



Chemical Evaluation of *"Mumu"* Formulated from Pearl Millet, Irish Potato and Sesame Flour Blends

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Abstract

An investigation into anti-nutrient composition *mumu*, a traditional dish in Benue State, Nigeria, was carried out. Formulation of blends was based on different levels of roasted pearl millet flour (RPMF), boiled Irish potato flour (BIPF), and roasted sesame seed flour (RSSF). The three ingredients were combined in the following ratios: RPMF 100 (control), 70:20:10, 65:25:10 and 65:20:15 of RPMF: BIPF: RSSF, respectively. Mineral bioavailability was evaluated by [Oxalate]/[Ca], [Phytate]/[Ca], [Phytate]/[Fe] and [Phytate]/[Zn] molar ratios of the different blends of *mumu* were investigated, to which only Fe showed limited absorption. The anti-nutritional constituents showed that concentration of phytates>alkaloids>oxalates>total phenols, with values ranging from 24.22-24.93 mg/100 g Phytates, 4.15-4.81 mg/100 g alkaloids; 0.39-0.78 mg/100g oxalates; 0.00-0.01 mg/100g total phenols. The consumption of *mumu* is encouraged as it contains minerals important to the body, nonetheless, Fe supplementation is suggested.

Keywords: Mumu; Blends; Anti-nutrient; Mineral Bioavailability evaluated

Introduction

Diet and nutrition are important factors in the promotion and maintenance of good health in the human body [1]. This means that the nutritional quality of food is critical as it determines to an extent the acceptance of food. The nutritional value of foods strongly depends on their nutritional and composition. anti-nutritional **Micronutrients** deficiency is a serious form of malnutrition affecting the world. Inadequate intake of micronutrients particularly the microelements contribute to the global disease burden, leading to increased rates of illness and death from infectious diseases; and prevalence of disabilities such as mental impairment [2].

Anti-nutritional factors are mainly associated with compounds or substances from natural or synthetic sources, which interfere with the absorption of nutrients, and act to reduce nutrient intake, digestion, and utilization. These substances may also produce other adverse effects such as allergic reactions. Anti-nutrients are frequently related to plant-based, uncooked or vegan diets and are naturally synthesized in plants [3]. Their highest concentrations are mainly found ingrains, beans, legumes and nuts, but can also be found in leaves, roots and fruits of certain species of plants.

It is important to determine anti-nutrients composition as well as the bioavailability of nutrient in all foods to ascertain their safety.

Materials and Methods

Sample Collection and Preparation

Pearl millet (*Pennisetum glaucum* (L.) R. Br.),Irish potato (*Solanum tuberosum* L.) and Sesame (*Sesamum indicum* L.) seeds were purchased from Wurukum market, in Makurdi, Benue state, Nigeria. The sample of food items were identified and authenticated by the botanist at the Department of Biological Sciences, Benue State University, Makurdi.

Preparation of Roast Pearl Millet Flour

Roast pearl millet flour was prepared according to the method described by Shar *et al.*, [5] with slight modification. The dried grains were sorted and winnowed to remove grain stalk, sticks and remaining cob parts and further subjected to visual screening to remove foreign particles such as stones. This was followed by washing with water to remove dust, soil particles and any over floats. Damaged, diseased or discoloured grains as well as immature or sprouted grains were discarded. Cleaned pearl millet grains were cooked in boiling water for 45 min and allowed to cool after draining it of excess water for 15 min. The grains were roasted in microwave oven at 150°C for 60 min. The roast grains were allowed to cool and then milled using a locally fabricated attrition mill, the obtained pearl millet flour was sieved using a 0.25 mm sieve and kept in an airtight polyethylene bag until when required for formulation of blends.

Preparation of Boiled Irish Potato Flour

The potato flour was prepared using the method described by Uzo-Peters and Akinola [6] with slight modification. Fresh Irish potatoes were washed thoroughly with water to remove adhering soil particles. The washed potatoes were then boiled for 45 min, allowed to cool and drained for 15 min and then peeled using sharp knives before being uniformly chipped into slices of about 2 mm thickness to facilitate drying using kitchen plantain slicer. The Irish potato chips were thinly spread on aluminum foil lined oven cabinet and dried at 65°C in an oven to a constant weight The dried potato chips were then milled into powder using a locally fabricated attrition mill, and sieved using 0.25mm sieve before being kept in an airtight polyethylene bag until when required for formulation of blends.

Preparation of Roasted Sesame Seed Flour

Roast sesame seed flour was prepared by combining the methods described by Uzo-Peters and Akinola [6] and Ganorkar and Jain [7] with little modification. The sesame seeds were cleaned, manually sorted and winnowed to remove all forms of foreign particles and defective seeds. The sesame seeds were then roasted in microwave oven at 150°C for 5 minutes to reduce cyanogenic glycosides (CG) content and to develop nutty flavour. The roasted sesame seeds were then ground to powder in a domestic mixer (M/s Sumeet Mixer Model DXE plus) for 2 min and sieved. The obtained meal was kept in airtight polyethylene bag until when required for formulation of blends.

Formulation of Blends

The blends of *mumu* were formulated from roast pearl millet flour (RPMF), boiled irish potato flour (BIPF), and roast sesame seed flour (RSSF) using the ratios of Aande *et al.*[8] as given in Table 1.

Table 1. Formulation of blends of roast pearl millet flour (RPMF), boiled irish potato flour (BIPF), and roast sesame seed flour (RSSF).

Blend	Ingredient	Ratio
А	RPMF	100:0:0 (Control)
В	RPMF : BIPF : RSSF	70:20:10
С	RPMF : BIPF : RSSF	65:25:10
D	RPMF : BIPF : RSSF	65:20:15

Determination of Anti-nutrients in Mumu

The levels of anti-nutrients like phytates, alkaloids, oxalates and phenolic compounds were investigated using standardized procedures.

Determination of Phytates [9]

Four grams of each of the food blends were weighed into a beaker and soaked with 100 mL of 2% hydrochloric acid for five hours and was filtered. Afterwards 25 mL of the filtrate were taken into a conical flask and 5 mL of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of iron (III) chloride until a brownish-yellow colour persisted for 5 min.

Determination of Alkaloids [10]

Five grams of each sample was weighed and dispersed into 50 mL of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for 4 h before being filtered. The filtrate was then evaporated to one quarter of its original volume on a hot plate. Concentrated ammonium hydroxide was added drop-wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60°C for 30 min, transferred into a desiccator to cool and then reweighed until a constant weight was obtained. The constant weight was recorded while the weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analysed.

Determination of Oxalates [11]

Two grams of each sample was digested with 10 mL of 6 M hydrochloric acid for 1 h and made up to 250 mL in a volumetric flask. The pH of the filtrate was adjusted with concentrated ammonium hydroxide solution until the colour of solution changed from salmon pink colour to a faint yellow colour. Thereafter, the filtrate was treated with 10 mL of 5% calcium chloride solution to precipitate the insoluble oxalate. The suspension was now centrifuged at 2500 rpm, after which the supernatant was decanted and the precipitate completely dissolved in 10 mL of 20% sulphuric acid. The total filtrate resulting from the dissolution in sulphuric acid was made up to 300 mL. An aliquot of 125 mL of the filtrate was heated until near boiling point and then titrated against 0.01 N of standardized potassium permanganate solution to a faint pink colour which persisted for about 30 seconds.

Determination of Total Phenolics [12]

The food blends were analyzed for total phenolics according to the Folin-Ciocalteu method. Two grams of the sample paste was extracted with 20 mL of ethanol 80% for 1 h. The mixture was centrifuged at 3000 rpm for 10 min and the supernatant collected. To a volume of 100 µL of samples extracts, was added 1.150 mL of distilled water and 250 µL of the Folin-Ciocalteu solution. After 6 min, 2.5 mL of a solution of sodium carbonate 7% was added and the volume was adjusted to 6 mL with distilled water. The mixture was allowed to stand for 90 min. Optical density was measured at 760 nm using a spectrometer. The calibration curve was obtained using gallic acid as standard and the concentration ranged from 20 to 600 mg/mL. The results were expressed as gallic acid equivalents/100 g of sample.

Determination of Mineral Composition Mumu [13, 14]

The food blends were analyzed for ash content according to the AOAC official methods of analysis. The crucible and lid were placed in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off. The crucible was kept in the desiccator for 30 minutes and then weighed to 3 decimal places. About 5g of

sample was weighed into the crucible and heated over low Bunsen flame with lid half covered. When fumes are no longer produced, crucible and lid were placed in the furnace and heated at 550°C overnight. During heating, the lid was not covered. After complete heating the lid was placed to prevent loss of fluffy ash and cooled down in the desiccator. The ash with crucible and lid was weighed when the sample turns to grey.

Calculation

Ash (%) = Weight of $ash \times 100$

Weight of sample

A whitish or greyish ash was obtained after the determination of ash. The ash was treated with concentrated hydrochloric acid transferred to a volumetric flask and made up to 100 mL before submission to atomic absorption spectrophotometry (AAS).

Determination of Minerals Bioavailability [15]

The molar ratios of phytate to calcium (phy: Ca), phytate to iron (phy:Fe), phytate to zinc (phy:Zn) and oxalate to calcium(Oxa:Ca) were calculated to estimate the relative bioavailability of calcium, iron and zinc in the presence of antinutrients. Molar ratios of anti-nutrient/minerals were used to predict the minerals bioavailability. The molar ratios were calculated as:

Mole of Anti-nutrient			
Molar ratio= $\frac{Mole of Anti-nutrient}{Mole of Mineral}$			
Mole of anti-nutrient= <u>Anti-nutrient(mg)</u>			
Atomic weight (g/mol)			
Mole of mineral = $\frac{Minerals(mg)}{minerals(mg)}$			
Atomic weight(g/mol)			

The molar masses used were phytate (660 g/mol), oxalate (88 g/mol) calcium (40 g/mol), iron (56 g/mol) and zinc (65 g/mol). The recommended critical values used to predict the bioavailability were phytate: calcium >0.24 [16], phytate: zinc >15 [13], phytate: iron < 1 [17], and Oxalate: calcium >1[18].

Statistical Analysis [19]

All analytical determinations were conducted in triplicates. The data was subjected to Analysis of Variance (ANOVA) followed by Duncan's multiple range test to compare treatment means; differences were considered significant at 95% (P \leq 0.05) using the IBM SPSS statistics software version 20.

Results and Discussion *Anti-nutritional and Mineral composition*

The result of anti-nutritional content and mineral composition of the blends is presented in Table 2 and 3 respectively. The concentrations of followed anti-nutrients the trend. Phytates>alkaloids>oxalates> total phenol with values ranging from 24.22-24.93mg/100g Phytates, 4.15-4.81 mg/100g alkaloids; 0.39-0.78 mg/100g oxalates; 0.00-0.01 mg/100g total phenols.It is necessary that they exhibit very low levels in food materials as a consequence of the deleterious effect they confer such as inhibition of some anti-oxidants enzymes and reproductive toxicities [20]. Antinutrients are the chemical compounds synthesized in natural food by normal metabolism which decreases the nutritive value of food [16]. The antinutrients (phytate and oxalate) have the ability to form insoluble complexes with positively charged food components such as protein, carbohydrate, minerals and trace elements. These complexes lead to reduced bioavailability of calcium, zinc and iron [21]. Significant losses in minerals could also be as a result of house hold preparation practices as observed by Hailu and Addis [22] in their study, bioavailability of mineral nutrients of selected wild and traditional edible plants. The same reason may apply to the minerals which showed enormous reduction in the boiled and peeled D. abyssinica as reported by Bhandari and Kawabata [18]. Reduction in zinc content was also reported in peeled and boiled tubers of Dioscorea cayenensis [23].

The minerals generally showed highest concentration for Ca followed by Zn and Fe the least, Cereals and legumes are important food sources of Iron, Zinc, and Calcium especially for young children in rural areas. Zinc is a component of every living cell and plays a significant role in several body functions, from helping in enzyme functions to blood clotting, and is essential to taste, vision, and wound healing. An iron deficiency called iron-deficiency anemia is very common around the world, especially for women and children in developing nations [6]. The low concentration of Zn and Fe when compared to daily requirements however indicates that fortification with suitable micronutrients or micronutrient-dense foodstuffs was essential.

Table 2: Anti-Nutrient Composition of Mumu [8].

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Anti-Nutrient (mg/100g)					
Blends	Alkaloids	Oxalates	Phytates	Total Phenolic	
Α	4.81±0.15 ^b	0.39±0.05 ^a	$24.93 \pm 1.29^{\circ}$	0.01±0.00 a	
В	4.74±0.12 ^b	0.69 ± 0.05^{b}	$24.91 \pm 0.95^{\circ}$	0.01±0.00 a	
С	4.63±0.25 ^b	0.72 ± 0.09^{b}	$24.66\pm0.82^{\text{b}}$	0.01±0.00 a	
D	4.15 ± 0.30^{a}	0.78 ± 0.10^{b}	24.22 ± 0.95^a	$0.01{\pm}0.00^{a}$	
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Values are means \pm SD of three replicate determinations. Figures in the same vertical column with the same superscript are not significantly different (P>0.05).

Table 3: Mineral Composition of Mumu [8].

Mineral Composition (mg/100g)					
Blends	Ca	Zn	Fe		
А	7.10±0.0 ^a	1.70±0.01 ^a	2.90±0.01 ^a		
В	206.90±0.01b	1.94±0.01 °	3.97±0.00 °		
С	207.11±0.01 °	1.87±0.01 ^b	3.55±0.00 ^b		
D	275.95±0.01 ^d	2.21±0.01 ^d	4.26±0.01 ^d		

Values are means \pm SD of three replicate determinations. Figures in the same vertical column with the same superscript are not significantly different (P>0.05).

Molar Ratios and Bioavailability of Minerals

The molar ratios along with the suggested critical values for the bioavailability calcium, iron, and zinc are presented in Table 4. The oxalate: Ca molar ratio ranges from 0.001 to 0.022. Phytate:Ca molar ratio ranges from 0.005 to 0.213. Phytate: Fe molar ratio ranges from 0.487 to 0.731 and phytate: Zn molar ratio ranges from 1.088 to 1.462. The oxalate:Ca, Phytate:Ca and phytate:Zn molar ratios fall below their critical limits 1, 0.24 and 15 respectively. Except for Phytate:Fe molar ratio that falls below the critical limit 1. The results indicated good calcium and zinc bioavailability but iron bioavailability was poor. Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed [24].

Oxalate can have deleterious effects on human nutrition and health, particularly by decreasing the calcium absorption to the body [18]. Plant product with the molar ratio of oxalate:Ca limits total dietary Ca availability when is greater than one [21, 25]. The oxalate:Ca molar ratio in all the formulated blends were lower than the reported critical value (1.0). These results indicate that the low level of oxalate in the blends have no adverse effects on bioavailability of dietary calcium.

Phytate:Ca molar ratio has been proposed as an indicator of Ca bioavailability as phytate decreases the absorption of Ca by forming insoluble complexes with them [25]. The critical molar ratio of phytate:Ca is reported to be < 0.24 for good calcium bioavailability [21, 25]. The results showed that, the molar ratio of phytate:Ca of all the blends were less than the reported critical value, which indicates that the absorption of calcium would not be adversely affected by the amount of phytate determined.

Phytate have inhibitory effect on iron absorption when phytate:Fe molar ratios are less than 1.0 [16]. The phytate:Fe molar ratios in all the blends had values less than the critical limits, which implies that the bioavailability of Fe in these blends are low and the absorption of iron may be inhibited by phytate found present.

Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH [18] and the formation of the chelates depends on relative levels of both zinc and phytic acid. Hence, the phytate:Zn molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone [25]. Phytate:Zn molar ratios >15 is indicative of poor zinc bioavailability [26]. The result show that all the blends are lower than the critical value indicating that the bioavailability of Zn in these blends was high and likely not to be affected by phytate present [21].

Table 4: Anti-Nutrient to Nutrients Molar Ratios of Mumu

Anti-Nutrient/Mineral Ratio					
Blends	[Oxalate]	[Phytate]	[Phytate]	[Phytates]/[Zn]	
	/[Ca]	/[Ca]	/[Fe]	-	
А	0.022	0.213	0.731	1.462	
В	0.002	0.007	0.535	1.267	
С	0.002	0.007	0.587	1.276	
D	0.001	0.005	0.487	1.088	
Critical	1	0.24	1	15	
value*					

Conclusion

The evaluated mineral bioavailability in the food blends shows considerable amounts of Ca and Zn, and a low level of anti-nutrients. Therefore, consumption of the *mumu* food samples is encouraged as a good source of nutrient to enhance growth and that can check malnutrition in the consumers. However, there is a low level of mineral bioavailability for Fe. Hence, there may be a need to supplement Fe to increase iron content in all the blends.

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