



Studies on the Rheological, Microbiological and Sensory Qualities of Weaning Food Formulated from Pearl Millet, Wheat, Cowpea and Groundnut

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Authors' contributions

This work was carried out in collaboration between all authors. Author HHL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MS and BM managed the analyses of the study. Author BM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Weaning foods were formulated in a cereal-legume combination using pearl millet, wheat, cowpea and groundnut. The pearl millet was fermented to produce "Akamu", the wheat was germinated while the cowpea and groundnut were roasted separately. The weaning foods were formulated as follows: Pearl millet (60%)-cowpea (20%)-wheat (10%)-groundnut (10%) (PCWG); Pearl millet (60%)-cowpea (30%)-wheat (10%) (PCW). Pearl millet (60%)-groundnut (30%)-wheat (10%) (PGW). Significant difference ($p \leq 0.05$) was observed in grain hardness of pearl millet (3.00 ± 0.01), wheat (4.03 ± 0.05), cowpea (2.96 ± 0.05) and groundnut (0.93 ± 0.05). Significant differences ($p \leq 0.05$) were also observed in the 100 grain volume, 100 grain weight and density of the cereals and grains. A decrease in pH with a concomitant increase in Titratable Acidity (TA) was observed during 72 hours fermentation of pearl millet. Low apparent viscosity PCWG (1441.66 ± 1.14 cps), PCW (1432.66 ± 1.36 cps) and PGW (1410 ± 1.15 cps) were observed in the three complementary weaning food blends. Low Water Absorption Capacity (WAC) for PCWG (0.86 ± 0.08 g/g), PCW (1.05 ± 0.12 g/g) and PGW (0.92 ± 0.14 g/g) were also observed. Predominant microorganisms

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(*Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Micrococcus lateus* and *Streptococcus lactics*) isolated during the production of “Akamu” from Pearl millet shows that the weaning foods are free from pathogenic microorganisms. The result of the sensory evaluation indicated that in terms of colour, odour, taste and texture there were no significant differences except in the overall acceptability where the weaning food PCWG was preferred to PCW and PGW.

Keywords: Viscosity; water absorption capacity; weaning foods; micoorganisms.

1. INTRODUCTION

Developing nutrient dense, fully cooked, ready to eat inexpensive supplementary foods from locally grown food ingredients has been strongly recommended as a viable and sustainable approach to address the problem of under nutrition in developing countries [1,2]. Acute infection is a key point of vulnerability in malnourished children, leading to increased susceptibility to further infection and in some cases death [3]. Although significant progress has been made over the past few decades in reducing the prevalence of malnutrition, recent data show that the prevalence of underweight and stunting has more than doubled in Africa and Asia [4].

Traditional weaning foods in Nigeria and most parts of West Africa consist of bulky monocereal grains prepared from either fermented millet, maize or sorghum into gruels referred to as “Akamu” or ‘Ogi’ which are inadequate in energy and nutrient content [5]. During the transition period from lactose based diet to semi-solid food, thin gruels with viscosities ranging from 1000 to 3000 cps were found ideal in feeding infants [6]. To reduce the bulk and increase the nutrient density of weaning food formulations, simple processing methods such as germination and fermentation are used.

The objective of this research was to prepare acceptable complementary weaning foods with low bulk and high nutrient from locally available materials such as pearl millet (*Pennisetum glaucoma* (L) R.BR.), wheat (*Triticum aestivum*), cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogae*) that will meet the nutritional requirement of infants.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Sources of raw materials

Improved varieties of pearl millet SOSAT C-88 and wheat Atilla Gan Atilla were obtained from

Lake Chad research institute (LCRI) while the cowpea seeds (Borno red) and groundnut (Dakar) were obtained through a seed breeder at LCRI. The grains and legumes were authenticated by a seed breeder at LCRI.

2.2 Methods

2.2.1 Sample preparation

The “Akamu” was prepared by method described by Akingbala et al. [7].

2.2.2 Preparation of wheat

One hundred grams (100 g) of wheat grains were cleaned to remove dirt. The grains were washed three times with water and then soaked (1:3 w/v) for 2 hours at room temperature after soaking the grains were drained and wrapped in the dampened cotton cloth. Germination was carried out at room temperature for 48 hours. The mouldy seeds were removed by hand and sprouted grains were washed before sun drying to a constant weight. The dried grains were ground into a fine powder and sieved using a 1mm pore sieve to obtain a fine powder [8].

2.2.3 Preparation of cowpea

Cowpea seeds were cleaned of dirt and soaked in water for 20 minutes. The cowpea seeds were dehulled using a mortar and a pestle. The seeds were washed to separate the coat and dried to a constant weight. The dried seeds were roasted at temperature of 120°C for 30 minutes. The seeds were continually stirred until a characteristic slightly brown colour was obtained. The seeds were allowed to cool and then ground into a fine powder. The ground seeds were sieved using a 1mm pore sieve [9].

2.2.4 Preparation of groundnut

The groundnut was cleaned of dirt, washed, soaked, dried, roasted at low temperature, dehulled to remove the testa and milled [10].

2.2.5 Preparation of the weaning food blends

The formulation of the weaning foods was done in the following ratios: (flow diagram for the preparation of the weaning foods is presented in Fig. 1).

1. 60 parts of pearl millet, 20 parts of roasted cowpea, 10 parts of germinated wheat and

2. 10 parts of roasted groundnut i.e. 60:20:10:10- PCWG
3. 60 parts of pearl millet, 30 parts of roasted cowpea and 10 parts of germinated wheat i.e. 60:30:10 -PCW
4. 60 parts of pearl millet, 30 parts of roasted groundnuts and 10 parts of germinated wheat i.e. 60:30:10- PGW.

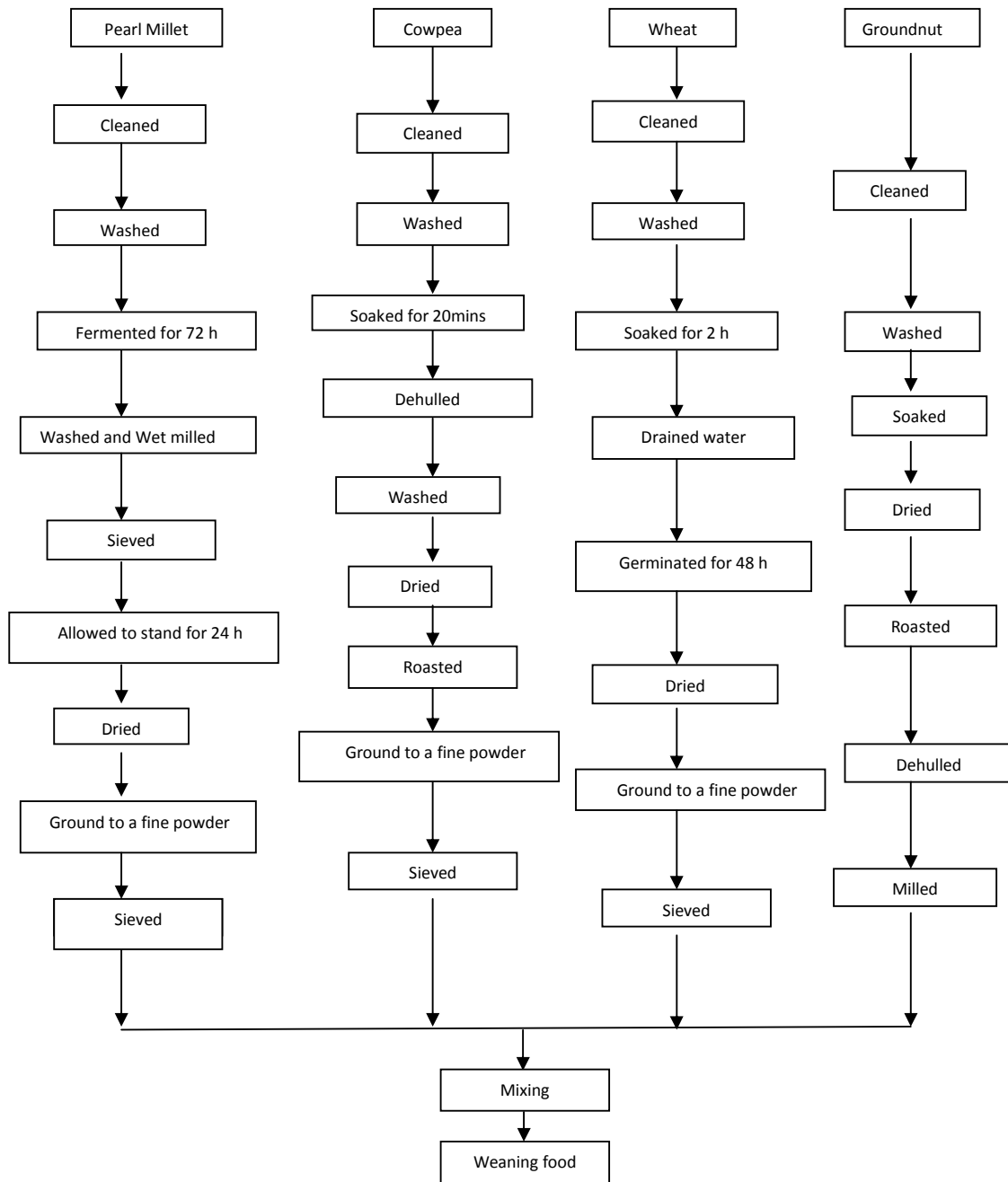


Fig. 1. Flow diagram for preparation of weaning foods

2.2.6 pH and titratable acidity

During 72 hours of fermentation, pH and titratable acidity were measured. The pH of the supernatant was taken using a pH meter, for the titratable acidity, 10 ml of sample was measured into a 50 ml beaker; 3 drops of phenolphthalein indicator was added and titrated against 0.1 M NaOH. The milliequivalent is the amount of NaOH consumed in each mill lactic acid equivalent which is equal to 90.08 mg [11].

Calculation:

$$\text{Titratable acidity (mg/ml)} = \frac{V \times N \times ME}{\text{Volume of sample used}}$$

Where

V= volume of sodium hydroxide.
N=molarity of sodium hydroxide.
ME = Mill Equivalent = 90.08 mg

2.2.7 Sun drying characteristics of “Akamu”

The sun drying characteristics of “Akamu” was determined according to the method described by Lescano [12]. A known weight of “Akamu” (5 mm in thickness) was spread on a weighed Petri dish. The petri dish was placed under the sun and the weight taken every hour until a constant weight was obtained. A graph of weight against time was plotted to obtain the drying curve (drying characteristics).

2.3 Physical Characteristics

2.3.1 100 g seed weight determination

The seed weight was determined by weighing 100 randomly selected raw seeds of each grain [13].

2.3.2 100 g seed volume

The 100 seed weight was transferred into a measuring cylinder containing a known volume of water. Increase in volume from displacement of the grains by the water was taken and the difference between the two readings was taken as the seed volume [14].

2.4 Seed Density

The density of the seed was calculated on the volume obtained from 100 seed weight and 100 seed volume [14].

Calculation:

$$\frac{\text{Mass (g)}}{\text{Volume (cm}^3\text{)}}$$

2.4.1 Grain hardness

The grain hardness was measured with hardness tester. The grains were placed into the machine and mechanical force applied on the grains and readings were recorded on the recorder [15,16].

2.5 Functional Properties

2.5.1 Water absorption capacity (WAC)

One gram (1 g) of each diet was weighed into a centrifuge tube and 10 ml of distilled water was added. Samples were vortexed for 5 minutes and allowed to stand for 15 minutes at room temperature before centrifuging (10,000 g) for 5 minutes. Excess water was allowed to drain by inverting the tube over absorbent paper. The weight of samples bound to water was determined by difference [17].

2.5.2 Apparent viscosity

Apparent viscosity was determined using torsion viscometer (Gallenkempt England). Water dispersions containing 10% (w/v) of each diet formulation was treated for 10 minutes at 100°C to form gruel. The gruels were cooled to 28°C before viscosity measurement was taken [18].

2.6 Microbiological Analysis

The microbial analysis of the samples before, during and after fermentation was done according to the method described by Harrigan and McCaine (1976). Appropriate dilution of samples was enumerated for counts of bacteria and yeasts using nutrient agar, Sabouraud dextrose agar and blood agar base. Inoculated plates were incubated at appropriate time and temperature combinations. Colonies of respective microbial types appearing in inoculated plates was counted and expressed as colony forming units (cfu/g). Colonies of bacteria and yeast were isolated and subcultured to obtain pure cultures.

2.7 Media Preparation

2.7.1 Nutrient agar

This is a good purpose medium which may be enriched with 10% blood or other biological fluid.

It supports the growth of a wide range of microorganisms and contains sufficient nutrients for the organisms.

2.7.1.1 Procedure

Twenty grams (20 g) of nutrient agar (oxid) was weighed and dissolved in one litre of distilled water in a clean conical flask. It was brought to boil to dissolve completely and then sterilized by autoclaving at 121°C for 15 minutes. It was allowed to cool to 50°C-55°C and then poured aseptically into sterile petri dishes and allowed to set.

2.7.2 Mac Conkey Agar

This is a differential medium for the isolation of coliforms and intestinal pathogens in water, dairy and biological specimens.

2.7.2.1 Procedure

Fifty two grams (52 g) of Mac Conkey agar was weighed into one litre of distilled water in a conical flask. This was brought to boil to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes. It was aseptically poured into sterile petri dishes. The surface of the gel was dried before inoculation.

2.7.3 Blood agar base

Forty two grams (42 g) of nutrient agar was dissolved in one litre of distilled water. It was dissolved and sterilised in an autoclave at 121°C for 15 minutes. On cooling, 10 ml of blood was added and poured aseptically into sterile petri dishes.

2.7.4 Sabouraud dextrose agar (SDA)

This is a general purpose medium for the cultivation of yeasts and moulds.

2.7.4.1 Procedure

Sixty five grams (65 g) of SDA was suspended in one litre of distilled water. It was boiled to completely dissolve and autoclaved at 121°C for 15 minutes and then cooled and aseptically poured into petri dishes.

2.8 Determination of Total Viable Count

After inoculation, the plates were incubated at 37°C for 24 hours. The colonies obtained were

counted on an electric colony counter (Gallen Kamp colony counter).

2.9 Isolation and Identification

A loopful of the sample was smeared over one corner of the solidified medium which was sufficiently dried. A nichrome wire loop was sterilized over a spirit lamp then cooled and used to make parallel streaks from the main inoculums. The plates were inoculated at 37°C for 24 hours.

2.10 Statistical Analysis

Data obtained from the research were in triplicates analysis was done using Analysis Of Variance (ANOVA). Duncan multiple range test was used to compare the differences between the means. Significance was accepted at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physical Characteristics

The results of the physical characteristics of pearl millet, wheat, cowpea and groundnut are presented in Table 1. Seed hardness of wheat, cowpea and groundnut varied between 0.93 ± 0.05 to 4.03 ± 0.05 . There were significant differences ($P \leq 0.05$) in the 100 g seed volume and 100 g seed weight. Significant differences ($P \leq 0.05$) were observed in the density of pearl millet and wheat while cowpea and groundnut did not show any significant difference ($P > 0.05$).

Grain hardness is a very important factor in determining grain quality. Milling quality is influenced by grain hardness. Harder grains give higher milling yield and also influence water absorption [14]. Grain weight provides information about the size and density of the grain. Grains of different density mill differently and are likely to retain moisture differently. Uniform grain weight is important for consistent grain quality. The differences observed in the hardness, weight, volume and density may be due to the differences in the genetic makeup of the cereals and legumes [19].

3.2 pH and Titratable Acidity (TA)

Table 2 presents the evolution of pH and titratable acidity during the fermentation of pearl millet for "Akamu" production. The initial pH (6.1 ± 0.15) dropped markedly during fermentation

to 3.5 ± 0.06 . Acidity increased with decreased in pH. The titratable acidity increased from 0.2 ± 0.01 to 7.4 ± 0.15 during the 72 hours fermentation of pearl millet.

Fermentation was found to cause a gradual decrease in pH with time. The reduction in pH of Pearl millet during 72 hours fermentation is similar to the result of Fatoumata et al. [20]; Singh et al. [21]. As a result of fermentation, acidity increases and pH falls and this enhances the keeping quality of foods by inhibiting microbial growth and also contributing to the flavour of processed millet [22]. The pH of fermented product is lowered due to the production of organic acid by the microflora; hetero fermentors were reported to convert glucose to equimolar mixture of lactic acid, ethanol and carbon dioxide [23]. A pH range of 3.6-4.1 is evidently favourable for eliminating undesirable microbial flora in fermented foods [24].

Concomitant with the drop in pH was a rise in Titratable Acidity (TA). This is in agreement with the work of Sripiya et al. (1997). High acidity is responsible for low microbial load in fermented product during storage period [25].

3.3 Drying Characteristics of “Akamu”

The drying characteristic of “Akamu” is presented in Table 3. A decrease in weight with increase in time was observed. The initial weight of the “Akamu” was 0.030 kg water/kg w.b at 0 hour. The weight of the “Akamu” after six hours of sun drying was 0.017 kg water/kg w.b. which remained constant to nine hours of sun drying.

Drying behaviour of solid can be described by measuring the function of moisture content loss verses time. A reduction in the moisture content of foods through proper drying increases the shelf life of the foods. The basic objective of drying food products is the removal of water in the solids up to a certain level at which microbial and deterioration chemical reactions are greatly minimised [26,27].

3.4 Functional Properties

Functional properties of the weaning food blends are presented in Table 4.

3.5 Apparent Viscosity

Significant differences ($P \leq 0.05$) were observed in the apparent viscosity of the weaning food blends. PCWG had the highest viscosity followed by cowpea based weaning food PCW and then the groundnut based weaning food PGW.

Low viscosity indicates increase in nutrient density. The groundnut based weaning food PGW had a lower viscosity, than the cowpea based weaning food PCW. A larger percentage of fat decreases the carbohydrate content, thereby reducing the amount of starch available for gelatinization [28]. Szczodrak and Pommeranz [29] found that lipids reduced swelling of starch granules. Amylose, the starch responsible for gelatinization formed insoluble complexes with lipids which reduced swelling capabilities upon heating.

Table 1. Physical characteristics of pearl millet, wheat, cowpea and groundnut

Parameters	Pearlmillet	Wheat	Cowpea	Groundnut
Grain hardness	3.00 ± 0.01^a	4.03 ± 0.05^b	2.96 ± 0.05^a	0.93 ± 0.05^c
100 grain volume (ml)	6.96 ± 0.03^a	4.93 ± 0.03^b	10.03 ± 0.08^c	22.90 ± 0.05^d
100 grain weight (g)	0.96 ± 0.03^a	3.56 ± 0.71^b	22.33 ± 0.03^c	50.00 ± 0.57^d
Density (g/ml)	0.13 ± 0.01^a	0.60 ± 0.01^b	2.23 ± 0.05^c	2.18 ± 0.01^c

Values are recorded as mean \pm SD of three determinations. Values in the same row with different superscript are significantly different ($P \leq 0.05$)

Table 2. pH and Titratable Acidity (TA) of 72 hours fermentation of pearl millet

	0	24	48	72
pH	6.1 ± 0.15^a	5.6 ± 0.20^b	4.7 ± 0.06^c	3.5 ± 0.06^d
Titratable Acidity (TA) mg/ml	0.20 ± 0.01^a	0.7 ± 0.03^b	3.4 ± 0.11^c	7.4 ± 0.05^d

Values are recorded as mean \pm SD of three determinations. Values in the same row with different superscript are significantly different ($P \leq 0.05$)

Table 3. Sun Drying Characteristics of “Akamu”

Time (h)	Moisture content (kg water/kg w.b)
0	0.030
1	0.029
2	0.026
3	0.022
4	0.019
5	0.018
6	0.017
7	0.017
8	0.017
9	0.017

Low Viscosity weaning diet with a high nutrient content is a desirable characteristic in weaning food [30]. The suggested semi-liquid consistency 1000-3000 cps is advocated for cereal-based gruels used for infant feeding. Low viscosity allows flexibility for manipulating the product's consistency by increasing the solids concentration. The processing methods of fermentation and germination evidently modified the rheological properties of the raw materials because low energy density is usually associated with weaning food formulations from unmodified starchy staples [31]. This provides a simple inexpensive means for increasing the products nutrient density [28].

3.6 Water Absorption Capacity (WAC)

The water absorption capacity of the three weaning food blends exhibited significant differences ($P \leq 0.05$). PCW had the highest WAC while PCWG had the lowest.

Water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain [32]. Water absorption capacity indicates the volume of water required to form gruel with suitable consistency for infant feeding [33].

The process of germination provides a simple inexpensive means of increasing nutrient density by reducing bulk. During fermentation, the pH decreases with an increase in titratable acidity with time. Reduction in pH to 3.8-4.0 decreases viscosity in cereals [28]. Apparently the process of fermentation influenced the ability of the weaning food blends' macromolecules to absorb water. Low water absorption capacity is a desirable functional property required to produce thin gruel that can be used in infant formulas [33].

Food products with low water absorption capacity tend to have their microbial activities reduced hence extending their shelf life [34].

3.7 Total Bacterial Count and Microorganisms Isolated

The total bacterial count during the production of “Akamu” is presented in Table 5. The total bacterial at 24 hours was 27×10^5 cfu/ml which dropped to 3×10^5 cfu/ml after 72 hours fermentation of Pearl millet. The bacterial count obtained from the slurry after 24 hours was 1×10^5 cfu/ml by the time the “Akamu” was sun dried to a constant weight, there was virtually no growth of bacteria recorded.

The microorganisms isolated from “Akamu” production are shown in Table 6. The water that was used for soaking of the grains and sieving of the milled Pearl millet did not show any significant growth of microorganisms. *Saccharomyces cerevisiae* and *Streptococcus lactics* were present throughout the 72 hours fermentation although *Lactobacillus plantarum* appeared after 48 hours fermentation. *Saccharomyces cerevisiae* isolated in the slurry after 24 hours. By the time the “Akamu” was sun dried to a constant weight, no growth of microorganisms were recorded.

Table 4. Functional properties of the weaning of food blends

	PCWG	PCW	PGW
Apparent Viscosity (10%) gruel concentration(cps)	1441.66±1.14 ^a	1432.66±1.36 ^b	1410.00±1.15 ^c
Water absorption capacity (g/g)	0.86±0.08 ^a	1.05±0.12 ^b	0.92±0.14 ^c

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

PCWG - 60 parts of Pearl millet, 20 Parts of Cowpea, 10 parts of wheat and 10 parts of groundnuts.

PCW - 60 parts of Pearl millet 30 parts of cowpea, 10 parts of wheat.

PGW- 60 parts of Pearl millet, 30 parts of groundnut, 10 parts of wheat.

Table 5. Total bacterial count during production of 'Akamu'

Sample	Total bacteria count (cfu/ml)		
	24 hours	48 hours	72 hours
Pearl millet			
Steep water	27x10 ⁵	11 x10 ⁵	3 x10 ⁵
Slurry	1 x10 ⁵	-	-
Dried "Akamu"	0	-	-

Table 6. Microorganisms isolated during production of 'Akamu'

Sample	Microorganisms isolated		
	24 hours	48 hours	72 hours
Pearl millet			
Sleep water	<i>Saccharomyces cerevisiae</i> , <i>Streptococcus lactics</i>	<i>Saccharomyces cerevisiae</i> , <i>Streptococcus lactic</i> , <i>Lactobacillus plantarum</i>	<i>Saccharomyces cerevisiae</i> , <i>Streptococcus lactics</i>
Slurry	<i>Saccharomyces cerevisiae</i> , <i>Streptococcus lactics</i> , <i>Micrococcus lateus</i> .	-	-
Dried "Akamu"	0	-	-

Table 7. Sensory Evaluation of weaning food blends

	PCWG	PCW	PGW
Colour	7.5±0.73 ^a	7.30±0.85 ^a	7.80±1.25 ^a
Odour	7.17±1.34 ^a	7.01±1.05 ^a	7.0±1.49 ^a
Taste	7.70±0.92 ^a	7.47±1.25 ^a	7.13±1.46 ^a
Texture	7.43±1.22 ^a	7.45±1.22 ^a	7.83±1.93 ^a
O/A	8.97±0.75 ^a	7.38±0.78 ^b	7.73±1.18 ^b

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

Based on a 9 point hedonic scale.

PCWG - 60 parts of Pearl millet 20 Parts of Cowpea, 10 parts of wheat and 10 parts of groundnuts

PCW - 60 parts of Pearl millet 30 parts of cowpea, 10 parts of wheat.

PGW - 60 parts of Pearl millet 30 parts of groundnut, 10 parts of wheat.

Lactic bacteria and yeasts are predominant in all the grains. This is as a result of a symbiotic relationship between lactate and yeast. It is assumed that the lactic flora provide an acidic condition for growth while yeast provide sufficient growth factors which enhances growth of lactate flora [35]. Lactic acid produced during fermentation increases acidity which helps in food preservation [36]. Fermentation has also been strongly suggested to have inhibition effects on the groups of microorganisms that can cause spoilage or food poisoning [37].

3.8 Sensory Evaluation

The sensory evaluation of the complementary weaning foods is presented in Table 7. Sensory

evaluation was conducted for the three complementary weaning foods by a 50 member untrained panellists consisting of nursing mothers. There were no significant difference (P≤0.05) in the colour, odour, taste and texture of all the three weaning food blends. Significant differences (P≤0.05) were observed in the overall acceptability of PCWG and PCW and PGW. While there were no significant differences (p>0.05) between PCW and PGW. PCWG was rated higher (8.97±0.57) in the overall acceptability than PCW (7.33±0.78) and PGW (7.73±1.18).

The result of the sensory evaluation indicates that in terms of colour, odour, taste and texture, all the three weaning food blends were accepted equally, but in the overall acceptability PCWG was accepted more than PCW and PGW.

4. CONCLUSION

The process of fermentation and germination modified the functional properties of the weaning food blends by reducing bulk and increasing nutrient density.

Predominant microorganisms (*Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Micrococcus lateus* and *Streptococcus lactics*) isolated during the production of "Akamu" from Pearl millet shows that the weaning foods are free from pathogenic organisms. The sensory evaluation showed that in terms of colour, odour, taste and texture there were no significant differences except in the overall acceptability where the

weaning food PCWG was preferred to PCW and PGW. The weaning food blends were formulated to meet the nutritional requirement of infants of 0-1 year.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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