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Full Length Research Paper

Quality assessment of starter- produced weaning food subjected to different temperatures and pH

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Weaning is a gradual stoppage of feeding a baby with the mother's milk and start feeding the baby with semi-solid food such as maize, sorghum or millet which can be fermented to increase their nutrient content and carbohydrate digestibility. The weaning food was formulated by fortifying cereal (Sorghum) with legume (Cowpea) which had been cleaned, steeped in sterile distilled water for 24 h and germinated for 48 h at 30℃. The rootlet was remove d and dried in oven at 60℃ for 24 h and milled separately. The product was formulated at the ratio of 70:30 and fermented with the use of starter, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in single and in mixed culture *Lactobacillus Saccharomyces.* The formulated blends were incubated at different temperatures and pH adjusted by the use of phosphate buffer and potentiometric confirmation. The analysis of the effect of temperature on nutrient composition showed that crude protein increases with fermentation while the ether extract, crude fibre and ash contents decreased. The highest crude protein content (19.5%) was recorded at 37℃ in mixed culture gruel. The vitamin content al so increase with temperature (25 to 37℃) and decreases at 40℃ and the highest vitamin content w as observed at 37℃ in *L. Saccharomyces* blend. The vitamin content increased with fermentation and pH, and the highest was observed at pH 6.The optimum environmental conditions for the development of nutritious weaning food is therefore a fermentation temperature of 37℃ at pH 6 for 48 h w ith a starter combination of *L. plantarum* and *S. cerevisiae*.

Key words: Weaning food, starter culture, fermentation, temperature and ph changes, nutritional composition.

INTRODUCTION

In many West African countries, exclusive breast feeding is usually adequate up to three to four months of age, but after this period it may become increasing inadequate to support the nutritional demands of the growing infants. Thus, in a weaning process there is always a need to introduced soft, easily swallowed foods to supplement the infant's feeding early in life (Onofiok et al., 1992). Weaning foods are traditionally composed of staple cereals and legumes prepared either individually or as composite gruels. Cereal form basis for most of the traditional weaning foods in West Africa. The protein content of maize and sorghum is of poor quality, low in lysine and tryptophan. These two amino acids are indispensable to the growth of the young child (Oyenuga 1968). Cereal gruel processing methods have resulted in loss of the original tryptophan in corn during the processing of 'ogi' (Makinde and Lachance, 1976). Indeed, Akinrele and Edward (1971) concluded that the protein content of 'ogi' was too low even to support the growth of rat. Another report noted that corn gruel can provide some energy, but not other nutrients needed for the growth of the baby (Ketiku and Ayoku, 1984). Food staples such as maize, sorghum, millet, rice can be fermented to increase the nutrient content, carbohydrate digestibility, and energy densities of gruels, increase the bio-availability of amino acids and also improve their shelf life under controlled environment.

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The product may be consumed by gelatinizing it into stiff gel or made into gruel or pap that is popular amongst infant as weaning food and breakfast meal amongst adults (Ozoh and Kuyanbana, 1995). Many researchers have worked extensively on cereal- legume combinations (Onilude et al., 1999; Sanni et al., 1999; Achi, 2005; Wakil and Onilude, 2009) which have been used to improve the protein quality of cereals. Spontaneous fermentation that is, process initiated without the use of a starter inoculum, have been applied in food preservation for millennia and were elucidated through trial and error perhaps over thousands of years. The spontaneous fermentation typically results from competitive activities of varieties of contaminating microorganism (Holzapfel, 2001). Spontaneous fermentation are difficult to control, not predictable in terms of length of fermentation and quality of the product or product with a short shelf life and sometimes not safe since they are liable to contamination by pathogen (Novellie and de Schaeprejver, 1986; Tamime, 1990; Nout, 1992).

To overcome this problem, the most predominant microorganism found in an acceptable product are isolated and purified (Marshall, 1987; Tamime, 1990) The medium used for the fermentation is then pasteurized to exclude most unknown microorganism and the purified microorganisms is introduced to initiate the fermentation (Marshall, 1987; Hesseltine, 1992). By so doing the fermentation can be manipulated in such a way that it is possible to predict the amount and the quality of the product formed and the length of the fermentation period (Tamime, 1990; Hesseltine, 1992). Such introduced cultures are termed starter culture. Process control could be achieved by environmental manipulation, which involves controlling temperature, pH, water activity of the fermented food and may involve the use of starter organism enrichment like the use of pure culture which result to multiple starters, usually starter cultures are to be selected according to the ability to ferment sugars, rate of organic acid, optimum temperature and flavor production (Nout and Rombout, 1992). In view of the above information, this research therefore aimed at determining the best temperature and pH for the development of highly nutritious starter-produced weaning food.

MATERIALS AND METHODS

Collection of samples

Brown sorghum *(Sorghum bicolor)* and cowpea (*Vigna unguculata)* used were bought from Bodija market, Ibadan, Oyo state in a clean sterile polythene bags and kept in the refrigerator until use.

Sample treatment and formulation

The collected samples were treated using the modified method of Wakil and Onilude (2009). The method involved sorting, steeping, malting, oven drying and milling. The cereal-legume formulation blends were done in a ratio of 70:30 (w/w) (Malleshi et al., 1989).

Starter development

The starters used, L. *plantarum* and *S. cerevisiae* in this work were collected from Microbial Physiology and Biotechnology unit, Department of Microbiology, University of Ibadan, Ibadan. Nigeria based on their recommendation by Kazeem (2009).

Fermentation of the sorghum-cowpea blend

Sorghum and cowpea flour (250 µm grit size) was reconstituted with sterile distilled water at a concentration of 30% (w/v) (Livingstone et al., 1993) and inoculated with 1ml (10^7) each of the washed cells of the starters singly and mixed (in combination) culture, then allowed to ferment for 48 h. Samples were taken at the initial stage (0 h) and 48 h for the purpose of the analysis of the nutritional content.

Effect of temperature and ph on nutritional content

The effect of different temperatures on the nutritional content of the fermenting blends was determined by incubating the Erlenmeyer flasks containing the fermenting mash in an incubator operating at 25, 30, 37 and 40°C. The pH of the mash was adjusted using phosphate buffer and further confirmation by potentiometric use of pH electrode.

Determination of proximate composition

The methods of analysis followed were those described by the Association of Official Analytical Chemists (AOAC, 1980). The ash was determined by incineration of known weight of samples at 550°C until ash was obtained. Protein (N x 6.25) was determined by the macro-Kjeldahl method. The fat composition was determined 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 (AOAC, 1980). Total carbohydrate was calculated by difference.

Determination of mineral contents

The effect of starter used and fermentation on the mineral contents (Mg, Zn, Fe and Ca) of the fermented blends were determined using atomic absorption spectrophotometric method as described by AOAC (1980).

Determination of vitamin contents

The vitamin contents of the starter produced fermented weaning blends was determined using spectrophotometric method of AOAC (1980). Statistical analysis of all the data obtained was done using analysis of variance (ANOVA) and Duncan's multiple tests.

RESULTS

The effect of the three starters used (*L. plantarum, S. cerevisiae* and combination of the two *L. Saccharomyces*) on the nutritional composition of sorghum-cowpea fermented blend at 25°C is as shown on Table 1. Fermentation at 25°C increased the protein content and the highest value (17.7%) was observed in

Table 1. Nutritional composition of starter-developed blends fermented at 25℃.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Ash	Crude protein	Ether extract	Crude fibre	Total carbohydrate	Iron	Calcium	Zinc	Magnesium
LP-0 h	90.70	4.26^{a}	15.10^{e}	5.35^{ab}	4.300^{a}	70.99^{b}	1.40	7.00^{a}	0.05	1.40
LP-48 h	90.0	4.00^{a}	15.90^{c}	4.00^{d}	3.00^{b}	73.10^{a}	1.50	7.00^{a}	0.05	1.40
SC-0 h	89.8	3.90^{a}	15.49^{d}	4.90^{c}	2.91^{b}	72.81^{a}	1.40	6.70^{ab}	0.05	1.30
SC-48 h	89.9	3.92^{a}	15.81^{cd}	4.71^{c}	2.92^{b}	72.66^{ab}	1.50	6.60^{b}	0.06	1.30
LS-0 h	90.66	3.34^{b}	16.62^{b}	5.516^{a}	4.41^{a}	70.14^{b}	1.50	6.80^{ab}	0.05	1.40
LS-48 h	90.40	4.12^{a}	17.70^{a}	5.00^{bc}	4.12^{a}	69.08^{b}	1.50	6.83^{ab}	0.05	1.50

LP-0 h, *Lactobacillus plantarum* inoculated in unfermented gruel; LP-48 h=*Lactobacillus plantarum* in fermented gruel after 48 h; LS-0 h, *Lactobacillus plantarum* and *Saccharomyces cerevisiae* inoculated in unfermented gruel; LS-48 h= *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in fermented gruel after 48 h; SC-0 h, *Saccharomyces cerevisiae* inoculated in unfermented gruel; SC-48 h, *Saccharomyces cerevisiae* in fermented gruel after 48 h.

Table 2. Nutritional composition of starter-developed blends fermented at 30℃.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Crude protein	Ash	Ether extract	Crude fibre	Total carbohydrate	Iron	Calcium	Zinc	Magnesium
LP-0 h	90.7	16.10^{d}	4.30^{b}	5.32^{a}	4.30^{a}	69.98^{b}	1.40	7.00	0.05	1.40
LP-48 h	90.6	17.00^{bc}	4.122^{bc}	4.02^{c}	3.13^{b}	71.73^{a}	1.50	6.90	0.05	1.30
SC-0 h	90.26	15.63^{d}	3.907^{c}	4.90^{b}	3.91^{b}	71.70^{a}	1.40	6.90	0.05	1.30
SC-48 h	90.06	17.31^{b}	4.027^{bc}	4.026^{c}	3.02^{cd}	71.70^{a}	1.40	6.95	0.05	1.40
LS-0 h	91.00	16.0^{c}	4.913^{a}	5.516^{a}	4.40^{a}	69.17^{d}	1.40	6.50	0.05	1.40
LS-48 h	91.00	19.1^{a}	3.310^{d}	4.567^{b}	3.31^{b}	69.73^{d}	1.40	6.90	0.05	1.40

Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

the mixed culture *L. Saccharomyces* gruel while the crude fat, crude fibre and the carbohydrate contents decreased. Statistical analysis showed that the crude protein and the ether extract contents of fermented gruels are significantly different from the unfermented starter – developed blends. Also, fermentation of the starter-developed blend at 25℃ increased the iron contents of the blends but the increase is not significant. Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

The result of the gruel fermented at 30℃ shows that fermentation increased the crude protein content with the highest content of 19.1% recorded in the mixed culture gruel *L. Saccharomyces* and the least content (17%) recorded in *L. plantarum* fermented gruel (Table 2). Fermentation decrease the moisture content, crude fibre and ether extract contents of the starter-developed gruels, and these decreases are significantly different ($p<0.05$) from the unfermented blends. Fermentation at 30℃ also increases the iron content and decreases the magnesium content of *L. plantarum* fermented blends while other starter showed no difference in content. Generally, both starter used and fermentation temperature does not affect the mineral content statistically. Table 3 shows the effect of fermentation at 37℃ temperature on the starter developed blends. From the table, fermentation increased the crude protein and total carbohydrate contents of *L. plantarum* fermented gruel, crude protein and ash contents of *S. cerevisiae* fermented gruel while increase was only observed in crude protein of the fermented mixed culture *L. Saccharomyces* gruel.

The proximate composition of the fermented starter-developed gruel are significantly different ($p<0.05$) from the unfermented gruel. The result of the analysis of the nutritional composition of the gruel fermented at 40℃ shows that fermentation increased the crude protein contents of *L. plantarum*, so also the crude protein, ash content and ether extract of *S. cerevisiae* fermented gruel. The mixed culture fermented gruel *L. Saccharomyces* had increase in the crude protein and ash contents. The proximate composition of the fermented starter-developed gruel are significantly different ($p<0.05$) from the unfermented gruel. Fermentation at 40℃ decreases the mineral contents of the starter-develop blends which were not significantly different from each other. Figure 1 shows the effect of different temperature (25 to 40℃) and different pH (3 to 6) ranges

Table 3. Nutritional composition of fermented starter-developed blends at 37℃.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Crude protein	Ether extract	Ash	Crude fibre	Total carbohydrate	Iron	Calcium	Zinc	Magnesium
LP-0 h	90.70	16.10^{d}	5.423^{a}	4.30^{a}	4.300^{ab}	69.90^{b}	1.40	7.00^{a}	0.05^{b}	1.40
LP-48 h	90.80	17.33^{b}	5.410^{ab}	3.22^{c}	2.120^{e}	72.36^{a}	1.36	6.90^{a}	0.05^{b}	1.30
SC-0 h	89.00	15.62^{e}	5.320^{ab}	4.30^{a}	3.910^{c}	70.87^{a}	1.40	6.90^{a}	0.05^{b}	1.30
SC-48 h	90.70	19.31^{a}	4.900^{c}	3.90^{b}	3.208^{c}	68.70^{b}	1.40	6.60^{b}	0.05^{b}	1.40
LS-0 h	90.66	16.62^{c}	5.516^{a}	4.10^{ab}	4.413^{a}	69.40^{b}	1.50	6.80^{ab}	0.50^{a}	1.40
LS-48 h	90.62	19.51^{a}	5.11^{bc}	3.31^{c}	4.107^{b}	67.99^{c}	1.50	6.73^{ab}	0.50^{a}	1.30

Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

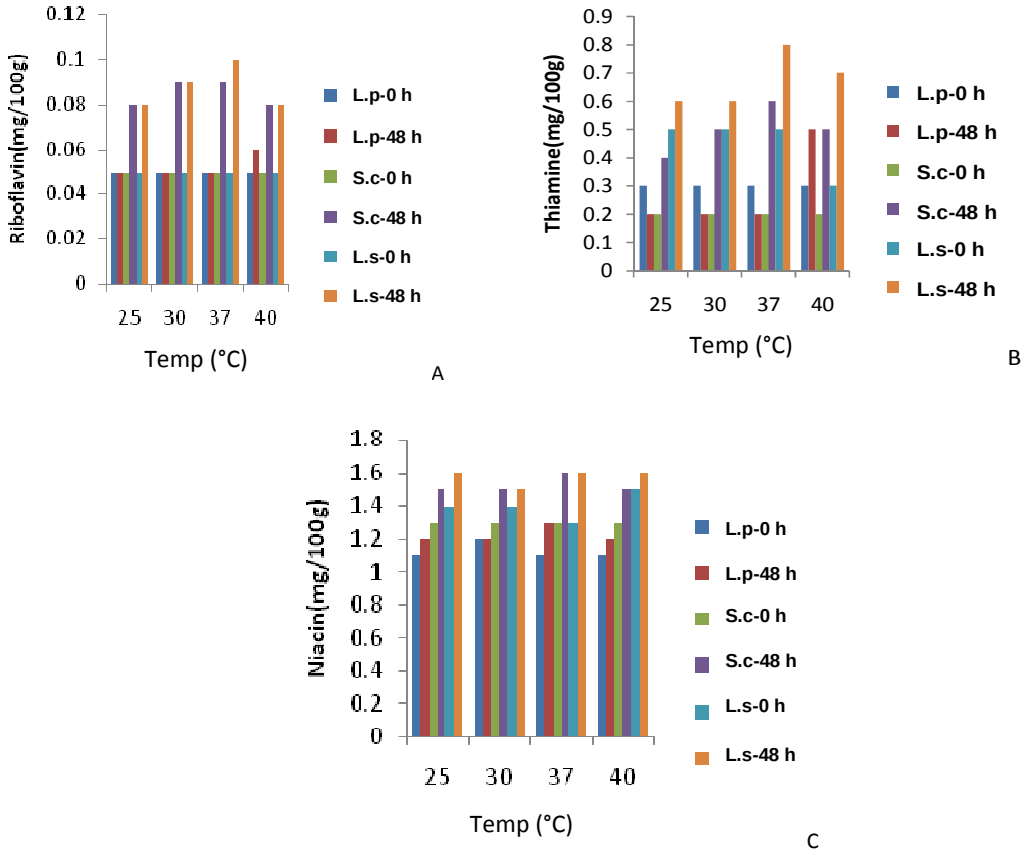

Figure 1A-1C. Effect of temperature on on vitamin content of fermented starter-developed blends/gruels.

on the vitamin contents of the starter- developed sorghum-cowpea blends. Figure 1A shows that riboflavin content of the fermented blends increased with increase in temperature (25 to 37℃) and later decreased at 40℃ except for *L. plantarum* developed blend. Also, the riboflavin content of all the unfermented blend is the same irrespective of the starter used while fermented mixed culture developed blends has the highest riboflavin content at all temperature used. The analysis of the thiamine contents of all the starter- developed blends shows that 37℃ is the optimum temperature for the production of the vitamins for all starters except with *L. plantarum* and mixed culture blend *L. Saccharomyces* having the highest thiamine content (0.8 mg/100 g).

The highest content of thiamine 0.5 mg/100 g was observed in the *L. plantarum* gruel at 40℃ (Figure 1B). The result of analysis of niacin content of different starter-developed blends fermented at different temperatures is as shown in Figure 1C. From the figure, the highest niacin contents of all the fermented starter gruel was

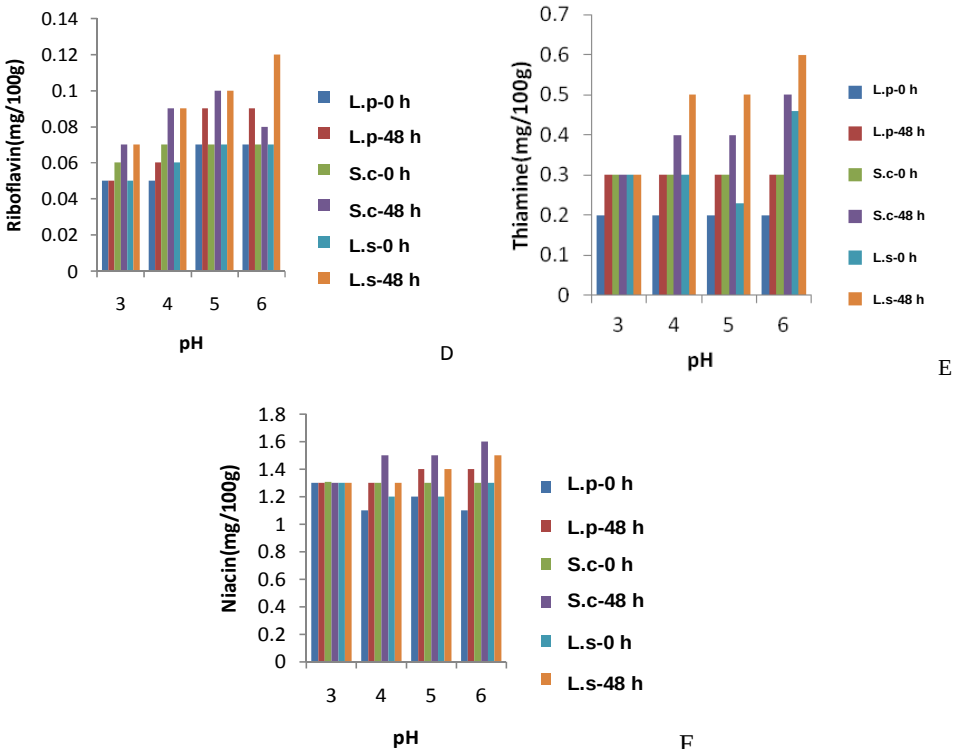

Figure 1D-F. Effect of temperature and on vitamin content of fermented starter-developed blends/gruels.

Table 4. Nutritional composition of the fermented starter-developed blends at 40℃.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Crude protein	Ether extract	Ash	Crude fibre	Total carbohydrate	Iron	Calcium	Magnesium	Zinc
LP-0 h	90.7	16.12^{b}	5.37^{a}	4.30^{b}	4.30^{a}	70.00^{b}	1.4	7.0	1.4	0.05
LP-48 h	89.9	17.82^{ab}	4.95^{c}	3.96^{c}	2.97^{d}	70.45^{d}	1.3	6.9	1.2	0.05
SC-0 h	89.8	15.68^{c}	5.31^{a}	5.31^{a}	3.94^{b}	69.76^{c}	1.4	6.9	1.3	0.05
SC-48 h	90.6	16.14^{b}	4.90^{c}	3.92^{b}	3.19^{c}	71.96^{a}	1.4	6.9	1.2	0.05
LS-0 h	91.0	16.66^{bc}	5.55^{a}	5.10^{a}	4.44^{a}	68.9^{c}	1.5	6.9	1.5	0.05
LS-48 h	90.2	17.92^{a}	5.10^{ab}	3.33^{c}	3.06^{c}	71.34^{a}	1.4	6.9	1.2	0.05

Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

observed at 37℃ except for mixed culture *L. Saccharomyces* starter gruel. Effect of different pH ranges on vitamin contents of the fermented starter blends is as shown on Figure 1D to F. Figure 1D shows that riboflavin content of all the fermented blends increased as the pH increases (except for *L. plantarum* blends) from 3 to 6 with the value ranging from 0.05 mg/100 g in fermented *L. plantarum* blend to 0.12 mg/100 g in fermented *L. Saccharomyces* blend. The highest riboflavin content in the *L. plantarum* blend (0.09 mg/100 g) is also observed at pH 5 and 6. The variation in the pH from 3 to 6 does not affect the thiamine production in the unfermented *L. plantarum* and *S. cerevisiae* and fermented *L. plantarum* blends while the thiamine content increased with increase pH in unfermented and fermented *S. cerevisiae* and *L. Saccharomyces* blends. At all pH, mixed culture blends have the highest value (Figure 1E). The result of the analysis of the niacin content of the starter blends at different pH is as shown in Figure 1F, decrease in acidity/ increase in pH increases the niacin contents of all the fermented starter blends with *S. cerevisiae* blends having the highest content. The result of the analysis of the effect of the variation in pH of the starter developed blends on the nutrient composition is as shown on Tables 4 to 7. Fermenting the blends at Ph 3 does not have any significant effect on the

Table 5. Nutritional composition of fermenting gruel adjusted to pH 4.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Crude protein	Fat content	Ash	Crude fibre	Total carbohydrate	Iron	Calcium	Magnesium	Zinc
LP-0 h	90.30^{d}	19.19^{b}	4.133^{b}	3.36^{b}	5.23^{b}	67.20^{a}	1.50^{b}	6.30^{d}	0.73	0.06
LP-48 h	90.40^{d}	19.26^{ab}	4.486^{b}	4.10^{a}	5.20^{b}	66.20^{ab}	1.50^{b}	6.40^{cd}	1.00	0.05
SC-0 h	90.50^{cd}	20.0^{ab}	5.253^{a}	4.20^{a}	5.20^{b}	65.08^{bc}	1.80^{a}	6.70^{a}	1.10	0.05
SC-48 h	90.70^{bc}	19.11^{b}	5.366^{a}	4.30^{a}	5.20^{b}	65.16^{bc}	1.70^{ab}	6.60^{ab}	1.10	0.05
LS-0 h	91.00^{a}	20.0^{ab}	4.400^{b}	3.31^{b}	6.60^{a}	65.16^{bc}	1.51^{b}	6.50^{bc}	0.73	0.05
LS-48 h	90.80^{a}	21.24^{a}	5.410^{a}	3.22^{b}	5.41^{b}	64.03^{c}	1.61^{ab}	6.50^{bc}	1.20	0.05

Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

Table 6. Nutritional composition of fermenting gruel adjusted to pH 5.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Crude protein	Fat content	Ash	Crude fibre	Total carbohydrate	Iron	Calcium	Magnesium	Zinc
LP-0 h	90.10	18.12^{c}	5.20^{ab}	3.03^{ab}	5.01^{a}	68.60^{a}	1.63	6.30^{b}	1.10	0.05^{b}
LP-48 h	90.13	19.73^{b}	5.06^{b}	3.10^{ab}	4.12^{ab}	68.00^{a}	1.50	6.40^{ab}	1.20	0.05^{b}
SC-0 h	90.90	19.72^{b}	5.59^{a}	3.22^{a}	5.43^{a}	66.18^{b}	1.60	6.46^{ab}	0.93	0.06^{a}
SC-48 h	90.26	19.58^{b}	5.41^{ab}	2.92^{b}	4.31^{b}	67.87^{b}	1.70	6.50^{ab}	1.10	0.06^{a}
LS-0 h	90.07	18.32^{b}	4.02^{c}	3.20^{ab}	4.32^{b}	70.18^{a}	1.60	6.60^{a}	1.10	0.05^{b}
LS-48 h	90.30	21.25^{a}	4.10^{c}	3.09^{ab}	4.11^{bc}	67.55^{a}	1.60	6.60^{a}	1.10	0.05^{b}

Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

Table 7. Nutritional composition of fermenting gruel adjusted to pH 6.

Organism	Proximate composition (%)						Mineral content (mg/100 g)			
	Moisture content	Crude protein	Fat content	Ash	Crude fibre	Total carbohydrate	Iron	Calcium	Magnesium	Zinc
LP-0 h	90.60	19.11^{c}	4.21^{bc}	3.13^{b}	4.21^{c}	69.40^{b}	1.56	6.60^{b}	1.10	0.05^{b}
LP-48 h	89.90	20.33^{b}	4.65^{ab}	3.03^{b}	4.10^{cd}	68.00^{bc}	1.70	6.60^{b}	1.20	0.05^{b}
SC-0 h	88.83	16.13^{d}	3.80^{c}	3.92^{a}	4.72^{b}	71.40^{a}	1.80	6.70^{a}	1.10	0.06^{a}
SC-48 h	89.30	19.80^{bc}	3.96^{bc}	2.81^{b}	3.92^{d}	69.54^{b}	1.80	6.70^{a}	1.03	0.06^{a}
LS-0 h	90.13	19.73^{bc}	3.08^{c}	4.02^{a}	5.20^{a}	68.10^{bc}	1.62	6.63^{a}	1.30	0.05^{b}
LS-48 h	90.06	21.65^{a}	5.27^{a}	3.10^{b}	4.02^{d}	66.10^{cd}	1.60	6.60^{b}	1.10	0.056^{a}

Values are mean of three replicates. Means that is within the same column with different superscript are significantly different ($p<0.05$).

proximate composition and the mineral contents from both within and among the starters except crude fibre and total carbohydrate (Data not shown).

The nutrient composition of the starter-developed blends at pH 4 is as shown on Table 5. Fermented mixed culture blend has the highest crude protein (21.24%), moisture content (90.8%), ether extract (5.41%) and crude fibre (5.41%) contents and the least, ash (3.22%) and total carbohydrate (64.03%). From the table, the mineral contents (Fe, Ca, Mg and Zn) of both fermented and unfermented blends were not significantly different from each other. The highest crude protein is observed in the fermented mixed culture *L. Saccharomyces* blend (21.25%), and the table also shows that adjusting the fermented gruel to pH 5 increases the crude protein, ether extract and the ash contents except for *S. cerevisiae* blends while crude fibre content decreases (Table 6). The result of the analysis of the mineral contents from the table also shows that fermentation pH and starter used has no significant effect on the blends. Table 7 shows the effect of pH6 on the nutrient composition of the three starter-developed blends. The result shows that the highest crude protein content (21.65%), ether extract (5.27%), ash (4.02%), crude fiber

(5.20%) and the least total carbohydrate (65.2%) was recorded in the fermented mixed culture blend *L. Saccharomyces.* The mineral contents (Fe, Ca and Zn) is the highest in the *S. cerevisiae* developed blend but in all, the mineral of both fermented and unfermented blends are not significantly different ($p<0.05$) both within and among the developed blends except for Calcium and Zinc contents of mixed cultured blend. Generally, at all the pH considered, the mixed cultured blends have the highest protein content and the least total carbohydrate.

DISSCUSION

There was reduction in the moisture content of the fermenting gruels for most of the temperatures and this could be probably due to the microorganism's adsorption of water for their microbial activities. The significant increase in the protein contents of the fermented gruel containing the mixed cultures *L. Saccharomyces* can be attributed to the proteolytic activity of the two microorganisms which involves a symbiotic interaction whereby *S. cerevisiae* stimulate the *L. plantarum* to produce high level of protein content (Hamad and Fields, 1979; Zamora and Fields, 1979, Chavan and Kadam, 1989). The protein contents increased as the temperature increases and later decreases at 40℃.The highest protein content was recorded at 37℃, this was probably the best optimum and favourable temperature for the growth and metabolic activities of the starters to perform their microbial activities. This finding is similar to the report of the Raja et al. (2009) who reported that the maximum growth temperature for *Lactobacillus species* at temperature between 35 and 40℃. Other researchers (Kneifel et al., 1993; Korbekande et al., 2009; Mortazavian and Sohrabvandi, 2006) also recorded that the optimum growth temperature for most probiotics are between 37 to 40℃. There was reduction in the percentage of crude fibre and carbohydrate content leading to the reduction of the gruel; this may be attributed to the fact that all the temperatures were favourable for the breakdown the starch or carbohydrate to sugar in the fermenting gruel by the enzyme amylase which hydrolysis starch granules for the growth of the organism.

This observation is also similar to the report of Raimbault and Tewe (2001) and Ojokoh (2007) who reported the reduction of fibre and total carbohydrate in the fermentation of mango peel. For the mineral content, there was little or no increase among the four temperatures, also statistical analysis shows no significant difference ($p<0.05$) in the mineral content of the fermented gruel developed with *L. plantarum*, *S. cerevisiae* and the mixed cultures *L. Saccharomyces,* this observation was in contrast with the report of Oladele and Oshodi (2008) who reported that the mineral composition increased with fermentation time, but the findings of Sahlin (1999) was different, who notice that mineral content is not affected by fermentation unless some salts are added to the product during fermentation. There was increase in the vitamin contents of the fermenting gruel generally; the highest values were recorded in the mixed cultures at 37℃ and which may be due to the fact that the mixed starter cultures consisting of lactic acid bacteria and fermenting yeast are important in achieving biological stability by assimilating all available polymer to produce simple more nutrients such as vitamins. An observation similar to that of Kneifel and Mayer (1991) who reported that the two cultures shows 40% increase in thiamine contents and riboflavin content of kefir.

These was also similar to Sanni et al. (1999) reports where *S. cerevisiae* and *L. plantarum* were used as starter cultures to ferment various cereals in the production of weaning foods, an increase in the contents of riboflavin, thiamine, niacin and ascorbic acid was reported during fermentation. The result of the analysis of the effect of pH on the starter produced gruel shows that the protein content of the mixed culture *L. Saccharomyces* fermenting gruel has the highest protein content at pH 6. The increase in the protein content may be as a result of the microorganism present in the gruel which breaks down complex protein to amino acid at optimum pH 6, which it seems to be favourable for most lactic acid bacteria and yeast, an observation similar to that of Loubiere et al. (1997). Furthermore, mixed culture of *L. plantarum* and *S. cerevisiae* had the highest protein content which may be due to the fact that yeast stimulates the growth of the lactic acid bacteria which is already known that activity increases while cells are growing exponentially and this correlate with the report of Khetarpaul and Chauhan (1990) who found out that mixed cultures of these organisms during the fermentation of pearl millet flour improves its biological utilization in rats.

There was reduction in the carbohydrate and the fibre content at all the pH and all the starter culture combination used. This may probably due to the microbial activities of the organisms, as a result of utilization of the available starch for metabolic activities for the growth of the microorganism, an observation similar to the work of Ejiofor and Okafor (1981) who confirmed the reduction of carbohydrate by the activities of amylase for the breakdown of starch to simple sugars.
The effect of pH on the mineral content of the fermenting gruel was little or not significantly different ($p< 0.05$) at all the different pHs, this may be due to the fact that the organisms might convert some compound of the fermenting gruel to a useable minerals. This observation was also reported by Sahlin (1999) who notice that mineral content is not affected by fermentation and Betschart (1988) also confirm the same result. The analysis of the fermenting gruel at different pHs shows that the highest vitamin content was recorded at pH6 of the fermenting gruel while the pH 3 shows no significant

difference ($p< 0.05$) despite the increase in value. pH 6 is most favourable amongst other pH, this is due to the fact the optimum pH of most lactic acid bacteria is pH 6 to 6.5 (Hyun and Zeikus, 1985; Leroy and deVuyst, 1999).

CONCLUSION AND RECOMMENDATION

This research work has been able to find out that fortification of Sorghum/Cowpea at the optimum environmental condition of 37℃ and pH 6 will be the best for the development of high nutritional cereal-legume weaning blend. We therefore, recommend formulation of high quality fermented weaning blend with mixed starter culture of *L. plantarum* and *S. cerevisiae* under the above environmental conditions.

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