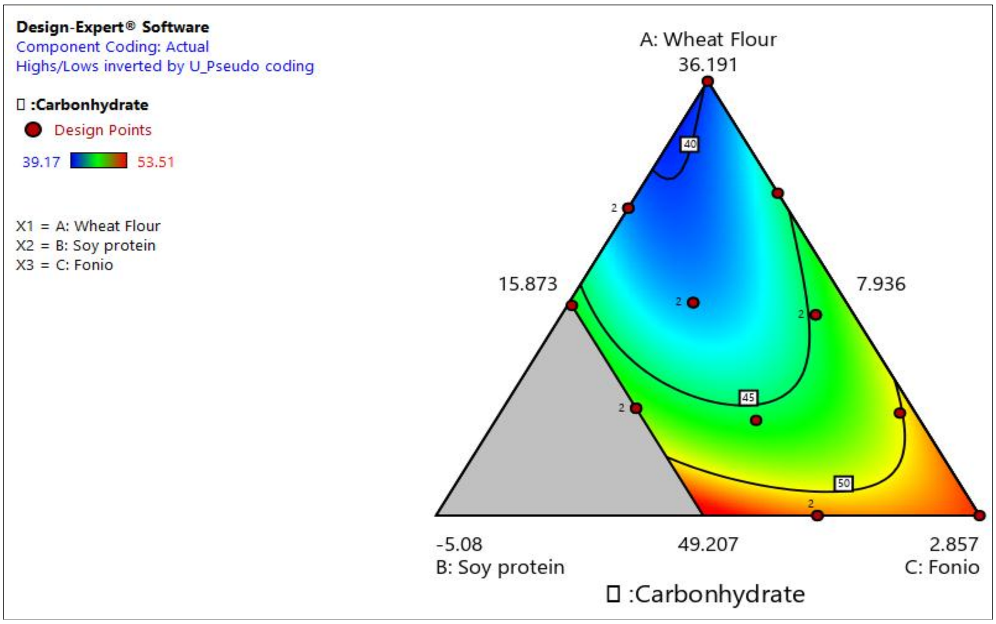

Fig 13: Mixture of wheat, soy protein isolate and fonio on Carbohydrate content of bread

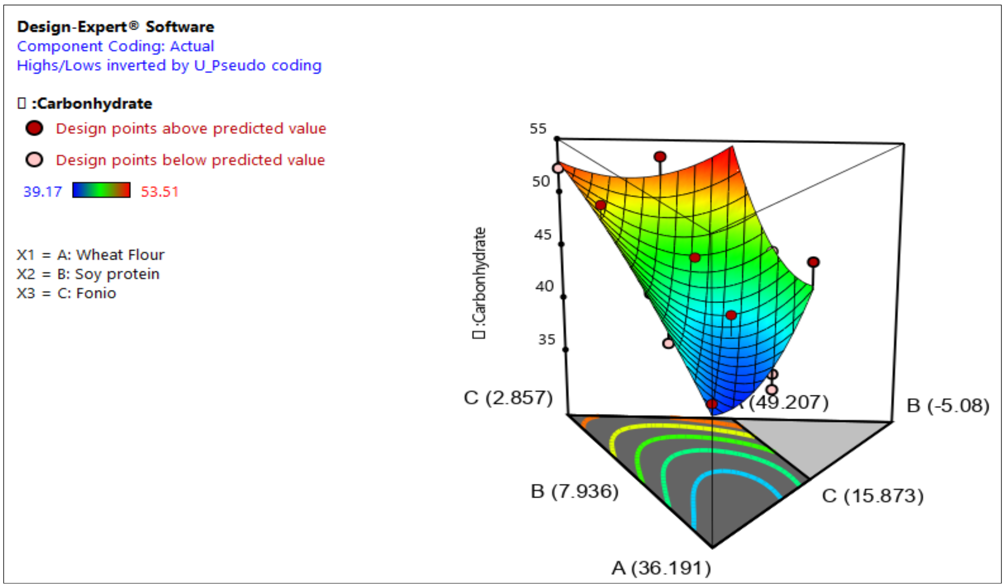

Fig 14: Three-dimensional surface plot of the carbohydrate content model

Conclusion

In this study, composite bread samples were produced with the aid of mixture design. The response surface methodology (RSM) was particularly effective in determining the effect of three independent variables; Wheat (A), Soy protein isolate (B) and Fonio (C) at constant temperature and time of 187.5 °C and 30min respectively on the proximate properties of composite bread. ANOVA indicated that the linear, quadratic and interactive effects of wheat (A), soy protein isolate (B) and fonio (C) significantly (p<0.05) affected all the composite bread properties evaluated. The emperical models fitted to the experimental data showed that the model was significant with the following: 13.03 (protein), 5.50 (fat content), 3.52 (crude fibre), 49.62 (moisture content), 6.13 (ash content) and 17.31% (carbohydrate). The bread with optimal quality indexes with overall desirability index of 0.568 was obtained from 39.42% wheat, 6.28% SPI and 13.76% Fonio. The properties of this optimal bread were: protein 17.25%, fat 8.83%, crude fibre 2.81%, moisture content 28.47%, ash content 2.80%, carbohydrate 41.35% and acceptability 3.56 respectively.

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