

## Functional Biscuits from Wheat, Soya Bean and Turmeric (*Curcuma Longa*): Optimization of Ingredients Levels Using Response Surface Methodology

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### ABSTRACT

This study aimed at producing composite biscuits from wheat flour, soya bean and turmeric (*Curcuma longa*) as a functional food. An experimental design was used to optimize the mixture of the variables by mixture response surface methodology. This produced eight experimental runs. The variables considered were wheat flour, soybean flour, and turmeric powder, while the responses were moisture content, protein content, fat content, DPPH free radical scavenging power, phenolic content, total antioxidant and reducing power. The colour characteristics and the sensory attributes were analysed and compared with those of wheat biscuit (100% refined wheat flour). Turmeric powder had the highest positive influence on the antioxidant properties while soybean had the highest effect in improving the nutritional composition (protein,) of the biscuits. The optimum composite flour blends for biscuit production consisted of 72.88%, 26.63% and 0.5% of wheat flour, soybean flour and turmeric respectively. The optimum combination consisted of 6.59% moisture, 21% fat, 13.01% protein, 48.77% DPPH, 0.74mgGAE/g phenolic, 3.78mgGAE/g total antioxidant and 1.59mg/ml reducing power. Regression analysis of the model indicates that significant interaction exists between the ingredients with high coefficient of determination (R<sup>2</sup>). The high R<sup>2</sup> values showed that the model developed for the responses appeared adequate for predictive purposes. The experimental and predictive values were closely related, showing that the models correctly predicted the response variables. From this study, it can be concluded that addition of turmeric into composite flour biscuit will improve its nutritional composition and functionality.

**Keywords:** Turmeric, Antioxidant activity, Biscuits, Variables optimization, mixture response surface.

### INTRODUCTION

Biscuits have become traditional and significant food in many countries. This may be as a result of variety in form and taste combined with long shelf life and convenience of use. Biscuits are eaten majorly not for any other reasons than that they are pleasant and provide a snack or complement to other food. They are thought of as a source of pleasure and energy but not a significant item of nutrition. Biscuit is also known as an excellent vehicle for incorporation of different nutritionally rich ingredients, thus making it a useful tool in meeting the nutritional requirements of the increasing population. Fortification of wheat flour with non-wheat proteins increases protein quality by improving its amino acid profiles. Biscuits enriched with protein, usually from soy flour and caseinate,

have been developed for special feeding programmes usually for children in developing countries. Work has been done by Akbar *et al.*, (2016), Apotiola and Fashakin (2013), Okoye *et al.*, (2008), and Banureka and Mahendran (2009) to improve the protein content of biscuit.

Soybean is among the major industrial and food crops grown in every continent and have long been recognized as a plant food that, when compared with other plants, is relatively high in protein. Soy protein is the only vegetable source of complete protein, of a quality comparable to meat and eggs, which contains all the essential amino acids required by humans and animals. It also contains linoleic (omega-6) and alpha-linolenic acid (omega-3), both polyunsaturated fatty acids that are essential nutrients but also increasingly recognised to reduce the risk of chronic age-related diseases such as cancer and

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cardiovascular disease.(FAO, 1999). Soybean has been used effectively in bakery products (e.g. biscuit, bread etc.) to improve protein content.

Interest in the use of spices for bakery products had grown rapidly because of the increasing consumers awareness on functional foods. Turmeric (*Curcuma longa*), a native of tropical South Asia. It is a perennial plant belonging to the ginger family, Zingiberaceae. Turmeric contains 6.3% proteins, 5.1% fat, 3.5% minerals, 69.4% carbohydrate, and 13.1% moisture. The major chemical compound in turmeric is (5V) diaryl-heptanoid colouring material known as curcuminoids (Tokusogluet *al.*.2015. Rohite:*al.*. 2014). Turmeric is also a good source of the co-3 fatty acid and  $\alpha$ -linolenic acid (2.5%) (Goudet *al.*, 1993). Curcuminoids are the most important chemical components of turmeric and it includes curcumin (diferuloylmethane), demethoxy curcumin, and bisdemethoxy curcumin. Curcumin, a yellow pigment from *curcuma longa*, is a major component of turmeric and is commonly used as a spice and food colouring material. It exhibits antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic properties. Turmeric has been reported to have a role in preventing human diseases such as cancer (as antioxidants and anti-carcinogenic) and cardiovascular diseases (Prathapaner *al.*, 2009). These medical properties have been credited mainly to the curcuminoids which are abundant in turmeric rhizome (Maheshwari *et al.*, 2006). The aim of the present study was to formulate a biscuit (using composite flour - wheat and soybean flour) with functional attributes as well as optimum nutritional and sensory attributes by incorporating *curcuma longa*.

### MATERIALS AND METHODS

#### Materials

Commercially available wheat flour, soybeans seed and other ingredients used for this study were procured from bodija market in Ibadan, Oyo state, Nigeria. Fresh turmeric rhizome used was procured from a local market in Akure, Ondo state, Nigeria. All chemicals used were of analytical grade.

#### Methods

##### Production of Soybean Flour

The soybean flour was prepared according to

the method described by Ihekoronye and Ngoddy (1985). During preparation, the soybean seeds were sorted to remove dirt and other foreign particles such as stones, sticks and leaves, washed and soaked in clean water for 8 hours. Thereafter, the seeds were drained, dehulled manually, boiled (100°C for 30min) and dried in cabinet dryer (65°C for 6hr). During drying, the dehulled seeds were stirred at intervals of 30 minutes to ensure uniform drying. The dried seeds were milled (attrition mill) and sieved to obtain soybean flour which was finally packaged in an air tight container for further use.

##### Production of turmeric powder

The fresh turmeric rhizome were cleaned under running tap water and then placed on a rack at room temperature for 2hr to drain. After which the cleaned turmeric rhizomes were sliced into thin layer and then sundried. The dried turmeric was then grinded into powder.

##### Experimental design for the preparation of composite flour

The experimental design for the production of wheat, soybean and turmeric composite flours were carried out using the optimum mixture design of response surface methodology (Design-Expert software version 7.0.0) which gave 8 experimental runs as shown in table 1. The variables were wheat (X1), soybean (X2) and turmeric(X3) flours while the responses were moisture content, fat content, protein content and antioxidant properties (DPPH, total antioxidant, phenolic and reducing power). The proportion of each flour was expressed as a fraction of the mixture and for each treatment combination giving the sum of the component proportion as 100.

##### Biscuit Production

The formulation used in the study was calculated for 100 g of flour mixture; 40 g of margarine, 30 g sugar, 1 g baking powder and 1 g salt were used. The margarine and the sugar were creamed together by a mixer to produce a creamy mixture. After mixing, the sieved composite flour, spice, baking powder and salt were slowly introduced into the mixture to form hard consistence dough. The dough obtained was thinly rolled on a flat rolling board to a uniform thickness using wooden rolling pin. Biscuit cutter was used to cut the dough into desired shapes of similar sizes, after which they were placed in greased

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baking trays. It was baked in a pre-heated oven at 180°C for 20 minutes. The biscuits were cooled immediately after baking and packaged individually in an air tight container and kept at

room temperature until used for analysis and sensory evaluation. Wheat biscuits were similarly baked as reference.

**Table1.** Experimental design used to produce the composite flour.

| Sample | Wheat (g) (X <sup>1</sup> ) | Soybean (g) (X <sub>2</sub> ) | Spice (g) (X <sub>3</sub> ) |
|--------|-----------------------------|-------------------------------|-----------------------------|
| A      | 89.49                       | 10.00                         | 0.50                        |
| B      | 89.25                       | 10.00                         | 0.75                        |
| C      | 70.00                       | 29.03                         | 0.97                        |
| D      | 79.33                       | 20.00                         | 0.97                        |
| E      | 74.79                       | 24.65                         | 0.55                        |
| F      | 86.63                       | 12.3"                         | 1.00                        |
| G      | 70.00                       | 29.50                         | 0.50                        |
| H      | 86.63                       | 12.3"                         | 1.00                        |
| I      | 100                         | -                             | -                           |

### Determination of Proximate Composition of Biscuit

The moisture content, fat content and protein content of the biscuit produced were carried out using AOAC (2010) methods.

Anti-oxidant properties of the biscuits DPPH radical scavenging activities of biscuit extracts The free radical scavenging ability of the sample against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was also evaluated (Ursini *et al.*, 1994). 0.2ml of the sample aliquot (0.5g of the biscuit sample homogenized in 20ml methanol) was mixed with 7.6ml, 0.4ml methanolic solution containing 1,1DPPH radical, the mixture was left in the dark for 30min before measuring the absorbance at 517nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\% inhibition)} = [(A_0 - A_e) / A_0] \times 100$$

Where A<sub>e</sub> was the absorbance of sample and A<sub>0</sub> was the absorbance of the control. The % inhibition value was plotted against concentration of the sample extraTotal antioxidant activity of extracts from the biscuitsTotal antioxidant activity of extract was evaluated by the formation of phosphomolybdenum complex (Prieto *et al.*, 1999) 0.1ml ethanolic solution of extract (100ug/ml) was added to 1.9ml of reagent solution (0.6M H<sub>2</sub>SO<sub>4</sub> 28Mm sodium phosphate and 4Mm ammonium molybdate). The blank solution contained only 2ml of reagent solution .The absorbance was measured at 695nm after 90 minutes.

### Total Phenolic Content of the Biscuits

The concentration of phenolic in the biscuit

sample powder was determined using spectrophotometric method. (Singleton *et al.*, 1999). Ethanol solution of the extract in the concentration of 1mg/ml was used in the analysis. The reaction of the mixture was prepared by mixing 1ml of ethanol solution of extract, 2.5ml of 10% Folin Ciocalteu reagent dissolved in water and 2ml of 7.5% NaHCO<sub>3</sub> is added, blank was prepared. The sample was left for 30min. The absorbance was determined using spectrophotometer at 610nm, the sample was prepared in triplicate for each analysis. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed, based on the measured absorbance, the concentration of phenolic was read (mg/g) from the calibration line; then the content of phenolic in extract was expressed in terms of gallic acid equivalent (mg GAE/g of extract).

### Reducing power of the Biscuit Samples

The reducing properties of the biscuit sample were determined by assessing the ability of the sample to reduce FeCl solution as described by Pulido *et al.*, (2000). 1ml of the sample aliquot (0.5g of the sample homogenized in 20ml ethanol) was mixed with 2.5ml, 200Mm sodium phosphate buffer (PH6.6) and 2.5ml of 1g/100ml potassium ferrocyanide, the mixture was incubated at 5°C for 20mins. 2.5ml, 10ml. and 100ml Trichloroacetic acid were added and subsequently centrifuged at 650rpm for 10min, 2.5ml of supernatant was mixed with equal volume of water and 0.5ml of ferric chloride .the absorbance was later measured at 700nm. a higher absorbance indicate a higher reducing power. Standard curve of iron (II) sulfate solution (200, 400, 600, 800 and 1000 ppm) was prepared using the similar procedure.

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The results were expressed as mg Fe (II) 100 g extract sample.

### Colour Determination of Biscuit Samples

Color determination was carried out on the biscuits using a handheld Chromameter (model CR-400, Konica Minolta, Japan). The obtained results were expressed in terms of L\* (lightness), a\*(redness to greenness-positive to negative values, respectively), and b\* (yellowness to blueness -positive to negative values, respectively) values.

### Sensory Evaluation

Sensory analysis of biscuits supplemented by different levels of substitutions of soybean flour and turmeric powder was conducted and compared with control biscuits (100% wheat flour). Sensory parameters such as appearance, taste, colour, crispiness, aroma and general acceptability were analysed, 20 panelists from within the university community were used for this test. The rating were on a 9-point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely).

### Statistical Analysis

The results of the *sensory* analysis were evaluated by analysis of variance (ANOVA) at a significant level of 5%. Data obtained from other analysis were analysed using the Response Surface Methodology so as to fit the quadratic polynomial equation generated by the Design-Expert software version 7.0.0. In order to correlate the response variable to the independent variables, multiple regressions was used to fit the coefficient of the polynomial model of the response. The quality of the fit of model was evaluated using analysis of variance (ANOVA).

## RESULT AND DISCUSSION

### Proximate Composition of the Biscuit Samples

Table 2 showed the proximate composition of the composite biscuit obtained mixture design. Moisture content

The moisture content of the composite biscuit ranged from 5.43% to 7.78% with sample A having the lowest moisture content while sample G had the highest content. The moisture content of a product is an index of the shelf life of that product. The moisture content of the composite biscuits studied was within the recommended range of 0-10% for storage of

biscuits without microorganism degradation of the triglyceride (Singh *et al.* 2000). The moisture content of all the composite biscuits confers reasonable extent shelf stability on the sample. Table 3 showed the coefficients estimate and adjusted regression coefficients ( $R^2$  adjusted) for the proximate composition of the biscuits, the analysis of variance (Anova) for the moisture content showed that the full quadratic model was significant in predicting the moisture content of the biscuits. The model which explained the relationship between the moisture content of the biscuits and the independent factors (A, B, and C) is shown in Equation 1:

$$\text{Moisture content} = 5.43A + 7.78B - 4.771.06C - 6.7AB + 4979.98AC + 4823.06 BC \quad (1)$$

From the equation, it was observed that increasing the linear and binary combination where turmeric was increased had a decreasing effect on the moisture content of the biscuits. This is however -good for the biscuits as it discourage microbial growth thereby increasing storage quality (Singh *et al.*,2000). Turmeric exhibited the highest effect in decreasing the moisture content of the biscuit than wheat and soybean flour. The  $R^2$  adj. for the moisture content was 0.9996. This high value suggests that the effect of the independent variables contributed 99.96% of the observed changes in the moisture content while the remaining 0.04% changes in the moisture content was caused by other variables which served as noise in the experiment.

### Fat Contents

The result of the fat content of the biscuits is presented in Table 2. The table revealed that the fat content ranged from 18.29% to 21.81% with the control sample (100% wheat flour) having the lowest fat content while sample G had the highest fat content. The high fat content of the biscuits might be due to the fat added as recipe in the production of the products. The high value recorded for sample G. C. and E could be attributed to increase in soybean flour in the composite flour as it had been reported that soybean, an oilseed plant possess high fat content (Enwere, 1998). The coefficient estimate,  $R^2$  adj., and lack of fit for the fat content are presented in Table 5. The final equation for the fat content is presented in Equation 2. The high fat content of the biscuits indicate high energy value. A disadvantage is that high fat content could affect the storage stability of the biscuit due to increased tendency

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of rancidity, hence, the merit in the choice of optimum fat content of 18.4% with the optimum condition of 89.5% wheat flour, 10% soybean flour, and 0.5% turmeric. From Table 3, it can be seen that increasing the linear and interaction terms of the spices lead to the reduction of the fat content of the biscuit. However, soybean

flour had the highest effect in increasing the fat content of the biscuits, followed by wheat flour. The decreasing effect of turmeric is an advantage as it reduces the onset of oxidative rancidity thereby reducing the risk of cardiovascular diseases (Kadiri and Olawoye, 2015).

**Table2.** Proximate composition of formulated biscuits

| Sample  | Moisture               | Fat                     | Protein                  |
|---------|------------------------|-------------------------|--------------------------|
| A       | 5.43±0.01 <sup>g</sup> | 18.39±0.12 <sup>1</sup> | 9.72±0.03 <sup>d</sup>   |
| B       | 7.23±0.03 <sup>c</sup> | 18.69±0.10 <sup>e</sup> | 9.64±0.03 <sup>d</sup>   |
| C       | 6.06±0.01 <sup>e</sup> | 20.98±0.16 <sup>b</sup> | 13.15±0.07 <sup>ab</sup> |
| D       | 5.68±0.02 <sup>1</sup> | 19.54±0.09 <sup>d</sup> | 11.89±0.02 <sup>c</sup>  |
| E       | 6.16±0.01 <sup>e</sup> | 20.48±0.21 <sup>c</sup> | 12.64±0.04 <sup>b</sup>  |
| F       | 6.49±0.10 <sup>d</sup> | 18.87±0.37 <sup>e</sup> | 10.23±0.04 <sup>d</sup>  |
| G       | 7.78±0.09 <sup>a</sup> | 21.81±0.20 <sup>e</sup> | 13.41±0.04 <sup>a</sup>  |
| H       | 6.46±0.05 <sup>d</sup> | 18.86±0.20 <sup>e</sup> | 10.27±0.01 <sup>d</sup>  |
| Control | 7.53±0.03 <sup>b</sup> | 18.29±0.19 <sup>1</sup> | 8.9±0.08 <sup>e</sup>    |

Values are mean ± standard deviation of triplicate samples.

Values on vertical row with the same superscript are not significantly different ( $P < 0.05$ )

**Table3.** Regression coefficient of predicted quadratic polynomial models for proximate composition of biscuits.

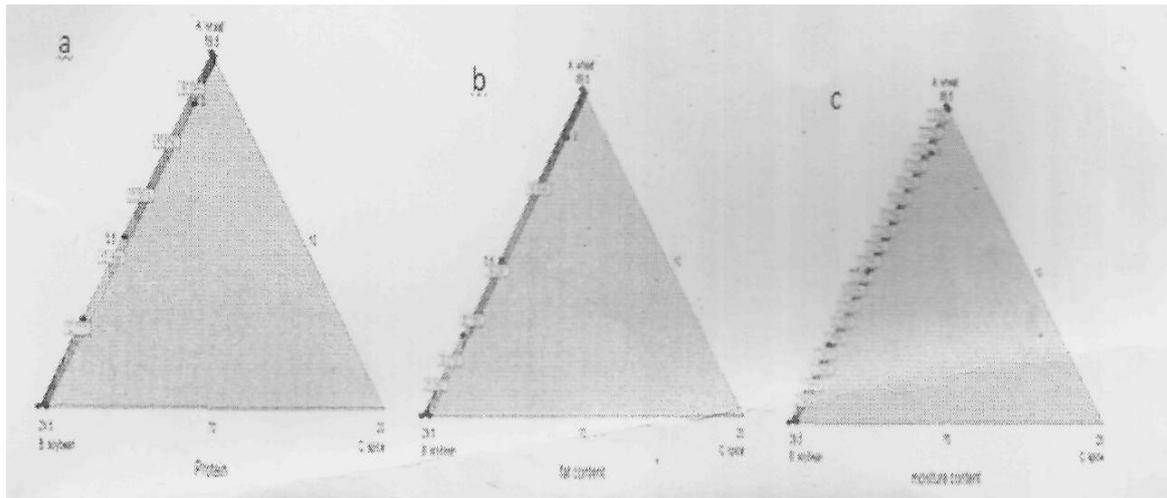
| Variable                | Moisture | Fat     | Protein |
|-------------------------|----------|---------|---------|
| A                       | 5.43     | 18.39   | 9.71    |
| B                       | 7.78     | 21.81   | 13.39   |
| C                       | -4771.06 | -341.37 | 263.42  |
| AB                      | -6.70    | -2.42   | 1.32    |
| AC                      | 4979.98  | 388.05  | -261.79 |
| BC                      | 4823.06  | 336.66  | -266.28 |
| R <sup>2</sup> Adjusted | 0.9996   | 0.9998  | 0.9989  |

### Protein contents

The result shown in table 2 implies that the biscuit samples were high in protein content and could be used as alternative protein source in protein deficiency. The protein content of the biscuits ranged from 8.9% to 13.41%. Sample G had the highest value of 13.41% while the least value was recorded for the control sample. There was significant difference ( $p < 0.05$ ) between the control and other samples in the experiment. It was observed from the table that the protein content of the biscuit increases with increase in the replacement level of the soybean flour. This observation is expected as soybean is a high protein legume, this form the basis of its use as an economical protein supplement in biscuit, bread, pasta, and other cereal products (Hegstad, 2008). Incorporation of soyflour into food has a greater potential in overcoming protein-caloric malnutrition in the world (Akubor and Ukwuru, 2005). Other studies have also reported a similar increase of protein content in soy composite biscuit (Ndifeet *al*,

2014; Singh *et al*, 2000). Table 3 showed that increasing the linear and binary combinations where soybean flour was incorporated increased the protein content of the biscuit, the binary combination of wheat and turmeric decreased the protein content of the biscuit. From the table it was seen that soyflour exhibited the highest effect in increasing the protein content of the biscuit as compared to wheat flour having the positive coefficient of 13.39. The linear effect of variable C is more pronounced in increasing the protein content of the biscuit than its binary combination (AC and BC) where a decreasing effect is observed with increase in the variables. Turmeric is said to contain 6.3% protein (Rohit *et al*, 2014), which can be a contributing effect to increase in the protein content of the biscuit. The final equation for the protein content of the biscuit is shown in equation 2 below.

$$9.71 A + 13.39B + 263.42C + 1.32AB - 261.79AC - 266.28BC \quad (2).$$



**Figure 1.** Model graph showing the protein, fat and moisture content of biscuit produced from composite blends of wheat flour, soybean flour and turmeric.

### Antioxidant properties

#### DPPH radical scavenging activity

The DPPH radical scavenging activities of processed biscuit samples were compared with that of known natural antioxidant (ascorbic acid (AA)). Table 4 Showed the DPPH radical scavenging activity of the biscuits. The value ranged between 24.34% and 55.87% with biscuits made from 100% wheat (control) having the highest radical scavenging activity while sample A had the lowest DPPH. There was a significant difference ( $p < 0.05$ ) in the DPPH radical scavenging of the formulated biscuit. It could be noted that as the level of soybean flour and spices substitution increases, the radical scavenging activities of the formulated biscuit increases. This could be attributed to high antioxidant activity of the spices and soybean. Soybean had been reported by Ogunwolu *et al.* (2009) to contain high amount of hydrophobic and hydrophilic amino acid, these amino acids are known to exhibit antioxidant activity. Table 5 showed the coefficient estimate and adjusted regression coefficients ( $R$  adjusted) for the antioxidant properties of the biscuits, the analysis of variance (ANOVA) for the DPPH showed that the full quadratic model was significant in predicting the radical scavenging activity of the biscuits. The model which explained the relationship between the DPPH radical scavenging activity of the biscuits and the independent factors (A, B, and C) is shown in Equation 3:

$$\text{DPPH} = 24.34A - 51.36B + 37536.46 +$$

$$11.13AB - 37734.38AC - 39129.84BC \quad (3)$$

According to this equation, it could be noted that the spices which is variable C had the highest effect on the radical scavenging activity of the biscuits. This is evidence with high positive value of the coefficient of estimate, thus confirming the report by Sharma *et al.* (2015) that turmeric increases the antioxidant potential of biscuit produce from blends of wheat flour and turmeric powder.

#### Total phenolic compounds

Table 4 showed the result of total phenolic compound of the formulated biscuits. The value ranged from 0.13mgGAE/g in control sample to 0.77mgGAE/g in sample G. There was no significant difference ( $p < 0.05$ ) in the total phenolic compound of sample E, F, G, and H, however, the total phenolic compound present in sample G was significantly ( $p < 0.05$ ) higher than that of control. It was noted that the total phenolic compound increased with increase in the level of substitution of soybean flour and turmeric powder. Zahra *et al.* (2014) also reported an increase in total phenolic compound of novel snack cracker when *Hibiscus sabdariffa* powder was incorporated. As revealed in table 7, the coefficient of estimate revealed that the spices had the profound positive effect on the total phenolic compound of the biscuits. The final equation for the total phenolic compound is given in Eq 4

$$\text{TPC} = 048A + 0.77B + 772.99C - 0.11 AB - 15.5AC - 5107.03BC \quad (4)$$

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**Table4.** Antioxidant properties of formulated biscuit;

| Sample  | DPPH(%)                  | Phenolic(mgGAE/g)      | Total                  | Reducing power         |
|---------|--------------------------|------------------------|------------------------|------------------------|
| A       | 24.34±0.01 <sup>h</sup>  | 0.48±0.04 <sup>b</sup> | 0.53±0.03 <sup>e</sup> | 4.05±0.04 <sup>c</sup> |
| B       | 27.77±0.03 <sup>g</sup>  | 0.49±0.07 <sup>b</sup> | 1.46±0.04 <sup>d</sup> | 4.68±0.04 <sup>b</sup> |
| C       | 34.42±0.01 <sup>f</sup>  | 0.51±0.03 <sup>b</sup> | 1.49±0.06 <sup>c</sup> | 4.87±0.04 <sup>a</sup> |
| D       | 35.62±0.04 <sup>e</sup>  | 0.53±0.08 <sup>b</sup> | 1.53±0.01 <sup>d</sup> | 1.03±0.04 <sup>g</sup> |
| E       | 43.44±0.03 <sup>d</sup>  | 0.67±0.03 <sup>a</sup> | 3.26±0.06 <sup>c</sup> | 1.25±0.04 <sup>f</sup> |
| F       | 43.51±0.04 <sup>cd</sup> | 0.74±0.06 <sup>a</sup> | 3.45±0.01 <sup>b</sup> | 2.04±0.01 <sup>e</sup> |
| G       | 51.24±0.06 <sup>b</sup>  | 0.77±0.03 <sup>a</sup> | 4.2±0.04 <sup>a</sup>  | 3.39±0.04 <sup>d</sup> |
| H       | 43.57±0.03 <sup>c</sup>  | 0.74±0.04 <sup>a</sup> | 3.45±0.03 <sup>b</sup> | 2.03±0.03 <sup>e</sup> |
| Control | 55.87±0.07 <sup>a</sup>  | 0.13±0.04 <sup>c</sup> | 0.27±0.03 <sup>f</sup> | 0.7±0.04 <sup>h</sup>  |

Values are mean ± standard deviation of triplicate samples.

Values on vertical row with the same superscript are not significantly different ( $P < 0.05$ )

**Table5.** Regression coefficient of predicted quadratic polynomial models for antioxidant properties of biscuits.

| Variable                | DPPH      | Phenolic | Total Antioxidant | Reducing Power |
|-------------------------|-----------|----------|-------------------|----------------|
| A                       | 24.34     | 0.48     | 0.56              | 4.05           |
| B                       | 51.36     | 0.77     | 4.36              | 3.38           |
| C                       | 37536.46  | 772.99   | 4868.59           | -5641.68       |
| AB                      | 11.13     | 0.11     | -0.14             | -14.93         |
| AC                      | -37734.38 | -781.50  | -4868.39          | 5769.14        |
| BC                      | -39129.84 | -802.13  | -5107.03          | 5847.85        |
| R <sup>2</sup> Adjusted | 0.9997    | 0.9909   | 0.9427            | 0.9995         |

### Total Antioxidant

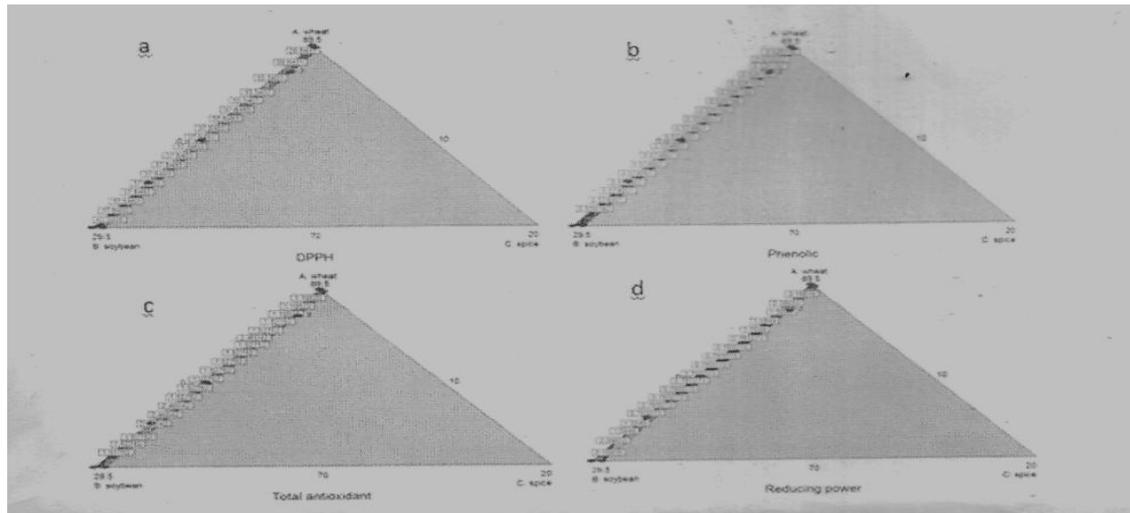
The total antioxidant of the biscuits varies between 0.27mgAAE/g and 4.2 mgAAE/g, with biscuit produced from 100% wheat flour (control) having the lowest antioxidant concentration while biscuits produce from 70% wheat, 29.5% soybean flour and 0.5% turmeric powder had the highest total antioxidant. There was significant difference ( $p < 0.05$ ) among the formulated biscuits. The increase in the total antioxidant of the biscuits with increase in the level of soybean flour and turmeric powder could be attributed to high antioxidant potential of the turmeric powder and high protein content of the soybean flour. Hegstad (2008) reported soybean contain various compound which can act as antioxidant and thus, increase the antioxidant potential of formulated food products. As it can be seen from the coefficient of estimate, turmeric also had the most profound positive effect on the total antioxidant followed by soybean flour. This report is in agreement with the report of Sharma et al. (2015) that turmeric significantly increased the total antioxidant of biscuits when compared with biscuits made from 100% wheat flour.

### Ferric Reducing Power Assay

The ability of biscuits to chelate and deactivate transition metals is shown in Table 4. The chelating ability of the extract measures how

effective the compounds in it can compete with ferrozine for ferrous ion. By forming a stable iron (II) chelate, an extract with high chelating power reduces the free ferrous ion concentration thus decreasing the extent of Fenton reaction which is implicated in many diseases (Pulido *et al.*, 2000). This reducing power activity, which may serve as a significant reflection of antioxidant activity was determined using a modified Fe (III) to Fe (II) reduction assay; the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of the samples. The presence of antioxidants in the samples causes the reduction of the Fe<sup>2+</sup>/Ferricyanide complex to the ferrous form. Therefore, Fe<sup>3+</sup> can be monitored by measuring the formation of Perls Prussian blue at 593 nm (Pulido *et al.*, 2000). There was significant difference ( $p < 0.05$ ) in the reducing power of the formulated biscuits with Sample C possessing the highest reducing power while biscuit made from 100% wheat (control) had the lowest reducing power. The high ferric reducing power assay (4.87 mg/ml) of sample C could be attributed to high proportions of protein and amino acids such as leucine, lysine, methionine, tyrosine, isoleucine, histidine and tryptophan (Hegstad *et al.*, 2008). Contrary to other antioxidant assay, turmeric powder had the least effect on ferric reducing power assay of the biscuits as shown in Table 5.

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**Figure 2.** Model graph showing the antioxidant properties of biscuit produced from composite blends of wheat flour, soybean flour and turmeric.

### Color Determination

The mean color values of biscuits chromatic parameters are shown in table 6. The chromatic parameters  $L^*$  (whiteness or brightness darkness),  $a^*$  (greenness-redness), and  $b^*$  (blueness-yellowness) have been widely used to describe color changes during thermal processing of agricultural products; they have been related to the types and quantities of some components present in those products (Bahloul et al., 2009). The chromatic parameters exhibited significant differences ( $P < 0.05$ ) between the control biscuit and other samples. The  $L^*$  value of the biscuits reduced from 62.88 in the control sample to 53.28 in sample C. All biscuits formulated with the flour blends had lower  $L^*$  values than the control, and therefore a darker color. This indicates that the control sample had a bright color as well as high luminosity as compared with biscuits formulated with the flour blends. The  $L^*$  value decreased with increase in the level of turmeric. This could

be due to the main bioactive components (curcumin) present in turmeric, which is responsible for the yellow color of turmeric (Tokusoglu et al., 2015). The decrease in  $L^*$  value could also be due to browning reaction that occurred during baking. Parthian et al. (2009) reported that the higher the degree of browning, the lower the  $L^*$  value of the sample. The  $a^*$  value gives an indication of the redness while  $b^*$  value is a measure of the yellowness of the sample and a high  $a^*$  and  $b^*$  values were found for all biscuits formulated with the flour blends in comparison to the control biscuit. The results showed that the control biscuit had the lowest  $a^*$  value while sample C had the highest value, and they were significantly different ( $P < 0.05$ ) from the other samples. Sample B and the control biscuit had the highest and lowest  $b^*$  values respectively and they were significantly different ( $P < 0.05$ ) from other samples. These results are in harmony with those obtained by Esmat et al., 2011; Mona and Hinar, 2015.

**Table 6.** Color characteristics of formulated biscuits.

| Sample  | L                        | A                      | b                       |
|---------|--------------------------|------------------------|-------------------------|
| A       | 61.46±0.025 <sup>b</sup> | 2.68±0.02 <sup>g</sup> | 23.03±0.02 <sup>b</sup> |
| B       | 58.43±0.23 <sup>d</sup>  | 3.00±0.01 <sup>f</sup> | 23.78±0.06 <sup>a</sup> |
| C       | 53.28±0.11 <sup>1</sup>  | 5.76±0.03 <sup>a</sup> | 18.77±0.02 <sup>f</sup> |
| D       | 59.40±0.45 <sup>c</sup>  | 4.60±0.20 <sup>b</sup> | 20.87±0.08 <sup>d</sup> |
| E       | 59.39±0.16 <sup>c</sup>  | 3.84±0.02 <sup>d</sup> | 20.96±0.04 <sup>d</sup> |
| F       | 56.82±0.26 <sup>e</sup>  | 4.22±0.02 <sup>c</sup> | 20.36±0.07 <sup>e</sup> |
| G       | 59.08±0.49 <sup>c</sup>  | 3.55±0.00 <sup>e</sup> | 22.59±0.16 <sup>c</sup> |
| H       | 56.73±0.21 <sup>e</sup>  | 4.21±0.01 <sup>c</sup> | 20.42±0.03 <sup>e</sup> |
| Control | 62.88±0.17 <sup>a</sup>  | 2.58±0.01 <sup>h</sup> | 15.14±0.10 <sup>g</sup> |

Values are mean ± standard deviation of triplicate samples.

Values on vertical row with the same superscript are not significantly different ( $P < 0.05$ ).

**Sensory Evaluation of Biscuit**

Table 7 showed the results of sensory analysis of the biscuits. It was observed that A and D samples are well accepted in terms of appearance, taste, crispiness and aroma compared to the control. Also in the fresh samples, it was observed that there was no significant difference in the overall acceptability among the biscuit made with flour blends, while

there was significant difference between the control biscuit and samples B, D, F, G and H. Incorporation of turmeric powder had significant influence on the surface colour characteristics of the biscuits, owing to the level of addition. In the biscuit samples, there were significant difference between the colour of the control biscuits and other samples.

**Table 7.** Sensory score for biscuit after production.

| Sample (Initial) | Appearance              | Taste                   | Colour                 | Crispiness              | Aroma                   | General Acceptability   |
|------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| A                | 7.20±0.92 <sup>ab</sup> | 7.10±1.37 <sup>ab</sup> | 6.80±1.62 <sup>a</sup> | 7.30±1.34 <sup>ab</sup> | 6.50±1.43 <sup>ab</sup> | 6.90±1.45 <sup>ab</sup> |
| B                | 6.60±0.07 <sup>ab</sup> | 6.50±1.35 <sup>a</sup>  | 6.10±1.88 <sup>a</sup> | 6.90±1.88 <sup>ab</sup> | 6.40±1.35 <sup>ab</sup> | 6.60±1.26 <sup>a</sup>  |
| C                | 6.90±0.99 <sup>ab</sup> | 6.70±1.57 <sup>a</sup>  | 7.10±1.88 <sup>a</sup> | 6.80±1.39 <sup>ab</sup> | 6.50±1.27 <sup>ab</sup> | 7.00±1.25 <sup>ab</sup> |
| D                | 6.50±1.35 <sup>ab</sup> | 6.90±1.37 <sup>ab</sup> | 6.60±1.17 <sup>a</sup> | 6.70±1.49 <sup>ab</sup> | 6.30±1.49 <sup>ab</sup> | 6.20±1.03 <sup>a</sup>  |
| E                | 6.50±1.17 <sup>ab</sup> | 6.70±1.82 <sup>a</sup>  | 6.30±1.66 <sup>a</sup> | 6.30±2.06 <sup>ab</sup> | 6.40±1.50 <sup>ab</sup> | 7.00±1.82 <sup>ab</sup> |
| F                | 6.10±1.52 <sup>a</sup>  | 7.10±1.10 <sup>ab</sup> | 6.10±1.73 <sup>a</sup> | 6.70±1.77 <sup>ab</sup> | 6.70±1.89 <sup>a</sup>  | 6.50±1.78 <sup>a</sup>  |
| G                | 6.10±1.85 <sup>a</sup>  | 6.70±1.77 <sup>a</sup>  | 6.70±1.50 <sup>a</sup> | 6.30±2.00 <sup>a</sup>  | 5.80±2.04 <sup>a</sup>  | 6.10±2.29 <sup>a</sup>  |
| H                | 6.10±1.42 <sup>a</sup>  | 7.00±1.00 <sup>ab</sup> | 6.10±1.60 <sup>a</sup> | 6.50±1.75 <sup>ab</sup> | 5.70±1.80 <sup>a</sup>  | 6.00±1.68 <sup>a</sup>  |
| Control          | 7.80±1.62 <sup>b</sup>  | 8.10±1.88 <sup>b</sup>  | 8.10±1.10 <sup>b</sup> | 8.10±1.58 <sup>b</sup>  | 7.50±1.18 <sup>b</sup>  | 8.20±1.03 <sup>b</sup>  |

Values are mean ± standard deviation of triplicate samples.

Values on vertical row with the same superscript are not significantly different ( $P < 0.05$ )

**CONCLUSION**

This study revealed that incorporation of turmeric improved the nutritional quality without adversely affecting the colour and sensory characteristics. Application of experimental design approach by Mixture Response Surface Methodology could be used to determine the effect of variations in levels of wheat flour, soybean flour and turmeric powder on proximate composition and antioxidant properties of eight biscuits formulations. The optimum composite flour blends for biscuit production consisted of 72.88%, 26.63% and 0.5% of wheat flour, soybean flour and turmeric respectively with a response of 6.59% moisture content, 21% fat, 13.01% protein, 48.77% DPPH, 0.74mgGAE/g phenolic, 3.75mgGAE.g total antioxidant and 1.59mg/ml reducing power. Prediction models obtained were adequate, hence the agreement between the experimental and predicted values. The formulated biscuit serves as a good source of antioxidant and protein when compared to the wheat biscuit (100% refined wheat flour). The prediction model can be used to develop baked goods using composite flours from indigenous sources in area where wheat flour is widely unavailable. The addition of turmeric gave an excellent antioxidative effect on the biscuit. Results from sensory evaluation revealed that turmeric can be incorporated into biscuit since it

had no adverse effect on the organoleptic properties of the biscuit. The final goal of obtaining a novel functional biscuit was achieved. From the study, it can be concluded that turmeric is suitable for use as natural antioxidant in biscuit manufacture

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