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Microbiological and Nutritional Qualities of Moringa Oleifera Supplemented and Fermented Weaning Blends

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Abstract
Infant weaning food was developed using pearl millet (*Pennisetum glaucum*), cowpea (*Vigna unguiculata*), and peanut (*Arachis hypogaea L*) flours. The flours were supplemented with *Moringa oleifera* leaf powder at different ratio to produce six weaning blends. The formulated blends were subjected to spontaneous fermentation for 72hr, during which the microbiological and nutritional qualities were studied. The results of the chemical analysis showed that the pH increases with increase in fermentation time while the total titratable acidity (TTA) decreases with little difference in 15g and 20g supplemented blends. The total bacteria count, aerobic count and lactic acid bacteria count increases throughout the fermentation period with count decreasing with increase in quantity of supplementation. Analysis of the proximate content revealed that increase in supplementation increased the crude fibre and ash contents and decreased the fat content. The crude protein and fat contents was highest in PCG (17.239%; 10.256%) and the least content was recorded in PC (13.025% and 5.091%) respectively. The least bulk density (1.00g/ml), highest gross energy (35.799kcal/g) and the least pH (4.7) was recorded in sample 3 (PCGM1). Statistically, the chemical composition, proximate content and the physicochemical qualities of the formulated blends were significantly different ($p \leq 0.05$) from each other. As a result of the low bulk density, high energy content, least pH value and relatively high proximate contents with a low carbohydrate content, we therefore recommend PCGM1 i.e. 5g *Moringa oleifera* supplementation for a high nutritional quality weaning blend.

Key words: Weaning food, Supplementation, Fermentation, *Moringa oleifera*, Nutritional quality.

Introduction

If a country really wants development, adequate nutrition/balanced diet of the children must be ensured. It is clear there is no other way to develop than to provide adequate nutrient for them and build up a formidable human resource of the country [1]. Commercial infant weaning foods available in most developing countries are often not sufficiently available to the poor families. This could be attributed to the high cost of the component ingredients [2]. The introduction of foods other than breast milk into infant's diet while slowly reducing breast feeding is called the weaning process [3]. It is therefore necessary to augment the mother's with nutritionally adequate but cheap infant weaning food during the weaning periods, milk which start as liquid foods and slowly progress to solid foods.

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Cereal form basis for most of the traditional weaning foods in West Africa. A number of studies have reported that the nutritional qualities of traditional weaning foods in developing countries, particularly in Nigeria, are low in protein content and also devoid of vital nutrients required for normal child growth and development [4]. Prevalence of under nutrition and micro-nutrients deficiency is therefore high among infants and young children of 6-23 months old [5] and malnutrition causes a great deal of human suffering and is associated with more than half of all deaths of children worldwide [6]. In many developing countries, infant malnutrition constitutes mostly in protein energy malnutrition and micronutrient under nutrition (which may occur together) and remains a serious problem that causes growth retardation, diseases of all sorts and eventual death of infants [1]. Amongst a list of diseases associated with nutritional deficiencies in infants, marasmus and kwashiorkor occur as a result of low calorie

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and protein intake respectively [7]. Both diseases can result in mental retardation in a case where the child survives or the infant immune system is impaired as a result of malnutrition which leads to incidence of diarrhoea which could be an acute watery type, dysentery and the persistent type [7]. In order to reduce high morbidity and mortality due to malnutrition, attention has been focused on the exploitation and utilization of plant resources [8] to supplement the liquid milk-based diet from mothers which becomes insufficient [9] after four months of age and above, when the child doubles and triples his/her birth weight.

Traditional West African weaning foods could be improved by combining locally available foods that complement each other in such a way that the new pattern of amino acids created by this combination is similar to that recommended for infants [10]. Combination of commonly used cereals with inexpensive plant protein sources like legumes can be used. Cereals are deficient in lysine but have sufficient sulphur containing amino acids which are limited in legumes [11, 12] whereas legumes are rich in lysine. Therefore, the combination of cereals and legumes has been found to produce amino acid patterns that adequately promote growth [13]. The effects of the supplementation are highly beneficial, since nutritive value of the product is also improved.

A number of cereals and legumes that are readily available have been found to have nutrient potentials that could complement one another if properly processed and blended [14] as no single food contains all the micronutrients (e.g. the use of *Ogi* alone as weaning food has a low nutritional value). Hence, several attempts have been made to improve upon the nutritional status by fortifying it with protein rich substrates that is similar to the new pattern of amino acids [15, 16]. Researchers have worked extensively on cereal-legumes combinations for development of nutritious weaning foods with or without fermentation process [17, 18, 19, 20, 21].

Moringa oleifera is a small, fast growing evergreen or deciduous tree that usually grows as high as 9m, with a soft white wood, corky and gummy bark [22]. All parts of the Moringa tree are edible and have long been consumed by humans [23]. One of the uses of Moringa is the use of powdered seeds to flocculate contaminants and purify drinking water [24]. The seeds are also eaten green, roasted, powdered and steeped for tea or used in curries [24]. *Moringa oleifera* tree in recent times has been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids suitable for utilization in many of the developing regions of the world where undernourishment is a major concern [23].

In view of the outstanding qualities of *Moringa oleifera* tree, this research work aimed at studying the effect of fortifying spontaneously fermenting cereal-legume blends with moringa leaf powder on the microbiological and nutritional attributes of developed weaning blends.

Materials and Methods

Collection of Sample

Pearl millet (*Pennisetum glaucum*), cowpea (*Vigna unguiculata*), and groundnut (*Arachis hypogaea L*) purchased from Bodija market Ibadan, Oyo State, Nigeria were packed in clean polythene bags and stored in the refrigerator at +4^{o}C until use. Fresh *Moringa oleifera* leaves obtained from the Botanical garden of the Department of Botany, University of Ibadan, Ibadan were similarly packed in clean polyethene bags.

Processing of Cereal, Legumes and Moringa oleifera Leaves Samples

The cereal and legume samples were manually sorted and washed with distilled water to remove extraneous materials; cowpea was dehulled. The cereal and dehulled cowpea were oven dried at 50^{o}C and

60°C respectively for 8hours and milled to fine flour with sterile blender, sieved to fine particle size flour while the groundnut seeds were roasted after drying and milled to fine particle size flour. The flours were packed into clean polyethylene bags and stored at 4°C. The *Moringa oleifera* leaves were washed with distilled water to remove dust and extraneous materials, drained and dried at room temperature for 7 days. Dried samples were ground to fine powder using a blender, sieved and stored in labelled Zip-lock bags at 4°C.

Formulation of Composite Blends

Blend Formulation: This was carried out by modified method of Malleshi et al. [25].

The flour blends were mixed in accordance to the ratios presented in Table 1.

Table 1: Formulation Ratios of the Weaning Blends

Sample code	Peal millet	Cowpea	Groundnut	*Moringa oleifera*
PC	70	30	-	-
PCG	70	20	10	-
PCGM1	70	15	10	5
PCGM2	70	10	10	10
PCGM3	70	10	5	15
PCGM4	70	5	5	20

Fermentation and Sampling

The various blend formulation was transferred into labelled sterile flasks for further analysis. The flour blends in the flasks were reconstituted with sterile distilled water at a concentration of 30% (w/v) [26] and allowed to ferment spontaneously at 30±2°C for 72 hours. Samples were taken from each of the fermenting blends at 0, 24, 48 and 72 hours to assess the nutritional qualities of the formulated blends.

Microbiological Analysis

Isolation of micro-organisms

One milliliter amount of the fermenting slurry was introduced into 9ml sterile distilled water in a clean, sterile 25ml bottle. This was properly mixed and serially diluted out (10^6-10^{14}) with sterile distilled water, 0.1ml of highest dilutions were pour-plated out onto appropriate media – de Mann Rogosa and Sharpe (MRS) medium for lactic acid bacteria (LAB), Nutrient agar for aerobic bacteria, MacConkey agar for enterobacteria, Plate count agar (PCA) for total viable count (TVC) of bacteria and Malt extract agar for moulds and yeast. The above media were all prepared according to manufacturer's specification and sterilized for 15 minutes at 121°C. All plates were incubated at 30±2°C for 24 hours (bacteria and lactic acid bacteria), and 96 hours (yeasts and moulds). After incubation period, the plates were observed for growth of colonies which were counted and randomly selected. Streaking of a single colony was done on a well solidified agar plate until pure cultures of the selected isolates were obtained.

Characterization of isolates: Isolates were characterized and identified on the basis of their cultural, morphological, physiological and biochemical properties using Bergeys Manual of Systematic Bacteriology [27].

Chemical Analysis

Determination of pH: The pH of the fermenting gruel was monitored 24 hourly for 72 hours using a pH meter (Model 3505 Jenway). The fermenting gruel (10mls) was aseptically removed and its pH was determined by direct insertion of the glass electrode of the pH meter into the fermenting samples.

Titratable Acidity (Lactic acid determination): The amount of lactic acid produced in the fermenting slurry was

determined 24 hourly for each fermenting samples. 10mls of the supernatant of the mixture of steep water and fermenting gruel was pipetted and titrated against 1N NaOH to phenolphthalein end point. Each ml of 1N NaOH is equivalent to 90.08mg of lactic acid. A colour change from colourless to pink indicated the end point [28].

Proximate Analysis of Fermenting Blends: The moisture content, crude protein, crude fat, crude ash and total carbohydrate were determined by the method of the Association of Official Analytical Chemists [28]. The ash was determined by incineration of known weight of samples at 550°C until ash was obtained. Crude Protein (N x 6.25) was determined by the macro-Kjeldahl method. The fat composition was deter-mined by exhaustively extracting a known weight of sample with petroleum ether. Moisture content was determined by drying the sample in the oven and cooled in desiccator overnight at room temperature and dry matter was then calculated. Total carbohydrate was calculated by difference.

Physicochemical Analysis: The physico-chemical properties determined include: the viscosity, bulk density and gross energy content. The bulk density of loose fermented flour was determined by transferring 50g flour into a 250ml graduate cylinder and the volume was measured after tapping the cylinder until the powder stopped settling [29].

The cooked paste viscosity of the samples was determined with Brabender Viscograph E (IDENT 80252, Duisburg- Germany) [30]. The gross energy value was calculated using the Atwater conversion factors of 4, 9 and 4 for multiplying crude protein, crude fat and carbohydrate respectively. The sum of these values is equal to the calorific values expressed in kilocalories per 100 gram of samples (Kcal/100g) [28].

Sensory Evaluation

Each of the samples was prepared into cooked porridge (10ml of fermenting gruel mixed with 50ml of boiled water), cooled to 40^{o}C until it formed a semi-solid paste. The samples were then evaluated by 10 untrained panelists (comprising of nursing and potential mothers).They were assessed using a 9-points hedonic scale ranging between 9 ("like extremely") to 1 ("dislike extremely") based on parameters such as color, texture, taste, flavour and general acceptability [19].

Analysis of Data

Data obtained were subjected to statistical analysis using a two-way ANOVA. Tests of significance were carried out using Duncan multiple range tests at $P \leq 0.05$.

Results

Microbiological Analysis

The total lactic acid bacteria (LAB) counts, increases with increase in fermentation time with count ranging from $\log_{10}5.15$ Cfu/ml in unfermented 20g *Moringa oleifera* supplemented blend (PCGM4) to $\log_{10}20.74$ Cfu/ml in 72hr fermented millet-cowpea (PC) blend.

Total aerobic count increases within 48hr of fermentation of all the blends and decreases by 72hr. The aerobic count at each sampling time decreases with increase in supplementation with highest count observed in millet-cowpea blends at all sampling times.

Total bacteria count increases throughout the fermentation time in all the blends with lower counts observed in supplemented blends. The higher the supplementation, the lower the total bacteria count. The least bacterial count of $Log_{10}4.78$ Cfu/ml was recorded in unfermented 20g *Moringa oleifera* supplementation blend (0hr) while the highest count ($Log_{10}17.01$ Cfu/ml) was observed in 72hr fermented millet-cowpea-groundnut (PCG) blends.

The isolated bacteria were characterized and identified based on their morphological, biochemical and physiological characteristics

as *Klebsiella sp, Escherichia coli, Bacillus sp*. *Lactobacillus species, L. fermentum, L plantarum, L. helviticus, L. casei,* and *L. bulgaricus.* The yeast isolates were identified on the basis of their assimilation and fermentation of carbohydrates as *Candida species* and *Saccharomyces cerevisiae* while the moulds were identified as *Penicillium species, Aspergillus niger,* and *Rhizopus species* based on mycelia color and structures, and the microscopic examination of mycelia.

Chemical Analysis

Determination of pH

The pH of all the samples decreased with increase in fermentation period. The highest pH value (7.53) was obtained with the unfermented millet-cowpea-groundnut blend (PCG) while the lowest (3.58) was obtained in millet-cowpea blend (PC) by 72 hours of fermentation time. Statistical analysis shows that the pH of all samples was significantly different ($p<0.05$) (Table 2).

Table 2: The pH of the Formulated Blends

Sample/Time (Hr)	0	24	48	72
PC	7.47^{a}	4.28^{b}	4.08^{c}	3.58^{d}
PCG	7.53^{a}	4.08^{b}	3.94^{c}	3.69^{d}
PCGM1	6.73^{a}	4.31^{b}	4.13^{c}	3.76^{d}
PCGM2	6.57^{a}	4.36^{b}	4.41^{b}	3.99^{b}
PCGM3	6.47^{a}	5.32^{b}	4.46^{c}	4.21^{d}
PCGM4	6.45^{a}	5.01^{b}	4.99^{b}	4.57^{c}

Titratable Acidity

The titratable acidity of all the samples increased within the first 48 hours of fermentation time and decreased by 72 hours except for millet-cowpea (PC) and 10g *Moringa oleifera* supplemented blend (PCGM2) which increased throughout the fermentation period. The highest lactic acid value (18.01mg/ml) was recorded by 72 hours in millet-cowpea blend (PC) while the lowest (1.70mg/ml) was obtained in unfermented millet-cowpea blend (PC). Statistical analysis shows that total titratable acidity in all samples was significantly different ($p<0.05$) among the samples during the fermentation periods (Table 3).

Table 3: The Titratable Acidity (mg/ml) of the Formulated Blends

Sample/Time (Hr)	0	24	48	72
PC	1.70^{d}	7.70^{c}	8.86^{b}	18.07^{a}
PCG	1.90^{c}	8.30^{b}	12.39^{a}	12.39^{a}
PCGM1	2.10^{d}	7.90^{c}	10.59^{a}	10.39^{b}
PCGM2	2.30^{d}	7.60^{c}	8.90^{b}	10.19^{a}
PCGM3	3.50^{d}	6.99^{c}	7.89^{a}	7.50^{b}
PCGM4	4.50^{d}	6.29^{b}	7.40^{a}	6.20^{c}

Proximate Composition of Fermented Blends

Fermentation times significantly affect the proximate contents. The crude protein and ash contents increased within the first 48hr and decreased by 72hr while the crude fibre, crude fat and carbohydrate decreased within the first 48hr and increased by 72hr in some samples (Samples PC, PCGM1, PCGM3, PCGM4) while the crude fat and carbohydrate decreases with increase in fermentation in PCG and PCGM2.

Increased protein content was obtained with *Moringa oleifera* supplementation up to 15g (17.21%), this was however decreased to 15.84% at 20g (PCGM4) supplementation (Table 4). Statistical analysis using a 2-way ANOVA revealed that the crude protein contents are significantly different ($p<0.05$) among the blends while 15g supplemented blend (PCGM3) was not significantly different ($p>0.05$) from millet-cowpea fortified groundnut blend (PCG). Supplementation with *Moringa oleifera* also increases the ash and crude fibre contents with a concomitant decrease in fat contents. The ash content ranges from 1.71% in unsupplemented millet-cowpea blend (PC) to 2.42% in 20g supplemented millet-cowpea-groundnut blend (PCGM4). The ash contents are significantly different ($p<0.05$) among the formulations except for 15g and 20g supplemented blends. The crude fat content decreases with increase in supplementation of the blends, and were significantly different ($p<0.05$) among the blends with PCG (millet-cowpea-groundnut blend) having the highest (10.26%) fat content and the least (5.09%) observed in millet-cowpea blend (PC). The higher the supplementation with *Moringa oleifera* the higher the moisture content, which were statistically different from each other except blends PCG and PCGM3.

Table 4: Proximate analysis of all fermented formulated samples

Sample code	% Moisture Content	% Protein	% Fat	% Ash	% Crude Fibre	% Carbohydrate
PC	62.13 ± 1.0^{c}	13.03 ± 0.3^{e}	5.09 ± 0.2^{f}	1.71 ± 0.1^{e}	1.89 ± 0.1^{d}	78.29 ± 0.6^{a}
PCG	62.38 ± 1.2^{b}	17.24 ± 0.7^{a}	10.26 ± 0.3^{a}	1.78 ± 0.1^{d}	3.08 ± 0.4^{c}	67.64 ± 0.9^{d}
PCGM1	58.52 ± 1.0^{e}	15.38 ± 0.4^{d}	9.84 ± 0.3^{b}	2.08 ± 0.1^{c}	3.95 ± 0.2^{b}	66.92 ± 0.5^{f}
PCGM2	60.00 ± 1.5^{d}	16.99 ± 1.1^{b}	9.16 ± 0.2^{c}	2.27 ± 0.1^{b}	4.85 ± 0.5^{a}	67.28 ± 1.2^{e}
PCGM3	62.41 ± 1.0^{b}	17.21 ± 0.4^{a}	8.59 ± 0.2^{d}	2.39 ± 0.2^{a}	4.30 ± 0.1^{b}	68.79 ± 0.5^{b}
PCGM4	67.02 ± 1.5^{a}	15.84 ± 0.4^{c}	8.28 ± 0.2^{e}	2.42 ± 0.1^{a}	4.84 ± 0.2^{a}	68.63 ± 0.5^{c}

Mean ± standard error based on three replications, all values expressed in dry weight basis except moisture content. Means within the same column with different superscript are significantly different using Duncans multiple range test at $p<0.05$.

Generally, the least proximate composition was observed in millet-cowpea blend (PC) and highest protein and fat contents in millet-cowpea-groundnut blend (PCG).

Physicochemical Composition of all Fermented Blends

Table 5 shows the results of the analysis of the physicochemical properties of the fermented blends. From the table, the least bulk density (1.00g/ml) was recorded in 5g supplemented *M. oleifera* blend (PCGM1) while the highest (1.10g/ml) was observed in 20g *M. oleifera* supplemented blend (PCGM4). The least viscosity (35.45B.U) and gross energy (31.49kcal/g) was observed in millet-cowpea blend (PC) while the highest value of 60.12B.U (Viscosity) and 35.79kcal/g (Gross energy) was recorded in 20g supplemented *M. oleifera* blend (PCGM4) and 5g supplemented blend (PCGM1) respectively. Supplementation decreased the acidity (increased the pH) and the lactic acid contents (titratable acidity) of the fermented blends. The least pH of 4.74 was recorded in 5g supplemented *M. oleifera* blend (PCGM1) while the least lactic acid content (TTA) was observed in PCGM4 (6.10g/ml). Statistical analysis of the physicochemical characteristics using 2-ways ANOVA reveals that the blends were significantly different ($p < 0.05$) from each other. Overall the highest gross energy (35.79kcal/g) content, least bulk density (1.00g/ml) and pH (4.74) and a relatively low viscosity (36.92B.U) was recorded in 5g supplemented blend (PCGM1).

Table 5: Physicochemical Analysis of Fermented Samples

Sample/Code	Bulk Density (g/ml)	Viscosity (B.U)	Gross Energy (kcal/g)	pH	Titratable Acidity(g/ml)
PC	1.02 ±0.01^{d}	35.45 ±1.4^{f}	31.49 ±1.6^{e}	4.85 ±0.5^{bc}	9.07 ±1.8^{a}
PCG	1.04 ±0.01^{c}	35.84 ±1.6^{e}	35.07 ±2.3^{c}	4.81 ±0.5^{d}	8.75 ±1.3^{b}
PCGM1	1.00 ±0.01^{e}	36.92 ±1.5^{d}	35.79 ±1.9^{a}	4.74 ±0.4^{e}	7.75 ±1.0^{c}
PCGM2	1.08 ±0.01^{b}	58.82 ±1.1^{b}	35.24 ±1.9^{b}	4.83 ±0.3^{cd}	7.25 ±1.0^{d}
PCGM3	1.09 ±0.02^{a}	57.05 ±1.0^{c}	33.45 ±1.2^{d}	5.12 ±0.3^{ab}	6.47 ±0.5^{e}
PCGM4	1.10 ±0.02^{a}	60.12 ±0.5^{a}	35.26 ±2.3^{b}	5.26 ±0.2^{a}	6.10 ±0.3^{f}

Means ± standard errors based on three replications, all values expressed in dry weight basis. Means within the same column with different superscript are significantly different using Duncans multiple range test at $p<0.05$.

Table 6 shows the results of analysis of the sensory attributes of the fermented weaning blends. The unsupplemented fermented blends (PC and PCG) were significantly ($p<0.05$) rated high in terms of texture, aroma, appearance, taste and overall acceptability compared to Moringa oleifera supplemented formulated and fermented blends. The rating of all the sensory characteristics assessed decreases with increase in supplementation except for texture.

Table 6: Analysis of the Sensory Qualities of the Fermented Formulated Blends

Samples/Code	Texture	Aroma	Appearance	Taste	Over-all acceptance
P C	6.38±0.25^{ab}	6.38±0.25^{a}	5.50±1.41^{ab}	5.87±0.75^{a}	6.13±0.85^{a}
PCG	6.75±0.29^{a}	6.38±0.48^{a}	6.00±1.08^{a}	5.50±0.71^{a}	6.13±1.18^{a}
PCGM1	6.13±1.55^{ab}	5.50±1.41^{ab}	5.00±1.08^{ab}	4.75±1.32^{ab}	5.75±0.96^{a}
PCGM2	4.50±1.47^{b}	4.00±1.83^{b}	4.00±0.91^{b}	4.75±1.32^{ab}	4.37±1.65^{b}
PCGM3	4.75±1.32^{b}	3.50±1.68^{b}	4.00±1.22^{b}	4.13±1.89^{b}	4.13±1.49^{b}
PCGM4	5.00±1.47^{ab}	3.50±2.08^{b}	4.25±0.96^{b}	3.87±1.89^{b}	4.13±1.49^{b}

Mean ± standard error based on three replications. Means within the same column with different superscript are significantly different using Duncans multiple range test at $p<0.05$.

Discussion

The types of micro-organisms isolated during the fermentation process include lactic acid bacteria, aerobic bacteria, mould and yeast. From the result, the fungi isolates were quickly eliminated during the fermentation period which is in accordance with the findings of Akinrele [31]. The predominance of *Lactobacillus plantarum* at all levels of fermentation agreed with the report of Wakil and Onilude [21] which may be due to production of lactic acid which inhibits the growth of other micro-organisms in fermenting medium. This is in agreement with Chavan and Kadam [32] who reported lactic acid bacteria as one of major micro-organisms involved in fermentation of fortified cereal blends.

The reduced/low counts of entero-bacteriacea and other gram-negative bacteria as observed in this study showed that their growth might have been inhibited due to the presence of lactic acid bacteria which produce lactic acid [33]. No enterobacteria were observed at the commencement of active fermentation in all the formulated blends. The total disappearance of enterobacteriaceae by 24 hours fermentation time agrees with the observation of Michodjehoun-Mestres et al. [34] and that observed by Hounhouigan et al. [35], portraying the decrease that culminated in total disappearance of the enterics population was parallel to pH decrease, which is expected in accordance with the death kinetic of Enterobacteriaceae due to low pH.

Phenotypic identification of all the isolates based on morphological, physio-logical and biochemical characteristics showed the prospective isolates on Nutrient agar, Plate count agar MRS agar, MacConkey and Malt extract agar to be *Lactobacillus platarum, L. brevis, L. helviticus, L. bulgaricus, L. fermentum, L. casei*, *Bacillus sp, Escherichia coli Klebssiella sp Aspergillus niger, Rhizopus sp, and Penicillium sp Saccharomyces cerevisiae*,

Candida sp. This observation is similar to that of Wakil and Onilude [21]. The viable and lactic counts of the fermenting organisms' increases as the fermentation period increased. A concomitant increase in lactic acid bacteria and its association with other micro-organisms' e.g. yeast and fungi has been noted in several cereal foods [36] as their development have been reported to be stimulated by yeast; which provides soluble nitrogen compounds and other growth factors e.g. CO_2, pyruvate, acetate etc. [37].

The result of the culture-dependent (plating) method, which was mainly characterized by an initial increase in total bacteria count, lactic acid bacteria and yeast count is similar to that reported by Wakil and Onilude [21]. Apart from the flora present on the surface of the grains, microbial flora may have also been established during milling thus explaining the higher initial culturable count in the weaning blends [21].

The changes in pH and titratable acidity observed in this study have been reported to be characteristic of the fermentation of carbohydrate rich plant material which is in line with the findings of Odunfa and Adeleye [36] and Sefa-Dedeh et al. [38]. The pH of the blends decreased as the fermentation time progresses. This decrease in pH is attributed to the production of organic acids in the fermenting slurries, a result similar with the observation of Sanni et al. [19] during fermentation of cereal –soybean weaning blend. The decrease in pH and increase in titratable acidity may be due to the microbial activity that resulted in the dominance of lactic acid bacteria which degrade carbohydrates resulting in acidification of the product [21].

Mensah et al. [39] reported that high titratable acidity reduce the incidence of diarrhoea in infants consuming fermented cereal porridge. The acidic pH obtained in this study may also reduce the amylase activities of the grains thus, limiting the conversion of starch to fermentable sugars.

In the supplemented samples, protein, carbohydrates, crude fibre and ash content were observed to increase while the crude fat decreased with increase in fermentation time. In very few samples, a decrease was observed with increase in fermentation time. As observed in this study, increase in protein content of the supplemented blends at the various fermentation levels is in agreement with the findings of other researchers who had reported natural fermentation to improve the protein contents of foods as a result of microbial activity [40, 41]. Sanni et al. [19] reported that the increase in protein content of the fermented blends could be attributed to the breakdown of nutrients present in the substrates especially cowpea (known to be a protein rich seed) by fermenting organisms. The increase could also be as a result of the synergy between *Moringa oleifera* reported to be highly nutritious [23], and other components that make up the formulated blends. The decrease in protein content observed in few samples (PCG, PCGM1) also agrees with the work of Sanni et al. [19] a report which also stated that the decrease could be attributed to the *Lactobacillus plantarum* utilizing the amino acids for growth.

A decrease in the fat content of most of the formulated blends as observed in this study is comparable with the findings of Sanni et al. [19] and Chinma et al. [42]. They reported that this decrease might be attributed to the increased activities of lipolytic enzymes during fermentation which hydrolyzes fat contents into fatty acid and glycerol. The crude fibre of the formulated blends in most samples was observed to decrease with increase in fermentation time in most samples. This is also in line with the report of Sanni et al. [19].

The ash content as observed in some samples increased with increase in fermentation time. This report is in line with that of Adebowale and Maliki [43] who observed same trend after the fermentation of pigeon pea seeds. Comparable to the observation in this study which might be as a result of supplementing with *M. oleifera* is that finding of Eka et al. [44] who reported an

increase in ash content among formulations with crayfish inclusion, an animal product in most of the diets indicating a higher level of mineral elements in the diets.

The moisture content of the formulated blends in wet form increased with increase in fermentation time in most of the samples, a report which is comparable with Adebowale and Maliki [43] who reported same trend with fermented pigeon pea. They suggested that this might be due to the low dry matter content of the component. In few formulated blends, a decrease in the moisture content level was observed which is in line with the report of Eka et al. [44]. A decrease in moisture content of weaning food implies they can be kept for long time without spoilage as a result of fungal growth as high moisture content encourages microbial growth [45]. This is a very important consideration for local feeding methods in Nigeria for example because mothers often prepare large quantities of dry infants foods and keep in containers, to avoid frequent processing in other to have spare time and energy for other domestic activities [44].

The carbohydrate composition of the formulated blends decreased with increase with fermentation time and reduced with increase in the quantity of *M. oleifera* supplementation. The unfermented samples were observed to possess the highest carbohydrate content. This report is comparable to the observation of Sefa-dedeh et al. [38] and Mbata et al. [46].

The significant increase in proximate content of the supplemented legume fortified millet based samples with *M. oleifera* as observed in this study is a pointer to the fact that it has exceptional nutritive values which agrees with the observation of [47], which reports that *M. oleifera* leaves rivals and even defeats commonly available natural edibles. An increase in fermentation time was also observed to cause a significant increase in the proximate content and sensory quality of supplemented samples which agree with reports of Odunfa, [48] and Sanni et al. [19].

The overall acceptability of the formulated supplemented food samples was significantly ($p<0.05$) rated low compared with the unsupplemented blends; however, there was no significant difference between the overall accept-ability of the 5g supplemented blend (PCGM1) and that of unsupplemented weaning blends (Table 5). The rating of fermented unsupplemented blends (PC and PCG) above the Moringa supplemented blends in terms of texture, colour, aroma, taste and overall acceptability could be attributed to the cultural belief and phytochemical constituents of the *Moringa oleifera* leaf used.

Conclusion

Fermentation and supplementation with *Moringa oleifera* which is known to possess high nutritional contents improved the nutritional profile of the blends and decreased consumer overall acceptability with increase in the quantity of *M. oleifera* powder. The study also revealed that fermentation of 5g supplemented millet-cowpea-groundnut blend resulted in weaning food with the least moisture and carbohydrate contents, bulk density and pH values, highest Gross energy content and comparable sensory qualities with the unsupplemented foods. In view of these attributes, we therefore recommended PCGM1 as a potential nutritious infant weaning food.

References

(1) Fugile, L.J. 2001. The Miracle Tree: Moringa Oleifera: Natural Nutrition for the Tropics Church World Service, Dakar. 68 pp.; revised in 2001 and published as The Miracle Tree: The Multiple Attributes of Moringa, 172 pp. *http/\:/ww.echotech.org/bookstore/advanced_search_result.php?keywords=Miracle+ Tree.*

(2) Johnson, O.A., Muyiwa, A., Gbenga, E.O., Christian, O. and Valentine, A.A. 2008. Nutritive evaluation of *Talferia Occidentales* Leaf protein concentrate in

infant foods. *Africa Journal of Biotechnology* 7(15): 2721-2722.

(3) WHO. 2000. Complementary Feeding: Family Foods for Breast Feeding Children. WHO Geneva.

(4) FAO/UN. 2004. Seed quest news section. Biotechnology application in food processing, Can developing countries benefit? Rome Italy.

(5) Oyarekua, M.A. 2011. Evaluation of the nutritional and microbiological status of co-fermented cereal/cowpea 'Ogi'. *Agriculture and Biology Journal of North America* 2: 1.61.73.

(6) Pelletier, D.L., Frongillo, E.A., Schroeder, D.G. and Habicht, J.P. 1995. The effects of malnutrition on child mortality in developing countries. *Bulletin of the World Health Organization* 73(4): 443–448.

(7) Steinkraus, K.H. 2002. Fermentation in world food processing. In: *Compre-hensive review in Food Science and Safety*. Institute of Food Technologist 1 pp. 23-32.

(8) Hassan, L.G. and Umar, K.J. 2005. Protein and amino acids composition of African locust bean (*Parkia biglobosa*). *Tropical Subtropical Agro ecosystem* 5: 45-50.

(9) Pollit, E.A. 1994. Early supplementary feeding, child development and health policy. Food Nutrition Bulletin **15**(3): 208-214.

(10) Uwaegbute, A.C. and Nnayelugo, D.O. 1989. Towards improving the nutri-tional value of traditional weaning foods. In: Fashakin J.B., ed. *Proceedings of the workshop on present knowledge on weaning foods in Nigeria*, held in Lagos, 29-30 August 1989. Federal Institute for Industrial Research Oshodi, Lagos: 63-85.

(11) Iqbal, A., Ateeq, N., Khalil, I.A., Perveen, S. and Saleemullah, S. 2006. Physico-chemical characteristics and amino acid profile of chickpea cultivars grown in Pakistan. *Journal of Food Science* 17: 94-101.

(12) Shewry, P. 2007. Improving protein content and composition of cereal grain. *Journal of Cereal Science* 46:239-250.

(13) Onofiok, N. and Nnanyelugo, D.O. 1992. Nutrient intake of infants of high and low socio-economic groups in Nsukka, Nigeria. Occasional Paper. Nsukka: Department of Home Science and Nutrition, University of Nigeria.

(14) Fernandez, D.E., Vanderjagt, D.J., Williams, M., Huarg, Y.S., Lut-te, C., Millson, M., Andrew, R., Pastuszyn, A. and Glew, R.H. 2002. Fatty acids, amino acids, and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plant Foods for Human Nutrition* 57: 257-274.

(15) Bamiro, F.O., Esuoso, K.O. and Adetunji, N.T. 1994. Fortified corn flour feed as infant formulae substitute. *Pakistan Journal of Science Industrial Research* 37: 163-166.

(16) Osungbaro, T.O., Esuoso, Y.A. and Esuoso, K.O. 2000. Nutritional and textural qualities of soy-ogi, (soy-maize porridge) and agidi, (fermented soy-ogi meal). La Rivista Italiana Delle Sostanze Grasse. Vol. LXXVII.

(17) Afoakwa, E.O. 1996. Storage characteristics and quality Evaluation of Cowpea-Fortified Traditional Foods. B.Sc. Dissertation, Department of Nutrition and Food Science, University of Ghana, Legon-Accra, Ghana.

(18) Ayo, J.A., Oluwalana, I.B., Idowu, M.A., Ikuomola, D.S., Ayo, V.A., Umar, A. and Yusuf, E. 2011. Production and Evaluation of millet-egg-soyabean hull composite flour: A Weaning food. *American Journal of Food and Nutrition* 1:1. 7. 13.

(19) Sanni, A.I., Onilude, A.A., and Ibidapo, O.F. 1999. Physicochemical Charac-teristics of Weaning Food Formulated from different blends of cereals and soyabean. *Food Chemistry* 65: 35-39.

(20) Onilude, A.A., Sanni, A.I. and Ighalo, M.O. 2004. Process upgrade and the microbiological, nutritional and con-sumer acceptability of infant weaning food from fermented composite blends of cereals and soybean. *Journal of Food, Agriculture and Environment.* 2(3 & 4) 64-68.

(21) Wakil, S.M. and Onilude, A.A. 2009. Microbiological and chemical changes during production of malted and fermented cereal-legume weaning foods. *Advances in Food Sciences* 31(3): 139-145.

(22) Mishra, G., Singh, P., Verma, R., Kumar, S., Srivastav, S., K.Jha, K. and Khosa, R. 2011. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. Scholars

Research Library *Der Pharmacia Lettre* 3(2): 141-164.

(23) Fahey, J.W. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1.*Tree for Life Journal*, 1:5.

(24) Gassenschmidt, U., Jany, K.D., Tauscher, B. and Neibergall, H. 1995. Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. *Biochimica Biophysica Acta* 1243: 4777-481.

(25) Malleshi, N.G., Daodu, M.A. and Chandrasekhar, A. 1989. Development of weaning food formulations based on malting and roller drying of sorghum and cowpea. *International Journal of Food Science and Technology* 24: 511-519.

(26) Livingstone, S.A., Feng, J.J. and Malleshi, G.N. 1993. Development and nutritional quality evaluation of weaning foods based on malted popped and roller-dreid wheat and chickpea. *International Journal of Food Science and Technology* 28: 35-43.

(27) Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holts, J.G. 1986. Bergeys Manual of Systematic Bacteriology. Vol. 2 Baltimore, Williams and Wilkins.

(28) A.O.A.C. 1990. Official methods of Analysis, (15th ed) Association of Analytical Chemists: Washington D.C ISBN 2-93 558-4420.

(29) Akpapunam, M.A. and Markakis, P. 1981. Physico-Chemical and Nutritional aspects of cowpea flour. *Journal of Food Science* 46: 972 – 973.

(30) Malleshi, N.G. and Desikachar, H.S.R. 1986. Influence of malting conditions on quality of finger millet malt. *Journal of the institute of Brewing* 92: 81-83.

(31) Akinrele, I.A. 1970. Fermented studies on maize during preparation of traditional African starch cake food. *Journal of Science and Agriculture*. 21: 619-625.

(32) Chavan, J.K. and Kadam, S.S. 1989. Nutritional improvement of cereals by fermentation. In: Critical Reviews. In: *Food Science and Nutrition* 28: 349-400.

(33) Omemu, A.M., Oyewole, O.B. and Bankole, M.O. 2007. Significance of Yeasts in the Fermentation of Ogi production. *Food Microbiology* 24(6): 571-576.

(34) Michondjehoun-Mestres, L., Hounhouigan, D.J., Dossou, J. and Mestres, C. 2005. Physical, chemical and microbiological changes during natural fermentation of "Gowe", a sprouted or nonsprouted sourghum beverage from West Africa. *Africa Journal of Biotechnology* 4(6): 487-496.

(35) Hounhouigan, D.J., Nout, M.N., Nago, C., Houben, J. and Rombout, F. 1993. Microbiological and physical attibutes of Mawe, a fermented maize dough from Benin. *International journal of Science and Technology* 28: 513-517.

(36) Odunfa, S.A. and Adeyele, S. 1985. Microbiology changes during the traditional production of ogibaba, a West Africa fermented sorghum gruel. *Journal Cereal Science* 3: 173-180.

(37) Leroi, F. and Pidoux, M. 1993. Characterization of interactions between *Lactobacillus higard* and *Saccharo-myces horentimus* isolated from suary kefir grains. *Journal of applied Bacteriology* 74: 54-60.

(38) Sefa-Dedeh, S., Sakyi-Dawson, E., Afoakwa, E.O., Andoh- Kumi, K. and Tawo-Debrah, K. 2001. Effect of drying method, packaging material and storage on the quality of cowpea-based weaning foods. Presented at the Annual Meeting of the Institute of Food Technologists, New Orleans, and 25–29 June.

(39) Mensah, P.P.A., Tomkins, A.M., Draser, B.S. and Harrison, T.J. 1990. Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. *Lancet*, 336: 140-3.

(40) Oshodi, A.A., Ogungbenle, H.N. and Oladimeji, M.O. 1999. Chemical composition, nutritionally valuable minerals and Functional properties of beniseed, pearl millet and quinoa flour. *International Journal of Food science and Nutrition* 50: 325-331.

(41) Wakil, S.M. and Onilude, A.A. 2010. Monitoring the effect of fortification on bacterial population dynamics in malted and fermented maize-based weaning foods using PCR-DGGE. *Journal of Applied Biosciences* 26: 1997-5902.

(42) Chinma, C.E., Adewuyi, O. and Abu, O.J. 2009. Effect of germination on the chemical, functional and pasting proper-ties of flours from brown and yellow varieties of tiger nut (*Cyperus esculentus*) *Food Research International* 42: 1104-109.

(43) Adebowale, O.J. and Maliki, K. 2011. Effect of fermentation period on the chemical composition and functional properties of pigeon pea (*Cajanus cajan)* seed flour. *International Research Journal* 18(4): 1329-1333.

(44) Eka, Essien B., Bene, W., Abbey, and Joyce. O. Akaninwor. 2010. Nutritional Evaluation of Some Traditional Weaning Foods from Akwa Ibom State, Nigeria. *Nigerian Journal of Bio-chemistry and Molecular Biology Research Journal* 25 (1) 65-72.

(45) Temple, V.J., Badamosi, E.J., Ladeji, O. and Solomon, M. 1996. Proximate and chemical composition of three locally formulated complementary foods. *West African Journal of Biological Science* 55: 134-143.

(46) Mbata, T.I., Ikenebomeh, M.J. and Ezeibe, S. 2009. Evaluation of mineral content and functional properties of fermented maize (Generic and specific) flour blended with Bambara groundnut (*Vigna subterranean*). *African Journal of Food Science* 3(4) pp. 107-112.

(47) Fugile, L.J. 2000. New Uses of Moringa Studied in Nicaragua. ECHO Development Notes #68.

(48) Odunfa, S.A. 1987. African fermented foods from Art to Science. *Journal of Science, Food and Agriculture* 1(3): 179-183.