Studies on the Proximate, pH, Titratable Acidity and Microbial Activity of Maize Fermented Meal Fortified with Soybean and Bambaranut to Produce Infant Weaning Meal

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Abstract

White maize was fermented to produce an infant weaning food fortified by soybean and bambaranut, soybean and Bambaranut were roasted. The research was carried out to determine the proximate composition, pH, Titratable acidity and Microbial activity. Infant weaning food was formulated in a 70:20:10 ratio i.e. 70(g) white maize 20(g): soybean: 10(g) bamabaranut. By Standard Procedures. Parameters analyzed were carried out using analysis of variance (ANOVA). Values were significant at P 0.05. The weaning food blend had a potent level of (12.71 ± 0.19) which was below the control cerelec® (15) but has met the RDA of infants 0 - 1 year (12.44). The fat (13.13 ± 0.11) carbohydrate (71.0 ± 0.18) and energy content (426.08 ± 0.17) of the weaning food blend compare favourably with the control cerelec®. A steady drop in pH with a total increase in titratable acidity was observed during the fermentation of white maize. Lactobacillus and saccharomyces cerevisiae were mostly present during maize fermentation. The weaning food blend met the RDA of infant 0 - 1 year in terms of protein, fat, carbohydrate and energy with a longer shelf life and low microbial activity.

Keywords: Maize; Soybean; Bambaranut

Introduction

Weaning is a the process of gradually introducing an infant to what will be its adult diet while withdrawing the supply of its mothers milk. The infant is being introduced to foods which are soft, thick, creamy, porridge made from staple foods [1]. The weaning process is a step by step process by which the infant slowly gets used to the family or adult diet. Other process of weaning includes direct introduction of the infant to the family diet. This later option creates a problem, as the child may not have enough of the family diet to meet his or her nutritional demands [2,3].

Protein-energy malnutrition is one of the critical problem in developing countries and is associated with many health challenges. Which includes infant mortality, poor physical and intellectual development of infants, as well as very low resistance to disease and consequently stifles development. Protein-energy malnutrition generally occurs when infants are weaned from mothers milk to the family diet [4]. Poverty and improper feeding practices are the major challenges of this nutritional problem [5].

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Locally produced infants foods are mostly prepared from either maize, millet or sorghum referred to as "Ogi or Akamu", which are deficient in some of the essential amino acids such as lysine and tryptophan [6]. Our locally available cereals when subjected to some thermal processing techniques can be supplemented with legumes (rich in essential amino acids) which are a good potential source of protein for weaning food in children due to its high digestibility and acceptability to children [7]. Bambara groundnut is important for human consumption, it contains required quantities of protein, carbohydrate and fat. Studies have been carried out on the nutritional content of the seed [8,9].

Bambaranut contain about 63% carbohydrate, 19% protein, and 6.5% oil. The value of protein in Bambaranut can be used to fortify our mostly starchy foods like "Ogi", made from maize [10]. Traditionally prepared foods from maize (Zea mays) lack the adequate micronutrients. In order to help alleviate the ever-increasing problems of malnutrition in developing countries, the need for fortification of popularly consumed low protein staple foods with inexpensive sources of plant proteins cannot be overemphasized.

Studies have been carried out by Tsai., et al. [11] which shows an increase in the lysine and tryptophan in germinated corn. Also, a high vitamin was observed in germinated sorghum and maize [12], with an increased amino acid and vitamins contents in fermented blends of cereals and soybeans. Many brands of low-cost proprietary weaning foods have been developed from locally available, high-calorie cereals and legumes in tropical Africa [13]. Cereals are deficient in minerals such as calcium, potassium, iron and zinc [6] and fortification of cereals with legumes rich in proteins and essential amino acids has also been perfected [14]. There is need to fortify fermented maize flours with legume protein like soybean and bambara groundnut, which will produce an infant weaning food. However, studies on the proximate, pH, titratable acidity and Microbial activity of maize fermented with soybean and bambaranut is scars.

Objective of the Study

The objective are to:

1. Determine the proximate composition of weaning food blend.
2. Determine the pH and Titratable Acidity of the weaning food blend.
3. Carry out microbial analysis of the weaning food blend.

Source of material

White maize, soybean and bambara-nut where purchased from the Maiduguri Monday Market and authenticated by a plant taxonomist at University of Maiduguri, Borno state, Nigeria.

Preparation of materials

The white maize, soybean and bambara-nut was cleaned by picking all the stones and other foreign particles.

Preparation of white maize (Fermentation)

The fermentation was carried out using the method described by Akinbala., et al [15]. Two thousand gram of white maize was cleaned and stepped into 4000 ml of distilled water in a ratio 1:2 for 72 hours. The top water was decanted and 4000 ml of distilled water was added and milled into slurry. The slurry was then sieved through a nylon cloth to separate the bran. The filtrate was then allowed to stand for 24 hours and the top water decanted. The akamu oven dried to a constant moisture and transferred to an air tight container and stored.
Preparation of soybean

The cleaned soybean was roasted on a frying pan using a kerosene stove for 15 minutes with constant stirring. The roasted soybean was then ground into a fine powder which was used for the analysis [16].

Preparation of bambara-nut

The cleaned bambara-nut was roasted on a frying pan using a kerosene stove for 15 minutes with constant stirring. The roasted bambara-nut was then ground into a fine powder which was stored in an air tight container and used for the analysis [16].

Formulation of weaning food blend

The food blend was formulated in the ratio of 70:20:10. The fermented maize flour was fortified with soybean (processed) and bambara-nut (processed), with the maize flour taking 70%, soybean 20% and bambara-nut 10%.

Methods

Proximate analysis

Chemical analysis (proximate analysis) the moisture content, protein, fat, ash, crude fibre and carbohydrate of and processed white maize, soybean and bambara-nut were determined using the methods of Association of Official Analytical Chemist [17].

Crude protein

Crude protein was determined as the method adopted by kjeldahl method (Okalebo., et al. 2002). 1g or 2g of samples was weighed into a digestion tube and 1 or 2g kjeldahl tablets were added, 10 or 20 ml of concentrated sulphuric acid (Conc. $H_2SO_4$) was added into the tube and digested at 420°C for 3 - 5 hours. After cooling, 80 or 90 ml of distilled water was added into digested solution. About 50 ml of 40% caustic soda (NaOH) was added into 50 ml of digested/diluted solution and placed on heating section of the distillation chamber, 30 ml of 4% boric acid, bromocresol green and methyl red as an indicator was put onto conical flask and placed underneath the distillation chamber for collection of ammonia (NH$_3$), the solution changed from orange to green colour. About 0.1 normal solution of hydrochloric acid (HCl) was weighed into burette. The solution changed from green to pink after titration then the burette reading was taken.

The crude protein was calculated using the formula below:

$$\%\text{Crude protein} = \frac{(A-B) \times N \times F \times 6.25}{Mg \text{ of sample}} \times 100$$

Where $A=$ ml of acid used for titrating the sample

$B=$ ml of acid used for titrating blank sample

$N=$ Normality of acid used for titration

$F=$ factor 14.007

Constant= 6.25

Conversion factor= 100.
Dry matter

The dry matter content of the samples was determined by weighing log of samples into petri dish while placed in hot oven at a temperature of 105°C for 24 hours and then removed and placed in a desiccator to cool, after cooling you reweight. The dry matter content was calculated using the formula:

\[ \text{Dry matter} = \frac{W_1 - W_2}{W} \times 100 \]

Where:

- \( W_1 \) = Weight of petri dish with samples before oven drying
- \( W_2 \) = Weight of petri dish with samples after oven drying
- \( W \) = Weight in grams of empty petri dish.

Crude fibre content

Crude fibre was determined by weighing 2g of samples and placed in a round or flat bottom flask and 50 ml of trichloroacetic acid reagent (TCA) was added, the mixture was boiled and refluxed for 40 minutes. Filter paper was removed and cooled to 100°C temperature. The filter paper is then used to filter the residue. The residue obtained was washed 4 times with hot water and once with petroleum ether then the filter paper plus were folded together and dried at 30°C - 60°C in an oven for 24 hours, reweighed and then ash at 650°C and then cooled and reweighed.

\[ \text{Crude fibre} = \frac{\text{Difference in weighing}}{\text{Weight of sample on DM basis}} \times 100 \]

Ether extract (fat) content

The ether extract was determined by using soxhlet apparatus, 1 or 2g of the sample was weighed into a thimble and 200 ml of petroleum ether was measured with measuring cylinder, the solution was put into round or flat bottom flask and heated at 45°C for 1 hour for 2 hours. The collecting flask was removed and cooled into desiccator for 15 minutes and percentage of fat in the sample was determined using the formula below:

\[ \%\text{fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100 \]

Ash content

To determine the ash content, 1 or 2g of sample was weighed into crucible and dried at 105°C for 24 hours then cooled in a desiccator for 15 minutes and then reweighed. It was the charred at 600°C or 650°C in a muffle furnace for 2 - 3 hours and then cooled in a desiccator for 15 minutes and reweighed.

\[ \%\text{ash} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100 \]

Carbohydrate content

Percentage carbohydrate was determined by indirectly computing the differences of the other parameters using the formula below:

\[ \%\text{carbohydrate} = 100 - (\%\text{mct} + \%\text{cp} + \%\text{cf}) \]
Energy content
The energy content of sample was calculated using a water factor method as described by Mahgoub [18]:
\[ \text{Energy (Kg)/100g} = 4.186 \times (\% \text{cp} \times 4) + (\% \text{fat} \times 9) + (\% \text{carbohydrate} \times 4). \]

Statistical analysis
All data collected were subjected to analysis of variance (ANOVA) using SAS (SAS institute inc, 1999) package. All determination were made in three replicates and the difference among the means were separated using the DUNCAN multiple range test, SAS (1999), with significant difference at 5% \((p < 0.05)\).

pH and titratable acidity
The pH and Titratable Acidity of the liquid and the slurry was carried out according to Egan., \textit{et al} [19].

Exactly 10 mls of sample was measured into a 50 ml beaker, drops of phenolphthalein indicator was added and titrated against 0.1N NaOH. the result is expressed in gram Tartaric acid/100 ml sample.

Calculation:
\[ \text{Titratable Acidity (gram Tartaric/100ml)} = \frac{V \times N \times \text{Meq.wt} \times 100}{1000 \times V} \]
Where:
\( V \) = Volume of NaOH.
\( N \) = Normality of NaOH.
Meq.wt. = Mill equivalent weight of standard.

Microbial evaluation
The microbial analysis was carried out according to Harrigane and McCaine [20].

Preparation of culture media
Blood agar
The commercial prepared powdered blood agar base was weighted thirty seven gram (37g) and transferred into a clean conical flask; one liter of distilled water was added and shaken to dissolve completely by heating to boil in a water bath. The media was then sterilized by autoclaving at 121\(^\circ\)C for 15 minutes and allowed to cool up to 55\(^\circ\)C. 50 mls of titrated sheep blood was added carefully and mixed and aseptically poured into sterilized petri dish and allowed to set.

Macconkey agar
The commercially prepared macconkey agar was weight 52g using sensitive balance and transferred into a clean conical flask. 1 liter of distilled water was added and shaken to dissolve completely and it was sterilized by autoclaving at 121\(^\circ\)C for 15 minutes. The media was allowed to cool to 55\(^\circ\)C and then poured into sterilized petri dish and allowed to set.
Eosin methylene blue agar (EMBA)

37g of the commercially prepared EMBA was weighted using sensitive balance and transferred into a clean conical flask and 1 litre of distilled water was added and shake to dissolve completely, the media was then sterilized by autoclaving at 121°C for 15 minutes. The media was allowed to cool to 55°C and then poured into sterilized petri dish and allowed to set.

Mannitol salt agar

The commercially prepared powdered mannitol salt agar was weighted 111g and transferred into a clean conical flask and 1 litre of distilled water was added and shake to dissolve completely, the media was then sterilized by autoclaving at 121°C for 15 minutes. The media was allowed to cool to 55°C and then poured into sterilized petri dish and allowed to set.

Nutrient agar

The commercially prepared powdered nutrient agar was weighted 28g and transferred into a clean conical flask and 1 litre of distilled water was added and shake to dissolve completely, the media was then sterilized by autoclaving at 121°C for 15 minutes. The media was allowed to cool to 55°C and then poured into sterilized petri dish and allowed to set.

Chocolate agar

The chocolate agar was prepared using the same procedure as blood agar but only differs in that after adding the blood it was reheated again gently to give the chocolate colour and poured into a sterile petri dish to set.

Preparation of normal saline

8.5 gram of sodium chloride (NaCl) was weighted and transferred into a conical flask, 1 liter of distilled water added shake well and autoclaved at 121°C for 30 minutes and allowed to cool.

Total bacterial count

The total bacterial count was carried out using tenth fold serial dilution with normal saline 9 ml each of the normal was dispensed in a sterilized test tube and 1 gram of the sample was weighted and transferred into a sterile universal container and 9 ml of normal saline is added and shake well. 1 ml was taken using sterile pipette and added to the 9 ml of normal saline inside the sterile tests to make 10 fold serial dilution, then 0.1 ml was transferred from the 3rd tube to a sterile dried nutrient agar and spread. It was incubated at 37°C for 24 hours. The colony counter was used to read results as colony forming unit per ml.

Identification of microorganisms

The presence of microbes was carried out by conventional methods starting with gram standing. Briefly, using a sterile wire loop, a drop of distilled water was put on the centre of grease free slide and a portion of colony was picked and emulsified into drop of sample and allowed to air dry before fixing to gram stain, crystal violet was then applied for 3 minutes.

It was then replaced with a gram's iodine for 1 minute prior to rinsing with water and application of 95% alcohol until no color appeared on the flow. Water was used to rinse the slides and safranin applied for 1 - 2 minutes. This was followed by rinsing and air-drying and then observed microscopically under x100 emersion oil objective.

Biochemical test

Sugar fermentation

The sugar fermentation test for carbohydrate fermentation was carried out on the isolated bacteria for their identification. The sugars were prepared by tyndallization process and the following sugars were used: glucose, lactose, mannitol, sorbitol, sucrose, maltose and...
xylose. A colony of the test organic was picked and inoculated the prepared sterilized sugar each using a sterilized were loop and was incubated at 37°C for 24 hours an acid and gas production was observed and result were recorded.

**Catalase test**

This test was carried out with a drop of 3% hydrogen peroxide put on a clean grease-free shade and a colony of the test organism was picked and emulsified (mixed) in the drop of the hydrogen peroxide and was examined for gas bubbles which indicates catalase positive. The test was used to differentiate between *Staphylococcus* spp. from *Streptococcus* spp. The gas bubble observed in this reaction was due to the breakdown of hydrogen peroxide to oxygen and water by enzyme called catalase.

**Coagulase test**

Coagulase test was carried out on all species of *Staphylococcus* using a drop of normal saline or physiological saline on a clean glass slide. A drop of rabbit plasma was added and mixed. Coagulase positive shows clumping or agglutination immediately, this test is used to differentiate pathogenic *Staphylococcus aureus* from non-pathogenic *Staphylococcus*.

**Methyl red test**

To 5 ml of the culture (incubated five days at 37°C in MR-VP bath) add five drops of methyl solution. A positive reaction indicated by a distinct red color indicates acidity (pH 4.9 - 6.0). A yellow color indicates negative reaction.

**Results**

Result of the proximate composition of unprocessed and processed white maize, soybean and bambaranut is presented in table 1. A significant decrease was observed in the raw and processed maize and Bambara nut in terms of their moisture content. The levels of protein of the fermented maize shows a significant increase after fermentation (8.12 ± 0.01) when compared to the raw maize (5.43 ± 0.02) and (5.23 ± 0.02) for the raw maize and Bambara nut for the processed maize and Bambara nut (4.4.2 ± 0.18) and (4.80 ± 0.23) respectively. The soybean had a high protein value after processing. The level of fat is lower in the raw maize (2.07 ± 0.01) than the processed maize (3.23 ± 0.01). Low level of fat was recorded for processed soybean and Bambara nut while and increase was recorded for unprocessed samples. There was a significance decrease in the carbohydrate and metabolizable energy content between the raw and processed maize and soybeans.

<table>
<thead>
<tr>
<th>Nutrient (%)</th>
<th>Sample</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maize</td>
<td>Raw</td>
<td>Fermented</td>
<td>Soybean</td>
<td>Roasted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>5.43 ± 0.02a</td>
<td>4.42 ± 0.18b</td>
<td>6.34 ± 0.02c</td>
<td>7.25 ± 0.12c</td>
<td>5.23 ± 0.08d</td>
</tr>
<tr>
<td>Crude protein</td>
<td>6.46 ± 0.01a</td>
<td>8.12 ± 0.01b</td>
<td>11.31 ± 0.05c</td>
<td>10.05 ± 0.02d</td>
<td>12.5 ± 0.12c</td>
</tr>
<tr>
<td>Crude fat %</td>
<td>2.07 ± 0.01a</td>
<td>3.23 ± 0.01b</td>
<td>12.07 ± 0.03c</td>
<td>10.15 ± 0.02d</td>
<td>4.0 ± 0.12b</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>81.92 ± 0.03a</td>
<td>84.24 ± 0.02b</td>
<td>67.29 ± 0.07c</td>
<td>62.20 ± 0.08c</td>
<td>48.2 ± 0.11c</td>
</tr>
<tr>
<td>Metabolizing (100g/kcal)</td>
<td>78.9 ± 0.07a</td>
<td>77.05 ± 0.02b</td>
<td>87.02 ± 0.11c</td>
<td>81.41 ± 0.14d</td>
<td>53.3 ± 0.17c</td>
</tr>
</tbody>
</table>

**Table 1**: Proximate composition of raw and processed white maize, soybean and bambara-nut. Values are recorded as mean ± SEM of three determinations. Values in the same row with different superscript are significantly different (P < 0.05).

The proximate composition of the formulated weaning food blend and the control cerelac® is presented in Table 2. The protein (12.71 ± 0.19) of the weaning food blend is lower than the control cerelac® (15). The energy (426.08 ± 0.17), fibre (9.48 ± 0.01), fat (13.13 ± 0.11) and carbohydrate content (71.01 ± 0.18) of the weaning food blend was higher than the commercial weaning food cerelac® (410), (2.0), fat, (10) and (65) respectively. The protein energy and fat content of the weaning food blend met the RDA of infant 0 - 1 year (12.44), (10) and (400) respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weaning food blend</th>
<th>Cerelac® Maize</th>
<th>RDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>4.30 ± 0.12</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>12.71 ± 0.19</td>
<td>15</td>
<td>12.44</td>
</tr>
<tr>
<td>Crude fat %</td>
<td>13.13 ± 0.11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>71.01 ± 0.18</td>
<td>65</td>
<td>-</td>
</tr>
<tr>
<td>Ash</td>
<td>1.03 ± 0.01</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>Fibre</td>
<td>9.48 ± 0.01</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Metabolizing energy (kcal/100g)</td>
<td>426.08 ± 0.17</td>
<td>410</td>
<td>400</td>
</tr>
</tbody>
</table>

Table 2: Proximate composition of weaning food blend.

*Values are recorded as means ± SEM of three determinations.*

*Cerelac® - Nestle Nigeria Plc. ND: Not Determinant.*

**Determination of pH and titratable acidity**

Change in pH and titratable acidity during production of Akamu is presented in Table 3. The pH dropped with an increase in time. The change in pH ranged from initial pH of 6.3 ± 0.05 to 5.0 ± 0.12 at 72 hours. An increase with Titratable acidity increased from 0.16 ± 0.01 to 0.5 ± 0.01 during the 72 hours of maize production.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0hrs</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.3 ± 0.06</td>
<td>5.7 ± 0.11</td>
<td>5.5 ± 0.15</td>
<td>5.0 ± 0.12</td>
</tr>
<tr>
<td>TA</td>
<td>0.16 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>0.5 ± 0.01</td>
</tr>
</tbody>
</table>

Table 3: Periodic pH and titratable acidity of steep water for 72 hours.

*Values are recorded as mean ± SEM of three determinations.*

**Total bacterial count and microorganisms isolated**

Microbial counts during the fermentation of "Akamu" is presented in Table 4.

The total bacterial count reduced from 22 x 10^3 cfu/ml which then dropped to 20 x 10^3, 13 x 10^3 and 10 x 10^3 at 0hrs, 24 hrs, 48hrs and 72hrs fermentation of maize respectively. The slurry recorded a Total Bacterial Count of 18 x 10^3 24 hrs, 15 x 10^3 for dried Akamu and 9 x 10^3 cfu/ml for the weaning food blend.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 hrs</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steep water</td>
<td>$22 \times 10^3$</td>
<td>$20 \times 10^3$</td>
<td>$13 \times 10^3$</td>
<td>$10 \times 10^3$</td>
</tr>
<tr>
<td>Slurry</td>
<td>$18 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dried Akamu</td>
<td>$15 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weaning food blend</td>
<td>$9 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4: Periodic total bacteria count during the production of “Akamu”.

Occurrence of microbes isolated from “Akamu” production are shown in table 5. Staphylococcus aureus, Bacillus subtilis and lactobacillus appeared after 24hrs of maize fermentation. The microbes present in the slurry are Lactobacillus, saccharomyces cerevisiae. Streptococcus lactis, lactobacillus were presented after “Akamu” was sun dried to a constant weight. Lactobacillus was present in the infant formulated weaning food blend.

<table>
<thead>
<tr>
<th>Sample</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Staphylococcus aureus, Bacillus subtilis, Lactobacillus</td>
<td>Lactobacillus, Saccharomyces, cerevisiae Streptococcus, Lactis E. coli</td>
<td>Lactobacillus, Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Steep water</td>
<td>Staphylococcus aureus, Bacillus subtilis, Lactobacillus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slurry</td>
<td>Saccharomyces cerevisiae, Lactobacillus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dried “Akamu”</td>
<td>Streptococcus lactis, Lactobacillus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weaning food blend</td>
<td>Lactobacillus</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5: Micro-organisms isolated during the production of “Akamu”.

Values are recorded as mean ± SEM of three determinations. Values in the same row with different superscript are significantly different (P < 0.05).

Discussion

Proximate composition

A significant differences was shown in the raw and processed white maize, the moisture content of raw (5.43%) and processed white maize (4.42%) shows significant difference (p < 0.05) with raw having higher value and this may be due to the method of processing (fermentation), which means it will keep longer than the raw [21], whereas in soybean there is a little increase in moisture content of the processed (7.25%) and raw (6.24%). There was no significant change in the moisture content of raw and processed bambara-nut. An high protein was observed during the fermentation of “Akamu” as it was earlier reported by Mahgoub [18] that natural fermentation of cereals improved protein level slightly and this may be due to the loss in dry matter. Crude protein was higher in soybean and bambara-nut compared with the white maize, the figure suggest that the soybean and bambara-nut are good source of protein and can be use in alleviating the problem of protein energy malnutrition (PEM) commonly prevalent in the developing countries. An increased in carbohydrate content in akamu produced from fermented white maize was noticed with similar report of Modu, et al. [7] and Bintu., et al. [1] which provide higher percentage of carbohydrate than both soybean and bambara-nut. Carbohydrate content of maize made it possible a good source of starch.

The weaning food blend had a moisture of 4.3%. Low moisture indicates that it will have a good keeping quality. Because food spoilage micro-organism strive where the moisture content of a food is high, this is in agreement with similar reports of Bintu., et al [1].

protein content of the weaning food blend (12.71%) is lower than the control cerelac (15) and recommended daily allowance of infants from 0 month to 1 year (protein is 14). Therefore, the result showed that there was substantial increase in the protein after blending of the “Akamu” prepared from white maize, soybean and bambara-nut. Tallies with findings were also reported by Modu., et al. [7], Nkama., et al. [22], Mbata., et al. [23,24], Amankwah [4] in a related work, where an increase in level of protein was observed. The infant weaning food blend had a fat content of 13.13% which is also in contrast to the 10% as recommended by protein advisory group (1972). Hence, food high in fat have short life span than one with a lower fat content. The level of carbohydrate of the weaning food blend is 71.01% which is slightly higher than the recommendation of protein advisory group (1972) which is 50 - 60%. The weaning food blend has high amount of energy, these implies that the product would supply the needed energy to meet infant growths demand.

**pH and titratable acidity**

The reduction in pH (from 6.3 ± 0.06 to 5.0 ± 0.12) observed in this study has reported to be due to the production of acid by fermenting microorganisms and the observed increase in Titratable acidity (from 0.16 ± 0.01 to 0.50 ± 0.01). Acidification may occur due to dominance of Lactic acid bacteria

High titratable acidity has been reported to reduce incidence of diarrhea in infants consuming fermented foods [25]. Therefore, the Maize, Bambaranut and Soybean blend will have two attributes, such as antimicrobial properties and high protein content.

**Microorganisms isolated**

The reduction in total bacterial count of the weaning food blend might be attributed to the presence of glycoamylase which degrades starch. Similar with report of Nout [26]. Fermentation has been suggested to have inhibits the growth of microorganisms that can cause spoilage of food poisoning [27-29].

**Conclusion**

In conclusion, it was observed that fermentation and supplementation of cereals (Maize) with legumes (soybean and bambara-nut) has improved the protein quality of the weaning food. The weaning food blend met the recommended dietary allowance (RDA) of infant 4 - 6 months and above in terms of protein, fat, carbohydrate and energy and also be used in the production of a weaning food blend enhanced with longer shelf life and low microbial activity.

**Recommendation**

There is a need to change the processing method of soybean as it has been found to decrease the nutritional content of the seed and also increase the sterility of the processing procedures so as to avoid contamination by microorganisms.

**Bibliography**


Studies on the Proximate, pH, Titratable Acidity and Microbial Activity of Maize Fermented Meal Fortified with Soybean and Bambaranut to Produce Infant Weaning Meal


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