
*Journal of Scientific Research & Reports*
*2(2): 665-681, 2013; Article no. JSRR.2013.015*

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# Optimisation of Plantain - Brewers' Spent Grain Biscuit Using Response Surface Methodology

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors OSO and AOA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OOA managed the analyses of the study. Author AIO managed the literature searches. All authors read and approved the final manuscript.*

***Research Article***

***Received 20th May 2013***
***Accepted 7th August 2013***
***Published 27th August 2013***

## ABSTRACT

**Aim:** To optimize the formulation of plantain – brewers' spent grain biscuits using Response Surface Methodology with the purpose of achieving the maximum quality and overall acceptability of produced biscuits.
**Study Design:** Central composite design model of Response surface methodology was employed to design the work. This produced thirteen experimental runs, the variables considered were Brewer's spent grain (94-97 %) and plantain flour (3-6 %) while the responses were percentage soluble dietary fibre, percentage insoluble dietary fibre and iron contents.
**Place and Duration of Study:** Department of Food science and Technology, Federal University of Technology, Akure, Nigeria, between January to December 2012.
**Methodology:** The variables considered for the research were Brewer's spent grain (94-97 %) and plantain flour (3-6 %) while the responses were percentage soluble dietary fibre, percentage insoluble dietary fibre and iron contents. Thirteen (13) runs were obtained.The optimization was carried out on plantain flour and brewer's spent grain in order to know which of the combinations will give best total dietary fibre and iron content.
**Results:** Optimized values, which are the highest total dietary fibre and iron values of 68.55 % and 0.80 mg /100g respectively, were obtained from blends of 95.5 % and 4. 5 % of plantain flours and Brewer's spent grains respectively (PS 4). The best response

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surface model for the insoluble dietary fibre was quadratic that of soluble dietary fibre was linear while it was quadratic also for iron content. The lack of fit, (p=*0.4208)* is non significant. A non significant lack of fit is required confirming that, the model of is adequate. The $R^2$ value of 52 % signifies a good model. This is in view of the fact that $R^2$ must be at least 50 % to be a good model.
**Conclusion:** Response Surface Methodology was effective in optimizing iron and dietary fibre in the formulation of plantain-spent grain biscuit. The nutritional composition of the plantain-spent grain biscuit indicated good levels of dietary fibre (IDF, SDF and TDF), Iron.

*Keywords: Response surface methodology; plantain flour; brewers spent grain; total dietary fiber; iron content.*

## 1. INTRODUCTION

The role of diet in the prevention of human ailments such as cancer, cardiovascular diseases and obesity has become more evident and many consumers are increasingly seeking functional foods to improve their diets. Consequently, there is a trend to search for natural raw materials rich in dietary fibre (DF) and high in antioxidant capacity as functional ingredients for the food industry [1]. Major health benefits associated with increased intake of dietary fibre includes reduce risk of heart diseases, diabetes, obesity and some forms of cancer [2]. Dietary fibre intake in Western countries is currently estimated to be 25g per person per day; however, nutritionists recommend an intake 30 of 35 g per person per day [3]. The development of fibre-enriched foods could help consumers to meet such recommendations. Since the roles of dietary fibre in preventing and treating some diseases have been well documented, the addition of purified dietary fibre to foods has become popular. Brewer’s spent grain (BSG) refers to the extracted residue of barley malt, alone or in mixture with other cereal grains such as corn and rice grits, resulting from the manufacture of wort. Although it is the main by-product of the brewing industry, it is considered as a lignocellulosic material .BSG has received little attention as a marketable commodity [4]. Due to its chemical and nutritional composition, it’s potential use in food industries, such as bakery industry, pastry, flour confectionery goods, confectionery products are been exploited [5]. It is rich in dietary fiber, which range between 60–71% [6] and has high protein content of about 20% [7]. The ingestion of BSG or products containing BSG can aid the prevention of gastrointestinal disorders and cardiovascular diseases [8] and has been associated with increased faecal weight, accelerated transit time, increase cholesterol and fat excretion [9]. Plantain and banana (*Musa Spp*. L.) is a major starchy staple in sub-Saharan Africa, providing more than 25% of the carbohydrates and 10% of the daily calorie intake for more than 70 million people in the continent, the yield estimated in Western Africa is 26.4 tonnes per hectare [10]. Plantain flour can be made from unripe plantain which can be used in bakery industry, instead of using the expensive all-purpose wheat flour. Plantain for local consumption plays a role in food and income security and has the potential to contribute to national food security and reduce rural poverty. Response Surface Methodology (RSM) is an effective statistical technique for optimizing complex processes; it has been successfully applied to determine the optimum formulation for various food products, while evaluating sensory or physicochemical attributes [11,12,13]. Many people are dependent on snack foods as part of their daily diet [14], biscuit as snacks could therefore be a good vehicle for fibre consumption. The cost of importation of wheat flour in the production of different kinds of baked goods has been of major concern, hence the need to explore the potential of plantain (our local staple) and spent grain, in the production of rich fibre gluten free biscuits.

Therefore, the main objective of this study is to optimize the formulation of plantain – brewer's spent grain biscuits using Response Surface Methodology with the purpose of achieving the maximum quality and overall acceptability of produced biscuits.

## 2. MATERIALS AND METHODS

### 2.1 Biscuit Ingredients

Plantain, margarine and sugar were purchased from a local market in Akure, Nigeria. The defatted soy flour was supplied by Jof Ideal Family Farms, Owo, Nigeria. Brewers' spent grain was obtained from Nigerian Breweries Plc Ibadan, Nigeria; while Xanthan gum was obtained from a commercial analytical laboratory in Lagos, Nigeria. All chemicals used were of analytical grade.

### 2.2 Preparation of Plantain Flour

Plantain flour was produced from matured plantain using the method [15]. Green matured plantain fruits were washed to remove adhering soil particles, peeled and sliced into thin thickness of about 2 cm, soaked in 0.03% of sodium metabisulphite for 20 min and oven-dried at 65ºC for 48 h. The dried plantain slices were milled into flour using a disc attrition mill and sieved through 250µm aperture sieve. The flour was kept in an airtight container at 4ºC prior to use.

### 2.3 Treatment of Spent Grain

The spent grains were treated to remove residual sugar and alcohol. 100 g of brewer's spent grain (BSG) was made into a suspension by dissolving in 400 ml of water and fermented with yeast (0.8 g) for 1hr to convert its residual sugar to alcohol. The fermented suspension was subjected to distillation to remove residual alcohol. The pH of the distilled suspension was adjusted to about 6.0 –7.0 by adding 5% (w/v) sodium hydroxide solution in a solid: liquid ratio of 1:20 g: g, and dried to a moisture content of 5.06 ± 0.05% [16].

### 2.4 Production of Plantain-Spent Grain Biscuits

Plantain flour was composited with brewers' spent grain. The brewers' spent grain used in compositing ranged from 3-6%, xanthan gum 0.2%; water 20% (on 100% weight basis of 94 mixed flour). Xanthan gum was prepared by mixing with water at 40 ± 2ºC in the ratio of 1:2 [17]. Sugar (58%) and fat (28%) were initially creamed in a Kenwood mixer (Model AT 220A) for 3 mins at low speed to produce a creamy mixture before the flour and other dry ingredients (flour - 225g, soy flour - 10%, and xanthan gum - 0.2%) were added and blended. Thereafter, the mixture was thoroughly mixed with little water to form hard consistent dough. The dough obtained was thoroughly kneaded on a smooth clean board for about 5mins. The dough was thinly rolled on a wooden board with rolling pin to uniform thickness of 5mm and cut out (using biscuit cutter) to desired shapes of similar sizes. The cut out biscuit dough pieces were placed on a greased baking tray and baked in an electric oven (MK 9368) at 150 ± 5ºC for 20mins. The biscuits were cooled immediately after baking and vacuum packed in polyethylene bags and kept at 4ºC prior to use for analysis and sensory evaluation.

## 2.5 Experimental Design for Optimization of Formulation

Face-centred central composite design (CCF) was used; it is one of the three types of Box-Wilson central composite design. Central composite design is the most popular of the many classes of Response Surface Methodology (RSM) designs [18]. The independent variables were percentage of plantain flour and spent grain, low and high levels of the independent variables used were 94% and 97% for percentage of plantain flour and 3% and 6% for spent grain respectively (on weight basis of mixed flour). The ranges of plantain flour and spent grain have been selected by conducting preliminary experiments. The experiments plans in coded and uncoded form of variables, along with responses are given in Tables 1 - 3.

## 2.6 Determination of Proximate Composition of Biscuit

The proximate composition of biscuits was determined according to the standard methods of AOAC method 922.06 [19]. Carbohydrate content was calculated by difference and calculated energy value (Kcal/100g) was calculated using the Atwater factor (6kcal/g protein, 9kcal/g fat, and 4kcal/g total carbohydrate) [20]. Kcal was converted to Kj by multiplying values with 4.18.

## 2.7 Determination of Mineral Composition of Biscuit

Mineral determination: Mineral contents of biscuits were determined by Atomic Absorption Spectrometry and flame photometry according to AOAC method 965.09 [19].

Wet digestion of sample: For wet digestion of sample, exactly (1 g) of the grinded biscuit sample was taken in digesting glass tube. Twelve milliliters (12ml) of $HNO_3$ was added to the biscuit samples and mixture was kept overnight at room temperature. Then 4.0 ml perchloric acid ($HClO_4$) was added to this mixture and was kept in the fumes block for digestion. The temperature was increased gradually, starting from 50ºC to 250-300ºC. The digestion was completed in about 70 - 85 minutes as indicated by the appearance of white fumes. The mixture was left to cool down and the contents of the tubes were transferred to 100 ml volumetric flasks and the volumes of the contents were made to 100 ml with distilled water. The wet digested solution was transferred to plastic bottles labeled accurately. The digest was stored and used for mineral determination.

**Table 1. Factors and their levels for central composite design**

| | **Symbol** | **Coded levels** | | |
|---|---|---|---|---|
| | | **-1** | **0** | **+1** |
| Plantain | A | 94.00 | 95.50 | 97.00 |
| Spent grain | B | 3.00 | 4.50 | 6.00 |

**Table 2. Experimental design matrix by central composite design for three-level-two-factors response surface study for Insoluble, Soluble and Total dietary fibre of biscuits**

| Samples | Run | Factor 1 A:plantain | Factor 2 B:spent grain | Insoluble dietary fibre (%) | | | Soluble dietary fibre (%) | | | Total dietary fibre |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Actual value | Predicted value | Residual | Actual value | Predicted value | Residual | |
| PS1 | 1 | 97.00 | 3.00 | 49.63 | 48.47 | -0.99 | 13.62 | 13.40 | -0.38 | 63.25 |
| PS2 | 2 | 96.95 | 3.25 | 48.75 | 50.92 | -2.17 | 13.73 | 12.32 | 1.41 | 62.48 |
| PS3 | 3 | 95.57 | 4.43 | 46.39 | 48.91 | -0.89 | 16.96 | 15.33 | -1.71 | 63.35 |
| PS4 | 4 | 95.50 | 4.50 | 55.53 | 47.46 | 2.17 | 13.02 | 17.49 | 1.40 | 68.55 |
| PS5 | 5 | 95.43 | 4.57 | 56.35 | 50.02 | -3.63 | 8.39 | 13.40 | -0.38 | 64.74 |
| PS6 | 6 | 94.17 | 5.83 | 48.53 | 45.73 | 2.80 | 18.89 | 14.48 | -8.44 | 67.42 |
| PS7 | 7 | 94.09 | 5.91 | 46.55 | 52.62 | 2.91 | 6.04 | 17.49 | 1.40 | 52.59 |
| PS8 | 8 | 94.03 | 5.97 | 47.48 | 54.91 | 1.44 | 13.3 | 12.31 | 1.41 | 60.78 |
| PS9 | 9 | 94.00 | 6.00 | 48.02 | 52.62 | 2.91 | 15.97 | 10.39 | -2.00 | 63.99 |
| PS1 | 10 | 97.00 | 3.00 | 49.63 | 50.02 | -3.63 | 13.62 | 13.40 | -0.38 | 63.25 |
| PS2 | 11 | 96.95 | 3.25 | 48.75 | 50.92 | -2.17 | 13.73 | 12.32 | 1.41 | 62.48 |
| PS3 | 12 | 95.57 | 4.43 | 46.39 | 47.48 | -0.93 | 16.96 | 15.33 | -1.71 | 63.35 |
| PS4 | 13 | 95.50 | 4.50 | 55.53 | 47.46 | 2.17 | 13.02 | 17.49 | 1.40 | 68.55 |

**Table 3. Experimental design matrix by central composite design for three-level-two-factors response surface study for iron content of biscuit**

| Samples | Run | A:plantain | B:spent grain | Iron (mg/100g) | | Residual |
|---|---|---|---|---|---|---|
| PS1 | 1 | 97.00 | 3.00 | 0.33 | 0.45 | -0.12 |
| PS2 | 2 | 96.95 | 3.05 | 0.28 | 0.27 | 0.010 |
| PS3 | 3 | 95.57 | 4.43 | 0.51 | 0.54 | -0.033 |
| PS4 | 4 | 95.50 | 4.50 | 0.80 | 0.51 | -0.12 |
| PS5 | 5 | 95.43 | 4.57 | 0.29 | 0.43 | -0.14 |
| PS6 | 6 | 94.17 | 5.83 | 0.29 | 0.19 | 0.10 |
| PS7 | 7 | 94.09 | 5.91 | 0.31 | 0.57 | -0.26 |
| PS8 | 8 | 94.03 | 5.97 | 0.25 | 0.26 | -0.010 |
| PS9 | 9 | 94.00 | 6.00 | 0.51 | 0.54 | -0.033 |
| PS7 | 10 | 94.09 | 5.91 | 0.51 | 0.54 | -0.033 |
| PS7 | 11 | 94.09 | 5.91 | 0.77 | 0.64 | 0.13 |
| S7 | 12 | 94.09 | 5.91 | 0.51 | 0.54 | -0.033 |
| PS4 | 13 | 95.50 | 4.50 | 0.80 | 0.51 | 0.29 |

**Procedure:** The digested sample was analyzed for mineral contents by Atomic Absorption Spectrophotometer (Hitachi model 170-10) in the Biochemistry Department of the Federal University of Technology, Akure, Nigeria. Different electrode lamps were used for each mineral. The equipment was run for standard solutions of each mineral before and during determination to check that it is working properly. The dilution factor for all minerals except Mg was 100. For determination of Mg, further dilution of the original solution was done by using 0.5 ml of the original solution and enough distilled water was added to make the volume up to 100 ml. Also for the determination of Ca, 1.0 ml lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentrations of minerals recorded in parts per million [ppm] were converted to milligrams [mg] of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

MW = absorbency (ppm) x dry wt. x D ⁄ Wt. of sample x1000

**Note:** Dilution factor for magnesium is 10000 and for other minerals including calcium, iron, zinc and sodium is 100.

Determination of Sodium (Na) and Potassium (K) by flame photometer

Na and K contents were determined using flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK).

**Principle:** The flame photometer measures the emission of radiant energy when atoms of an element return to their ground state after their excitation by the high temperature of the flame. The degree of emission is related to the concentration of the element in the solution.

**Procedure:** Na and K analysis of the sample were done by flame-photometric method. The same wet digested food sample solutions as used in AAS were used for the determination of Na and K. Standard solutions of 20, 40, 60, 80 and 100 milliequivalent/l were used both for Na and K. The calculations for the total mineral intake involve the same procedure as given in AAS.

## 2.8 Determination of Dietary Fibre Content of Biscuits

The soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) of biscuit samples were determined using the enzymatic-gravimetric procedure [21].

## 2.9 Determination of Textural Characteristics of Biscuits

The breaking strength (load at break), hardness (energy at break) and toughness (extension at break) of the biscuits was measured by the triple beam snap (also called three-point break) technique using an Instron texture analyzer according to the method [22].

## 2.10 Determination of the Colour of Biscuits

The color of the biscuit was measured according to the method described by [23], with a hand held tri stimulus reflectance color meter. The color was recorded using a CIE–L*a*b* uniform color space (–Lab), where L* indicates lightness, a* indicates chromaticity on a green (-) to red (+) axis, and b* chromaticity on a blue (-) to yellow (+) axis.

## 2.11 Determination of the Physical Characteristics of Biscuits

The diameter was measured with a calibrated ruler as described by [24]. The thickness (T, mm) of biscuits was measured by stacking six biscuits on top of one another and taking the average value. The weight of the biscuits was measured by weighing on a weighing balance (model Mettler). The spread ratio was calculated as Weight/Thickness [25].

## 2.12 Sensory Evaluation of biscuits

Sensory evaluation of the samples was carried out for consumer acceptance and preference using ten (10) untrained panelists selected at random from the Federal University of Technology, Akure campus. The plantain-spent grain biscuit samples were evaluated for overall impression, taste, aroma, crispiness, colour and general acceptability using 9-point hedonic scale where 9 and 1 represent extremely like and extremely dislike respectively.

## 2.13 Statistical Analysis

The data obtained in the experiments (Table 1) were analyzed using response surface methodology, 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). In addition, analysis were carried out in triplicates and results subjected to analysis of variance (ANOVA) using the Statistical Package for Social Statistics (SPSS version 15). Means were separated using the Duncan's New Multiple Range Test (DNMRT).

## 3. RESULTS AND DISCUSSION

### 3.1 Statistical Analysis and Optimization

The effect of plantain flour and brewer's spent grain on the insoluble dietary fibre is shown in Fig. 1. Insoluble dietary fibre reduces with increasing plantain flour content and vice versa for BSG. A quadratic model was obtained from the ANOVA which had a final equation shown in eq. 1:

$$Insoluble\ dietary\ fibre = +52.62 - 2.89A + 0.85B + 0.43AB - 0.14A^2 - 0.86\,B^2 \quad (1)$$

This final equation also buttressed Fig. 1. Plantain flour (A) had negative effect on the insoluble dietary fibre as compared to brewer's spent grain (B) which had positive effect.

The effect of plantain flour and brewer's spent grain on the soluble dietary fibre was shown in Fig. 2. They both had positive effect on the soluble dietary fibre though plantain seemed to have higher effect on the soluble dietary fibre. This could be due to the higher percentage of plantain in the blend. The final equation obtained from ANOVA (eq.2) supported this also.

$$Soluble\ dietary\ fibre = +12.86 + 3.01A + 0.54B \quad (2)$$

The model was linear. 'A' and 'B' can be seen from the equation as having positive effects on the soluble dietary fibre. The contribution of plantain flour to the soluble dietary fibre was significant (*P= .05).*

The effect of plantain flour and brewer's spent grain on the iron content was shown in Fig. 3. As the amount of both increased so also was an increase observed in the iron content, the ANOVA gave a quadratic model with final equation shown in eq. 3.

$$Iron\ content = +0.58 + 0.20A + 0.21B - 0.081AB - 0.24A^2 - 0.076\,B^2 \quad (3)$$

Contribution of brewer's spent grain was however significant (*P= .05).*

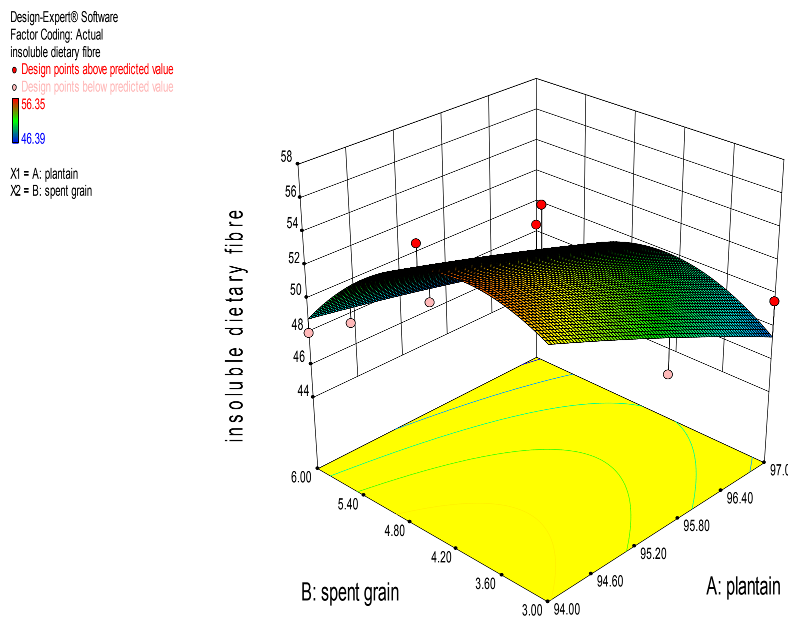


**Fig. 1. Effect of plantain and spent grain on the insoluble dietary fibre content of the biscuit**

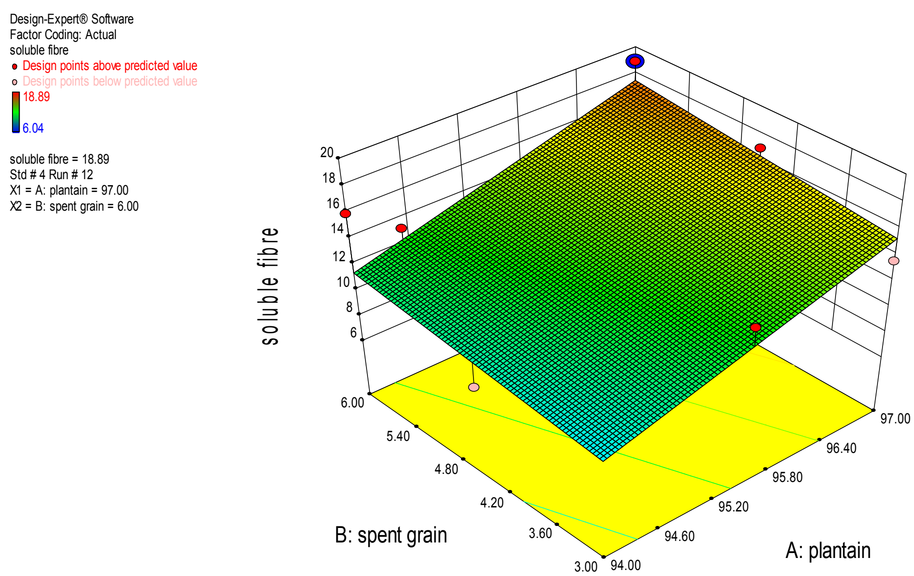


**Fig. 2. Effect of plantain and spent grain on the soluble fibre content of the biscuit**

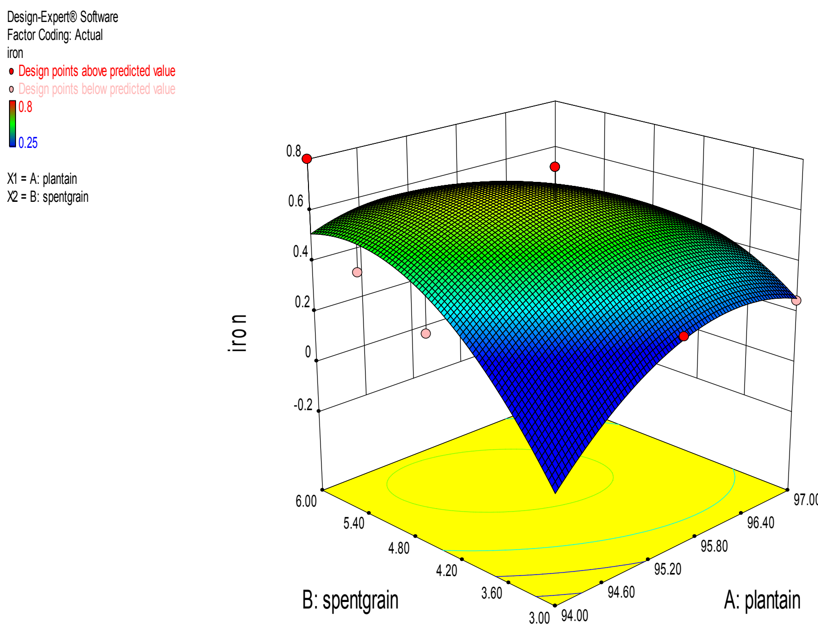


**Fig. 3. Effect of plantain and spent grain on the iron content of the biscuit**

## 3.2 Proximate Composition

The proximate composition of the plantain-spent grain (PS) biscuit obtained from different ratios of plantain flour and brewers' spent grain are presented in Table 4. The moisture content of the PS biscuit samples ranged between 2.87 to 4.93% with PS2 having the highest value and PS4 the lowest value. The low moisture content obtained implies that the product (plantain-spent grain biscuit) will be shelf stable. Biscuits differ from other products like bread and cake because of its low moisture content which makes it comparatively free from microbial spoilage thereby enhancing long shelf life of the product [26]. The values obtained in this study favourably compares with those reported by [27] for different types of commercial biscuits. The protein content of PS biscuits ranged between 7.33 to 9.09% with PS5 having the highest value, these value obtained could be attributed to the low protein content of plantain. Fat content of biscuits ranged between 15.14 to 19.64% with PS2 having the highest value, these values are within the recommended range of 10 – 25g oil per 100g [28] which is the guidelines for formulation of supplementary foods for young children. Biscuits could also be of good benefits to children. The crude fibre ranged between 0.69 to 2.11% with PS2 having the highest value. These values are within the recommended range of not more than 5g fibre and other non - absorbable carbohydrates per 100g dry matter [28]. The high carbohydrate values might be attributed high carbohydrate content of plantain; [29] reported a carbohydrate content of 78.72% for plantain flour. The energy value ranged between 444.00 kcal to 487.07 kcal, values obtained ranged within the recommended value for estimated energy intake of children of age range 1-24 months which is 479 - 1148Kj/ day [30]; recommended daily energy intake varies with height and age. The energy value of food is very important as it helps in determining the fuel value of food.

**Table 4. Proximate composition of plantain-spent grain (PS) biscuit in g/100g and energy value (kcal/100g) (*Dry matter basis*)**

| Sample | Moisture | Fat | Ash | Protein | Carbohydrate | Crude fibre | Energy value |
|---|---|---|---|---|---|---|---|
| PS1 | 4.13 | $18.14^{abc}$ ±1.22 | 1.39 ±0.60 | $7.61^{ef}$ ±1.10 | 71.37 ±2.25 | $0.87^{ab}$ ±0.30 | 479.18 |
| PS2 | 4.93 | $19.64^{a}$ ±1.30 | 2.46 ±1.62 | $7.55^{ef}$ ±0.04 | 68.26 ±3.92 | $2.11^{a}$ ±1.06 | 480.00 |
| PS3 | 4.60 | $18.87^{abc}$ ±2.11 | 2.45 ±0.61 | $7.64^{e}$ ±0.11 | 70.33 ±2.85 | $0.70^{b}$ ±0.30 | 481.71 |
| PS4 | 2.87 | $17.85^{abc}$ ±2.37 | 1.37 ±0.60 | $7.33^{g}$ ±1.12 | 72.27 ±3.01 | $1.20^{ab}$ ±0.79 | 479.05 |
| PS5 | 3.13 | $15.14^{c}$ ±2.38 | 1.72 ±0.60 | $9.09^{a}$ ±1.13 | 73.18 ±2.90 | $0.86^{ab}$ ±0.30 | 465.34 |
| PS6 | 4.40 | $15.34^{bc}$ ±4.33 | 2.09 ±0.01 | $8.17^{c}$ ±0.11 | 68.42 ±4.70 | $1.57^{ab}$ ±1.05 | 444.42 |
| PS7 | 3.00 | $17.18^{abc}$ ±1.19 | 2.41 ±0.60 | $7.46^{fg}$ ±0.11 | 71.36 ±0.51 | $1.03^{ab}$ ±0.89 | 469.90 |
| PS8 | 3.07 | $16.51^{abc}$ ±0.08 | 2.07 ±1.04 | $8.61^{b}$ ±0.07 | 71.97 ±0.87 | $0.86^{ab}$ ±0.30 | 470.91 |
| PS9 | 3.53 | $19.35^{ab}$ ±1.20 | 1.73 ±1.19 | $7.98^{d}$ ±0.03 | 70.25 ±0.86 | $0.69^{b}$ ±0.30 | 487.07 |

*Values are mean ± standard deviation of triplicate samples.*
*Values on vertical row with the same superscript are not significantly different at P = .05*

## 3.3 Mineral Composition

The Calcium, Magnesium, Zinc, and Iron contents of biscuits, are comparably high (Table 5), Potassium (K) ranged between 41.80 and 53.90 mg/100g; Sodium (Na) ranged between 55.50 and 73.30mg/100g; Iron (Fe) ranged between 0.25 and 0.80mg/100g; calcium (Ca) ranged between 4.20 and 7.70mg/100g; Magnesium (Mg) ranged between 1.49 and 2.14mg/100g; Zinc (Zn)ranged between 0.12 and 0.30mg/100g while Copper (Cu) ranged between 0.01 and 0.09mg/100g. K and Na are reported to be the most abundant mineral in the biscuit. Both sodium and potassium are required to maintain osmotic balance of the body fluids, the pH of the body, to regulate muscle and nerve irritability, control glucose absorption, and enhance normal retention of protein during growth [31]. The Fe content of biscuits are higher than values reported by [16], in spent grain enhanced cookies, this may be due to the high iron content of plantain, which is the basic raw material, biscuits from this study therefore would have a positive effect on the packed cell volume (PCV) of the blood, which has direct relationship with the heamoglobin content of the blood.

**Table 5. Mineral composition of plantain-spent grain (PS) biscuit (mg/100g)**

| Biscuits | K | Na | Fe | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|
| PS1 | 51.10 | 72.60 | 0.25 | 4.50 | 2.14 | 0.30 | 0.05 |
| PS2 | 52.60 | 73.30 | 0.28 | 6.60 | 2.14 | 0.14 | 0.05 |
| PS3 | 51.20 | 64.60 | 0.33 | 6.90 | 2.14 | 0.14 | 0.09 |
| PS4 | 50.20 | 59.60 | 0.77 | 5.50 | 1.49 | 0.15 | 0.04 |
| PS5 | 50.20 | 55.50 | 0.29 | 4.30 | 2.12 | 0.20 | 0.07 |
| PS6 | 48.70 | 61.50 | 0.29 | 7.70 | 1.78 | 0.13 | 0.05 |
| PS7 | 50.70 | 59.00 | 0.51 | 4.20 | 2.14 | 0.12 | 0.01 |
| PS8 | 53.90 | 72.20 | 0.31 | 6.60 | 1.58 | 0.16 | 0.10 |
| PS9 | 41.80 | 57.00 | 0.80 | 5.30 | 2.11 | 0.23 | 0.05 |

*Values are mean values of triplicate readings*

## 3.4 Total Dietary Fiber

The total dietary fibre (TDF), which is the summation of soluble fibre (SDF) and insoluble fibre (IDF) of PS biscuits are shown in Table 2, Figs. 1 and 2. TDF ranged from 52.59 to 68.55% with PS4 having the highest value and PS7 with the lowest value, SDF ranged from 6.04 to 18.89% with PS6 having the highest value while PS7 had the lowest value, IDF ranged from 46.55 to 56.35% with PS5 having the highest value while PS7 has the lowest value. The values obtained are fairly comparable to the values reported by [25], when10% white grape pomace was used as a source of dietary fibre in wheat biscuit (TDF=64.86, SDF= 9.46 and IDF=55.41)and higher than those reported by [32], who made dietary fibre enriched biscuit from cassava based composite flour (TDF=1.61-3.40, IDF=1.21-2.18) and also higher than values obtained by [33], who reported maize and distillers spent grain for 'kokoro' production. The high values obtained might be due to the fact that both plantain and spent grain used in biscuit production are rich sources of dietary fibre. A high intake of DF is positively related to different physiological and metabolic effects [33]. Insoluble fibre prevents constipation, soluble fibre helps to reduce the cholesterol level in the blood, slows down digestion and sudden release of energy, thus making blood level stable. The IDF/SDF ratio, which is nutritionally significant, agrees with the report of [25] that the proportion of SDF and IDF in the diet should be between 1:4 and 1:3. Soluble fibre reduces the risk of heart diseases and lowers blood levels of cholesterol, triglycerides and glucose. On the other hand, the insoluble fraction has a positive influence on the colon. Food containing at least 3g⁄100 g dietary fiber (DF) can be referred to as a source of DF; it is high in DF when it contains at least 6 g⁄100 g dietary fibre [34]. The consumption of biscuits will meet the WHO recommendation for dietary fiber intake of about 25 g $day^{-1}$ [35].

## 3.5 Colour Characteristics of Biscuits

The biscuit colour is shown in Table 6. The L*, a*, b* are not significantly different (P =.05) from each other. L* value ranged between 57.76 and 68.51, a* ranged from 5.70 – 7.01, while b* ranged from 15.41 – 18.05, the variation in the level of plantain and spent grain seems to have significant effect (*P= .05*) on the lightness, chromaticity on a green (-) to red (+) axis, and chromaticity on a blue (-) to yellow (+) axis of the biscuit. Food product colour is a very important characteristic which influences the consumer acceptability.

**Table 6. Colour characteristics of plantain-spent grain biscuit**

| Sample | L* | a* | b* | Hue | Chroma | Whiteness index |
|---|---|---|---|---|---|---|
| PS1 | $64.84^{bc}$ ±0.71 | $7.01^{b}$ ±0.30 | $17.94^{a}$ ±0.23 | 68.66 | 19.26 | -306.14 |
| PS2 | $68.51^{a}$ ±0.29 | $5.70^{e}$ ±0.17 | $18.05^{a}$ ±0.27 | 72.47 | 18.93 | -289.78 |
| PS3 | $66.01^{b}$ ±0.18 | $6.47^{cd}$ ±0.09 | $17.71^{a}$ ±0.36 | 69.93 | 18.85 | -289.50 |
| PS4 | $64.20^{c}$ ±0.97 | $6.57^{c}$ ±0.13 | $17.84^{a}$ ±0.76 | 69.78 | 19.01 | -297.23 |
| PS5 | $60.73^{d}$ ±0.53 | $7.47^{a}$ ±0.24 | $17.80^{a}$ ±0.73 | 67.23 | 19.30 | -311.91 |
| PS6 | $61.02^{d}$ ±0.34 | $7.35^{a}$ ±0.07 | $17.04^{a}$ ±0.55 | 66.67 | 18.56 | -283.36 |
| PS7 | $57.76^{e}$ ±0.31 | $7.38^{a}$ ±0.17 | $15.41^{b}$ ±0.12 | 64.41 | 17.09 | -234.17 |
| PS8 | $65.02^{bc}$ ±1.55 | $6.53^{c}$ ±0.13 | $17.25^{a}$ ±0.48 | 69.27 | 18.44 | -275.18 |
| PS9 | $63.93^{c}$ ±0.79 | $6.16^{d}$ ±0.31 | $17.13^{a}$ ±0.93 | 70.22 | 18.20 | -267.45 |

*Values are mean ± standard deviation of triplicate samples*
*Values on vertical row with the same superscript are not significantly different (P= .05)*

## 3.6 Physical Parameters of Biscuit

The physical parameters of the biscuits are shown in Table 7. The thickness and diameter of the biscuits ranged between 0.40 to 0.48cm and 5.67 to 5.77cm respectively. The thickness and diameter obtained for PS biscuit fairly compares with values reported for biscuits by [17], 0.66 to 0.68 cm and 5.12 to 5.46cm (for wheat and rice bran); 0.66 to 0.68cm and 5.48 to 5.60cm (wheat and oat bran) and 0.54 to 0.60cm and 5.12 to 5.39cm reported for wheat and barley bran. No significant difference (*P=.05*) was observed in the thickness and the diameter of the biscuits. The weight of the biscuits ranged between 7.33 and 9.07g, no significant difference was observed in the weight of the biscuits. The volume of the biscuits ranged between 10.02- 11.94$cm^3$. The spread ratio ranged between 11.89 and 14.18, values were higher than values reported by [17]. The spread ratio could have been affected by the competition of ingredients for the available water and other functional properties of proteins and fat, which may invariably, affect the texture and eating quality of the biscuit.

**Table 7. Physical parameters of plantain-spent grain biscuit**

| Sample | Thickness(cm) | Diameter(cm) | Weight(g) | Volume(cm$^3$) | spread ratio | Density(g/cm$^3$) |
|---|---|---|---|---|---|---|
| PS1 | $0.48^a$ ±0.01 | 5.67 ±0.06 | $9.07^a$ ±1.17 | $11.94^a$ ±0.14 | $11.89^c$ ±0.23 | $0.75^{abc}$ ±0.11 |
| PS2 | $0.43^{bc}$ ±0.01 | 5.67 ±0.06 | $8.37^{ab}$ ±0.35 | $10.69^{bc}$ ±0.15 | $13.28^b$ ±0.28 | $0.78^{abc}$ ±0.05 |
| PS3 | $0.43^{bc}$ ±0.01 | 5.67 ±0.06 | $9.37^a$ ±1.78 | $10.69^{bc}$ ±0.15 | $13.28^b$ ±0.04 | $0.88^a$ ±0.16 |
| PS4 | $0.43^{bc}$ ±0.01 | 5.67 ±0.06 | $9.20^a$ ±0.26 | $10.69^{bc}$ ±0.15 | $13.28^b$ ±0.27 | $0.86^{ab}$ ±0.03 |
| PS5 | $0.44^b$ ±0.01 | 5.67 ±0.06 | $7.73^{ab}$ ±0.60 | $11.03^b$ ±0.25 | $12.88^b$ ± 0.19 | $0.70^c$ ±0.05 |
| PS6 | $0.40^d$ ±0.02 | 5.67 ±0.06 | $8.20^{ab}$ ±0.61 | $10.02^d$ ±0.50 | $14.18^a$ ±0.59 | $0.82^{abc}$ ±0.08 |
| PS7 | $0.40^d$ ±0.02 | 5.67 ±0.06 | $8.40^{ab}$ ±0.62 | $10.02^d$ ±0.50 | $14.18^a$ ±0.59 | $0.84^{abc}$ ±0.04 |
| PS8 | $0.41^{cd}$ ±0.01 | 5.77 ±0.06 | $7.33^b$ ±0.72 | $10.27^{cd}$ ±0.25 | $14.07^a$ ±0.47 | $0.71^{bc}$ ±0.08 |
| PS9 | $0.44^b$ ±0.02 | 5.77 ±0.06 | $8.53^{ab}$ ±0.55 | $10.94^b$ ±0.38 | $13.22^b$ ±0.57 | $0.78^{abc}$ ±0.03 |

*Values are mean ± standard deviation of triplicate samples*
*Values on vertical row with the same superscript are not significantly different at P = .05*

### 3.7 Sensory Parameters

The mean score for all the parameters analyzed are shown in Table 8. Taste, aroma, colour, crispiness and general acceptability ranged from 7.40 to 7.60, aroma from 7.30 to 7.90, colour from 8.40 to 8.50,crispiness from 8.40 to 8.60 and the general acceptability was rated 7.60 for all the biscuit samples. This implies that all the biscuit samples are generally and equally acceptable with no significant difference in all the sensory parameters analyzed (taste, colour, aroma, crispiness, and general acceptability) which is in agreement with the report of [16]. The result is fairly comparable to the report of [16] for 3 and 6% level of incorporation of spent grain for cookies formulation where the mean values ranging between 6 and 8 were obtained for all sensory parameters checked. Consumers quality is a major factor for selecting a product and among the main characteristics related to quality are texture, taste and surface colour of a biscuit. The result shows that plantain and spent grain can be combined in any of the ratio to produce a sensorially acceptable biscuit with high nutritional quality.

**Table 8. Sensory parameters of plantain-spent grain biscuits**

| Sample | Taste | Aroma | Colour | Crispiness | General acceptability |
|---|---|---|---|---|---|
| PS1 | 7.50 ±0.71 | 7.40 ±1.26 | 8.50 ±0.53 | 8.50 ±0.53 | 7.60 ±0.70 |
| PS2 | 7.40 ±0.52 | 7.30 ±0.67 | 8.50 ±0.53 | 8.50 ±0.53 | 7.60 ±0.63 |
| PS3 | 7.40 ±0.52 | 7.80 ±0.92 | 8.50 ±0.52 | 8.60 ±0.52 | 7.60 ±0.67 |
| PS4 | 7.60 ±0.52 | 7.90 ±0.74 | 8.50 ±0.53 | 8.50 ±0.53 | 7.60 ±0.57 |
| PS5 | 7.40 ±0.52 | 7.60 ±0.84 | 8.50 ±0.53 | 8.40 ±0.52 | 7.60 ±0.67 |
| PS6 | 7.50 ±0.53 | 7.70 ±0.48 | 8.50 ±0.71 | 8.50 ±0.53 | 7.60 ±0.70 |
| PS7 | 7.60 ±0.52 | 7.80 ±0.42 | 8.40 ±0.52 | 8.50 ±0.53 | 7.60 ±0.48 |
| PS8 | 7.50 ±0.53 | 7.50 ±0.53 | 8.40 ±0.70 | 8.60 ±0.52 | 7.60 ±0.52 |
| PS9 | 7.50 ±0.53 | 7.60 ±0.70 | 8.40 ±0.70 | 8.50 ±0.53 | 7.60 ±0.70 |

*Values are mean ± standard deviation of triplicate samples. Values on vertical row with the same superscript are not significantly different at P =.05*

## 4. CONCLUSION

Response Surface Methodology was effective in optimizing iron and dietary fibre in the formulation of plantain-spent grain biscuit. Optimized values, with the highest total dietary fibre and iron values of 68.55 % and 0.80 mg /100g respectively, were obtained from blends of 95.5 % and 4. 5 % of plantain flours and Brewer's spent grains respectively (PS 4). The nutritional composition of the plantain-spent grain biscuit indicated good levels of dietary fibre (IDF, SDF and TDF), Iron. Plantain-spent grain biscuits will serve as vehicle for increasing intake of dietary fibre and iron. The utilization of plantain-spent grain will not only reduce post harvest losses of plantain it will increase utilization of spent grain and invariably reduce the burden of waste disposal in brewery.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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