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Full Length Research Paper

Production, *in vitro* protein digestibility, phytate content and acceptability of weaning foods prepared from pearl millet (*Pennisetum typhoideum*) and cowpea (*Vigna unguiculata)*

H. H. Laminu[1], S. Modu[1]* and A. I. Numan[2]*

[1]Department of Biochemistry, Faculty of Science, University of Maiduguri, Maiduguri, Borno State, Nigeria.
[2]Department of Human Anatomy, University of Maiduguri, Maiduguri, Borno State, Nigeria.

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Weaning food blends prepared from fermented pearl millet/roasted cowpea in 70:30 and 60:40 ratios were evaluated for their nutritional values. The pearl millets used were SOSAT C-88 (an improved variety) and a local variety (unimproved), while the cowpea used was the Borno red. Standard methods were used for the analysis. Processing of the grains resulted in lower levels of phytic acid and higher *in vitro* protein digestibility of the weaning food blends. The predominant microorganisms isolated during the production of "Kamu" were *Streptococcus tactics, Saccharomyces cerevisae, Micrococcus lateus* and *Lactobacillus plantarum.* Sensory evaluation results showed that there were no significant differences (P>0.05) in the weaning food blends. Even though the 70:30 ratio was used in most literatures, the 60:40 ratio used in this study was superior to the 70:30 ratio.

Key words: Millet, *in vitro* protein digestability, phytate, sensory.

INTRODUCTION

In many West African countries, exclusive breast feeding is usually adequate for about three to four months of age, but after this period, it may become increasingly inadequate to meet the nutritional demands of the growing infant. Thus, there is need to introduce soft, easily swallowed and nutritionally adequate foods to supplement the infants feeding in life (Onofiok and Nnanyelugo, 1992; Modu et al., 2005). Traditional weaning foods in Nigeria constitute single monocereal grains made either from fermented millet, maize or sorghum referred to as 'Ogi' (Kamu) which are of poor nutritional value (Hellstrom et al., 1981). Protein energy malnutrition is a major problem which frequently occurs during the important transitional phase of weaning infants thereby affecting the physical and mental growth of many infants in developing countries. This is as a result of inflation, ignorance and the exorbitant cost of animal source of protein, thereby making it out of reach for the common man. The low income group of people constitutes a major part of the population and is therefore at the risk of such situation (Patrick, 1998).

The weaning food therefore needs to be developed from locally available resources which are economical, easily digestible and acceptable to children. This can be achieved by the supplementation of our locally available cereals with legumes. Legumes are a good source of dietary protein rich in lysine and tryptophan and are cheaper than animal protein (Marero et al., 1988).

With millet and cowpea being staple foods in this part of the country, there is need to produce supplementary cereal/legume weaning foods that would be close to infant formula with respect to nutrient bioavailability.

Objectives of the study

The objectives of this study are: first, to prepare a high protein weaning food from pearl millet and cowpea that will be adequate to meet the nutritional needs of infants during weaning period; secondly, to determine the level of phytic acid and in *vitro* protein digestibility of the

*Corresponding author. E-mail: shemodu@yahoo.com.

weaning food blends; and thirdly, to determine the overall acceptability of the weaning food.

MATERIALS AND METHODS

Source of pearl millet and cowpea

The improved variety of pearl millet SOSAT C-88 was obtained from the Lake Chad Research Institute Maiduguri, while the local (unimproved) variety of pearl millet and cowpea seed (Borno Red) were purchased from the Maiduguri Monday Market. All the grains were authenticated by a seed breeder.

Preparation of 'Kamu'

'Kamu' (Ogi) was prepared by the method described by Akingbala et al. (1981). One hundred grams of the dirt free pearl millet (raw grain) was cleaned and steeped in 200 ml of distilled water (1:2 ratio) for 72 h. At the end of the 72 h, the top water was decanted and 200 ml of the distilled water was added and milled with a warring blender for 4min at rheostat setting of 120. The slurry obtained was sieved through a nylon cloth to separate the bran after adding 600 ml of distilled water. The 'Kamu' was then allowed to stand for 24 h for the starchy part to settle, after which the water was decanted and the Kamu was sun dried to a constant weight.

Preparation of the cowpea

One hundred grams of the cowpea was cleaned of dirt and soaked in distilled water for 20 min. The cowpea was then dehulled using a pestle and a mortar. It was then washed to separate the husk, after which it was dried to a constant weight. The cowpea was roasted and then ground into a fine powder.

Preparation of the weaning food blend

The blending of the weaning meal was done in two ratios, 70:30 and 60:40, as described by Akpapunam (1984), that is, 70 parts of fermented pearl millet ('Kamu') and 30 parts of roasted cowpea, and 60 parts of fermented pearl millet ('Kamu') and 40 parts of roasted cowpea.

In this study, the following methods were used: Determination of the *in vitro* protein digestibility was done by the method of Nills (1979); determination of phytic acid was done by the method of Davies and Reid (1979); microbiological analysis was done by the method of Harrigan and McCaine (1976); and sensory evaluation was by the method of Williams (1981).

Determination of *in vitro* protein digestibility (Nills, 1979)

One milliliter of 11% trypsin was introduced into 3 test tubes. Subsequently, 4 ml of phosphate buffer with pH 7.5 was added to each test-tube and 1 ml of 0.1 NHC1 was also added and allowed to stand to equilibrate, after which 1 ml of 1% 'Kamu' was added to all the test tubes (labeled as digestibility at 1 and 6 h). The reaction in each of the test tube was stopped with 5 ml of neutralized formalin at 60 min and 6 h. The content of the test tubes were then filtered using filter paper.

The filter papers were dried in an oven at 108°C for 3 h. The nitrogen of the undigested sample was determined by the Kjedahl method:

$$\% \textit{ in vitro} \text{ protein digestibility} = \frac{CP1 - CP2}{CP1}$$

Where CP1 = Total protein of unprocessed grain; CP2 = Total protein after digestion with tryspin.

Determination of phytic acid (Davies and Reid, 1979)

One gram of sample was extracted into 40 ml of 0.5 M nitric acid for 1 h. The sample was filtered and 5 ml of 0.08 M ferric chloride was added. It was then boiled for 20 min and then filtered. The free iron (Fe^{3-})remaining in the solution was then determined colorimetrically by adding 2 ml of 0.005 M ammonium thiocyanate and the iron-binding capacities of the extracts was determined by difference. The results were then expressed in terms of mg Fe bound g^{-1} sample extracted.

Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA), and SPSS for windows (Version 9.0) was used to separate the means.

RESULTS

In vitro protein digestibility

Table 1 shows the *in vitro* protein digestibility of the weaning food blends. The digestibility of the weaning food blends ranged between 87.9 and 89.7% in 1 h with the 70:30 ratio having 89.5% and the 60:40 ratio having 87.9% for SOSAT C-88.

The unimproved variety had 89.7 and 88.2% for 70:30 and 60:40 ratios, respectively. The differences recorded in the two varieties of pearl millet were insignificant. Digestibility at six hours for SOSAT C-88 was 90.9% for 70:30 and 89.9% for 60:40, while the unimproved variety had 90.9% for 70:30 and 89.9% for 60:40.

Phytic acid

The phytic acid content of the raw pearl millet and the 'Kamu' is presented in Table 2. A percentage decrease of 60.4% was observed between the raw grain (745 mg/g) and 'Kamu' (295 mg/g) of the improved variety, while a decrease of 67.27% was observed for the unimproved variety with the raw grain having 825 mg/g and 'Kamu' having 270 mg/g. The unimproved variety showed a higher percentage decrease than the improved variety SOSATC-88.

Total bacterial count and microorganisms isolated

Tables 3 and 4 show the total bacterial count and the microorganism isolated in the production of 'Kamu' from the two varieties of pearl millet.

The total bacterial at 24 h for the unimproved pearl

Table 1. *In vitro* protein digestibility of the weaning food blends.

Digestibility	SOSAT C-88 millet/Cowpea		Unimproved millet variety/Cowpea	
	70:30*	60:40**	70:30*	60:40**
Digestibility at 1 h (%)	89.5 ±0.11	87.9 ± 0.91	89.7 ± 0.23	88.2 ± 0.33
Digestibility at 6 h (%)	90.9 ± 0.66	89.9 ± 0.71	90.9 ± 0.33	89.9 ± 0.79

Values are recorded as means ± SD of three determinations. Values in the same row with different superscript are significantly different (P<0.05).
* 70 parts of millet to 30 parts of cowpea; ** 60 parts of millet to 40 parts of cowpea.

Table 2. Phytic acid content of the raw pearl millet grain and 'Kamu'.

Item	Pearl millet-SOSAT C-88		Pearl millet-unimproved variety	
	Raw	'Kamu'	Raw	'Kamu'
Phytic acid (mg/g)	745:00 ± 4.61	295:00 ± 8.66	825:00 ± 4.21	270:00 ± 1.66
Percentage decrease	60.4		67.27	

Values are recorded as means ± SD of three determinations. Values in the same row with different superscript are significantly different (P0<0.05).

Table 3. Total bacterial count during production of 'Kamu'.

Sample	Total bacterial count (cfu/ml)		
SOSAT C-88 pearl millet	**24 h**	**48 h**	**72 h**
Steep water	28×10^5	11×10^5	4×10^5
Slurry	1×10^5	-	-
Dried 'Kamu'	0	-	-
Unimproved pearl millet variety	**24 h**	**48 h**	**72 h**
Steep water	35×10^5	22×10^5	10×10^5
Slurry	1×10^5	-	-
Dried 'Kamu'	0	-	-

Table 4. Micro-organisms isolated during 'Kamu' production.

Sample	Fermentation time (days)		
	24 h (day)	48 h (2 days)	72 h (3 days)
SOSAT C-88 pearl millet			
Steep water	*Lactobaccilus plantarum* and *Micrococcus lateus.*	*Streptococcus lactic* and *Saccharomyces cerevisae*	*Streptococcus lactics, Lactobacillus plantarum* and *Saccharomyces cerevisae.*
Slurry	*Streptococus lactic*	-	-
Dried 'Kamu'	No growth	-	-
Unimproved pearl millet			
Steep water	*Streptococcus lactic* and *Saccharomyces cerevisae.*	*Streptococcus lactics, Saccharomyces cerevisae* and *Lactobacillus plantarum.*	*Streptococcus lactics, Lactobacillus plantarum* and *Saccharomyces cerevisae.*
Slurry	*Streptococcus lactics, Lactobacillus plantarum, Saccharomyces cerevisae* and *Micrococcus lateus.*	-	-
Dried 'Kamu'	No growth	-	-

Table 5. Sensory evaluation of the weaning food blends.

Parameters	A	B	C	D
Colour	3.11 ± 1.31	3.21 ± 1.4	3.25 ± 1.9	3.10 ± 1.10
Odour	3. 47 ± 1.49	3.57 ± 1.23	3.51 ± 1.16	3.82 ± 1.21
Taste	3.03 ± 1.31	3.08± 1.61	3.12 ± 1.56	3.15 ± 1.73
Texture	3.25 ± 1.05	3.03 ± 1.41	3.25 ± 1.30	3.20 ± 1.76
Overall acceptability	3.22 ± 1.50	3.52± 1.14	3.21 ± 1.58	3.61 ± 1.21

Based on a 9 point hedonic scale: A - SOSAT C-88 -cowpea (70:30); B - SOSAT C-88-cowpea (60:40); C - Unimproved variety-cowpea (70:30); D - Unimproved variety -cowpea (60:40).

millet variety presented in Table 3 was 35 ×105, which dropped to 22 ×105 cfu/ml by the second day (48 h) and 10 ×105 cfu/ml after 72 h. The bacterial count obtained from the slurry after 24 h was 1 ×105 cfu/ml. By the time the 'Kamu' was sun-dried to a constant weight, there was virtually no growth of bacteria recorded. The peak of the growth of bacteria was recorded at 24 h. Almost the same trend was recorded for the improved variety SOSAT C-88 where the highest bacterial growth was recorded at 24 h (28 ×105 cfu/ml). The total bacterial count that was obtained after 48 h was 11 ×105 cfu/ml, while 4 ×105 cfu/ml was obtained after 72 h. After grinding and sieving of the grain, 1 ×105 cfu/ml was obtained. There was no significant growth recorded after drying of the 'Kamu' to a constant weight.

The microorganisms isolated from the 'Kamu' production are shown in Table 4. The water used for soaking of the grains and sieving did not show any significant growth of microorganisms. Through out the steeping of the unimproved variety, *Streptococcus lactics* and *Saccharomyces cerevisae* appeared, while *Lactobacillus plantarum* appeared on the second and third day. *S. lactics, l. plantarum, S. cerevisae* and *Micrococcus lateus* were all present after the grain was ground to obtain the slurry. However, *Micrococcus lateus* appeared after the slurry was left for 24 h to settle. There were no bacteria or yeast growth after the 'Kamu' was dried to a constant weight. Almost the same trend was observed for the improved variety where *L. plantarum* and *M. lateus* were isolated after 24 h, although they both disappeared after 48 h. *S. lactics* and *S. cerevisae* were isolated after 48 h and were still there after 72 h. *L. plantarum* reappeared after 72 h. Only *S. lactics* was isolated after the grain was ground and sieved and left for 24 h to settle. By the time the 'Kamu' was sun-dried to a constant weight, no growth of microorganisms was recorded.

Sensory evaluation of the weaning food blends

The results obtained from the sensory evaluation of the four weaning food blends presented in Table 5 show no significant differences (P>0.05) in the parameters that were evaluated.

The weaning food blends prepared from the pearl millet/cowpea showed no significant differences (P>0.05). This therefore suggests that any of the varieties of pearl millet can be used for the preparation of the weaning food blend.

DISCUSSION

Improvement in the protein digestibility of the weaning food blends may be attributed to the modifications that occur in protein during natural fermentation. The lactics of natural fermentation have been reported to contain proteolytic bacteria which degrade proteins into simple proteins, peptides and amino acids (Au and Fields, 1981). Reduction in contents of ant nutritional factors during the process of fermentation of pearl millet (Charu and Sehgal, 1991) and roasting of cowpea (Oshodi et al., 1997) may also be partly responsible for the increase in the protein digestibility of the weaning food blends.

Reduced level of phytic acid in cereals signifies a better nutritional value. The decrease in the level of phytic acid in the 'Kamu' prepared from the two varieties of pearl millet is due to the fermentation process. The inherent phytase activity on pearl millet grains believed to be activated during fermentation (Svanberg and Sanberg, 1988), hydrolyze phytic acid content of the 'Kamu'. The acidic pH and temperature provide favorable conditions for phytase activity. Similar reductions in phytic acid during fermentation and germination (Charu and Sehgal, 1991; Sripriya et al., 1997; Neelam and Chauhan, 1990) have been reported.

Lactic bacteria and yeasts are predominant in all the fermented grains as shown in Table 4. This is as a result of a symbiotic relationship between the lactate bacteria and yeast. It is assumed that the lactate flora provide an acidic condition for yeast growth, while yeast provide sufficient growth factors which enhances growth of lactate flora (Ikemefuna, 1998).

Lactate bacteria and yeasts were predominant in the fermented samples isolated. In many food and beverage fermentation, a symbiotic relationship between lactate

bacteria and yeast is common (Ikemefuna, 1998). It is assumed that the lactate flora provide an acidic condition for yeast growth, while yeast provide sufficient growth factors which enhance growth of lactate flora.

The microorganisms that were predominant were *Streptococcus lactics*, *Lactobacillus plantarum*, *Saccharomyces cerevisae* and *Micrococcus lateus*. This is in agreement with the findings of Chavan and Kadam (1989) and Ikemefuna (1998).

SUMMARY AND CONCLUSION

The results obtained indicate that a 90% increase in the *in vitro* protein digestibility showed by the weaning food blends from the two varieties of pearl millet is as a result of 60% reduction in the level of phytic acid in the raw grains during fermentation.

The predominant microorganisms (*S. cerevisae, L. plantarum, M. lateus and Streptococcus lactics)* isolated during the production of 'Kamu' from the two pearl millet varieties were consistent with the findings of other researchers. The result of the sensory evaluation showed that there was no significant difference (P>0.05) in the acceptability of the weaning food blends.

Thus, cereal/legume blends, such as millet cowpea, have been observed to meet the nutritional needs of infants of 0 to 12 months as depicted by the results of this study. Sensory evaluation and microbiological studies have revealed that the weaning food blends are acceptable and safe for feeding infants between 0 and 12 months of age. The 60:40 ratios were however found to be superior to the 70:30 ratios.

SUGGESTIONS AND RECOMMENDATION

Cereals make a substantial contribution to the intake of vitamins, particularly the B-group of vitamins which show that there is need to know the effect of fermentation on the B-group of vitamins. *In vivo* studies will also provide more information on the feeding value of the weaning food blends.

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