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Development of Maize-Tigernut Fortified Weaning Food Using Starter Cultures

Sherifah Monilola Wakil*, Joshua Opeyemi Ola

Department of Microbiology, University of Ibadan, Ibadan, Nigeria
Email: *shemowak@yahoo.com, *wakilola@gmail.com

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Abstract

The effects of starter fermentation on the nutritional qualities of maize-tigernut fortified weaning foods were investigated. The dry-milled, malted maize grains fortified with dry-milled roasted tigernut flours (70:30) were subjected to 48 hrs of spontaneous and starter fermentation (singly and as combined starters). Nutritional, sensory characteristics and feeding trials of the weaning foods were evaluated. Four fermented weaning blends were formulated: FMT (spontaneously fermented maize-tigernut), SFMT1 (*Lactobacillus plantarum* F2C fermented maize-tigernut), SFMT2 (*Lactobacillus plantarum* U2A fermented maize-tigernut) and SFMT3 (combined starter-fermented maize-tigernut). SFMT2 had the highest crude protein, fat, fibre, ash and least carbohydrate content among the blends. The highest energy content (456.84 Kcal/100 g) was observed in blend SMFT2 which was also higher than that of both negative (Nutrend) and positive (Conventional animal feed) controls. The least antinutrient and vitamin contents were recorded in SFMT2. Blend SFMT1 had the highest Vitamin B1 (0.67 mg/100 g), Vitamin A (472.60 ug/100 g), phosphorus (75.45 mg/100 g) and zinc (1.05 mg/100 g) contents while the highest calcium (17.17 mg/100 g) and iron (22.82 mg/100 g) were recorded in SFMT2. Sample SFMT2 was rated the highest in all of the sensory characteristics except colour and the highest overall acceptability (6.00) which was not different significantly from all other starter produce blends. Biological evaluation showed blend SFMT2 fed animals having the highest weight by 28 days (73.14 g), mean weight gain (5.46 g), mean feed intake (18.71 g) and mean protein efficiency ratio PER (3.65). However, all the PER values including that of controls (2.30) were higher than the value of 2.10 recommended by the Protein Advisory Group (PAG) for complementary foods. The RBC, WBC and PCV of the trial groups were within the rat hematologic reference ranges. Blend SFMT2 (*L. plantarum* U2A fermented blend) gave the best performance after rat feeding trials.

Keywords

Fortification, Tigernut, Nutritional Evaluation, Biological Evaluation, Starter-Fermentation

1. Introduction

The arrival of a new born is accompanied by the secretion of the highly nutritive, thick yellowish pre-milk substance, referred to as colostrum. The colostrum contains the very essential nutrients as well as antibodies and it helps in boosting the immunity of the infant to infections [1] [2] [3] [4]. Breast milk usually provides all the energy, nutrients and fluids that baby needs in order to grow and develop healthily during the first six months of life [5] [6]. Infants when exclusively breastfed for the optimal duration of six months are significantly protected against the major childhood diseases' conditions such as diarrhea, gastrointestinal tract infection, allergic diseases, diabetes, obesity, childhood leukaemia and lymphoma, inflammatory and bowel disease [5] [6].

The breast milk alone remains sufficient for an infant up until the sixth months after birth. Its supply of energy, protein, vitamin A and B6 as well as calcium, meets the requirements of the infant, it is however clear that the breast-milk is insufficient in its supply of vitamin D, iron and zinc [7] [8]. This inadequacy becomes more elaborate after 6 months of birth due to the increase in the nutrient requirements of the infant. There is therefore the need to mitigate the inadequacies with the use of supplements in the mother and child and complementary feeding of the infant if malnutrition occurrence is to be avoided.

The digestibility of the weaning food and the absorption capacity of the gastrointestinal tract are some of the factors that determine how efficient the contributions of the foods are to the overall health, growth and development of the infants [9] [10]. The vast majority of the infants are weaned most times with the low-cost traditional weaning food and weaning practices which resulted in increased susceptibility of infants to infections, mortality and diseases such as kwashiorkor, marasmus, beriberi, rickets and several other protein-energy malnutrition and micro-nutrient under-nutrition [4] [11] [12] [13].

Traditional weaning foods of West Africa are mostly based on the family staple foods which include cereals, starchy tubers, legumes and vegetables [14] [15]. Of these, cereals based weaning foods are mostly utilized and this has been a challenge due to their low-nutritive value, including inadequate quantity of the essential amino acids (lysine and tryptophan) that are important in the infants' growth [16] [17].

There have been several approaches aimed at addressing the challenges associated with the infants' consumption of the traditional weaning foods. These include the use of processing steps such as fermentation, malting, liquefaction, dry-milling, cereal-legume co-fermentation, fortification with selected additives while adhering to guidelines and regulations, addition of sugar and oil to cereal gruels as well as hygienic processing [18] [19] [20].

Supplementation of maize with legumes such as cowpea, Bambara-nut and soy-bean, has been reported to contribute significantly to the improvement of the protein content of the cereal based foods [21]-[26]. Tigernut suitability in formulation of weaning food as a result of its high energy, resulting from the rich protein, fat and sugar content, as well as minerals (phosphorus, potassium) and vitamins C and E constituents has been reported [27] [28]. Tigernut is gluten and cholesterol free and has very low Sodium Content, making it ideal for infants. There has been an increased awareness for the utilization of tigernut as nutritional source in recent times [29] [30] [31] [32] [33], however, tigernut remains underutilized in weaning food formulations. This work aimed at developing a good quality weaning blend having adequate protein-energy contents, necessary to promote growth and enhance nitrogen retention in infants.

2. Materials and Methods

2.1. Collection of Sample and Starter Organisms

Suwan-1, a variety of Maize grain (*Zea mays*) used in this study was obtained from the Institute of Agricultural Research and Training (IAR & T), Moor plantation, Ibadan, Oyo state, Nigeria. Tigernut (*Cyperus esculentus*) was purchased from Bodija market, Ibadan, Oyo state, Nigeria.

Pure cultures of *Lactobacillus plantarum* F2C and *Lactobacillus plantarum* U2A were obtained from the Industrial and Biotechnology laboratory of the Department of Microbiology, University of Ibadan, Oyo state. The cultures of *L. plantarum* strains were routinely maintained on de Man Rogosa and Sharpe (MRS) agar slant at 4˚C.

2.2. Sample Processing

The Maize grains (*Zea mays*) were sorted manually to remove dirt, stones, broken or moldy grains, washed and steeped in distilled water (1:3 w/v) at ambient temperature for 18 hrs. The steeped grains were malted and germination was halted by oven drying at 80˚C to 10% moisture. The root and shoot portions were removed and malted grains were dry milled to a fine particle size using a sterile blender (Philips kenwood, UK) followed by sieving to obtain a finer particle size flour.

Tigernut tubers (*Cyperus esculentus*) were sorted to remove stones, pebbles, dirt, damaged tubers and other extraneous materials followed by thorough washing in series of clean water to remove adhering soils. The cleaned tubers were then drained, dried and roasted in an oven at 85˚C for 24 hrs. The burnt tubers were removed and the rest of the bulk was dry milled into flour and sieved to obtain fine flour.

2.3. Formulation, Fermentation and Sampling of Composite Blends

Blend formulation (maize-tigernut in a ratio of 70:30) was carried out by the method of Malleshi *et al.* [34]. The blends in the different flasks were reconstituted with sterile distilled water at a concentration of 30% (w/v) [35] followed by fermentation.

Fermentation

The formulation was subjected to spontaneous fermentation at 30˚C ± 2˚C for 48 hours and also to starter fermentation using 10^8 cfu/ml pure cultures of starter singly and in combination [36]. Four fermented weaning blends were formulated: FMT (spontaneously fermented maize-tigernut), SFMT1 (*Lactobacillus plantarum* F2C fermented maize-tigernut), SFMT2 (*Lactobacillus plantarum* U2A fermented maize-tigernut) and SFMT3 (combined starter-fermented maize-tigernut). The developed blends were analyzed for their *in vitro* and *in vivo* nutritional qualities.

2.4. Analysis of Fermented Formulations

2.4.1. Determination of Proximate Contents

The proximate analysis of moisture content, crude protein, crude fat, crude fibre and ash were determined by the methods of the Association of Official Analytical Chemists [37] and total carbohydrate was determined by difference. The gross energy density of each formulated blend was determined using the Atwater factor.

$$\text{Energy value}\left(\text{KCal/100g}\right)=\left(\text{carbohydrate}\times 4\right)+\left(\text{fat}\times 9\right)+\left(\text{protein}\times 4\right)$$

2.4.2. Determination of Anti-Nutrient Contents

The anti nutrients that were determined include tannin, oxalate and phytic acid. Tannin content was determined spectrophotometrically by the method of AOAC [38], Oxalate using the method of Sanchez-Alonso and Lachica [39] while percentage phytic acid was determined titrimetrically using the formula described by AOAC [38].

2.4.3. Determination of Vitamin Contents

Thiamine (Vitamin B1), riboflavin (Vitamin B2), and (Vitamin A) were determined by the methods of the Association of Official Analytical Chemists [39].

2.4.4. Determination of Mineral Contents

The method of the Association of Official Analytical Chemists [38] was used in the determination of the mineral contents. Mineral analysis was carried out by ashing of 5 g of the sample into white ash at a temperature of 550˚C in a muffle furnace for 2 hours, followed by acid wash and filtration. The mineral elements (iron, magnesium, calcium, zinc, and phosphorus) were been determined using spectrophotometric methods (Atomic Absorption of Spectrophotometry AAS, 1 Nodel Phillips Pil 9100 X).

2.4.5. Sensory Evaluation of Formulated Blends

Sensory attributes of the best four fermented weaning foods were assessed by 15 untrained panelists of nursing mothers in Kajorepo community, Akinyele local government, Ibadan, Oyo state using the method described by Wakil and Alao [40].

2.5. Biological Evaluation of Formulated Blends

Animal feed experiment was carried out using male albino rats (*Rattus norvegicus*) of weaning age (three to four weeks old and with weight varying between 18 - 34 g) in order to carry out an assessment of the nutritional quality of the formulated weaning food and the overall health effect of the formulated weaning diet.

The four (4) formulations were used as feeding diet while Nutrend (a commercial weaning food brand) fed treatment group of the albino rats served as a negative control and a group fed with the commercial conventional diets of the rats served as positive control.

Animal Experiment

Twenty-four (24) young male albino rats of weaning age procured were randomly distributed into groups based on the experimental diet formulations to be used. The animals were kept in standard cages and subjected to the same environmental conditions. Each group comprised of four (4) rats per cage making a total of six (6) experimental groups. The rats were allowed to acclimatize for a period of one week before commencement of feeding with the experimental diets and the trials was carried out within duration of 28 days. Data were collected to determine the feed intake, body weight changes, feed conversion ratio (FCR) and protein efficiency ratio (PER) using the method of Addass *et al.* [41]. Determination of hematological parameters (RBC, WBC and PCV) was carried out using the method of Bull *et al.* [42].

2.6. Statistical Analysis

All the data obtained were subjected to statistical analysis using analysis of variance (ANOVA) and the mean separated by Duncan's multiple test range using the Gen Stat Software.

3. Results

Table 1 shows the proximate analysis of all the weaning diets fed to the experimental animal groups. The least protein (7.95%) and ash (0.79%) contents observed were obtained in the experimental weaning diet of group 1 which was fed with spontaneously fermented maize-tigernut blend (FMT) while group 2 weaning diet (SFMT1) had the least (1.40%) moisture and highest (78.75%) carbohydrate contents. Proximate analysis of group 3 diet (SFMT2) shows that the experimental diet had the highest (15.21%) content of crude fat and 456.84 KCal of energy, among the experimental diets. The SFMT2 blend is composed of 3.42% moisture, 8.39% crude protein, 15.21% crude fat, 4.56% crude fibre, 1.38% ash and 71.59% carbohydrate. The proximate contents of the experimental weaning diet for group 4 (SFMT3) had 3.32% moisture, 8.25% crude protein,

Table 1. Proximate analysis of the weaning diets supplied to the albino rats (per 100 g).

Group	Experimental Diet	Moisture %	Protein %	Fat %	Fibre %	Ash %	Carbohydrate %	Energy KCal/100 g
1	FMT	6.71 ± 0.01^{b+*}	7.95 ± 0.07^{d}	9.19 ± 0.01^{d}	4.36 ± 0.03^{d}	0.79 ± 0.01^{f}	75.25 ± 0.08^{c}	415.92 ± 0.12^{d}
2	SFMT1	1.40 ± 0.03^{f}	8.03 ± 0.07^{d}	10.51 ± 0.01^{c}	3.78 ± 0.01^{e}	1.31 ± 0.02^{d}	78.75 ± 0.13^{a}	441.68 ± 0.17^{b}
3	SFMT2	3.42 ± 0.02^{c}	8.39 ± 0.07^{c}	15.21 ± 0.01^{a}	4.56 ± 0.02^{c}	1.38 ± 0.01^{c}	71.59 ± 0.06^{d}	456.84 ± 0.04^{a}
4	SFMT3	3.32 ± 0.01^{d}	8.25 ± 0.07^{c}	10.68 ± 0.02^{b}	2.57 ± 0.02^{f}	1.25 ± 0.00^{e}	76.50 ± 0.09^{b}	435.14 ± 0.06^{c}
5	Nutrend	2.50 ± 0.02^{e}	15.00 ± 0.02^{a}	9.00 ± 0.02^{e}	7.00 ± 0.02^{b}	2.30 ± 0.02^{b}	64.20 ± 0.02^{f}	398.00 ± 0.02^{e}
6	CAF	8.90 ± 0.01^{a}	13.98 ± 0.03^{b}	5.48 ± 0.01^{f}	8.41 ± 0.01^{a}	3.20 ± 0.00^{a}	68.43 ± 0.03^{e}	379.00 ± 0.08^{f}

*Mean ± standard error based on three replicates. +Means within the same column with different superscript are significantly different at P ≤ 0.05. Key: FMT:- spontaneously fermented maize-tigernut blend, diet supplied to group 1; SFMT1: -maize-tigernut blend fermented using *Lactobacillus plantarum* F2C supplied to group 2; SFMT2: -maize-tigernut blend fermented using *Lactobacillus plantarum* U2A supplied to group 3; SFMT3: -maize-tigernut blend fermented using combined starter supplied to group 4; Nutrend: -commercial weaning food (group 5); CAF: -conventional animal feed (group 6).

10.68% crude fat, 1.25% ash, 76.50% carbohydrate and the least (2.57%) content of crude fibre.

Group 5 weaning diet (Nutrend) was observed to contain 2.50% moisture, 9.00% fat, 7.00% fibre, 2.3% ash, the least (64.20%) carbohydrate content and the highest protein content (15.00%) among the experimental weaning diets. The proximate composition of the experimental diet for group6 (CAF; conventional animal feed) also revealed to be made up of 8.90% moisture, 13.98% crude protein, 5.48% crude fat, 8.41% crude fibre, 3.20% ash and 68.43% carbohydrate. Among the experimental diets, the weaning diet supply of group six had the highest moisture and ash contents but the least energy content of 379.00 KCal/100 g. Statistical analysis revealed that differences observed in the moisture, crude fat, ash, carbohydrate and energy contents of all the experimental diets were significantly different at P ≤ 0.05. Statistical analysis also showed that FMT and SFMT1, and SFMT2 and SFMT3 diets were not significantly different (P ≤ 0.05) from each other in terms of protein contents but differ significantly from group 5 (CAF) and group 6 (Nutrend) diets.

The unfermented samples (UMT) had the least phytate (0.226%) and oxalate (0.104%) contents while least tannin content (0.028%) was observed for SFMT3 (Table 2). Among the fermented formulated samples, blend fermented using *Lactobacillus plantarun* U2A (SFMT2) had the least phytate (0.236%) and oxalate (0.193%) contents. Statistical analysis of the antinutrient contents revealed that the antinutrient compositions of the formulations were significantly different (P ≤ 0.05) among the blends.

3.1. Vitamin Composition of the Maize-Tigernut Formulated Blend Samples

Table 3 shows vitamin A, B1 and B2 contents of the maize-tigernut formulated blends. From the table, the unfermented maize-tigernut blend (UMT) had the least vitamin A, B1 and B2 contents. Among the formulated fermented samples, blend SFMT1 had the highest vitamin A (472.60 μg/100 g) and vitamin B1 (0.67 mg/100 g) contents while the least vitamin A, B1 and B2 contents of 460.0

Table 2. Antinutrient composition (%) of all formulated blend samples.

Sample code	Phytate	Oxalate	Tannin
UMT	0.226 ± 0.009^{d}	0.104 ± 0.002^{e}	0.042 ± 0.001^{a}
FMT	0.266 ± 0.002^{ab}	0.207 ± 0.001^{a}	0.042 ± 0.001^{a}
SFMT1	0.262 ± 0.005^{b}	0.204 ± 0.001^{b}	0.039 ± 0.002^{b}
SFMT2	0.236 ± 0.002^{c}	0.188 ± 0.001^{d}	0.036 ± 0.002^{c}
SFMT3	0.269 ± 0.002^{a}	0.193 ± 0.002^{c}	0.028 ± 0.002^{d}

Mean ± standard error based on duplicate values. Means within the same column with different superscript are significantly different using Duncans multiple range test at $p < 0.05$. Sample code as in Table 1 (UMT-unfermented blend).

Table 3. Vitamin contents of the maize-tigernut formulated blend samples.

Sample code	Vitamin B1 (mg/100 g)	Vitamin B2 (mg/100 g)	Vitamin A (ug/100 g)
UMT	0.52 ± 0.02^{c+*}	0.10 ± 0.01^{d}	452.75 ± 0.25^{e}
FMT	0.84 ± 0.02^{a}	0.28 ± 0.01^{a}	467.70 ± 0.10^{b}
$SFMT_1$	0.67 ± 0.02^{b}	0.18 ± 0.01^{b}	472.60 ± 0.10^{a}
$SFMT_2$	0.58 ± 0.01^{c}	0.14 ± 0.01^{c}	460.00 ± 0.20^{d}
$SFMT_3$	0.63 ± 0.04^{b}	0.20 ± 0.01^{b}	464.05 ± 0.25^{c}

*Mean ± standard error based on duplicate values. $^{+}$Means within the same column with different superscript are significantly different using Duncans multiple range test at $P \leq 0.05$.

μg/100 g, 0.58 mg/100 g and 0.14 mg/100 g respectively was recorded for blend SFMT2. Statistical analysis showed a significant difference ($P \leq 0.05$) in the vitamin contents of the maize-tigernut blends.

3.2. Mineral Contents of the Maize-Tigernut Formulated Blend Samples

Table 4 shows the mineral composition of the maize-tigernut formulated blends. From the table, there is an observed variation in the effect of fermentation on the mineral content of the samples when compared with the unfermented sample (UMT). The phosphorus contents of all the fermented samples decreased with fermentation with the highest reduction observed in the spontaneously fermented sample FMT (59.57 mg/100 g) while the least reduction was observed in sample SFMT1 (75.45 mg/100 g) and also with highest Zinc content of 1.05 mg/100 g. The highest amount of calcium and iron contents of 17.17 mg/100 g and 22.82 mg/100 g respectively was recorded in blend SFMT2 while the least calcium (14.05 mg/100 g), magnesium (1.57 mg/100 g) and zinc (0.59 mg/100 g) contents was observed in the sample SFMT3. Statistical analysis of the blends revealed that the magnesium contents of SFMT1 and SFMT2, so also the iron content of FMT and SFMT1; zinc contents of the samples FMT and SFMT2 were not significantly different ($p > 0.05$) from each other.

Table 4. Mineral contents (mg/100 g) of the maize-tigernut formulated blend samples.

Sample code	Phosphorus	Calcium	Magnesium	Iron	Zinc
UMT	78.33 ± 0.21[a]	16.23 ± 0.08[b]	2.70 ± 0.10[a]	13.53 ± 0.07[c]	0.64 ± 0.01[c]
FMT	59.57 ± 0.07[d]	17.20 ± 0.05[a]	2.75 ± 0.04[a]	17.27 ± 0.08[b]	0.81 ± 0.02[b]
SFMT1	75.45 ± 0.05[b]	15.38 ± 0.07[c]	2.47 ± 0.01[b]	17.28 ± 0.06[b]	1.05 ± 0.04[a]
SFMT2	73.67 ± 0.69[c]	17.17 ± 0.07[a]	2.40 ± 0.03[b]	22.82 ± 0.06[a]	0.76 ± 0.05[b]
SFMT3	74.42 ± 0.08[c]	14.05 ± 0.05[d]	1.57 ± 0.03[c]	13.70 ± 0.03[c]	0.59 ± 0.04[c]

*Mean ± standard error based on duplicate values.

3.3. Sensory Evaluation of the Maize-Tigernut Formulated Blend Samples

Table 5 shows the results of the sensory evaluation of the maize-tigernut formulated weaning blend among 20 untrained panelists made up of nursing mothers. The weaning blend fermented using the combined starter (SFMT3) had the highest rating in terms of the color with a mean score of 5.90 while sample SFMT2 was rated best in terms of the texture (5.40) and taste (6.10) whereas, the spontaneously fermented sample (FMT) had the least of ratings in all sensory attributes. Overall, blend SFMT2 had the highest acceptability rating of 6.00, statistically the starter fermented blends were significantly different ($P \le 0.05$) from the spontaneously fermented blend (FMT).

3.4. Biological Evaluation of Formulated and Standard Weaning Blends

Table 6 shows the result of biological evaluation of the formulated blends over the 28 days of feeding trials. The highest mean weight gain (6.77 g) and mean feed intake (19.49 g) was observed in the group that fed on the commercially available weaning food (Nutrend) while among the formulated blends, the highest was observed in blend SFMT2 with mean weight gain of 5.46 g and mean feed intake of 18.71 g. The highest mean feed conversion ratio (FCR) was recorded in blend SFMT1 (5.42) and the least value (3.31) recorded in Nutrend fed group. The statistical analysis shows that the mean weight gain, mean feed intake and mean FCR of the groups were not significantly different ($P > 0.05$) from each other. However, highest mean protein efficiency ratio (PER) of 3.65 was observed for blend SFMT2 which is significantly different ($P < 0.05$) from both the positive (CAF) and negative (Nutrend) controls.

3.5. Haematological Analysis of the Albino Rats

Table 7 shows the results of hematological analysis of the albino rats fed with the experimental weaning diets for the duration of 28 days. The SFMT3 group fed had the least value ($6.95 \times 10^6/mm^3$) for the Red Blood Cell (RBC) count while the highest count ($9.50 \times 10^6/mm^3$) was observed in the SFMT1 group. Also, the White blood cell (WBC) count was observed to be highest in the group fed with the blend fermented using *Lactobacillus plantarum* U2A (SFMT2) as

Table 5. Sensory evaluation of the maize-tigernut formulated blends.

Sample code	Color	Texture	Taste	Flavor	Overall acceptability
FMT	5.30 ± 0.213^{a}	4.50 ± 0.167^{b}	4.00 ± 0.258^{b}	4.80 ± 0.20^{c}	4.30 ± 0.26^{b}
SFMT1	5.60 ± 0.16^{a}	4.70 ± 0.15^{b}	5.60 ± 0.16^{a}	6.30 ± 0.23^{a}	5.60 ± 0.16^{a}
SFMT2	5.60 ± 0.16^{a}	5.40 ± 0.16^{a}	6.10 ± 0.18^{a}	6.30 ± 0.26^{a}	6.00 ± 0.14^{a}
SFMT3	5.90 ± 0.23^{a}	5.20 ± 0.20^{a}	5.70 ± 0.21^{a}	5.60 ± 0.16^{b}	5.50 ± 0.17^{a}

*Mean ± standard error based on ten replicates.

Table 6. Biological evaluation of formulated and standard weaning blends.

Experimental weaning diet	Initial weight (g)	Weight at day 28 (g)	mean weight gain (g)	Mean feed intake (g)	Mean FCR	Mean PER
FMT	24.65 ± 1.28^{a}	66.77 ± 0.35^{c}	4.64 ± 0.43^{a}	17.16 ± 0.89^{a}	4.22 ± 0.66^{a}	3.40 ± 0.31^{ab}
SFMT1	26.64 ± 1.07^{a}	70.41 ± 0.94^{d}	4.92 ± 0.76^{a}	17.94 ± 1.26^{a}	5.42 ± 1.63^{a}	3.42 ± 0.54^{ab}
SFMT2	24.00 ± 0.47^{a}	73.14 ± 0.62^{c}	5.46 ± 0.74^{a}	18.71 ± 1.06^{a}	4.27 ± 0.83^{a}	3.65 ± 0.51^{a}
SFMT3	25.80 ± 1.41^{a}	71.89 ± 0.38^{cd}	5.16 ± 0.48^{a}	18.69 ± 0.96^{a}	4.28 ± 0.71^{a}	3.36 ± 0.31^{ab}
Nutrend	26.21 ± 0.46^{a}	87.21 ± 0.50^{a}	6.77 ± 0.80^{a}	19.49 ± 1.08^{a}	3.31 ± 0.48^{a}	2.29 ± 0.25^{b}
CAF	25.20 ± 0.88^{a}	79.48 ± 0.74^{b}	6.09 ± 0.71^{a}	18.39 ± 1.29^{a}	3.42 ± 0.42^{a}	2.30 ± 0.32^{b}

Mean ± standard error based on three replicates. Means within the same column with different superscript are significantly different using Duncans multiple range test at $p < 0.05$. Key: FCR: -Feed Conversion Ratio; PER: -Protein Efficiency Ratio; CAF: -Conventional Available Feed.

Table 7. Haematological analysis of experimental animal group.

Experimental diet Group	Red blood cell (RBC) ×10^{6}/mm^{3}	White blood cell (WBC) ×10^{3}/mm^{3}	Packed cell volume (PCV) %
FMT	8.75 ± 0.25^{ab+*}	6.78 ± 0.09^{bc}	38.67 ± 0.33^{b}
SFMT1	9.50 ± 0.12^{a}	6.90 ± 0.06^{ab}	34.67 ± 0.67^{c}
SFMT2	8.83 ± 0.33^{ab}.	7.03 ± 0.03^{ab}	40.00 ± 0.58^{b}
SFMT3	6.95 ± 0.06^{d}	7.03 ± 0.09^{ab}	38.33 ± 1.20^{b}
Nutrend	7.78 ± 0.32^{c}	7.22 ± 0.12^{a}	43.00 ± 0.58^{a}
CAF	8.17 ± 0.23^{bc}	6.44 ± 0.21^{c}	39.33 ± 0.88^{b}
Rat Haematologic reference ranges	6.76 - 9.75	6.6 - 12.6	37.6 - 50.6

*Mean ± standard error based on triplicate values. $^{+}$Means within the same column with different superscript are significantly different using Duncans multiple range test at $P \leq 0.05$.

well as the blend fermented using combined starter (SFMT3), with a value of 7.03 × 10^{3}/mm^{3} while the least WBC (6.78 × 10^{3}/mm^{3}) was recorded for the group fed with the spontaneously fermented blend (FMT). Statistical analysis of the white blood cell count showed that SFMT1, SFMT2 and SFMT3 fed groups were not significantly different ($p < 0.05$) from each other but were significantly different from FMT, Nutrend and Conventional animal fed groups.

The least packed cell volume (PCV) value of 34.67% and highest value of 40.00% were observed in the groups fed with sample SFMT1 and sample SFMT2 respectively. Packed cell volume (PCV) of group one (FMT), group three (SFMT2), group four (SFMT3) and group six (conventional animal feed) were not significantly different ($P \leq 0.05$) from each other but different from the PCV of group two (SFMT1) and group five (Nutrend). Generally, the RBC, WBC and PCV of the trial groups were within the rat haematologic reference ranges.

4. Discussion

The observed lower moisture contents of the fermented products will improve the storage period or keeping quality of the product because low moisture content would prevent the growth of mould and reduce moisture dependant biochemical reactions [43]. Higher protein contents of *Lactobacillus* fermented blends compared to the spontaneously fermented blend may be due to an increase in the proteolytic activities of the starters during fermentation which might have resulted in increased production of essential amino acids, an observation similar to that of Saleh *et al.* [44] and Apaliya *et al.* [45]. Thus improve in the protein quality of the starter products [46] resulting in an upgraded nutritious products which can prevent Protein Energy Malnutrition in developing countries. The higher ash contents recorded in starter fermented blends compare to the spontaneously fermented blend is an indication of increased mineral contents of the starter developed blends. Similar observation was reported by Wakil and Alagbe [28] on a related study on tigernut fortified weaning food. The starter fermented food formulations can serve as a very good source of energy with values ranging from 435.14 to 456.84 kcal/100 g when compared with commercial and conventional feeds for babies and animals, although the recommended daily allowance (RDA) for infants is 3000 kcal [47]. However higher energy value than those recorded in this study was recorded by Shiriki *et al.* [48] in their findings on nutritional evaluation of a complementary food formulation.

The higher mineral contents recorded in the SFMT2 sample could be of nutritional advantage to the weaning infants as minerals play important roles in body metabolism. The high calcium contents helps in the regulation of muscle contractions and transmission of nerve impulses, bone and teeth development [49] while the iron helps in heamoglobin formation. This could also be the reason for higher PCV recorded for this sample. This increase in the iron content also agrees with the findings of Oyarekua [50]. Iron deficiency can impair physical and cognitive development and immune response.

The improvement in organoleptic properties observed with the use of starter fermentation is similar to the report of Wakil and Kazeem [19]. However findings indicated that differences in sensorial properties were not significantly ($p > 0.05$) associated with the type of starter (lactic acid bacteria) used in the fermentation. This variation may be due to the subjective nature of sensory analysis using human beings. Generally, LABs contribute to the aroma and taste of fermented products [51] by acidifying food and gives it a tangy lactic acid taste.

The animal group fed with SFMT2 had the highest weight by 28 days (73.14 g), mean weight gain (5.46 g), mean feed intake (18.71 g) and mean protein efficiency ratio, PER (3.65). However, all the PER values including that of controls

(2.30) were higher than the value of 2.10 recommended by the Protein Advisory Group (PAG) for complementary foods. The higher PER and FCR values of fermented food products could be due to utilization of the increased protein and micronutrients from the tigernut supplement by the experimental animals. An observations similar with earlier reports of significant increase in PER in rats as a result of improved nutritional composition [48] [52]. The Red Blood Cell (RBC), White Blood Cell (WBC) and Packed Cell Volume (PCV) of the trial groups were within the rat haematologic reference ranges.

5. Conclusion

In Conclusion, blend SFMT2 (*L. plantarum* U2A fermented blend) has higher nutritional contents and gives the best performance after rat feeding trials, therefore, can be used as starter in weaning food development.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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