Available online at www.worldscientificnews.com

World Scientific News

WSN 24 (2015) 75-87 EISSN 2392-2192

Microbial profile and nutritional quality during the fermentation of cereal based weaning food fortified with soya bean and tiger nut using starter culture

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ABSTRACT

To develop a protein rich sorghum weaning food in a country with high poverty rate, the effects of adding soybeans and tiger-nut, two different ratios was formulated, R_1 and R_2. R_1 was formulated in the ratio 5:3:2 (comprising of 50% sorghum, 30% soybean and 20% tiger nut flours) while R_2 was formulated in the ratios 4:4:2 (40% sorghum, 40% soybeans and 20% tiger nut flours), respectively. Two fermentation processes was done for the ratios, starter (single) base fermentation ratios (SBFR) and spontaneous fermentation ratios (SPFR). Microbial analysis (total bacteria, yeast count, lactic acid bacteria count and enteric bacteria counts) were done after every 6 hrs of fermentation. The highest total bacterial count obtained was (4.4×10^5) in SPFR2 while the lowest count was 2.4×10^2 in SBFR1. Lactic acid bacteria range from 6.2×10^5 to 1.0×10^3. The highest enteric bacteria count was observed in SPFR1 (9.0×10^4) while highest yeast count (1.3×10^5) was observed in SPFR2. No growths were recorded for yeast and enteric bacteria after 12 to 24 hrs of fermentation for the starter based products. The results showed that the overall nutritional quality of sample SBFR1 was superior than sample SBFR2. Sample SBFR1 ~~and~~ had the higher total energy, protein content and reasonable amount of carbohydrates. The fermentation improves the nutritional qualities of locally produced sorghum weaning foods. Fortification of these foods with soybeans and tiger nut can be an added advantage as this may likely be a remedy to solving the menace of protein energy mal-nutrition in the developing countries and it was also confirmed that lactic acid bacteria used as starter, control the growth of yeast and enteric bacteria there by increasing the safety and shelf life of the product.

***Keywords*:** Weaning food, fermentation, Lactic acid bacteria, Starter-Development, Mixed-Culture, enteric bacteria, tiger nut, nutritional qualities

1. INTRODUCTION

In developing countries, one of the greatest problems affecting millions of people, particularly children are lack of adequate protein intake in term of quality and quantity. As cereals are generally low in protein, supplementation of cereals with locally available legume that is high in protein increases protein content of cereal-legume blends. Several traditional fermentations have been upgraded to high technology production systems and this has undoubtedly improved the general well-being of the people as well as the economy (Achi, 2005). Weaning is a gradual process of introducing solid food to an infant's diet alongside breast milk from the age of three to four months, since the breast feeding alone can not meet the infant' nutritional requirements (Oduro *et al*., 2007). In Nigeria, traditional weaning food consist of monocereal grains prepared form either millet, sorghum, maize, referred to as "Ogi" or "Akamu" which is of poor nutritional value (Rombouts and Nouts, 1995). The major problem associated with infant during transitional phase of weaning is protein energy malnutrition (PEM) which results into condition such as marasmus or kwashiorkor (Salmon *et al*., 2008).

The supplement of these local available cereals with legumes which are good sources of protein will gives raise to weaning food that gives the infant enough energy and it's nutritional requirements (Umeta *et al.,* 2003). The widespread problem of infant malnutrition in developing countries has stirred effort in research development and extension by both local and international organization (Umeta *et al.,* 2003). As a result, the formulation and development of nutritious weaning food from local and readily available raw-material, this have receive a lot of attention in many developing countries and it contributes to infant mortality, poor physical and intellectual development of infant as well as lowered resistance to disease and consequently stifles development (Umeta *et al.,* 2003). Protein energy malnutrition (PEM) generally, occurs during the crucial transitional phase when children are weaned from liquid, semi-solid or fully adult foods in addition to mother milk, because of the increasing nutritional demands of the growing body (Sajilate *et al.,* 2002).

Apart from protein and energy lysine but it has sufficient sulphur contain amino, weaning diet's of infant in developing countries requires more calcium, vitamins A and D, Iron and some important trace elements, which can be obtain by combining the local available material (Sajilate *et al.,* 2002). Combination of commonly used cereals with in-expensive plant protein source like legumes can be used, cereals are deficient in acid which are limited in legumes, where as legumes are highly rich in lysine. The effects of the fortification are highly beneficial since nutritive value of products is also improved (Wang and Daun, 2006).
Legumes are nutritious food and a substitute for animal protein arises from the knowledge of the functional properties of the seed flour and other products.

I Africa, malnutrition is prevalent due to lack of sufficient animal protein, hence the starch for alternative sources of protein form lesser known legume in lieu of expensive and scarce animal protein (Harper, 2003). The use of legumes seeds may be the beginning of a series of formulation which will lead to a substantial drop in dependency of animal source for nutritious foods. Unfortunately, legumes seeds contain anti nutritional factors like enzymes inhibitors phylates, oxalate, saponin and polyphenolic compound, all of which limit their utilization (Salunkhe, 1997). Although, remarkable improvement in the nutritive value and quality of legumes seeds have been achieved through de hulling, heat treatment, germination, fermentation, soaking and partial hydrolysis of proteolytic enzymes (Akinrele, 2003).

Fermentation of cereals is believed to increase protein and amino acid levels in the end product. Tiger nuts (*Cyperus esculentus*) are cultivated throughout the world including Nigeria, especially in the northern part, and other West Africa Countries like Guinea, Cote d'Ivore, Cameroon, Senegal, America and other parts of the World (Belewu and Abodunrin 2006). The nuts are valued for their highly nutritious starch content, dietary fiber and carbohydrate (mono, di and polysaccharides). The nut has also been reported to be rich in sucrose (17.4-20.0%), fat (25.50%), protein (8%), and minerals such as sodium, calcium, potassium, and magnesium (Umerie and Enebeli 1997). Tiger nut also known in Nigeria as "Ayaya" in Hausa, "Ofio" in Yoruba and "Akiausa" in Igbo has three varieties (black, yellow and brown) which are underutilized due to lack of information on their nutritional potentials (Omode *et al.,* 1995). It can be eaten raw, roasted, dried, baked or be made into milk (Oladele *et al*., 2007).

Breast-milk satisfies the nutrient and energy requirements of the infant for the first 6 months. As the child grows, the nutrient composition of milk increasingly becomes inadequate to meet the infant's requirements. The nutrients most affected are some essential minerals and vitamins especially vitamins A. Therefore, to be able to meet the changing requirements of the infant's development, there is the need to supplement the breast milk with a nutritious diet, which could be a proprietary formula or locally prepared at home, while breastfeeding continues for at least two years (Okoye, 1992).

Millions of children in most developing countries are suffering from malnutrition and weaning infection; starter based fermentation of fortified cereal with legumes and nuts to wean can improve the safety and nutritional balance of wean children. There is, however, still a need for more information on the nutritional, microbiological qualities during the fermentation of cereal based weaning food fortified with soya bean and tiger nut using starter culture.

2. MATERIALS AND METHODS

2. 1. Source of materials

Sorghum, soybeans, tiger nut, were obtained from New-Market Brining Kebbi, Kebbi State, Nigeria. They were taken to the laboratory in a clean polythene bag for processing and analysis. Starter cultures were obtained from the Department of Science Laboratory, Microbiology Unit, Waziru Umaru Federal Polytechnic, Brinin Kebbi, Nigeria.

2. 2. Preparation of Sorghum Flour

The sorghum grains were sorted out for stone and other physical defects. The grains were soaked for one hour (1hr) to allow easy separation of button and top settler. Again, the grains were cleaned and transfer into clean container and kept for 2 days to allow germination of grain, the sprouted grain was solar dry to about 8.95% moisture content. The sprouted grains were milled and sieve with 75 micron mesh to obtained sprouted sorghum flour.

2. 3. Preparation of Soybean Flour

The soybeans were sorted out for stone, rot and other physical defects. The cleaned beans were dried to obtain about 9.59% moisture content. The beans were roasted on hot-plate until golden brown, the beans were de-hulled immediately after roasting and allow to

cool, the roasted beans were milled with Hammer miller (Model GG-300, Henan Gelgoog commercial and Trading co., China) and sieved with 75 micron mash to obtained the soybean flour (Amankwah *et al*., 2009).

2. 4. Preparation of Tiger Nut Flour

The tiger nut seed were sorted for stone rot and other physical defect. The cleaned seeds were dried to about 9.60% moisture content. The seeds were roasted and allow to cool, the roasted seed was milled with miller and sieved to obtain the fine powder of tiger nut seeds (Amankwah *et al.,* 2009).

2. 5. Formulation of Weaning Choice Mix

Blend A was formed in the ratios 5:3:2 (50% sorghum, 30% soybean and 20% tiger nut flours) while blend B was formulated in the ratios of 4:4:2 (40% sorghum, 40% soybeans and 20% tiger nut flours). The blends were varied to check the one that will give the best sensory and nutritional quality.

2. 6. Fermentation of Prepared Weaning Food

From blends A and B, 10 g of the blend was dissolved in 5 ml of sterile water to form a slurry (Ogi). The slurry was inoculated with Lactobacillus plantarum starter culture using half strength of 0.5 MacFanland standard (1.5×10^4), the slurry was allow to ferment for 24 hrs. (Omafuvbe, 2006). The blends were left to also left to ferment spontaneously without the addition of starter culture.

2. 7. Microbiological Analysis

Nutrient agar (NA), De Man Rogosa and Sharpe (MRS), EMB agar and Malt extract agar (MEA) was used for microbial culturing of total viable bacteria, lactic acid bacteria, enteric bacteria and yeast, respectively. All media were prepared according to the manufacturers specifications and sterilized at 121 °C for 15 min. 1.0 ml from appropriate dilutions (10^1 to 10^7) was pour plated on sterile molten agar, swirled and allowed to set. NA and EMB plates were incubated aerobically at 37 °C for 24 hrs, while MRS agar was incubated at 37 °C for 48 hrs anaerobically and MEA at 30 °C for 2 - 5 days. Determinations were carried out in triplicates and counts were expressed in logarithmic of colony-forming unit per mL of sample (log CFU/mL).

3. NUTRITIONAL ANALYSIS

The nutrient compositions of the imported commercial weaning foods and as well as the prepared weaning food that was prepared in our laboratory were carried out as follows:

3. 1. Proximate analysis

Moisture content, protein (N x 6.25), fat, ash and crude fibre were determined by standard procedures (AOAC, 2005). The carbohydrate content of each sample was determined by difference.

3. 2. Determination of total energy

The total energy value of the food formulation was calculated according to the method of Harland and Oberleas (1986) using the formula as shown in the following equation:

Total energy (kcal/100 g) = [(% available carbohydrates × 4) + (% protein × 4) + (% fat × 9)

Sensory evaluation

The fermented weaning foods were evaluated for acceptability by 25 semi-trained panelists. The panelists were asked to score the products for appearance, color, flavor, taste, odor and overall acceptability using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The samples were labeled with a three digit code and a side-by-side presentation protocol was followed for all the four samples.

3. 3. Statistically Analysis

The data obtained from the proximate analysis and sensory evaluation were statistically analyzed using Stat graphics (Graphics Software System, STCC, Inc. U.S.A). Comparisons between sample treatments and the indices were done using analysis of variance (ANOVA) with a probability $p < 0.05$.

4. RESULTS AND DISCUSSION

Table 1 shows bacterial counts at different fermentation period. The fermentation was done for 24 hrs. The counts obtained varies, there were decreases in bacterial population for both starter base fermentation ratio R_1 and R_2, as the fermentation period progresses; for $SBFR_1$ the bacteria count obtained is as follow (3.0×10^5, 3.5×10^3, 3.9×10^3, 2.8×10^2, 2.5×10^2) while for $SBFR_2$ (2.5×10^5, 4.3×10^5, 3.2×10^3, 9.0×10^2, 4.4×10^2), this agree with results obtained by Adriana *et al.* (2002) that studies on the effect of lactic acid bacteria in microbiological quality of fermented food. For spontaneous fermentation R_1 and R_2 there were decrease in bacteria growth for the first 6 hrs of fermentation period but later increase as the fermentation progresses to 24 hrs the bacteria counts obtained for $SPFR_1$ is (4.0×10^5, 2.2×10^3, 1.6×10^5, 4.0×10^5) while $SPFR_2$ (4.4×10^5, 2.2×10^3, 3.5×10^3, 1.4×10^5, 4.2×10^5), this result is also agrees with the works of Adriana *et al*. (2002) and Amankwah *et al.* (2009).

Table 1. Total bacterial counts (CFU/ml) at different fermentation period.

Blend code	Fermentation period (hrs)				
	Zero time	6	12	18	24
SBFR1	3.0×10^5	3.5×10^3	3.9×10^3	2.8×10^2	2.5×10^2
SBFR2	2.5×10^5	4.3×10^5	3.2×10^3	9.0×10^2	2.4×10^2
SPFR1	4.0×10^5	2.0×10^2	3.2×10^3	1.6×10^5	4.0×10^5
SPFR2	4.4×10^5	2.2×10^3	3.5×10^3	1.4×10^5	4.2×10^5

Table 2 shows lactic acid bacteria counts at different fermentation period, the lactic acid bacteria counts was done at six hours interval, the total lactics count were as follows $SBFR_1$ (1.6×10^4, 3.9×10^5, 4.8×10^5, 6.2×10^5, 5.7×10^5) $SBFR_2$ (1.5×10^4, 4.0×10^5, 5.4×10^5, 4.8×10^5) $SPFR_1$ (2.5×10^5, 1.2×10^5, 1.6×10^5, 1.0×10^5) $SPFR_2$ (2.5×10^5, 1.2×10^5, 2.4×10^5, 1.4×10^5, 1.0×10^5), at first 6 hrs of the fermentation period, there were decreased in population of lactic acid bacteria in $SBFR_1$, $SPFR_1$, SFR_2, throughout the fermentation period, this result is in line with the results obtained by Hubert *et al.* (2011).

Table 2. Total lactic acid count (CFU/ml) at different Fermentation period.

Blend code	Fermentation period (hrs)				
	Zero time	6	12	18	24
SBFR1	1.6×10^4	3.9×10^5	4.8×10^5	6.2×10^5	5.7×10^5
SBFR2	1.5×10^4	4.0×10^5	5.8×10^5	5.4×10^5	4.8×10^5
SPFR1	1.5×10^4	1.0×10^3	1.2×10^5	1.6×10^5	1.2×10^5
SPFR2	2.5×10^5	1.2×10^5	2.4×10^5	1.4×10^5	1.0×10^5

Table 3 shows yeast count at different fermentation periods the yeast count obtained is as follow $SBFR_1$ (7.0×10^4, 8.0×10^3) $SBFR_2$ (7.0×10^5, 8.0×10^3) $SPFR_1$ (1.0×10^5, 1.0×10^4, 1.5×10^3 1.7×10^3, 2.4×10^2) $SPFR_2$ (1.3×10^5, 1.1×10^5, 1.6×10^5, 1.8×10^2, 2.4×10^2) at first 6 hrs of the fermentation there were decreased in yeast population but later reduced into <10 at 12 to 24 hrs of fermentation period but for $SPFR_1$ and $SPFR_2$ there were growth at the end of fermentation period this result is not in satisfy with the results obtained by Amankwan *et al* (2009) which numerous growth was observed at the end of the fermentation period.

Table 3. Yeast count (CFU/ml) at different Fermentation period.

Blend code	Fermentation period (hrs)				
	Zero time	6	12	18	24
SBFR1	7.0×10^4	8.0×10^3	<10	<10	<10
SBFR2	1.0×10^5	9.0×10^3	<10	<10	<10
SPFR1	1.1×10^5	1×10^4	1.5×10^3	1.7×10^3	2.4×10^2
SPFR2	1.3×10^5	1.1×10^5	1.6×10^5	1.8×10^2	2.4×10^2

Table 4 shows enteric bacteria count obtained during the fermentation period is as follow: $SBFR_1$ (7.6×10^4, 6.0×10^2), $SBFR_2$ (8.0×10^4, 6.0×10^2), $SPFR_1$ (9.0×10^4, 5.0×10^4, 1.5×10^3, 1.2×10^2, 4.0×10^2), $SPFR_2$ (1.5×10^5, 2.6×10^5, 2.4×10^3, 3.6×10^2, 2.0×10^2) the values obtained from enteric bacteria a count shown that there were decreased in enteric bacteria, the

growth is reduced up to <10 in $SBFR_1$ and $SBFR_2$ at fermentation period of 18-24 hrs fermentation in $SBFR_1$ and $SBFR_2$ also reduced $SPFR_1$ and $SPFR_2$. Akinrele (2003) used lactic acid bacteria fermentation for the preservation of food, due to acidic nature of the bacteria the author suspected that non acidophile bacterial and fungal growth can be inhibited by low acidic environment produced by lactic acid bacteria. Nwokoro and Chkwu (2012) highlighted the importance of adequate hygiene during the preparation of food and also link between infection and nutrition. The microbial count obtained from this study is in line with results obtained by Amankwah *et al.* (2009). The international microbiological standard recommended that limit of contamination for fermented food should be less than 1.5×10^6 CFU/g (http://www.salford.gov.uk). The low microbial count obtained from this present study could be as a result of higher standard of personal hygiene and quality maintenance of manufacturing practice observed during the food preparation.

Table 4. Enteric bacterial count (CFU/ml) at different Fermentation period.

Blend code	Fermentation period (hrs)				
	Zero time	6	12	18	24
SBFR1	7.0×10^4	6.0×10^2	<10	<10	<10
SBFR2	8.0×10^4	6.0×10^4	<10	<10	<10
SPFR1	9.0×10^4	5.0×10^4	1.5×10^3	1.2×10^2	4×10^2
SPFR2	1.5×10^5	2.0×10^5	2.4×10^3	3.0×10^2	2.0×10^2

Problem of malnutrition in children continues to be critical in most underdeveloped and developing countries like Nigeria and India. This problem associated with inadequate protein and amino acids supply to the growing child.

Malnutrition and poor growth during infancy affect a large portion of the world's population; more than 800 million children under 5 years of age suffer from malnutrition and growth failure. Such morbidity is responsible for more than 10 million deaths per year in this age group (Deshpande and Poshadri, 2011).

Malnutrition accounts for the higher infant mortality rate in most developing countries of the world including Nigeria, India and other countries of the world compared to that in developed countries (Deshpande and Poshadri, 2011).

The protein deficiency is a major public health problem affecting an estimated 190 million preschool–age children, mostly from Africa and South-East Asia. Inadequate intakes of protein at infants developing age could lead to Protein Energy Malnutrition (PEM), which, when severe, may cause death or increase the risk of illness and mortality from childhood infections such as measles and those causing diarrhea (WHO, 2011).

The present work carried out to provide different food blends that can use as a complementary weaning food for kids after the six months. In this study, the sensory attributes and nutritional quality of fermented sorghum weaning food fortified with soy bean and tiger nut were evaluated in a quest to seek out measures that could be applicable in

reducing or preventing infant malnutrition which is one of the major causes of infant mortality in the most developing nations and the world at large. This study is aimed to develop a protein rich sorghum weaning food in a country with high poverty rate, the effects of adding soybeans and tiger-nut. The nutritional characteristics of the formulated weaning foods after 36 hrs and 48 hrs fermentation as presented in Table 5 and 6, respectively.

Table 5. Proximate compositions (%) of fermented weaning food produced after 36 hour fermentation.

Blend code	Protein	Carbohydrate	Moisture	Ash	Crude Fibre	Fat
SPFR1	19.80	33.30	7.99	23.38	2.02	13.51
SPFR2	23.61	28.85	7.83	19.08	2.42	18.21
SBFR1	26.61	26.24	8.60	23.84	1.38	13.33
SBFR2	28.22	24.62	8.92	21.56	2.24	13.45

Table 6. Proximate compositions (%) of fermented weaning food produced after 48 hour fermentation.

Blend code	Protein	Carbohydrate	Moisture	Ash	Crude Fibre	Fat
SPFR1	22.13	30.06	9.10	23.62	1.33	13.76
SPFR2	23.88	28.26	6.80	24.46	2.17	14.43
SBFR1	33.46	19.98	4.83	25.40	2.03	14.33
SBFR2	35.63	22.11	5.22	23.10	2.12	11.87

The proximate compositions of all the starter fermented formulated sorghum soya, bean and tiger nuts blends indicates that the protein content increase with increase in fermentation. The highest protein content (35.63%) was observed in samples SBFR2 after 48 hrs fermentation time, while the lowest protein content of 19.80% was observed in sample SPFR1 at 36 hrs fermentation time.

The result of the crude protein content on Table 7 shows an increase in values with respect to increase in fermentation period. The result also shows that the crude fat content ranges from 11.87% to 14.43%.

For all combinations, the spontaneously fermented blends had the highest carbohydrates content with no observable difference after 48h of fermentation.

The crude fibre contents of all the starter-produced weaning blends were low in all cases, ranges from 1.33 to 2.17. The results of the proximate composition were not in agreement with the study of Wakil and Kazeem (2012) in which the authors noticed a decreased with fermentation time on moisture content, fat, ash content, crude fibre and total carbohydrate content.

Table 7. Sensory evaluation of fermented weaning food produced after 36 hrs of fermentation.

Blend	Color	Aroma	Taste	Mouth feel	Total Acceptability
SPFR1	3.00	2.20	2.20	3.00	2.20
SPFR2	3.12	2.22	2.30	2.20	2.32
SBFR1	4.18	4.23	4.0	4.00	4.55
SBFR2	4.22	3.22	3.11	3.22	3.22

Although, the spontaneously fermented formulations had the highest total energy (Fig. 1) and reasonable amount of carbohydrates content but they both scored low in taste, aroma and general acceptability (Tables 7 and 8) compared to starter based fermented formulations in which they were generally accepted. SBFR1 scored the highest in general acceptability and mouth feel.

Table 8. Sensory evaluation of fermented weaning food after 48 hrs of fermentation.

Sample	Color	Aroma	Taste	Mouth feel	Total Acceptability
SPFR1	2.21	2.22	2.00	3.10	2.22
SPFR2	2.22	2.00	2.12	2.32	2.24
SBFR1	4.22	4.00	3.52	4.00	4.22
SBFR2	4.24	3.54	3.22	3.56	3.22

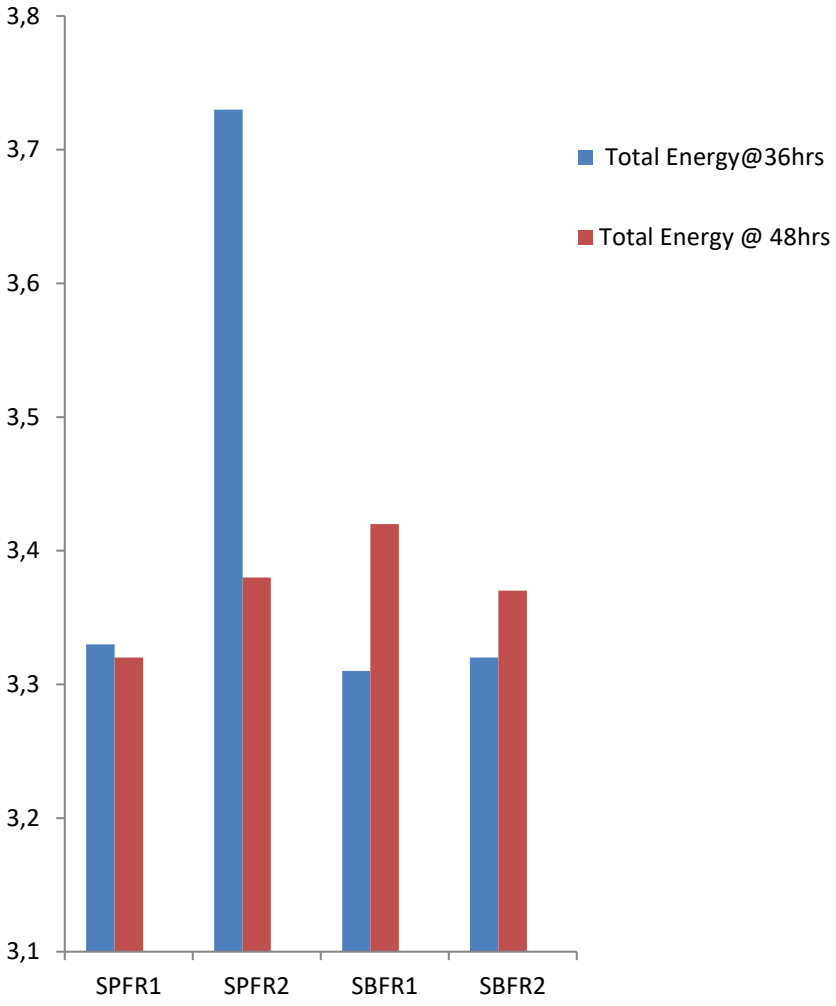

Fig. 1. Total Free energy (Kcal.) of weaning food produce after 36 and 48 hours of fermentation.

Key = ($SBFR_1$) Starter Base Fermentation Ratio One
($SBFR_2$) Starter Base Fermentation Ratio Two
($SPFR_1$) Spontaneous Fermentation Ratio One
($SPFR_2$) Spontaneous Fermentation Ratio Two

5. CONCLUSION

This study showed that formulation of cereal based fermented weaning food fortified with tiger nut, and soybean using lactic acid bacteria as starter culture will provides an alternative to the weaning food in Nigeria market as well as other developing countries in the world. This started based weaning food fortifications has produced improved protein content, organoleptic quality and safety weaning food which can be used to feed weaning children. Further study is needed to determine the minerals and vitamin content of the blends.

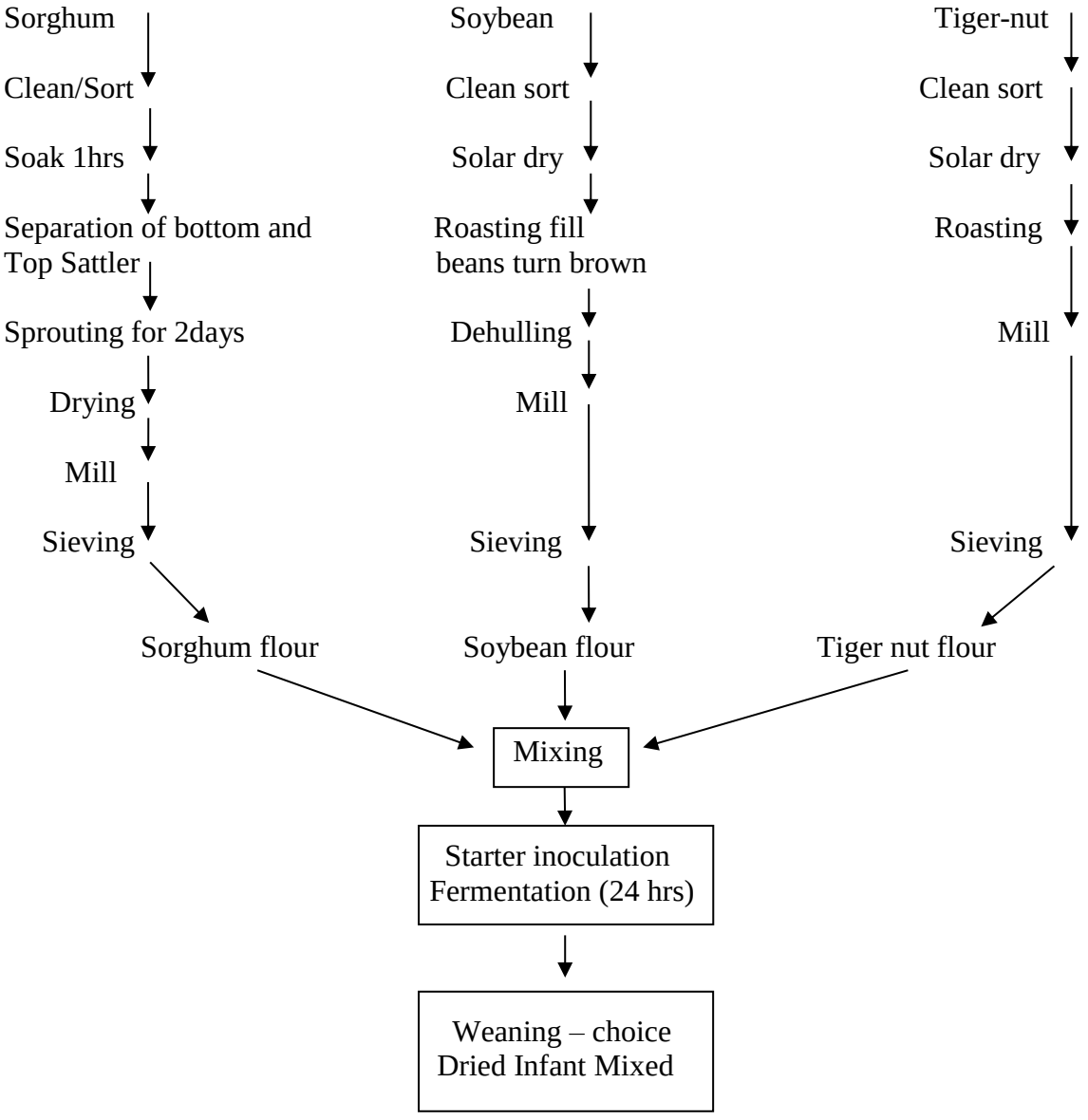

Fig. 2. Flow chart of prepared weaning – choice infant mix.

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(Received 08 October 2015; accepted 26 October 2015)