Effect of Processing on Bioavailability of Macronutrients and Minerals in Complementary Food Formulated from Cereals and Supplemented with Legumes

Gogonte Hezekiah Amah1*, Boluwatife Florence Sode2, Babafemi Tosin Ogunbiyi3, Oluwaseyi Adegoke Adetunji4, Odutola Osilesi5

Abstract
Low bioavailability of protein and minerals in plant-based foods has contributed to malnutrition challenge confronting young humans in developing settings. Therefore, roasting, germination, fermentation and boiling were investigated as possible household processing methods that could improve bioavailability of a cereal-based complementary food supplemented with soy bean and groundnut. Standard methods were adopted in evaluating the bioavailability of these nutrients in male weaning albino rat model. The results obtained showed that the bioavailability of macronutrients in the food range from 67.68±12.36% to 98.17±0.10%, 67.71±5.29% to 92.41±1.99%, 63.58±8.50% to 83.98±3.57% and 71.66±7.33% to 78.60±4.00% for protein, fat, carbohydrate and crude fiber, respectively. The highest bioavailability of macronutrients was observed in protein of roasted food. Calcium (Ca) and Potassium (K) in roasted food and in the unprocessed food showed the highest and lowest bioavailability of 94.10±1.65% and 51.85±5.63%, respectively when considering the macro minerals. Boiling and roasting promoted bioavailability of Zinc (Zn) (96.61±0.57%), Iron (Fe) (83.52±4.08%) and Copper (Cu) (80.72±3.81%), respectively. The present study demonstrated that some of the processing methods promoted bioavailability of the different nutrients considered variably though, roasting seemed much more promising in the final analyses. Again, the household processing methods considered can also be proportionately relevant in improving bioavailability of macronutrients and minerals in the plant-based complementary food considered if applied appropriately.

Keywords: Food processing, bioavailability, complementary food, macronutrients, minerals

INTRODUCTION
Combination of pulses with other plant-based protein sources such as cereal grains in producing complementary food can generate a more complete protein and also provide vitamins and minerals not found in either on itself [1]. Plant-based complementary foods often contain high levels of anti-nutrients such as phytate and oxalate, to mention but two, which inhibit the absorption of important nutrients required by infants for growth and development [2]. Hence, low bioavailability of nutrients results from such unhealthy levels of anti-nutrients and lowers the nutritional quality of such plant-based infant foods [3].

Food processing methods such as soaking, fermentation, roasting and germination have been...
reported to enhance the bioavailability of micronutrients in plant-based diets by decreasing their anti-nutrients contents and improving total digestibility, nutrient quality and absorption of essential nutrients [4]. Germination enhances the nutritive value of legumes by the biosynthesis of enzymes, such as phytase and α-galactosidase among others, which eliminates or reduces the anti-nutritional factors in the substrate [5]. Germination also decreases the level of condensed tannins in cereals [6, 7].

Fermentation of cereal/legume blends has been found to remove the anti-nutritional factors as well as to increase the nutritive value and total nutrient density of such blends [8]. Low-molecular-weight organic acids such as citric acid, malic acid and lactic acid are also produced during fermentation with the potential to improve Iron (Fe) and Zinc (Zn) absorption [9].

Roasting employs dry heat at a temperature of at least 150 °C to reduce the level of anti-nutrients in grains, including tannin and phytate. It also improves the flavor through browning of the surfaces of the foods [10].

Boiling has being demonstrated to increase the consumption of legumes, especially among children [11]. Cooking has been described to occur at temperature range that modifies the appearance, flavor and texture of the food cooked while certain nutrients (such as folate) seemed to be better retained in the food by boiling [12].

Bioavailability describes the absorbability of nutrients and their availability for metabolic utilization. Although several methods of measuring bioavailability exist [13], the World Health Organization (WHO) [14] observed that balanced studies usually evaluate the difference between intake and disappearance in faeces as bioavailability, which is the approach adopted for the present study.

Notwithstanding, the effect of certain conventional processing methods on the bioavailability of nutrients in legume–cereal based locally formulated infant foods of this sort has not been reported. Such knowledge gap necessitates the present study. Therefore, it is needful to determine the bioavailability of selected dietary nutrients present in this prototype, cereal–legume based complementary food that has been subjected to certain traditional processing methods.

**MATERIALS AND METHODS**

**Acquisition and Grouping of Experimental Food**

Fresh, viable and good quality grains of yellow maize (*Zea mays*), groundnut (*Arachis hypogea*), wheat (*Triticum aestivum*), guinea corn (*Sorghum bicolor*) and soybean (*Glycine max*) were bought from the Ilshan main market, Ogun State, Nigeria in adequate amounts. The grains were sorted by removing unwanted particles and were separated into differently labeled containers. Each grain type was divided into five subsumes of 2 kg each as shown below:

- Subsume 1: Subjected to roasting
- Subsume 2: Subjected to germinated
- Subsume 3: Subjected to fermentation
- Subsume 4: Subjected to boiling
- Subsume 5: Left in the raw form

Each of the subsumes of grains was rinsed, following sorting, and oven dried for 30 min at 30 °C to remove water absorbed by the grains during rinsing. Subsequently, each subsume of grains was processed as categorized previously.

**Food Processing**

**Fermentation**

Sorted soybean, groundnut, yellow corn, guinea corn and wheat were rinsed with water and soaked in different containers and respectively covered. Fermentation was allowed to take place for three days. The fermented grains were rinsed and oven dried in hot air oven at 50 °C until they were dry.
**Cooking**
Sorted soybean, groundnut, yellow corn, guinea corn and wheat were rinsed with water. Water was allowed to boil and the grains were poured into separate cooking pots containing boiling water each. The grains were boiled under the supervision of food preparation expert. The cooked grains were oven dried in hot air oven at 50 °C to constant weight.

**Roasting**
After sorting, the grains were separately rinsed with water thoroughly. They were then roasted using a hot air oven at previously determined temperatures for different duration as previously determined. The roasted grains were allowed to cool and were stored in different containers for formulation and subsequent analyses.

**Germination**
Sorted yellow corn, groundnut, soybean, guinea corn and wheat were separately rinsed with water and spread between a layers of jute bag, kept wet by 12-hourly spraying of water for three days to allow for sprouting. The germinated seeds were washed with water and oven dried at 50 °C until constant weight was obtained before being formulated into complementary food.

**Formulation and Compounding of the Experimental Diet (Composite Blend)**
The differently processed grains and the raw samples were formulated each in ratio 2:1:1:1:1 for soybeans, yellow maize, guinea corn, groundnut and wheat into complementary food. The formulated foodstuffs were labeled as fermented, roasted, germinated, cooked and raw according to the processing method each category was subjected to. These samples were thoroughly milled and a fraction of each type was made into pellets for animal studies and the other used for *ex vivo* analyses.

The five food types were analyzed for their proximate and mineral nutrients composition and then fed to laboratory rats in order to determine the percentage nutrient absorbed.

**Experimental Animals**
Male Wistar strain weaning rats weighing between 27–45 g, obtained from the Animal Facility of Babcock University Ilishan-Remo Ogun state, Nigeria were used for the *in vivo* study to determine the fraction of each nutrient absorbed from the gut.

**Ethical Consideration**
Experimental animals were handled according to the international regulations guiding the use of animals in research and testing and as approved by appropriate local authority.

**Chemical Analysis of the Diets**

**Proximate Analyses of Compounded Complementary Foods and their Corresponding Faecal Matters**
Percentages of moisture, ash, crude fibre and crude fat in each of the samples were determined using methods 934.01, 942.05, 978.10 and 920.39, respectively [15]. Protein content of each sample was analyzed by using method 990.03 [16]. Carbohydrate by difference was determined as recommended by experts [17].

**Mineral Analysis**
The method of Twyman [18] was adapted as detailed forthwith to digest the samples for mineral nutrient analyses. About 10 ml of the di-acid mixture (concentrated nitric acid and perchloric acid in ratio 2:1) was added to 2 g of each homogenized sample in a 250 ml pyrex glass conical flask. The flask content was boiled to clarity. On cooling, it was filtered into calibrated sample bottle. Double distilled deionized water was added to make up 25 ml volume in each case. The filtrate obtained was used as stock solution for the various mineral analyses. Potassium, sodium and calcium were analyzed...
by flame photometry (Model 210VGA, Buck Scientific, Inc., UK) while magnesium, manganese, iron, zinc and copper were analyzed using an atomic absorption spectrophotometer (Model PFP7/C, Jenway, Staffordshire, UK) [19].

**Animal Experimentation**

**Animal Grouping**

The rats were randomly distributed into five groups of five rats each and caged metabolically in well ventilated housing facility. Water and food were given *ad libitum*. The rats were acclimatized for five days and allowed to fast overnight (12 h) before commencement of feeding with the experimental diets.

Animal groups were assigned the following diets during the period of feeding experimentation:
- Group 1: Diet 1 (placed on roasted complementary food)
- Group 2: Diet 2 (placed on germinated complementary food)
- Group 3: Diet 3 (placed on fermented complementary food)
- Group 4: Diet 4 (placed on cooked complementary food)
- Group 5: Diet 5 (placed on raw complementary food)

**Feeding of Animal**

Known quantity of each diet type and water *ad libitum* were served to each experimental animal group daily for a period of 20 days. Faeces and urine were collected separately to avoid mixing with the feed. Records of feed served, left over feed, weights of faecal matter egested, volumes of urine produced and weights of rats were kept daily.

**Statistical Analyses**

Data were expressed as mean ± SEM. Analysis of Variance (ANOVA) was carried out to determine heterogeneity of data using SPSS 23.0. Values were considered to be significantly different at 95% confidence. The differences in means were compared using least square deviation.

**Funding**

The present research was funded by the Babcock University through Directorate of Research, Innovation and International Cooperation (RIIC) with Research Grant number BU/RIIC/2018/13.

**Conflict of Interest**

The authors of the present work declared that there was no conflict of interest whatsoever associated with this report and the study reported herein.

**RESULTS**

Observations made on the extent to which roasting, germination, fermentation and cooking affected the bioavailability of proximate parameters and minerals in the complementary food formulated from ratio 2:1:1:1:1 of soybeans, groundnut, yellow corn, guinea corn and wheat are presented in Tables 1–3, respectively. Generally, the result demonstrated that the processing methods studied had positive effects on bioavailability of the food studied.

**Effect of Processing on Bioavailability of Macronutrients in the Food**

Table 1 shows the effects of processing methods studied on the bioavailability of macronutrients in the complementary food studied using male Wistar albino rats. Roasting significantly increased the percentage bioavailability of protein in the complementary food from 71.66±7.32% to 98.17±0.10%. None of the processing methods studied affected percentage bioavailability of fibre consumed in the food, which ranged from 71.66±7.33% to 78.60±4.00%. Carbohydrate bioavailability was significantly reduced from 83.98±3.57% in the unprocessed food to 68.41±6.45%, 66.64±19.66% and 63.58±8.50% in the roasted, boiled and fermented foods, respectively. Germination had no significant
impact on bioavailability of carbohydrate in the complementary food studied. Roasting and boiling increased fat bioavailability to 91.71±0.93% and 92.41±1.99%, respectively from 89.63±2.41% observed in the raw food, while germinating reduced it significantly to 67.71±5.29%. Roasting seemed a good method to improve percentage bioavailability of protein and fat.

Table 1. Average percentage bioavailability of macronutrients in the complementary food using male Wistar Albino Rats.

<table>
<thead>
<tr>
<th>Food</th>
<th>Crude protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasting</td>
<td>98.17±0.10b</td>
<td>91.71±0.93b</td>
<td>68.41±6.45a</td>
<td>78.23±1.51</td>
</tr>
<tr>
<td>Germination</td>
<td>67.71±5.29a</td>
<td>67.71±5.29a</td>
<td>77.75±3.05b</td>
<td>73.44±3.00</td>
</tr>
<tr>
<td>Fermentation</td>
<td>70.97±7.29a</td>
<td>87.63±3.11b</td>
<td>63.58±8.50a</td>
<td>76.41±3.10</td>
</tr>
<tr>
<td>Boiling</td>
<td>67.68±12.36a</td>
<td>92.41±1.99a</td>
<td>66.64±20.66a</td>
<td>78.60±4.00</td>
</tr>
<tr>
<td>Raw</td>
<td>71.66±7.32a</td>
<td>89.63±2.41b</td>
<td>83.98±3.57b</td>
<td>71.66±7.33</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM: Values carrying different alphabet in the same column are significantly different (p<0.05). ‘Raw’ indicated food type not subjected to any form of processing.

Table 2. Average percentage of Macrominerals absorbed by experimental animal placed on the complementary food.

<table>
<thead>
<tr>
<th>Food</th>
<th>Ca</th>
<th>Mg</th>
<th>K</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasted</td>
<td>94.10±1.65c</td>
<td>83.02±2.87b</td>
<td>74.36±4.19b</td>
<td>72.06±5.30b</td>
</tr>
<tr>
<td>Germinated</td>
<td>78.24±9.72b</td>
<td>87.75±3.5b</td>
<td>79.58±3.78b</td>
<td>71.87±3.63b</td>
</tr>
<tr>
<td>Fermented</td>
<td>83.93±7.78b</td>
<td>81.27±5.2b</td>
<td>58.76±7.08a</td>
<td>57.47±7.36a</td>
</tr>
<tr>
<td>Boiled</td>
<td>80.18±6.01b</td>
<td>70.50±6.70b</td>
<td>72.28±3.67b</td>
<td>56.80±7.43b</td>
</tr>
<tr>
<td>Raw</td>
<td>68.73±5.93a</td>
<td>68.27±4.88b</td>
<td>51.85±5.63a</td>
<td>52.16±9.94a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM: Values carrying different alphabet in the same column are significantly different (p<0.05).

Table 3. Average percentage Microminerals absorbed by experimental animals placed on the complementary food.

<table>
<thead>
<tr>
<th>Food</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasting</td>
<td>62.05±6.27b</td>
<td>83.52±4.08b</td>
<td>80.72±3.81b</td>
<td>78.81±5.18b</td>
</tr>
<tr>
<td>Germination</td>
<td>70.97±4.56b</td>
<td>71.49±5.21b</td>
<td>70.35±4.90b</td>
<td>54.29±5.89b</td>
</tr>
<tr>
<td>Fermentation</td>
<td>76.35±4.35b</td>
<td>76.53±4.65b</td>
<td>74.02±6.38b</td>
<td>79.17±5.06b</td>
</tr>
<tr>
<td>Boiling</td>
<td>75.20±3.69b</td>
<td>73.92±5.72b</td>
<td>74.31±4.13b</td>
<td>96.61±0.57b</td>
</tr>
<tr>
<td>Raw</td>
<td>51.26±6.76a</td>
<td>60.14±6.99a</td>
<td>57.97±7.28a</td>
<td>53.91±7.19a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM: Values carrying different alphabet in the same column are significantly different (p<0.05).

Effect of Processing on Bioavailability of Macrominerals in the Complementary Food

Table 2 shows the percentage bioavailability of macrominerals in the differently processed complementary food when fed to the experimental animals. The processing methods studied variedly improved the percentage bioavailability of calcium, magnesium, potassium and sodium generally. Specifically, percentage bioavailable calcium ranged from 78.24±9.72% in the unprocessed food to 94.10±1.65% in roasted food. Roasting improved the bioavailability of calcium in the complementary food (significantly) the most, although germination, fermentation and boiling also increased the percentage bioavailability of calcium significantly. Percentage bioavailability of magnesium was significantly improved from 68.27±4.88% for the raw food to 81.27±5.2%, 83.02±2.87% and 87.75±3.5% for fermented, roasted and boiled complementary foods, respectively. Although fermentation has no significant impact on boiling, roasting and germination increased the percentage bioavailability of potassium content in the complementary food of the rats. Only germination and
roasting were able to increase the percentage bioavailability of sodium significant from 52.16±9.94% observed in the raw food to 71.87±3.63% and 72.06±5.30%, respectively.

**Effect of Processing on Bioavailability of Microminerals in the Complementary Food**

The processing methods investigated, significantly increased the bioavailability of manganese, iron, copper and zinc. Fermentation, boiling, germination and roasting significantly increased the percentage bioavailability of manganese in the complementary food investigated from 51.26±6.76% in the raw food to 76.35±5.35%, 75.20±3.69%, 70.97±4.56% and 62.05±6.27%, respectively. Bioavailability of iron was increased the most by roasting (83.52±4.08%), followed by fermentation (76.53±4.65%), boiling (73.92±5.72%) and then germination (71.49±5.21%); all of which were significantly higher than the value observed in the raw sample (60.14±6.99%). Percentage bioavailability of copper increased significantly when the sample was subjected to roasting, boiling, fermentation and germination from the baseline value of 57.97±7.28% to 80.72±3.81%, 74.31±4.13%, 74.02±6.38% and 70.35±4.80%, respectively. Germination did not have any significant impact on the bioavailability of zinc (54.29±5.89%) observed in the complementary food, as compared to the baseline value of 53.91±7.19% observed in the unprocessed food. However, boiling brought about the highest significant increase in percentage bioavailable zinc (96.61±0.57%) in the complementary food followed by fermentation (79.17±5.06%) and then roasting (78.81±5.18%).

**DISCUSSION**

The present study demonstrated that determination of nutrient contents alone is not sufficient to predict the *in vivo* effect of food as faecal matter analyses showed that there are varied levels of macronutrients and minerals that were not absorbed during digestion in the gastrointestinal tract. It has been observed that data on nutrient contents of food are accurately applied when considered in the light of *in vivo* bioavailability [20]. Since plant-based foods form the major staple in developing economies and many of such foods have reduced bioavailable minerals in their natural states, Gibson and colleagues opined that ‘household strategies that reduce the content or counteract the inhibitory effects of these factors on micronutrient bioavailability are urgently needed in developing-country settings’[21]. It is evident that there are various ways such as germination, fermentation and cooking among others, by which bioavailability of nutrients in food can be improved [12, 21].

Protein bioavailability was improved up to 98.17±0.10% in the prototype complementary food by roasting, suggesting roasting to be an effective processing method that could be adopted in processing household food for older infants and young children if healthy protein nutrition is of prime interest. The foregoing corroborated the observation made by other independent opinions [22]. These results also suggested that heat treatment of roasting is effective in destroying factors that interfere with protein absorption as evidenced in the approximately 27% bioavailable crude protein difference observed between the raw food and in the roasted food. Fermentation does not seem effective in improving protein bioavailability, possibly due to protein utilization by the natural fermenters of the medium to support their protein needs. Protein-energy under-nutrition is still a public health nutrition challenge found among children in developing economies [23, 24] and could also be addressed with potent interventions such as feeding with highly bioavailable protein-rich foods. Direct relationship exists between fibre content of consumed foods and certain disease conditions [25, 26]. All the processing methods studied did not affect bioavailable fibre in the food suggesting that bowl movement upon consumption of the food will not be influenced when the food is so processed.

Micronutrients such as iron, zinc and calcium have been noted to have poor bioavailability when consumed in plant products [14, 27]. Therefore, it becomes compelling to explore simple household approaches that could optimize the bioavailability of these minerals if healthy nutrition of infants and children fed plant based is the focal point. About 23% and 26% improvement in bioaccessibility of iron, zinc, copper and calcium, respectively were brought about by roasting suggesting that roasting is an ideal processing method for complementary food targeted towards healthy mineral nutrition. Iron
and calcium have been implicated for haematological and bone and teeth well-being of young humans [14]. Zinc and copper are associated with brain and immunological development in humans of 0–2 years of age [28]. Zinc deficiency has been suspected in developing countries since their diets are predominantly made of plant-based foods (which are high in phytate) and low in animal products [14, 29]. The foregoing further necessitated the need for strategies to improve zinc bioavailability in cereal-legume based complementary food, which the results in the present report demonstrated effective intervention there to.

CONCLUSION

The present study demonstrated that roasting, germination, fermentation and boiling improved bioavailability of both macronutrients and minerals in the complementary food studied variedly, suggesting that these method could be applied to household processing of certain cereal-legume based complementary foods. Such processing method, if rightfully applied, could prove veritable intervention to relatively address malnutrition caused by poor bioavailability of nutrients in plant-based foods.

REFERENCES


