Formulation and Evaluation of Cookies Containing Germinated Pigeon Pea, Fermented Sorghum and Cocoyam Flour Blends using Mixture Response Surface Methodology

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Abstract: Cookies were produced from blends of germinated pigeon pea, fermented sorghum and cocoyam flours. The study was carried out to evaluate the effects of varying the proportions of these components on the sensory and protein quality of the cookies. The sensory attributes studied were colour, taste, texture, crispiness and general acceptability while the protein quality indices were Biological Value (BV) and Net Protein Utilization (NPU). Mixture response surface methodology was used to model the sensory and protein quality with single, binary and ternary combinations of germinated pigeon pea, fermented sorghum and cocoyam flours. The sum of the component proportions was always equal to 100%. Results showed that BV and NPU of most of the cookies were above minimum recommended levels. With the exception of cookies containing high levels of pigeon pea flour, cookies had acceptable sensory scores. Increase in pigeon pea flour resulted in increase in the BV and NPU. Regression equations suggested that the ternary blends produced the highest increase in all the sensory attributes (with the exception of colour).

Key words: Cocoyam flour, cookies, fermented sorghum flour, germinated pigeon pea flour, mixture response surface methodology

INTRODUCTION

Cookies are widely consumed throughout the world. In fact, they represent the largest category of snack foods in most parts of the world (Lorenz, 1983). Wheat flour, which is the flour of choice for producing cookies, is unavailable or uneconomical in many regions of the world. Therefore to produce baked goods, regions with limited supplies of wheat flour must rely on imports or exclude wheat products from the diet (Holt et al., 1992). The consumption of cereal based foods like cookies require the development of an adequate substitute for wheat (Eneche, 1999). The substitute should be one that is readily available, cheap and able to replace wheat flour in terms of functionality.

Flours produced from only either cereals, legumes or tubers will have a nutritional value inferior to those produced from a combination of cereals, legumes or tubers. For instance, composite flours produced from cereals and legumes have the advantage of improving overall nutrition (FAO, 1995) while composite flours produced from legumes and tubers will have high protein content and will also have high calorific value (Chimma et al., 2007). In selecting the components to be used in composite flour blends, the materials should preferably be readily available, culturally acceptable and provide increased nutritional potential (Akobundu et al., 1998).

Pigeon pea (Cajanus cajan) is a legume which has a relatively high amount of protein (Tiwari et al., 2008). Sorghum (Sorghum bicolor) is a cereal. World food consumption of sorghum has remained stagnant mainly because though nutritionally, sorghum compares favourably with other cereals, it is regarded in many countries as an inferior grain (FAO, 1995). Cocoyam (Xanthosoma sagittifolium) is a tuber. It has fine granular starch which has been reported to improve binding and reduce breakage of snack products (Huang, 2005). These three crops are grown in large quantities in the tropics but are underutilized.

Plant proteins have been reported to have limiting amino acids (Ihekoronye and Ngoddy, 1985) and it is necessary to combine these plant proteins in proportions that will improve the protein intake of such consumers. Many processes are available for improving the nutritional quality of plant foods. These methods include traditional processing methods like cooking, soaking, dewatering, fermentation, germination, smoking, salting, curing etc., (Teutonico and Knorr, 1985). Germination has been reported to induce an increase in free limiting amino acids and available vitamins with modified functional
properties of seed components (Hallén et al., 2004). It has also been shown to decrease antinutritional factors and also increase the protein digestibility, crude fibre and protein contents of chick pea (El-Adawy, 2002). Fermentation improves amino acid composition and vitamin content, increases protein and starch availabilities and lowers levels of antinutrients (Chavan and Kadam, 1989). The germination of pigeon pea and fermentation of sorghum should therefore be beneficial to the nutritional quality of the seeds.

Mixture response surface methodology (MRSM) is a statistical technique that can be used to systematically determine the effects of multiple variables in a mixture on quality attributes while minimizing the number of experiments that must be conducted (Cornell, 1979). It is a special response surface experiment in which the design factors are the components or ingredients of a mixture, and the response depends on the proportions of the ingredients that are present (Myers et al., 2009).

No literature is available on the use of germinated pigeon pea, fermented sorghum and blanched cocoyam flour blends in the production of cookies. This work seeks to produce cookies from blends of germinated pigeon pea, fermented sorghum and cocoyam flour blends (hereafter known as components) and use MRSM to study the effects of varying the proportions of these components on the sensory and protein quality of the cookies.

MATERIALS AND METHODS

This study was carried out from July 2009 to January, 2010 in the laboratory of the Food Science and Technology Department, Ebonyi State University, Abakaliki, Nigeria.

Materials: The white variety of pigeon pea (Cajanus cajan), the white variety of sorghum (Sorghum bicolor) and the tannia variety of cocoyam (Xanthosoma sagittifolium) were purchased from a retail outlet in Abakaliki, Ebonyi State. Wheat flour and all other baking ingredients such as eggs, baking powder, fat, milk and flavourings were also obtained from the same source. Corn starch was purchased from a retail outlet in Enugu, Enugu State; casein was bought from a chemical store in Nsukka, Enugu State while vitamin and mineral premixes were bought from Bio-organics Nigeria Plc, Lagos.

Cocoyam Flour (CF): Cocoyam flour was produced using the method described by Ijeoma (1983). The corms were washed, peeled, sliced and blanched at 80°C for 4 min. They were dried and milled to pass through 100 μm mesh sieve.

Germinated pigeon pea flour (GPF): Pigeon peas were germinated using the modified method of Hallén et al. (2004). Cleaned grains were soaked in 0.1% sodium hypochlorite solution for 30 min to prevent mould growth. After that time, the grains were thoroughly washed and soaked in water (10 h). The hydrated seeds were spread on jute bags and allowed to germinate for 4 days after which they were dried at 50°C. Thereafter, formed roots and testa were rubbed off before milling and sieving through 100 μm mesh sieve.

Fermented sorghum flour (FSF): The sorghum flour was subjected to natural lactic acid fermentation using the method of Hallén et al. (2004). Washed and dried grains were ground into fine flour. The flour was mixed with water (1:5 wt/wt) to form slurry followed by the addition of 5% sugar by weight of flour. The slurry was left to ferment in trays at room temperature until the pH of the slurry reached 5.5. The fermented slurry was dried at 50°C and then ground through a 100 μm mesh screen. Both of the flour samples were kept in airtight containers until needed for analysis.

Experimental design: A three-component augmented simplex centroid design was used as described by Scheffé (1965). The three mixture components evaluated in this study were cocoyam flour (x1), fermented sorghum flour (x2) and germinated pigeon pea flour (x3). The proportions for each ingredient were expressed as a fraction of the mixture and for each treatment combination, the sum of the component proportions was equal to one, where:

\[ \sum X_i = x_1 + x_2 + x_3 = 1 \]

In this design, the number of points (n) necessary to run a mixture experiment is:

\[ n = 2^q - 1 \]

where q is equal to the number of components being studied (3). This design resulted in 7 flour mixtures. It is usually desirable to augment the simplex centroid with additional points in the interior of the design where the blends will consist of all of q mixture components. Three additional points were therefore included to provide extra points within the mixture triangle. Four runs were replicated to give an internal estimate of error (Table 1).

Cookie preparation: Fat and sugar were mixed until fluffy. Whole eggs and powdered milk were added while mixing and then mixed for a total of about 30 min. Vanilla flavour, nutmeg, flour, baking powder and salt were mixed thoroughly and added to the cream mixture where they were all mixed together to form a dough. The dough was rolled and cut into shapes of 5 cm diameter. Baking
Table 1: Experimental design used to develop and analyze cookies produced from germinated pigeon pea, fermented sorghum and cocoyam flours and some protein quality parameters

| Formulations | Cocoyam Germinated pigeon Fermented orghum Biological Net protein Formulations (CF) (%) pea (GPF) (%) (FSF) (%) value (%) utilization (%) |
|--------------|---------------------------------------------------|---------------------------------------------------------------------|-------------|-------------------|----------------|
| 1            | 100                                               | 0                                                                   | 0           | 78.16de           | 70.08de        |
| 2            | 0                                                 | 100                                                                | 0           | 92.53abc          | 89.74abc       |
| 3            | 0                                                 | 0                                                                  | 100         | 78.98de           | 72.95de        |
| 4            | 50                                                | 0                                                                  | 50          | 95.46e            | 91.98e         |
| 5            | 0                                                 | 50                                                                | 50          | 88.11f            | 85.25e         |
| 6            | 50                                                | 50                                                                | 0           | 90.64abc          | 88.11abc       |
| 7            | 33.3                                              | 33.3                                                               | 33.3        | 86.64e            | 84.60e         |
| 8            | 16.7                                              | 66.6                                                               | 16.7        | 88.79e            | 86.68e         |
| 9            | 16.7                                              | 16.7                                                               | 66.6        | 91.28e            | 89.05e         |
| 10           | 66.6                                              | 16.7                                                               | 16.7        | 86.79f            | 83.99f         |
| 11           | 100                                               | 0                                                                  | 0           | 73.86e            | 65.09e         |
| 12           | 0                                                 | 100                                                                | 0           | 90.27e            | 87.59e         |
| 13           | 0                                                 | 0                                                                  | 100         | 80.14e            | 75.34e         |
| 14           | 50                                                | 0                                                                  | 50          | 93.74abc          | 88.45abc       |

Formulations 11-14 are replicates runs

was carried out at 185°C for 15-25 min. Cookies samples were cooled and stored in polyethylene bags until needed. Cookies were made from wheat to serve as a control.

**Rat feeding protocol:** The diets were adjusted to 10% protein for those diets with protein contents above 10%. For those diets with protein contents ranging from 6-9.99%, the test diets were adjusted to provide 6 and 8% protein. There were three reference protein diets of casein at 6, 8 and 10% protein levels for comparison of the protein quality of these test diets (Prabhavat et al., 1991). Other ingredients such as vegetable oil, vitamins, minerals and cornstarch were added to balance the diets. The diets were thoroughly mixed, pelleted and stored in polyethylene bags labeled with designated names. The polyethylene bags were kept in airtight containers until ready for use.

Eighteen groups (fourteen assays + three casein control groups + one group receiving a protein-free diet) of five male adult albino rats of the Wistar strain with average initial weight of 120-210 g were used. They were randomly divided and housed in individual screened bottomed cages designed to separately collect faeces and urine. Experimental animals received corresponding group diets and water ad libitum. The temperature of the laboratory was 28±1°C with alternate 12 h periods of light and dark. These animals were used to assess the Biological Value (BV) and Net Protein Utilization (NPU) of the diets based on casein. Following the method described by Al-Numair and Ahmed (2008), a 9-day balance study which included a four-day adjustment and five-day nitrogen (N) balance period was carried out. There was a preliminary feeding period of four days followed by a balance period of five days during which complete collection of faeces and urine was performed for each rat. Food intake was monitored daily and final body weights were recorded. Urine was collected in sample bottles, preserved in 0.1N HCl to prevent loss of ammonia and stored in a refrigerator until analyzed for urinary nitrogen. Faeces of individual rats were pooled, dried at 85°C for 4 h, weighed before being ground into fine powder and stored for faecal N determination. The concentration of nitrogen in the diet, faeces and urine was estimated by the Kjeldahl method (AOAC, 2000).

**Sensory evaluation:** A consumer acceptance test was conducted on the cookies. A total of twenty untrained panelists were recruited from staff and students of the Ebonyi State University, Abakaliki. A randomized complete block design was used whereby each panelist evaluated all the samples prepared for each treatment. Criteria for selection of panelists were that panelists were regular consumers of cookies and were not allergic to any food. Panelists were instructed to evaluate colour, flavour, texture, crispness and general acceptability of the cookies. A 9-point hedonic scale was used (Ihekoronye and Ngoddy, 1985). Samples were identified with 3-digit code numbers and presented in a random sequence to panelists. The panelists were instructed to drink water after every sample and not to make comments during evaluation to prevent influencing other panelists. They were also asked to comment freely on samples on the questionnaires given to them.

**Statistical analysis:** Analysis of variance (ANOVA) was carried out on data from sensory and protein quality tests followed by Duncan’s Multiple Range test (SPSS v. 16.0 for windows, SPSS Inc, Illinois, USA). A p-value below 0.05 was considered significant. The experimental data from sensory and protein quality tests were also evaluated using MRSMS. The generation of contour plots and statistical analysis were performed using Design-Expert (Version 8.0.3, State-Ease, Inc. Minneapolis, 2010) software. ANOVA was also performed using this software and model significance (p<0.05), lack of fit and adjusted regression coefficients...
The multiple regression analysis showed that the special cubic model was significant in predicting the BV of the cookies (p=0.0005) and it could explain 89.55% of all variance in the data. Furthermore, it did not present significant lack of fit (p=0.34). A non-significant lack of fit in the models makes them as predictive models (Yağcı and Göğüş, 2008). If a model has a significant lack of fit, it is not a good indicator of the response and should not be used for the prediction (Myers et al., 2009).

The regression model obtained for the BV of cookies was:

\[ Y = 75.85x_1 + 80.08x_2 + 91.17x_3 + 68.33x_1x_2 + 26.09x_1x_3 + 73.67x_2x_3 - 170.03 \]

(2)

The positive (+) sign in the equations means that the response value increased with increase of the variables, whether it is linear, binary or ternary combinations while the negative (-) sign means that the response value decreased with increase of the variables thereby producing an antagonistic effect. From the equation, it was observed that increasing the linear and binary blends resulted in higher BV while increasing the ternary blends resulted in lower BV. Among the single-component mixtures, pigeon pea flour produced the highest increase in BV followed by sorghum flour and cocoyam flour.

The MRSM application on NPU data showed that quadratic model was significant (p = 0.0003), no lack of fit was obtained (p = 0.25) and it could explain 86.90% of all variance in the data. The components significantly increased the NPU as seen in the equation below:

\[ Y = 67.78x_1 + 74.89x_2 + 88.50x_3 + 73.67x_1x_2 + 28.92x_1x_3 + 7.64x_2x_3 \]

(3)

As was seen with BV, pigeon pea flour produced the highest increase in the NPU. Contour plots generated for BV and NPU are shown in Fig. 1.
Table 3: Mean consumer acceptance scores observed for cookies produced from germinated pigeon pea, fermented sorghum and cocoyam flours

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blends</th>
<th>Texture</th>
<th>Taste</th>
<th>Colour</th>
<th>Crispiness</th>
<th>General Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100:0:0</td>
<td>7.60a</td>
<td>7.80b</td>
<td>7.60a</td>
<td>7.50c</td>
<td>7.40e</td>
</tr>
<tr>
<td>2</td>
<td>0:100:0</td>
<td>6.30cd</td>
<td>6.50bd</td>
<td>6.55bcd</td>
<td>6.15cde</td>
<td>7.05b</td>
</tr>
<tr>
<td>3</td>
<td>0:0:100</td>
<td>5.90e</td>
<td>5.00f</td>
<td>5.90e</td>
<td>5.25e</td>
<td>5.00d</td>
</tr>
<tr>
<td>4</td>
<td>50:50:0</td>
<td>7.05bc</td>
<td>7.10bc</td>
<td>6.95bcd</td>
<td>6.90bcd</td>
<td>7.10b</td>
</tr>
<tr>
<td>5</td>
<td>0:50:50</td>
<td>5.85cd</td>
<td>5.95cd</td>
<td>6.35cd</td>
<td>5.10d</td>
<td>5.00d</td>
</tr>
<tr>
<td>6</td>
<td>50:0:50</td>
<td>5.90d</td>
<td>5.85cd</td>
<td>6.65bcd</td>
<td>5.35e</td>
<td>5.50e</td>
</tr>
<tr>
<td>7</td>
<td>33.3:33.3:33.3</td>
<td>7.10bc</td>
<td>6.85bcd</td>
<td>6.90bcd</td>
<td>6.75bde</td>
<td>6.75bc</td>
</tr>
<tr>
<td>8</td>
<td>16.7:16.7:66.6</td>
<td>6.55cd</td>
<td>5.60bc</td>
<td>6.75bcd</td>
<td>6.00bcd</td>
<td>5.80cd</td>
</tr>
<tr>
<td>9</td>
<td>16.7:66.6:16.7</td>
<td>6.65bcd</td>
<td>6.65bcd</td>
<td>6.85bcd</td>
<td>6.45bcd</td>
<td>6.50bcd</td>
</tr>
<tr>
<td>10</td>
<td>66.6:16.7:16.7</td>
<td>7.50ab</td>
<td>7.50ab</td>
<td>7.60ab</td>
<td>7.10abcd</td>
<td>7.15b</td>
</tr>
<tr>
<td>11</td>
<td>100:0:0</td>
<td>8.00a</td>
<td>8.00a</td>
<td>7.30ab</td>
<td>7.65a</td>
<td>8.25a</td>
</tr>
<tr>
<td>12</td>
<td>0:100:0</td>
<td>5.25e</td>
<td>5.25e</td>
<td>5.75d</td>
<td>5.30e</td>
<td>5.15d</td>
</tr>
<tr>
<td>13</td>
<td>0:0:100</td>
<td>6.00b</td>
<td>6.70bcd</td>
<td>6.25cd</td>
<td>6.35bcd</td>
<td>6.95b</td>
</tr>
<tr>
<td>14</td>
<td>50:50:0</td>
<td>7.25ab</td>
<td>7.40ad</td>
<td>7.20ab</td>
<td>6.40bcd</td>
<td>7.05b</td>
</tr>
</tbody>
</table>

*: Scores are based on a 9-point hedonic scale with 1, dislike extremely; 5: neither like nor dislike; 9: like extremely; CF: Cocoyam flour; FSF: Fermented Sorghum flour; GPF: Germinated Pigeon pea flour Mean values within a column with the same superscript are not significantly different (p>0.05)

Fig. 1: Contour plots for BV and NPU of cookies produced from cookies made with blends of germinated pigeon pea, fermented sorghum and blanched cocoyam flours
Design - Expert software
Component coding : Actual
**Texture**

**Design points**
X1 = A: cocoyam
X2 = B: fer sorghum
X3 = C: ger pigeon pea

(b) Design - Expert software
Component coding : Actual
**Colour**

**Design points**
X1 = A: cocoyam
X2 = B: fer sorghum
X3 = C: ger pigeon pea

(c) Design - Expert software
Component coding : Actual
**Taste**

**Design points**
X1 = A: cocoyam
X2 = B: fer sorghum
X3 = C: ger pigeon pea
Fig. 2: Contour plots for the sensory attributes of cookies made with blends of germinated pigeon pea, fermented sorghum and blanched cocoyam flours: a) texture; b) colour; c) taste; d) crispiness and e) general acceptability

Sensory quality evaluation: Mean values for consumer acceptance scores for the attributes tested for cookies produced are shown in Table 3. It was observed that while the addition of cocoyam flour improved ratings for the cookies, the addition of germinated pigeon pea flour had the opposite effect. Almost all the cookies containing at least 50% GPF received low scores (≤6) for texture, taste, crispiness and general acceptability. Panelists described cookies containing high levels of GPF as having a bitter aftertaste and lacking the characteristic crispiness and texture associated with cookies. Except for those formulations containing above 50% GPF, all the cookies were at least slightly liked for the parameters studied. Cookies made with 100CF did not differ significantly (p>0.05) from the control (cookies made with 100% wheat flour) for all the attributes tested. The most acceptable of the binary combinations was the one produced with 50CF:50FSF. Among the ternary blends, 66.6CF:16.7FSF:16.7GPF was the one with the highest acceptability and it did not differ significantly from cookies made with 50CF:50FSF and 100CF (p>0.05).

Results of the regression analyses listing the coefficients estimates, adjusted regression coefficients ($R^2_{adj}$) and the results of model significance and lack of fit for all the sensory attributes evaluated for cookies made with blends of germinated pigeon pea, fermented sorghum and blanched cocoyam flours are presented in Table 4. The special cubic model was significant in predicting the texture of the cookies produced. Apart from the binary combination of CF and GPF, all the mixtures significantly
increased the texture of the cookies as can be seen in the equation below:

\[
Y = 7.81x_1 + 6.12x_2 + 5.62x_3 + 0.66x_1x_2 + 2.83x_1x_3 + 0.022x_2x_3 + 27.76x_1x_2x_3
\]

(4)

The model could explain 88.74% of the observed variations and did not present a significant lack of fit (p > 0.05). The equation suggests that the ternary blend produced the highest increase in sensory scores for texture.

The regression model obtained for the colour of the cookies was:

\[
Y = 7.70x_1 + 6.55x_2 + 5.99x_3
\]

(5)

There was significant influence of the linear terms on the colour of the cookies. The equation suggests that the cocoyam flour produced the highest increase in sensory scores for colour. This was followed by sorghum and pigeon pea flours. The model could explain 80.56% of the variations in the colour observed. Thus, 19.44% of the variation was attributed to factors not included in the model. The lack of fit test showed that model error and replicate error were small, meaning that there was no lack of fit (p = 0.43) which further validated the suitability of the model for prediction purposes.

With regards the taste of the cookies, the equation obtained from the data is as follows:

\[
Y = 8.16x_1 + 6.58x_2 + 5.10x_3 + 0.05x_1x_2 + 2.57x_1x_3 + 0.04x_2x_3 + 14.84x_1x_2x_3
\]

(6)

The equation suggests that the combination of CF and GPF (x_1, x_3) contributed to an antagonistic effect on the taste of the cookies. According to the multiple regression analysis, the special cubic model for taste data was significant statistically (p < 0.0001) and could explain 96.54% of all variance of the hedonic results. No lack of fit was obtained from the data (p = 0.43). The equation suggests that the ternary blend produced the highest increase in sensory scores for taste.

The scores obtained for crispiness differed statistically and it was observed that all of the binary combinations resulted in lower hedonic scores. Cookies made with 100CF did not differ statistically (p < 0.05) from the control. The MRSM application on crispiness scores showed that the special cubic model was significant (p < 0.0001), no lack of fit was obtained (p = 0.56) and it could explain 94.85% of all variance data. Thus, about 5.15% of the variation was due to other factors not included in the model.

The model obtained for the crispiness of the cookies was:

\[
Y = 7.58x_1 + 6.24x_2 + 5.31x_3 + 1.07x_1x_2 + 4.08x_1x_3 - 2.54x_2x_3 + 37.06x_1x_2x_3
\]

(7)

As was observed with texture and taste, it appeared that the crispiness of cookies was increased mostly by the ternary blend.

The regression model obtained for the general acceptability of the cookies was:

\[
Y = 7.82x_1 + 6.97x_2 + 5.11x_3 + 1.41x_1x_2 - 3.58x_1x_3 - 4.11x_2x_3 + 31.40x_1x_2x_3
\]

(8)

Statistical analysis indicated that the special cubic model was significant in predicting the general acceptability of the cookies (p < 0.0001). The model could explain about 97.86% of the observed variations and did not present significant lack of fit (p = 0.22). The equation shows that all of the mixtures except for the binary blends increased the general acceptability of the cookies. The equation also suggests that the ternary blend produced the highest increase in sensory scores for general acceptability.

Response surfaces representing plots produced from the parameter estimates for each of these attributes were also generated and shown in Fig. 2. The figures show that for most of the sensory attributes, ternary blends resulted in increased sensory scores.
CONCLUSION

Mixture response surface methodology could be used to determine the effect of variation in the levels of cocoyam, sorghum and pigeon pea flours on sensory scores and protein quality of cookie formulations. This research has also shown that the modelling of experimental data allowed the generation of useful equations which may be used to predict effects of various blends of germinated pigeon pea, fermented sorghum and cocoyam flours on the sensory and protein quality of cookies without preparing samples. The technique employed in this study can be used to develop novel foods.

REFERENCES


