Fortification Of Sorghum With Legumes Using *Lactobacillus Plantarum* As Starter For "Ori-Ese" Production.

Olonila Omolola Toyin, Adebayo-Tayo Bukola Christianah and Akinola Gbemisola Elizabeth

Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria.
bukola_tayo@yahoo.com, damselinioluwa@yahoo.com

Abstract: Sorghum (*Sorghum bicolor*) is one of the most important food crops in Africa but due to the deficient in certain amino acids there is a need for fortification of these cereals with legumes to improve the nutritional quality and acceptability. "Ori-ese", a traditional fermented Sorghum, tough porridge prepared by the Yoruba's in Ekiti state, western Nigeria."Ori ese" was produced using Sorghum (brown and white varieties) with different blends: Sorghum only (WC and BC) Sorghum-Bambara-nut (WBO and BBO), Sorghum-Soybeans (WSO and BSO), Sorghum-Cowpea (WCO and BCO), and Sorghum-groundnut (WGO and BGO), with *Lactobacillus plantarum* as a starter. The pH, lactic acid, diacetyl and hydrogen peroxide production during slurry fermentation ranged from 3 - 7, 0.125 - 6.125 g/l, 0.042 - 0.85g/l and 1.0 - 6.4g/l respectively. There was a significant difference in protein content of the samples, It ranged from 10.0^{h} - 13.3^{a} % in which BBO had the highest and WC sample had the least. The crude fat ranged from 2.08^{e} - 3.03^{a} % in which BSO had the highest while WGO had the least. Samples from WBO had the highest crude fiber ($3.22\%^{a}$) while WC has the least ($2.26^{fg}\%$). There was a significant difference ($P \geq 0.05$) in mineral composition of the blends, BSO had the highest Na content (0.108^{a} mg/kg) and K content (0.1221^{a} mg/kg) while WBO had the highest Fe content (2.5^{a} mg/kg) and Ca content(0.15^{a} mg/kg). There was a significant difference in P content of the samples except for WGO and WSO. Fortification and the use of starter for "Ori-ese" production enhanced the nutritional quality of the food. Fortification with Bambara-nut is highly recommended and it will be suitable in the total amelioration of protein –energy –malnutrition in the developing countries.

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1. Introduction

Food is an essential ingredient for sustenance of life either of plants or animals. Its demand can therefore not overemphasize. In countries like Nigeria, people depend mostly on indigenous technology for food preparations especially food of plant origin. Some of the food that is of plant origin are the cereal based foods, beverages, vegetable protein etc.

Cereal grains had been one of man earliest sources of food (Matz, 1971). One way of processing the grains into food is through fermentation (Akinrele, 1970; Akingbala *et al*., 1981; Fields *et al*., 1981; Adeyemi and Beckley, 1986). "Ori ese" or ''Ori-kati''is a porridge prepared from fermented Sorghum by Yoruba's in Ekiti state, western Nigeria. It is a staple of that region and serves as food for both infants and adults. The traditional method of preparing "Ori ese" involves steeping of Sorghum grains in water for 3 days followed by wet milling. Fermentation of slurry for 24hours after which the slurry is cooked in leaves and tied properly with rope. Further cooking for 1hour and the resulting in tough porridge is "Ori ese". Traditional production of ''Ori-ese'' is a wild process and effect of microorganism is not controlled (Banigo and Miller, 1972).

Sorghum has poor nutritional quality due to the deficiency of certain essential amino acids, such as methionine, Isoleucine and lysine (Neucere and Sumrell,1979) low starch availability (Hall *et al*.,1968) and low elated to the protein digestibility (Hamaker,1986; Maclean,1981),and this could also be related to the presence of certain anti nutritional factors, such as tannins (Price and Bultler,1977).

According to Malleshi and Desikachar (1982), it is quite possible to improve the nutrient quality and acceptability of cereals. Therefore attempts have been made to fortify the cereal with legumes or other cereals to make nutritionally superior and acceptable product (Nti and Plahar, 1995; Mbata *et al*., 2009; Afoakwa *et al*., 2002).

Mbata (2009) reported that Bambara-nut fortification increase acid production which could be due to availability or more nutrients for microbial proliferation and enhances metabolic activities. Cowpea fortification is also reported by Afoakwa *et al*., 2007 has had a great significance of the influence of acid production and enhanced the development of the characteristics sour taste and flavor of cereals based food. In order to accelerate fermentation and its predictability, process control is required and this can be achieved through ecological control involves the enrichment of starter organisms in food by natural selection (Holzapfel, 1997) Modern starter cultures are selected either as a single strain or multiple stains, specifically for their adaptation to a substrate or raw materials (Hansen, 2002)

This study was therefore designed to determine the effect of fortification and addition of starter on physiochemical and proximate/mineral composition of starter produced "Ori ese" using different blends (Sorghum, Sorghum-Soybeans, Sorghum-Cowpea, Sorghum-groundnut and Sorghum-Bambara nut) respectively.

2. Material and Methods

Sample collection

White and brown variety of Sorghum (*Sorghum bicolor* and *Sorghum vulgare*), Cowpea (*Vigna unguiculata*), Soybeans (*Glycine max),* Groundnut (*Arachis hypogaea*) and Bambara-nut (*Voandzeia Subterranea L. Verde*) were obtained from Bodija market, Ibadan, Oyo state. Broken and moldy seeds were removed manually.

Starter preparation

L. plantarum strain previously isolated during steeping of Sorghum for "Ori –ese" production in our previous work in the Department of Microbiology, University of Ibadan was used as a starter. Pure cultures of the isolates (*L. plantarum*) was routinely maintained on MRS agar (Oxoid, Basingstroke England). 18-hours old cultures were utilized in all aspects of the current work.

Inoculum preparation and determination of the Inoculums Size

The working culture was prepared by transferring 0.5ml of the stock frozen culture to 10ml of MRS broth and incubated for 16h at 30°C an anaerobically. The microbial load was determined by plating 0.1ml each of the broth culture in MRS agar to determine broth cultures containing about 2×10^{8}cfu/ml. Broth cultures containing the required concentration of viable cells was centrifuged, washed in sterile distilled water and re-centrifuged. The washed cell was used as inoculum in the fermentation of Sorghum for "Ori ese" production (Lonner *et al.,* 1986).

Fortification of Sorghum with legumes (Bambara-nut, Cowpea, Soybean and Groundnut)

The Sorghum and legumes were washed using 5% sodium bisulphate solution, each of the substrates were oven-dried and then dry-milled into flour using blender. The dry-milled Sorghum and legume flours were allowed to cool before use. The Sorghum and legumes (Bambara-nut, Cowpea, Soybean and Groundnut) flours were mixed together in the ratio 3:7. 700g each of Sorghum flour was mixed with 300grams of legume flours (Bambara nut, Cowpea, Soybean and Groundnut). Sterile distilled water was added to make a slurry and 10ml of the *L. plantarum* (2.0 $x10^{8}$ cfu/ml) was inoculated as starter and allowed to ferment for 24hrs. The fermenting samples were boiled for 30minutes respectively. The resulting dough was molded into a ball shape, wrapped in polyethylene nylon and cooked properly for 40 - 60hrs. The resulting thick porridge is "Ori- ese" (Sorghum "Ori- ese" (BC,WC); Sorghum-Bambara nut "Ori-ese" (BBO,WBO); Sorghum - Cowpea "Ori-ese" (BCO,WCO); Sorghum-Soybean "Ori-ese" (BSO,WSO) and Sorghum- Groundnut "Ori-ese" (BGO,WGO).

100g (Sorghum grains)+200mldistilledd water

↓

Washing

↓

Sorghum grains+200mldistilledd water + sodium metabisulfite

↓

Drying

↓

Dry milling (UsingMarlexx blender for 5mins)

↓

Fine flour

↓

700g of flour + 300g each of (Cowpea, Bambara- nuts, and groundnut and Soybean) + 200ml distilled water

↓

Fermentation (1 day)

↓

Boiling (20 minutes)

↓

Molding of dough into a ball-shaped

↓

Wrapping in polyethylene bag

↓

Cooking (1 hour)

↓

Fortified "Ori ese"

Figure 1: flow chart for production of "Ori ese".

Physicochemical Analysis:

Samples were taken during slurry fermentation at 6hrs interval for physicochemical analysis. pH, lactic acid concentration, hydrogen peroxide and diacetyl production by the isolates, these parameters were determined according to the method of AOAC (1990). The data obtained were subjected to statically analysis.

Proximate analysis:

Proximate and mineral compositions were carried out according to the method of A.O.A.C (1990). This includes determination moisture content, ash content, crude protein, and fiber, fat, total carbohydrate contents and mineral content such as potassium, calcium, phosphorus and iron.

3. Results

Different types of "Ori-ese" were prepared from Sorghum and different blends of legumes using *Lactobacillus plantarum* as starter. The blends were: Bambara-nut, Soybeans, Cowpea and Groundnut. During slurry fermentation for the White and Brown variety of Sorghum the pH ranged from 2.9-5.3 and 2.9 -7.1. The lowest pH was recorded in WGO and BGO samples. shown in Figure 2.

The result of lactic acid production during slurry fermentation is shown in Figure 3. It ranged from 0.153 – 5.945mg/l and 0.125 – 6.125mg/l for white and brown variety. The highest lactic acid production was recorded in WSO and BC respectively. A sharp increase in lactic acid production was observed in all the samples to increase in fermentation time.

Diacetyl production ranged from 0.212 – 0.77mg/l and 0.042 – 0.853mg/l for ''Ori-ese'' from white and brown variety. WSO and BSO had the highest production as shown in Figure 4. The peak production of diacetyl was recorded at 12hr in BSO (0.853mg/l) and WSO (0.741mg/l). The least production was recorded at 24hr by BBO. There was no specific trend followed in the production of diacetyl by all the samples.

Hydrogen peroxide production ranged from 1 x10^{-3} – 6.8 x10^{-3} mg/l in which WSO and BCO had the highest. The least was recorded in WGO and BSO as shown in Figure 5. The highest production was recorded in at 18hr after incubation and least was recorded at 24hr Figure5.

The proximate composition of the starter produced fortified "Ori-ese" samples is shown in Table 1. There was a significant differences in proximate composition of the blends. The protein content ranged from 10.07^{i} - 13.37^{a} % in which BBO had the highest followed in order by WBO (13.12^{b}) and the least was recorded in sample WC.

There was a significant difference in crude fat of the fortified samples. The control samples had higher crude fat than most of the starter produced fortified samples. Highest crude fat was recorded in BSO (3.03^{a}%) while the least was recorded in the WGO (2.08^{g} %). The crude fibre ranged from 2.26 -3.22^{a} % in which WBO had the highest followed in order by WSO and BSO while WC had the least. There was no significant difference in crude fibre of samples WCO, WGO and BC and BGO respectively.

The carbohydrate content ranged from 76.70h – 81.07^{a} in which WC had the highest followed by BC (79.14^{b}) while BSO had the least.

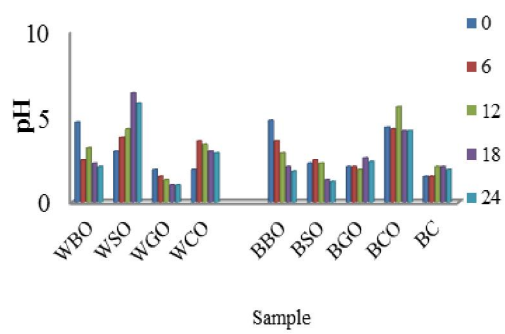

Figure 3.1: pH changes during slurry fermentation of fortified"Ori-ese"

WBO=white Sorghum-Bambara –nut, WSO= white Sorghum- Soybeans, WGO= white Sorghum - groundnut, WCO=White Sorghum-Cowpea,BBO= Brown Sorghum - Bambara –nut, BSO= Brown Sorghum -Soybeans, BCO= Brown Sorghum - Cowpea, BC=Brown Sorghum control.

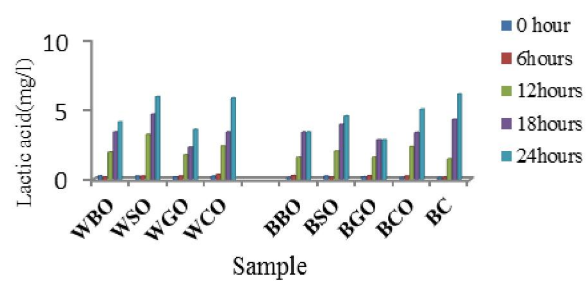

Figure 3: Lactic acid production during slurry fermentation of fortified "Ori-ese"

WBO=white Sorghum-Bambara –nut, WSO= white Sorghum- Soybeans, WGO= white Sorghum - groundnut, WCO=White Sorghum-Cowpea, BBO= Brown Sorghum - Bambara –nut, BSO= Brown Sorghum -Soybeans, BCO= Brown Sorghum - Cowpea, BC=Brown Sorghum control.

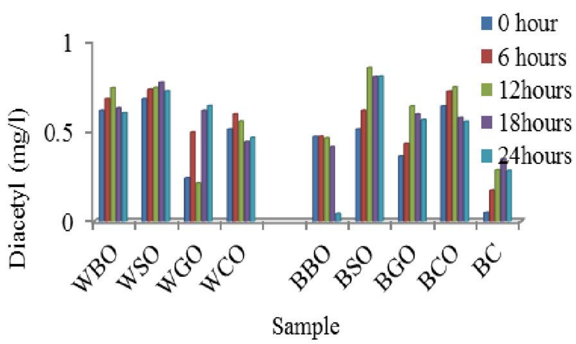

Figure 4: Diacetyl production during slurry fermentation of fortified "Ori-ese" WBO=white Sorghum-Bambara –nut, WSO= white Sorghum-Soybeans, WGO= white Sorghum - groundnut, WCO=White Sorghum-Cowpea, BBO= Brown Sorghum - Bambara –nut, BSO= Brown Sorghum - Soybeans, BCO= Brown Sorghum - Cowpea, BC=Brown Sorghum control.

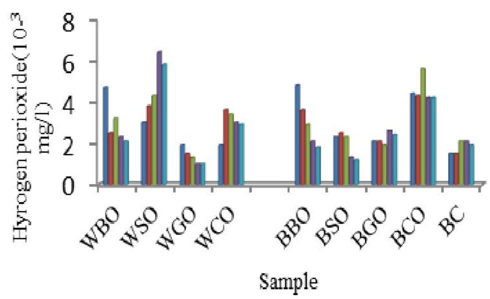

Figure5: Hydrogen perioxide during slurry fermentation of fortified "ori-ese".
WBO=white Sorghum-Bambara –nut, WSO= white Sorghum- Soybeans, WGO= white Sorghum - groundnut, WCO=White Sorghum-Cowpea, BBO= Brown Sorghum - Bambara –nut, BSO= Brown Sorghum -Soybeans, BCO= Brown Sorghum - Cowpea, BC=Brown Sorghum control.

Table 1: Proximate analysis (%) of fortified "Ori-ese".

PROXIMATE COMPOSITION (%)		SAMPLES FROM FORTIFIED WHITE VARIETY				SAMPLES FROM FORTIFIED BROWN VARIETY				
	WBO	WCO	WGO	WSO	WC	BBO	BCO	BGO	BSO	BC
Crude Protein	13.12^{b}	12.33^{d}	11.55^{f}	11.09^{h}	10.07^{i}	13.37^{a}	12.19^{e}	12.83^{c}	12.19^{e}	11.21^{g}
Crude Fat	2.44^{c}	2.19^{f}	2.08^{g}	2.60^{b}	2.31^{de}	2.26^{e}	2.19^{f}	2.36^{d}	3.03^{a}	2.48^{c}
Crude Fibre	3.22^{a}	2.94^{cd}	2.94^{cd}	3.09^{b}	2.26^{f}	2.90^{d}	2.70^{e}	2.77^{e}	3.03^{bc}	2.98^{bcd}
ASH	4.46^{c}	4.09^{e}	4.40^{c}	4.17^{e}	4.29^{d}	4.14^{e}	4.57^{b}	5.12^{a}	5.05^{a}	4.19^{de}
Carbohydrate	76.76^{h}	78.45^{d}	79.03^{c}	79.05^{bc}	81.07^{a}	77.33^{f}	77.95^{e}	76.92^{g}	76.70^{h}	79.14^{b}
Moisture	27.94^{e}	28.92^{b}	29.36^{a}	28.47^{c}	29.35^{a}	28.29^{d}	18.64^{h}	23.85^{g}	24.66^{f}	28.33^{d}

WBO=white Sorghum-Bambara –nut, WSO= white Sorghum- Soybeans, WGO= white Sorghum - groundnut, WCO=White Sorghum Cowpea,BBO= Brown Sorghum - Bambara –nut, BSO= Brown Sorghum -Soybeans, BCO= Brown Sorghum - Cowpea, BC=Brown Sorghum control.

Table 2: Mineral composition (%) of fortified "Ori-ese"

MINERALS (%)	SAMPLES FROM FORTIFIED WHITE VARIETY					SAMPLES FROM FORTIFIED BROWN VARIETY				
	WB0	WCO	WGO	WSO	WC	BBO	BCO	BGO	BSO	BC
Na	0.10^{ab}	0.051^{d}	0.105^{a}	0.094^{ab}	0.074^{c}	0.09^{b}	0.087^{bc}	0.096^{ab}	0.108^{a}	0.074^{c}
K	0.12^{a}	0.108^{bc}	0.118^{a}	0.101^{c}	0.081^{d}	0.106^{bc}	0.113^{ab}	0.114^{ab}	0.122^{a}	0.0805^{d}
P	0.062^{cd}	0.071^{bc}	0.082^{a}	0.082^{a}	0.064^{bcd}	0.07^{bc}	0.069^{bc}	0.057^{e}	0.064^{bcd}	0.064^{bcd}
Fe (Mg/Kg)	2.50^{a}	1.90^{c}	1.40^{e}	1.30^{e}	2.00^{c}	1.65^{d}	1.95^{c}	1.10^{f}	2.30^{b}	2.00^{c}
Ca	0.15^{a}	0.11^{b}	0.06^{de}	0.120^{b}	0.05^{e}	0.08^{cd}	0.10^{bc}	0.08^{cd}	0.120^{b}	0.05^{e}

WBO= white Sorghum - Bambara –nut, WSO= white Sorghum - Soybeans, WGO = white Sorghum-Groundnut, WCO=White Sorghum-Cowpea, BBO= Brown Sorghum - Bambara –nut, BSO= Brown Sorghum -Soybeans, BCO= Brown Sorghum - Cowpea, BC=Brown Sorghum control

The mineral composition of the starter produced fortified ''Ori-ese'' samples is shown in Table 2. There was a significant difference in mineral composition of the samples. BSO had highest Na content (0.108^{a}) while the least was recorded in BC (0.051^{d}). There was a significant difference in K content in which BSO had the highest (0.122^{a}) followed in order by BGO (0.114^{ab}) while the least was recorded in BC (0.0805^{d}). The highest P content was recorded in WSO and WGO (0.082^{a}) while the least was recorded in BGO (0.0805^{d}). WBO (2.50^{a}) had the highest Fe content, followed in order by BSO

(2.30[b]) and the least was recorded in BGO (1.10[f]). The highest Ca content was recorded in WBO (0.15[a]), there was no significant difference in BBO and BGO (0.08[cd]) while the least was recorded in the control sample.

4. Discussions

There was a steady decrease in the pH (Figure 2) during the fermentation of Sorghum meal-legume blends while there was a significant increase in the lactic acid (Figure 3) production in all the Sorghum slurry samples. This might be as a result of production of lactic acid by fermentative *L. plantarum* used for the fermentation of the slurry and chemical composition of the legumes used. This observation is in agreement with the report of previous studies (Odunfa and Adeyele, 1985; Adesokan *et al*., 2010). Other factors such as diacetyl production and hydrogen peroxide production increased with time. Several works reported the same trend in cereal-based fermentations (Akinrele, 1970; Oyeyiola, 1990; and Sanni *et al.,* 1998). The early rise in titratable acidity is important to avoid proliferation of undesirable organisms resulting in poor fermentation.

Significant differences that were observed in proximate and mineral composition of the fortified samples (Table 1 and 2), Crude protein content showed a significant difference between the fortified and unfortified samples (controls).

The high protein content recorded in brown Sorghum fortified with Bambara-nut can be as a result of the chemical composition of the seed and Mbata and others recorded thesame observation (Mbata *et al.,* 2009). Adu-Dapaah and Sangwan (2004) reported that Bambara-nut is very rich in iron and the protein with high lysine and methionine and that the seed is known to be a balanced food when compared to most food legumes. In addition, Bambara -nut is known to contain 63% carbohydrates, 18% oil and the fatty acid content is predominantly linoleic, palmitic and linolenic acids (Minka and Bruneteau, 2000). This is in accordance with the finding of Mbata *et al*. (2009).

The highest crude Fat, crude Fiber and Ash content were recorded in Sorghum fortified with Soybeans (BSO), white Sorghum fortified with Bambara-nut (WBO) and brown Sorghum Fortified with Bambara-nut (BGO) respectively. The higher carbohydrate content observed in the unfortified samples could be as a result of absence of blend for fortification. Since the products provided one third of the recommended dietary allowance RDA with respect to protein 10 to 12% and as recommended by food and agriculture organization FAO, 1985 and national institute of nutrition (1992) for children and rural mothers. The proximate characteristics of the fortified food were within the range reported for weaning and supplementary food (FAO, 1985).

The low moisture content of the product indicate that it would have a good keeping quality. This is because food spoiling micro-flora thrives well where there is adequate moisture (Ene-Obong and Carnovale, 1992).

In conclusion, “Ori-ese” fortified with different blends are hereby recommended for children of the weaning age and adults alike due to its high quality and acceptability. Sorghum flour made by dry milling is also recommended for easy handling, better keeping quality, convenience, faster preparation and its easy and adequate blending during fortification.

Dry milling is also preferred because it is more hygienic and retains the nutrients in cereals. “Ori-ese” has poor biological value thus; the majority of people feeding entirely on “Ori-ese” are known to suffer from protein-energy malnutrition (PEM). So, a good supplemental relationship thus exists between “Ori-ese” and Bambara. The addition of 30% Bambara supplemented “Ori-ese” improves the protein content of “Ori-ese”.

The study has revealed that fortification of Sorghum meal (cereal) with Bambara –nut (legume) is able to alleviate problems of protein energy malnutrition (PEM).

Furthermore, the introduction of appropriate starter culture techniques constitutes one of the major steps towards improving the safety, quality and security of traditional production of Bambara-nut Sorghum fermented meal.

Corresponding Author:
Adebayo-Tayo Bukola Christianah
Department of Microbiology, Faculty of Science,
University of Ibadan, Ibadan, Oyo state, Nigeria.
Email: bukola_tayo@yahoo.com
Tel: 234 803 5522409

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