Production and evaluation of chemical composition, tanin, in vitro protein digestibility and microbiological studies of fermented local variety of sorghum, fortified with cowpea and ground nut.

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The fermented sorghum (yafi moro) based cereal/legume blend was produced from 70 parts of fermented local sorghum flour, 20 parts of dehulled cowpea and 10 parts of roasted ground paste (FSCG). The chemical composition, mineral elements, in vitro protein digestibility and microbiological was determined using standard methods. The result showed that the protein of the samples (FSCG), raw and fermented sorghum were 12.46%, 8.65 and 9.5% respectively. The Moisture content 4.0%, 3.6%, 3.8%; Ash content was 2.0%, 2.2% and 1.5% while Carbohydrate 70.1%, 74.3% and 68.54%. The calculated energy value is 351Kcal, 332.8Kcal and 353.2 Kcal respectively. A significant decreased (p≥0.05) in the level of phytate, mineral element levels with a concomitant increased in in vitro digestibility in both fermented and fermented fortified blend compared to the unprocessed samples. The microorganism isolated and characterized were lactic acid bacteria. It can thus be concluded that; processing methods such as fermentation and subsequent fortification of the fermented samples with legume has greatly enhanced its nutritional value as reported in this study.

Key words: Sorghum, fermentation, cowpea, ground nut.

The present trend in population growth indicates that the protein gap may continue to increase in the future unless well- planned measures are taken too, to tackle the situation. Provision of adequate proteins of animal origin is difficult and expensive. An alternative for improving nutritional status of the people is to supplement the diet with plant proteins. Attention therefore has to be directed to the nutritional evaluation of proteins from plant species. Legumes (poor man’s meat) play an important role in human nutrition since they are rich sources of protein, calories, minerals, and vitamins and therefore can be good supplements (Deshpande, 1992).

Sorghum (sorghum bicolor L., Moench) is used for human consumption mostly is fermented sour drinks, sour porridge, and beer (Ankirele, 1967; Banigo, 1969). In Nigeria, Ogi porridge (yoruba), kamu (hausa), akamu (igbo), prepared from fermented grain sorghum, is an important traditional food for weaning infants and is also the major breakfast cereal for adults. Unfermented sorghum grain is also consumed by humans in Ethiopia, sudan, Uganda, and Nigeria as well as in Guatemala and Nicaragua (Murty and Kumar, 1995).

Sorghum like other cereals, has some limitations due both to its low protein content and to limitations in some essential amino acids found in sorghum flour occur in limited amount; lysine, methionine, isoleucine, tryptophan, and threonine, with lysine the most limited in protein. Sorghum also has a high leucine/ isoleucine ratio (5:1) and high consumption of this grain is known to cause pellagra in humans (an excess of leucine in the diet inhibits conversion of tryptophan to nicotinic acid).

In many African countries, sorghum is...
milled into flour before fermentation and cooking. High tannin content in some varieties of sorghum makes it difficult to digest (McGinty, 1968), and the astringency of the milled product may make it unpalatable. However, from an economic standpoint, high tannin content in sorghum may be an advantage because it renders the grain resistant to birds and molds.

Fermentation however, is an ancient method of food processing aimed at prolonging shelf-life and improving palatability. It may also improve digestibility and nutritional value of food and feed. The preservation by fermentation of vegetables and cereals is mostly due to the lactic acid bacteria, often in combination with yeasts. However, other types of bacteria, example, Bacillus Spp. are involved (Nout and Rombouts, 1992). It is important to consider the quality, quantity and availability of the nutrients in the grain. Sorghum like other cereals has some limitation in some essential amino acids particularly lysine, while legumes and oil seeds are high in both protein quality. Cooking reduce the protein digestibility of sorghum grain, when sorghum is cooked, enzymatically resistant protein polymers are formed through disulphide bonding of beta and gamma- kafirins (Oyus et al, 1985). This is perhaps one of the most important factors contributing to reduced protein digestibility of cooking sorghum.

The objective of present study was to determine the proximate composition, mineral element and in vitro protein digestibility, before and after processing of the sorghum variety (Yafi Moro), fortified with ground nut and cowpea.

MATERIALS AND METHODS

Raw Sorghum

The sorghum local variety (Yafi moro) was bought from a local market in the northern part of Nigeria, Maiduguri, Borno State. The cowpea was bought from local market in Maiduguri called Monday market including groundnut. Four formulation were produced, one containing fermented sorghum with processed groundnut and cowpea, the second containing raw sorghum with processed groundnut and cowpea, the third containing raw sorghum only and the fourth containing fermented sorghum only.

Chemical analysis

The procedures were analyzed using established methods of the AOAC (2000) for moisture, Ash, Fat and carbohydrate.

Determination of Crude protein;

Crude protein content was analysed using Kjeldeldall method.

Determination of crude fibre

A 2g of the sample was placed in a 450ml conical flask and 50mls of tri-chloroacetic acid digestion reagent (TCA) was added, the mixture was boiled and refluxed for 40 minutes. The flask was removed and cooled to room temperature. Filter paper was 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 the sample were folded together and dried at 30 – 600C in an oven 24 hours, reweighed then washed at 6000 and reweighed.

\[
\% \text{ CF} = \frac{\text{difference in weighing}}{\text{Weight of sample on DM basis}} \times 100
\]

Determination of Energy Value

This was obtained using the Atwater factors (physiological fuel values) of 4kcal, 4kcal and 9kcal per gram of carbohydrate, protein and fat content of the samples and respectively.

Determination of In vitro protein digestibility (Nills, 1979).

A 1ml of 11% trypsin was introduced into 3 test tubes. 4ml of phosphate buffer of pH 7.5 were added to each test tubes and 1ml of 0.1N HCL was added and allowed to stand to equilibrate 1ml of 1% ‘kamu’ was added to all the test tubes (labeled as digestibility at 1 hour and 6 hours). The reaction in each of the test tube was stopped with 5ml of neutralized formalin at 60 minutes and 6 hours. The content of the test tubes were then filtered using filter paper. The filter papers were dried in an oven at 1080C for 3 hours. The nitrogen of the undigested sample was determined by the Kjedahl method.

Protein digestibility (%) = \( \frac{\text{CP1–CP2}}{\text{CP1}} \times 100 \)

Where CP1 = total protein of unprocessed grain.

CP2 = total protein after digestion with trypsin

Tannin content Determination

The tannin content of the raw and
Figure 1: flow diagram for the production of sorghum – cowpea – groundnut – blend and traditional fermented sorghum flour samples.

Table 1: The proximate composition of the raw and processed local sorghum (yafi moro) and the fermented sorghum supplemented with cowpea and groundnut.

<table>
<thead>
<tr>
<th>%</th>
<th>Raw Sorghum</th>
<th>Fermented Sorghum</th>
<th>Fermented Sorghum supplemented with cowpea and groundnut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>90.6 ± 3.73a</td>
<td>85.7 ± 2.30b</td>
<td>89.8 ± 3.38a</td>
</tr>
<tr>
<td>Moisture content</td>
<td>3.6 ± 0.87a</td>
<td>3.8 ± 0.98a</td>
<td>4.0 ± 1.08a</td>
</tr>
<tr>
<td>Protein</td>
<td>8.6 ± 1.12a</td>
<td>9.5 ± 1.32b</td>
<td>12.46 ± 2.11c</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>13.5 ± 2.17a</td>
<td>8.9 ± 0.85b</td>
<td>10.0 ± 1.73c</td>
</tr>
<tr>
<td>Ash</td>
<td>2.2 ± 0.34a</td>
<td>1.5 ± 0.3b</td>
<td>2.0 ± 0.32a</td>
</tr>
<tr>
<td>Fat</td>
<td>2.0 ± 0.38a</td>
<td>2.0 ± 0.36a</td>
<td>3.0 ± 0.50b</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>70.1 ± 1.9a</td>
<td>74.3 ± 2.29b</td>
<td>68.54 ± 1.85a</td>
</tr>
<tr>
<td>Energy Kcal</td>
<td>332.8</td>
<td>353.2</td>
<td>351</td>
</tr>
</tbody>
</table>

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

Processed local sorghum (sorghum bicolor (L) Moench) was determined by vanillin – hydrochloric acid using quantitative analysis as described by burns 1963, Maxton and Rooney 1972, price et al 1978.

RESULTS AND DISCUSSION
Proximate Composition:
The table 2 shows the proximate composition of raw sorghum compared with fermented sorghum and fermented blends. The moisture, fat and protein of the fermented sorghum blends showed higher values (P > 0.05) than that of the raw and fermented sorghum.

The carbohydrate and energy values of the fermented blends improved compared to the raw sorghum and when compared with the fermented only, the difference is not significant.
Table 2. Mineral element composition in mg/l

<table>
<thead>
<tr>
<th>Mineral elements</th>
<th>Raw Sorghum</th>
<th>Fermented Sorghum</th>
<th>Fermented sorghum supplemented with cowpea and groundnut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>1.79 ± 0.22^a</td>
<td>1.81 ± 0.75^b</td>
<td>1.81 ± 0.33^b</td>
</tr>
<tr>
<td>Ca</td>
<td>1.73 ± 0.10^a</td>
<td>1.64 ± 0.38^b</td>
<td>1.98 ± 0.56^c</td>
</tr>
<tr>
<td>Fe</td>
<td>0.4 ± 0.02^a</td>
<td>0.01 ± 0.002^b</td>
<td>0.12 ± 0.02^c</td>
</tr>
<tr>
<td>Na</td>
<td>2.22 ± 0.01^a</td>
<td>2.24 ± 0.65^a</td>
<td>1.78 ± 0.38^a</td>
</tr>
<tr>
<td>K</td>
<td>9.23 ± 2.24^a</td>
<td>4.63 ± 1.19^b</td>
<td>6.35 ± 0.78^c</td>
</tr>
<tr>
<td>Cu</td>
<td>0.05 ± 0.01^a</td>
<td>0.003 ± 0.001^b</td>
<td>0.04 ± 0.01^a</td>
</tr>
<tr>
<td>P</td>
<td>0.49 ± 0.01^a</td>
<td>0.91 ± 0.11^b</td>
<td>1.12 ± 0.23^c</td>
</tr>
</tbody>
</table>

Values are recorded as mean ± SD of three determinations

Values in the same row with different superscript are significantly different (P < 0.05)

Mineral element composition:
The table 2 shows the mineral element composition of the raw and fermented local sorghum supplemented with cowpea and groundnut in the ratio of 70:20:10.

Values in the same row with different superscript are significantly different (P < 0.05)

The level of magnesium and calcium was higher than the raw sorghum. The level of potassium, phosphorus, iron and sodium were significantly low compared to the raw sorghum.

Tanin Contents
The tanin contents are presented in table 3. The tanin concentration of the raw local sorghum (yafi moro) which is a low tanin sorghum was higher than the processed fermented sorghum with their respective concentrations in mg/g as shown in the table 3.

Table 3. The tanin contents in raw and fermented sorghum.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tannin concentration (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sorghum</td>
<td>1.862</td>
</tr>
<tr>
<td>Fermented sorghum</td>
<td>1.812</td>
</tr>
</tbody>
</table>

It was observed that the pH of the fermenting material decreased sharply with a concomitant increase in the titrable acidity (figure 2). This result is in consistency with the result earlier reported by Faparusi et al, 1973.
Table 4. Different microorganism in fermented sorghum

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>Microorganism identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bacillus Spp, Corynebacteria Spp</td>
</tr>
<tr>
<td>24</td>
<td>Corynebacteria Spp, Lactobacillus, Saccharomyces cerevisiae.</td>
</tr>
<tr>
<td>48</td>
<td>Saccharomyces cerevisiae, Bacillus Subtilis, Streptactis</td>
</tr>
<tr>
<td>72</td>
<td>Saccharomyces cerevisiae, Bacillus Subtilis, Streptactis</td>
</tr>
<tr>
<td>Top water after milling</td>
<td>Saccharomyces cerevisiae, Bacillus Subtilis</td>
</tr>
<tr>
<td>After squeezing (slurry)</td>
<td>Saccharomyces cerevisiae, Bacillus Subtilis</td>
</tr>
<tr>
<td>Fermented flour</td>
<td>Saccharomyces cerevisiae</td>
</tr>
</tbody>
</table>

Table 5. In vitro protein digestibility

<table>
<thead>
<tr>
<th>%</th>
<th>Raw sorghum</th>
<th>Fermented sorghum</th>
<th>Fermented sorghum supplemented with cowpea and groundnut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestibility at 1 hour</td>
<td>88.37 ± 1.53</td>
<td>90.52 ± 1.57</td>
<td>94.42 ± 2.01</td>
</tr>
<tr>
<td>Digestibility at 6 hours</td>
<td>90.69 ± 1.75</td>
<td>94.73 ± 2.04</td>
<td>96.78 ± 2.27</td>
</tr>
</tbody>
</table>

Values are recorded in mean ± SD of three determinations

Values in the same row with different superscript are significantly different (P < 0.05)

Microbial analysis:

The microbial analysis determined the number of bacteria and their different types were identified from the top water during and after fermentation as given below; Total bacteria count: 0hr = 115 X 10 CFU : 24hrs = 336 X 10 CFU
48hrs = 240 X 10 CFU; 72hrs = 160 X 10 CFU
After milling = 30 X 10 CFU
After squeezing = 27 X 10 CFU: Fermented flour = 5 X 10 CFU
The CFU = colony forming unit
These are the dominant microorganism in fermented sorghum (table 4). They reduce consecutively because the acidity increases as the number of day’s increases and some cannot survive in severe acidic medium. This is in consistency with the early report from Adeyemi and Umar, 1994.
Table 5 presents the invitro protein digestibility of the raw sorghum and fermented sorghum compared with the fermented blends. The protein digestibility invitro at 1hr and 6hrs of the fermented blends is significantly higher than the recorded the raw and fermented sorghum samples.

Discussion

There was a considerable increase in the protein content of the fermented sorghum and the fermented sorghum supplemented with cowpea and groundnut which might be due to the lactic acid fermentation, because of the proteolytic activity of the microorganisms like Saccharomyces cerevisiae, Candida tropicalis and Corynebacteria species. It has been reported also by Reddy (1981) and Kazanas and Field (1981) that natural fermentation of cereals improves protein content and this is attributed to the loss in dry matter mainly carbohydrates.

The fat content was constant as earlier reported by Sathie et al, (1996), that fermentation does not increase the fat content in cereals. It was observed that the fat content of fermented blends increased which might be due to the addition of roasted groundnut in the blend.

This therefore shows that the improved amino acid content is due to the activity of the proteolytic enzyme that degrades protein to amino acids and since cowpea has high content of isoleucine, it thus balances the amino acid imbalance of sorghum (Oyus and Bender, 1985). It has been reported that sorghum has high leucine and when taken in excess causes pellagra due to amino acid imbalance but when supplemented with legumes like cowpea and groundnuts, it becomes balanced.

The loss in potassium, sodium and phosphorous observed in the fermented...
sorghum could be attributed to the loss in the ash and dietary fiber content during fermentation (Modu et al, 2009). Akingbala et al, 1981 reported that more than 50% of the ash in sorghum was leached into the steep and wash water and consequently, the level of phosphorous was reduced. The phosphorous is reduced due to the reduction of the phytic acid during fermentation. The fermentation process provides optimal pH conditions for the degradation of phytate (Svanberg and Sandberg, 1988).

However, supplementing the fermented sorghum with cowpea and groundnut improves the mineral content in magnesium, calcium, potassium and iron.

As earlier reported by Nkama et al. (2001), Ikemefuna (1998), Marero et al. (1998), Modu et al. (2010) that protein content increases on fermentation, the in vitro protein digestibility of the fermented blends were significantly higher than the raw and fermented sorghum. This also shows that amino acid imbalance from excessive intake of raw sorghum was greatly reversed when fermented and supplemented with cowpea and groundnut in the ratio of 70:20:10, since cowpea has higher concentration of isoleucine, methionine and lysine which is deficient in raw sorghum (Oyus and Bender, 1985). Consequently, cowpea can make a useful contribution towards infant food formulations.

The microbial analysis is observed in fermented food products to check the safety of the food in both human and animal life. Faparusi et al. (1973), the dominant microorganisms are Candida species and acetobacter species. The lactic acid bacteria count increased significantly while yeast count remained constant throughout fermentation. Determination of colony forming units (CFU) was used to verify growth of the starter cultures used.

CONCLUSION

The production of cereal-legumes blends was achieved by mixing fermented sorghum with dehulled cowpea and roasted groundnut in the ratio of 70:20:10. The results observed gave a good indication that the three formulations could provide the nutrients needed by both infants and adults. It also showed that food blends can be of high nutritional value with a balanced status than single meal (mono cereal meal). The fermented sorghum supplemented with cowpea and groundnut has high protein and carbohydrate quality than the raw sorghum. Due to the simplicity of the processing methods and availability of the blend components, low socioeconomic class of individuals can be able to meet up with the nutritional requirement from this class of food stuff.

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