The Chemical Composition of Bread from Composite Flours of Germinated Maize (Zea mays L.), Soybean (Glycine max L Merrill) and Morgina (M.stenopetala) Leaf

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Abstract

Bread is among the staple foods of the Ethiopian population. In most parts of the country, bread is prepared from wheat flour. The objective of this study was to improve the nutritional composition bread from germinated BH-660 maize by blending with soybean and moringa leaf powder. The sample diets were formulated with different proportions and the formulation were prepared using design expert (D-optimal design) with 16 runs. The bread formula were (flour 400g, dry yeast 1.6g, salt 4g, sugar 12g, fat 8g, bread improver 8g, water 400ml). Fermentation and proofing time were 1½hr at 30-32°C and 1hr at 30°C respectively; baking was done for 45 min at 240°C. Standard procedures were used to determine the principal parameters in formulated bread. The study shows that significant differences on ash, fat, zinc and tannin contents at(p≤0.5). Addition of soybean 10-30% showed positive effect by increasing protein and fat content and addition of moringa 5-10% improved mineral and protein content. For the formulated bread the optimum nutritional content was obtained at constraint combination of maize 74.89%, soybean 15.55% and moringa 9.56%, which gives ash 6.23%, fat 9.75%, fiber 11.79%, protein 12.2%, calcium 26.4%, iron 71.44%, zinc 4.3% and carbohydrate 40.03%.

Keywords: Maize; Soybean; Moringa; Germination; Fermentation; Bread; Formulation.

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1. Introduction

A critical yet often overlooked component of food security is diet quality [1], even households who have access to sufficient amounts of food and calories may still lack essential micronutrients, increasing their risk for both short- and long-term health and development consequences.

Maize, which is a cereal widely grown in Ethiopia, is not usually utilized for bread making except in some areas of the country [2]. In Ethiopia, maize grows from moisture stress area to high rain fall areas and from lowland to the high lands [3].

The soybean seeds contain high quantity of protein and its amino acid composition is approximate to composition of animal proteins, therefore is often used as replacement component of meat protein. Soybean seeds are used in oil industry. The leaves of the *Moringa oleifera* tree are very nutritious. They can be consumed as fresh, cooked or dried. Moringa leaf powder is an excellent nutritional supplement and can be added to any dish [4].

Any maize-based diet lacks in complementary protein that contain greater levels of lysine and tryptophan[5], which is found in meat, pulses, and dairy products, and maize diets are considered protein-deficient. Protein deficiency, especially in children, causes kwashiorkor, a potentially fatal syndrome characterized by initial growth failure, irritability, skin lesions, edema, and fatty liver. The increase in protein during germination and fermentation might also be due to the fact that some amino acids are produced in excess of the requirement during protein synthesis and these tend to accumulate in free amino acid pool [6]. Addition of Soybean and Moringa can improve the iron content and increases the protein content of the diet. The objective of this study was to improve the nutritional composition of Maize for consumers and to introduce new food preparation methods by determining the optimum formulation point for bread preparation from Maize, Soybean and Moringa for better nutrition.

2. Materials and Methods

2.1. Experimental Design

The formulations were obtained based on a constrained mixture D-optimal design. Table 1 presents sample codes and actual proportion of ingredients used. Totally there were 16 sets of experimental combinations.

![Figure 1: Experimental region and points determined based on constraints](image-url)

<table>
<thead>
<tr>
<th>Constraints region</th>
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<tr>
<td>(65 \leq A \leq 82.2)</td>
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<tr>
<td>(10 \leq B \leq 30)</td>
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<tr>
<td>(5 \leq C \leq 10)</td>
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Table 1: Constraints used for formulation

<table>
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<tr>
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<th>Component B: Soy bean (%)</th>
<th>Component C: Moringa (%)</th>
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<td>6</td>
<td>69.10</td>
<td>20.90</td>
<td>10.00</td>
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</table>

The food formulation work and the subsequent quality analysis were conducted at the post harvest, animal nutrition and chemistry laboratories of Jimma University located at Jimma, Ethiopia.

2.2. Sample Collection and Preparation

Maize variety BH-660 and soybean variety "Clark" were purchased from Jimma maize and soybean seed producer farmers who working in contact with Jimma Agricultural research center. The leaves of Moringa stenopetalea tree were obtained from Alagae College of Agricultural situated at Shashemene, Ethiopia.

Maize

Maize samples were prepared following the procedure outlined by [7]. Cleaning of maize was done to remove dusts, stones, debris, broken kernels dead insects and other contaminants manually. Clean Maize grains were washed in a 5% (w/v) sodium chloride (NaCl) solution to suppress growth of moulds. Then steeped using tap water at room temperature (30±2°C) in a ratio of 1:3 (w/v) grains: water in a plastic bucket for a total of steeping time of 12hr with minor modification of soaking time. Germination was achieved by spreading the soaked seeds on moistened jute bags placed under a growth chamber set at 32°C for 24hr. The germinated grains were dried under electric oven at 70°C for 18hr and milled using (heavy duty cutting mill, SM200/698upm, Germay).
Soybean

The preparation of soybean sample was in accordance with the work of [8]. Soybean grain was boiled for 30min, followed by washing to remove the husk from the beans. Oven drying was done at 60°C for 16hr with minor modification of time and temperature used for boiling and drying. The dried sample was milled using (heavy duty cutting mill,SM200/698upm,Germay) miller.

Moringa

The fresh leaves were washed and dried on a clean sheet under the shade by occasionally turning it to obtain uniform drying and to prevent browning and off odor. After the leaves were dried the stems removed and milled using (heavy duty cutting mill,SM200/698upm,Germay) in the laboratory.

2.3. Bread Making

The method used by [9] was followed with minor modification of baking and fermentation time. 400g of germinated Maize flour, Soybean flour and Moringa leaf powder were blended together to prepare formulated bread. Dough was prepared by blending the composite flours with dry yeast 1.6 g, salt 4g, sugar 12g, fat 8g, bread improver (baking powder) 8 g and water 400 ml. Dry ingredients were mixed together by placing in a mixer, and then the dough were prepared by kneading the mixed ingredients with water for 15min.

Proofing was under taken for 1h and 30 min under oven at 32°C. The dough was divided, knocked back and shaped. After the dough rise it was turned onto a lightly floured surface and gently degassed by pressing the dough. Then dough was divided into the required size pieces, the pieces were shaped into round balls. Then dough was covered and allowed to rest for final proofing. It was left to rise under oven (for 1hr at 30°C). The bread was baked in a hot oven set at a temperature of 240 °C for 45 min until the crust turns golden and baked well.

2.4. Proximate composition and Chemical analysis

The proximate composition and chemical analysis of the bread and individual flour samples of Moringa, Soybean and Maize were done using standardized analytical procedures of (AOAC, 1990; AACC, 2000).

Moisture

Method number 925.05 of the AOAC 2000 was used to determined the moisture contents of the samples.

Where

\[ \text{Moisture(\%)} = \frac{M_2 - M_1}{M_2 - M_1} \times 100\% \]

\[ M_1 = \text{weight of dish} \]
\[ M_2 = \text{weight of dish plus fresh sample} \]
\[ M_3 = \text{weight of dish plus dry sample} \]
Total ash

AOAC 2000, method number 941.12 was used to determine the total ash.

\[
\text{Ash(\%)} = \frac{M_3 - M_1}{M_2 - M_1} \times 100\%
\]

Where \( M_1 \) is mass of crucible in g, \( M_2 \) is mass of crucible and sample in g before ashing and \( M_3 \) is mass of crucible, the sample and ash after ashing in g.

Dietary fiber

Crude dietary fiber was determined following (AOAC 2000, 920.169).

\[
\text{Crude fiber(\%)} = \frac{M_1 - M_2}{\text{weight of sample}} \times 100\%
\]

Crude protein

Crude protein was determined using Kjeldahl method as described in AOAC (1990).

\[
N (\%) = \left[ \frac{V_{HCl} \times N_{HCl}}{\text{Sample weight in g on dry matter basis (db)}} \right] \times 100
\]

Where \( V_{HCl} \) is volume of HCl in liter consumed to the end point of titration, \( N_{HCl} \) is the normality of HCl used. The nitrogen percentage obtained was expressed on dry matter basis and the resulting value was multiplied by a factor of 6.25 to calculate percentage crude protein of each sample. Urea sample was used in the analysis as control.

Crude Fat

The crude fat content was determined according to method number 4.5.01 of the AOAC 2000.

\[
\text{Crude fat(\%)} = \frac{M_1 - M_2}{\text{weight of sample}} \times 100\%
\]

Crude carbohydrate

Total carbohydrate was determined by difference according to AOAC (1990) with the exclusion of crude fiber.

\[
\text{Total CHO} = \text{Total sample of food} - (\text{Crude protein} + \text{Total Fat} + \text{Moisture} + \text{Ash})
\]
Minerals

The contents of Ca, Zn and Fe were determined by Wet digestion of the food products.

Metal content (mg/100g) = [(a-b) x V]/10 W
Where:  W= Weight (g) of samples 
V= Volume (ml) of extract
a = Concentration (μg/mL) of sample solution
b = Concentration (μg/mL) of blank solution

2.5. Method of data analysis

The data were analyzed using minitab15 software package. Significant means were separated at (P<0.05).

3. Result and Discussion

Proximate composition

The protein content of Maize, Soybean and Moringa was low as compared with the literature values of Maize 7.37, Soybean 39.99 and Moringa 28.29 [10]. On the other hand the fiber was high in soybean compared to the literature values of 5 % [11]. Maize gave higher fiber content compared to literature value of 2.2 % [12].

Table 2: Proximate composition of Maize, Soybean and Moringa used as composite flour components for bread formulation

<table>
<thead>
<tr>
<th>Proximate composition</th>
<th>Samples</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Maize</td>
<td>Soybean</td>
<td>Moringa</td>
</tr>
<tr>
<td>Moisture %</td>
<td>6.00</td>
<td>15.00</td>
<td>5.40</td>
</tr>
<tr>
<td>Ash %</td>
<td>1.36</td>
<td>8.46</td>
<td>10.09</td>
</tr>
<tr>
<td>Fiber%</td>
<td>6.34</td>
<td>8.22</td>
<td>15.09</td>
</tr>
<tr>
<td>Protein%</td>
<td>6.39</td>
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<tr>
<td>Fat%</td>
<td>1.68</td>
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<td>5.766</td>
</tr>
<tr>
<td>Carbohydrate%</td>
<td>84.57</td>
<td>18.01</td>
<td>69.49</td>
</tr>
<tr>
<td>Zinc mg/100g</td>
<td>2.72</td>
<td>3.52</td>
<td>6.519</td>
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<tr>
<td>Iron mg/100g</td>
<td>16.73</td>
<td>73.50</td>
<td>75.25</td>
</tr>
<tr>
<td>calcium mg/100g</td>
<td>27.94</td>
<td>20.15</td>
<td>22.55</td>
</tr>
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</table>

The percentage of moisture content increased with the level of soybean in the formulated bread(Figure 5). There was no significant difference (p>0.05) of moisture content to show the effect of soybean on the moisture content. This was related with the work of [13].

Similarly the lack of fit for the model was not significant (p>0.05), the model equation for moisture content was shown on Eq.1.
According to soy food association of North America (2010), soy flour derived from ground soybean was found to bring up the moisture content for the baked products, high moisture content reduces the shelf life of foods by creating suitable environment for microorganisms growth and replication.

\[ MC = 26.9A + 47.8B + 167.2C + 10.2AB - 205.6AC + 1.4BC \]  

(1)

**Figure 2**: Surface plot for moisture content of composite flour bread

High ash content in a food sample means high total mineral content. Moringa was known for its rich mineral content such as iron and calcium content. The ash content was increased with the level of Moringa proportion goes from low to high(Figure 6). Ash content have a significant difference at \( p < 0.05 \) to show the effect of Moringa on ash content. The lack of fit for the fitted model was not significant \( (p>0.05) \), the fitted model value for ash was shown on Eq.2.

\[ Ash = 1A - 4.43B + 600C + 21.9AB - 605AC - 700.8BC \]  

(2)

**Figure 3**: Contour plot for ash content for composite flour bread

The lack of fit for the fitted model was not significant \( (p>0.05) \), the fitted model for fiber content was shown on Eq.3 . The level of fiber increased with the level of moringa (Figure 7). Fiber content was not significant \( (p>0.05) \). The crude fiber content of Moringa leaves was higher and this makes it a more favorable food since high fiber foods help in digestion and prevention of colon cancer [14 & 15]. Non-starchy foods were the richest sources of dietary fiber [16] addition of fiber was employed in the treatment of diseases such as obesity, diabetes and gastrointestinal disorders.
Table 3: Mean values for proximate composition of formulated mixes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Maize</th>
<th>Soybean</th>
<th>MC%</th>
<th>Ash%</th>
<th>Fiber%</th>
<th>Protein%</th>
<th>Fat%</th>
<th>CHO%</th>
<th>Zinc (mg/100g)</th>
<th>Fe (mg/100g)</th>
<th>Ca (mg/100g)</th>
<th>Phytate (mg/g)</th>
<th>Tannin (mg/g)</th>
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<td>3.19</td>
<td>69.10</td>
<td>42.29</td>
<td>85.65</td>
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</table>

CHO=Carbohydrate  Zn= zinc content  Fe= iron content  Ca= calcium content MC= moisture content
Fiber = 20.2A + 42.4B + 398.1C - 64.9AB - 480.8AC - 405.8BC \quad (3)

**Figure 4**: Surface plot for fiber content for the formulated bread

**Protein**

Increased in time of fermentation and germination slightly increases the protein content of the food [17]. Moringa was also used as protein source by substituting soybean for fish feed [18]. The contour plot for protein was shown on (Figure 8). Protein content was not significant (p > 0.05). The lack of fit for the fitted model was not significant (p > 0.05), the fitted model for protein level was shown on Eq.4. This was indicated that supplementation of maize bread with soybean flour and germination process of maize can improve the protein content of the formulated bread. This result was similar with the work of [13]. Soybean seeds were also rich in lysine content which was more deficient in most cereals [19].

Protein = 6.3A + 95.8B - 836.8C - 100.4AB + 974.5AC + 975.9BC \quad (4)

**Figure 5**: Contour plot for protein content for composite flour bread

**Crude Fat**

Figure 9 shows that large fat content was observed on high level of soybean in the formulation. Fat content have significantly increases at p < 0.05 to show the effect of soybean on fat content for the formulated bread. The Analysis of Variance (ANOVA) shows the lack of fit for the fitted model was not significant (p > 0.05), the fitted model was shown on Eq.5. The soybean seed is rich in its fat content in different from other crops, it contains approximately 47% [20].
Fat= -2.3A-38.2B+123.5C+130.3AB-78.4AC-264.1BC \hspace{1cm} (5) \\

**Figure 6:** Contour plot for fat content for composite flour bread

**Carbohydrate**

The surface plot for carbohydrate in Figure 10 shows the high carbohydrate content associated with high level of maize in composite flour bread. Carbohydrate was not significant (p >0.05) from the constraints used for the formulation to show the effect of maize. The lack of fit for the fitted model was highly significant (p<0.01), the fitted model for carbohydrate is shown in Eq.6.

CHO=67.2A-110.3B-823.5C+58.4AB+719.1AC-1836.2BC \hspace{1cm} (6) \\

**Figure 7:** Surface plot for carbohydrate content for composite flour bread

**Minerals**

The human body requires a daily intake of more than 100 mg of major minerals and less than 100 mg of minor minerals [21]. Minerals are required for the normal growth, activities of muscles and skeletal development of our body.
a) Zinc

The value for zinc content was in between 0.519 mg/100 and 6.917 mg/100 g as shown in Figure 11. Zinc had a significant difference (p < 0.05). The zinc content was not directly related with the level of components used for the formulation. The lack of fit for the fitted model was highly significant (p<0.01), the fitted model for zinc was shown on Eq.7.

\[
\text{Zinc} = 36A + 131B + 2522C - 226AB - 3000AC - 2899BC \quad (7)
\]

Figure 8: Contour plot of zinc for composite flour bread

b) Iron

The level of iron content was increased with the proportion of moringa in the formulation (Figure 12). Iron content was not significant (p >0.05) to show the effect of moringa on iron content in the formulated bread. The lack of fit for the model was highly significant (p<0.01), the model equation for iron is shown with Eq.8. The iron content of moringa leaves was reported to be 25 times more than that of iron content of spinach [22&23].

\[
\text{Fe} = 34A - 281B - 5790C + 558AB + 7420AC + 6565BC \quad (8)
\]

Figure 9: Contour plot of iron content for composite flour bread
c) Calcium

The mean values for calcium content varied from 6.044 mg/100 g to 42.294 mg/100 g (Figure 13). The increment of calcium content was not related with variation constraints used for the formulation. The leaves of moringa shows a very high concentration of calcium this is similar with the work of [24], Calcium content was not significant (p >0.05) to show the effect of constraints on calcium content. The lack of fit for the model was highly significant (p<0.01), the model equation for calcium is shown with Eq.9.

\[ Ca = -67A - 514B - 8394C + 696AB + 9794AC + 11904BC \]  

(9)

![Figure 10: Contour plot of calcium content for composite flour bread](image1)

Overall Nutritional composition of bread from composite flour was determined by overlaying the different contours for the respective parameters. Accordingly the best combination of the three components in terms of nutritional and anti-nutritional aspects lies in the sweet area which lies at maize74.8%, soybean16% and moringa9.1% and as shown on the figure16 below.

![Figure 11: Overlaid contour plot of sweet/optimum point for proximate analysis](image2)
5. Summary and Conclusion

For the formulated bread from maize, soybean and moringa the optimum point for proximate composition were obtained at components combination point of 74.84% maize, 16% soybean and 9.1% moringa, which was resulted chemical composition of 5.8% ash, 9.8% fat, 30.3% moisture content, 11.9% fiber content, 12.5% protein, 27.7 mg/100g calcium, 71.2 mg/100g iron, 3.72 mg/100g zinc, 40.4% carbohydrate.

Several studies on the effect of germination on grains found that germination can increase protein content and dietary fiber, increased mineral bioavailability [25; 26&27]. Germination also was reported to be associated with increased amount of vitamin concentrations and bioavailability of trace elements and minerals [28& 29] found that germination improves calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid contents.

Further analysis for micro nutrients of the formulated bread, improving the color of bread to make attractive the color of baked bread due to the addition of moringa leaf powder, to remove the bitter taste of moringa which was detected in the bread.

References


