

IMPROVEMENT OF THE PRODUCTION PROCESS AND NUTRITIONAL QUALITY OF NIGERIAN TOFU (BESKE) THROUGH FERMENTATION

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Abstract

Tofu (Nigerian Beske) is a soybeans based food that is highly rich in protein. It is made from curdling of hot soymilk using a coagulant. The food has a short shelf life of about two to three days. This work is aimed at improving the nutritional quality and shelf life of tofu by using starter cultures of lactic acid bacteria (LAB) for fermentation of soy milk. A total of 52 LAB were isolated from spontaneously fermented soymilk and they were; *Leuconostoc mesenteroides* (11.5%), *Pediococcus pentosaceus* (7.7%), *Lactococcus lactis* (13.5%), *Lactococcus cremoris* (9.6%), *Lactobacillus casei* (11.5%), *Lactobacillus curvatus* (9.6%), *Pediococcus acidilactic* (7.7%), *Lactobacillus delbrueckii* (5.8%), *Streptococcus sp* (11.5%), *Lactobacillus acidophilus* (3.8%) and *Enterococcus faecalis* (7.7%). The fermenting soymilk recorded a pH of 6.5 and a total titratable acidity of 0.74% at 0hr and pH of 4.8 and a total titratable acidity of 1.96% at 18hrs. The soymilk fermented with *Lactococcus lactis* SO51 (SYL₂) had the highest protein and ash content with a mean score of (4.06 ± 0.01) and (0.34±0.01) respectively while the unfermented soymilk had the highest carbohydrate content (1.99±0.00) and fat content (1.81±0.00). The fried fermented tofu with combined starters (FFTL₁L₂) recorded the highest mean score in protein (24.81±0.13), fat (11.02 ±0.05) and ash content (1.58±0.01). The fried fermented tofu using *Lactobacillus casei* SO36 (FFTL₁) had the least phytate and lectin content with a mean score of (0.03±0.00) % and (11.24±0.02) HU/mg respectively. This study showed that fermentation process increased the nutritional quality and shelf life of tofu, reduced the anti-nutritional factors and also brought about a new variety of the food product.

Keywords: Tofu; soymilk; starters; fermentation; shelf life

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1. INTRODUCTION

Soybeans (*Glycine max*) are leguminous crop which belongs to the family of leguminosae meaning the fruit of the flowering plants (Hymowitz, 1970). It is one of the oldest cultivated crops and has its origination from East Asia specifically Northern and Central China, with USA being the largest producer of the crop followed by Brazil, Argentina and China (Tas, 2003). There has been a significant increase in the demands for soybeans and this due to its nutritious and medicinal values (Steinkraus, 2004). In Nigeria, soybeans is one the important food crops consumed by humans and livestock, and incorporated as secondary crops in most farming systems for sustenance and maintenance of soil structure and fertility (Adebayo 2001; Randall *et al.*, 2006). The average nutritional composition of this leguminous crop reveals that it is extremely high in protein content. On the average,

soybeans contains roughly 40% of protein, 35% carbohydrate, 20% soybean oil and 5% ash (non-aqueous, metal oxides) content (Lui, 2004). Soybean meal is rich in mineral particularly Calcium, Phosphorus, and Iron and excellent content of vitamins, theomine, miacine and riboflavin (Ihejirika *et al.*, 2010). Soybean (*Glycine max*) (L.) Merrill is the world's most valuable oil-seed legume. Fermentation is one of the most ancient and most important food processing technologies. It is a relatively efficient, low energy preservation process, which increases the shelf life and decreases the need for refrigeration or other forms of food preservation technology. Traditionally, the essence of fermentation was majorly for food preservation and such as been used for centuries until present. This preservation effect is due to the formation of inhibitory metabolites such as organic acid (lactic acid, acetic acid, formic acid, and propionic acid), ethanol, bacteriocins, etc.,

often in combination with decrease of water activity (by drying or use of salt). It has also improved food safety through the inhibition of growth of pathogenic and spoilage bacteria in food or removal of toxic compounds. Fermentation processes has also led to improved nutritional value and organoleptic quality of food products (Odunfa, 1988; François, 2012).

Soymilk, a water extract of whole extract of whole soybean, which is rich in water soluble protein, carbohydrate and oil, contains no cholesterol or lactose and only a small quantity of saturated fatty acid. Soymilk is more beneficial in terms of cost effectiveness and quantity produced when compared with cow milk. It can be produced either traditionally, by whole bean method, defatted method or extruder method (Shurtleff and Aoyegi, 2000). Tofu is a traditional, oriental gel-like soybean food fundamental of Asian culture. The food is principally composed of protein and oil. Its preparation generally includes soaking and grinding of soybeans in water, filtering, boiling and coagulation of soymilk, molding and pressing. Fresh tofu has 86.7% water, 7.2% protein, 3.4% fat, 2% carbohydrate and 0.7% ash (inorganic residue) (Tee *et al.*, 1988). This highly rich in protein-based food can be used in soups, salads, pastries, sandwiches, etc., because it is easy to digest and it can be substituted for meats, cheeses and certain dairy products in diets for milk intolerance individuals, vegetarians and the elderly.

Lactic acid bacteria (LAB) are group of bacteria that are of high importance in fermentation processes. They are gram positive rods and cocci that occur naturally in different niches, also in the gastrointestinal tract, plants and fermented foods such as meat, dairy products and alcoholic beverages (Hammes and Hertel, 2006; Mohania *et al.*, 2008).

These groups of bacteria have been associated with production of fermented foods and feeds for many centuries by playing important role of preservation either as the natural microflora or as starter cultures added under controlled conditions.

The aim of this research is to improve the nutritional quality and shelf life of tofu using lactic acid bacteria as starter cultures for the fermentation of soy milk.

2. MATERIALS AND METHODS

Sample Collection

Soymilk samples were obtained from four different locations in Ibadan namely; Ajibode, Bodija, Ojoo and University of Ibadan. Two soymilk samples were collected from each location and the samples were collected in sterile plastic bottles, properly labelled and transported to the Laboratory for microbiological analysis. The samples were placed on the work bench and allowed to ferment overnight before isolation was done. The soybean (*Glycine max*) sample, seasonings, spices and vegetable oil used in this research were obtained from Bodija market in Ibadan, Oyo State.

Preparation of Culture Media

All culture media used were prepared by adjusting them into their appropriate pH, and then sterilized in an autoclave at 121°C for 15mins. The culture media used were; Nutrient agar (Biotech Laboratories Ltd, Ipswich, UK), MacConkey agar (Biotech Laboratories Ltd, Ipswich, UK), Malt extract agar (LAB M, Lancashire, UK), Potato dextrose agar (LAB M, Lancashire, UK), de Man Rogosa Sharpe agar and broth (LAB M, Lancashire, UK) and peptone water.

Isolation of Lactic Acid Bacteria from Sample

Soymilk samples obtained from different locations were made to ferment overnight. One millilitre (1mL) of each sample was aseptically taken from the representative duplicate sample bottle and dislodge aseptically into 9mL of sterile distilled water in test tubes and shaken together. The solution was serially diluted 10-fold in the sterile distilled water. Selected colonies were picked and repeatedly streaked on fresh agar plates until pure cultures were obtained.

Identification of Lactic Acid Bacteria

Identification of each colony which was considered as selected LAB was conducted by conventional method based on morphological, biochemical and physiological characteristics of isolate. The morphologically test based on the formation of colony, size, colour, edge form and texture of colony, then the selected colony was Gram stained and then viewed using the microscope. Profile matching methods based on Bergey's Manual of Systematic Bacteriology with some modification were used for characterization and identification of LAB isolates (Wang *et al.*, 2008).

Preparation of cell suspension of starter cultures

The cell suspension of each of the test organisms was prepared following the method described by Abiose *et al.* (1988). The starter culture (18 – 24h old) of each isolate in MacCartney bottle slants was washed with 10ml of sterile 0.9% NaCl solution. The cell suspension was transferred into 40mL of sterile 0.9% NaCl solution in a 100mL conical flask. The suspension was diluted to give an absorbance of 0.03 at 540nm in a spectrophotometer. The cell population of the resultant suspension of each isolate was determined using the pour plate method. The laboratory prepared soymilk was inoculated following the methods of Barber and Achinewu (1992). This involved the aseptic mixture of the sterile soymilk in each Erlenmeyer flask with 0.5mL of the appropriate cell suspension to give approximately 10^6 cells per ml as single isolates, in combinations of mixed isolates. All the flasks were incubated at room temperature to ferment for up to 18hrs. Duplicate flasks were removed for analyses at selected intervals.

Production of Tofu with Starter Culture

150g of sorted soybeans was weighed into 3 different labelled plastics buckets, washed and soaked with sterile distilled water in a ratio of 1:3 (w/v) each for a period of 12hours at room temperature. The soaked soybeans was then drained, washed and then blended using a

Binatone blender with the addition of sterile distilled water in a ratio 1:10 (w/v). The resulting slurry was sieved using a muslin cloth (0.5mm pore size) to extract the soymilk. The soymilk was then boiled for about 4minutes after which it was brought down to cool to about 45°C. 1ml of the selected 2 LAB pure culture (in saline suspension) in singles and 0.5mL each in combination was inoculated aseptically into the corresponding soymilk sample and then incubated at 30°C for 18hours for fermentation to take place. A duplicate sample was done in order to monitor the pH, TTA and Microbial Count at 6hrs intervals during the fermentation process.

After fermentation, the fermented soymilk was then heated at 80°C for 4minutes and a suspension of 4.5g (2.75tsp) Magnesium Chloride in 20mL distilled water was added into the hot fermented soymilk. The mixture was stirred in a spiral direction and then left to coagulate for 10minutes. The coagulated curd was scooped carefully into a muslin cloth and then placed in a plastic mold perforated with holes. The muslin cloth was covered and a weight of 1kg was applied to the curd to press out the whey. This was done for about 15minutes until the whole whey was out of the curd. The curd was then transferred into distilled water for about 15minutes to make the Tofu firm. The block of Tofu formed was then cut into pieces, seasoned with spice and then fried using vegetable oil. The traditionally prepared Tofu was used as the control

Determination of Titratable Acidity

The amount of lactic acid produced in the fermenting soymilk was determined at 6, 12, and 18hrs for each fermenting samples over the period of fermentation using the standard procedure according to A.O.A.C. (1990). The supernatant obtained from the pH determination above was pipetted and titrated against 1N NaOH to phenolphthalein end point.

Each millilitre of 1N NaOH is equivalent to 90.08mg of lactic acid. A color change from colourless to pink indicated the end point.

Determination of pH

The change in pH of the fermenting soymilk was monitored hourly using a pH meter. Ten millilitres of fermenting soymilk were collected at 0hr, 6hr, 12hr, and 18hr into sterile bottles and mixed with 5 times volume of distilled water. The mixture was allowed to stand for 15 minutes, shaken at 5 minutes interval and filtered with Whatman No. 4 filter paper. The pH of the filtrate was measured using the pin electrode of pH and Conductivity Meter (Jenway 3540) (AOAC 1990).

Proximate Analysis of Tofu

Samples of the fermented tofu and traditional tofu were analyzed by the standard procedures as adopted by AOAC (1990) for moisture content, ash content, crude fibre content, ether extract content, crude protein content and the carbohydrate content was estimated by the difference in value obtained when all the chemical composition values were subtracted from 100%. All analysis was done in triplicates.

Sensory Evaluation of Tofu

The sensory evaluation of fermented tofu and traditional tofu samples was carried out to determine the acceptability of the products. The products were subjected to organoleptic assessment by a 15-member panel (post-graduate students who were familiar with the

food). Each panellist was requested to eat the food samples one after the other and to indicate their degree of likeness or preference for the samples on the questionnaires provided. The panellists were also asked to rinse their mouth with drinking water so as to get the assessment of each food sample eaten. The samples were evaluated based on colour, aroma, taste, mouth feel and overall acceptability.

They were required to score each parameter on a 9 point hedonic scale ranging from 9 indicating like extremely to 1 indicating dislike extremely (Larmond, 1977).

3. RESULTS AND DISCUSSION

Lactic acid fermentation has attracted increased attention with regards to the improvement of nutritional value of foods and overall product safety. Several studies have been done on fermented milk products especially those from the 'animal protein' origin (nono/nunu maziwala, iben, raib, garris) and the fermented soy products (tempeh, miso, and soy sauce) but there seems to be a dearth of information on the improvisation of tofu (a soybean curd) through fermentation

Fig 1 shows the percentage occurrence of LAB isolates from spontaneously fermented soymilk.

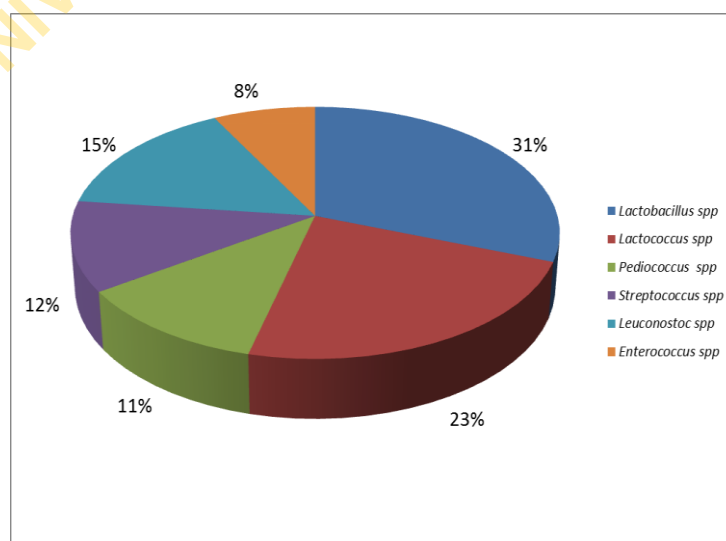


Figure 1. Percentage occurrence of lactic acid bacteria isolated from spontaneously fermented soy milk

The *Lactobacillus* spp had the highest percentage occurrence with 31% having 16 isolates; this is closely followed by *Lactococcus* spp having 23% with 12 isolates, *Pediococcus* spp having 15% with 8 isolates, *Streptococcus* spp and *Leuconostoc* spp both having 12% with 6 isolates and the lowest occurrence was found with the *Enterococcus* spp having 8% with 4 isolates. Soybean has been examined as a substrate for lactic acid bacteria (LAB) such as *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus reuteri* and *Bifidobacterium* (Garro *et al.*, 1999; Tzortis *et al.*, 2004).

In this study, the LAB isolates obtained from the spontaneous fermentation of soymilk include; *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Lactococcus lactis*, *Lactococcus cremoris*, *Lactobacillus casei*, *Lactobacillus curvatus*, *Pediococcus acidilactici*, *Lactobacillus delbrueckii*, *Streptococcus sp*, *Lactobacillus acidophilus* and *Enterococcus faecalis*.

The observed diversity of the LAB isolates with the dominance of *Lactobacillus* species is in agreement with the reports of Chumchueve and Robinson, (1999); Abdel-Moreim *et al.*(2006).

Fig 2 shows the change in total titratable acidity (TTA) of soymilk during the fermentation process. There was a general increase in the total titratable acidity as fermentation time increased. The highest TTA was produced at 18hours with the soymilk fermented with *Lactobacillus casei* SO36 (SYL₁) with the value 1.96% while the least TTA occurred at 0hour with all the samples having 0.74%.

Fig 3 shows the change in pH during the controlled fermentation of soymilk and from the graph, there was a general decrease in the pH value from 6.5 at fermentation time of 0hour to pH value of 4.8 at fermentation time of 18hours. The soymilk inoculated with single starter had a lower pH than the soymilk inoculated with combined starter. The starter culture composition and fermentation temperature could have influenced the pH and TTA of the fermenting soymilk as the physicochemical analysis revealed that a pH range of (6.5 to 4.8) and a TTA of (0.74 to 1.96) which is still within considerable limits when compared with the reports of Angeles and Martha (1971) and Opara *et al.* (2013).

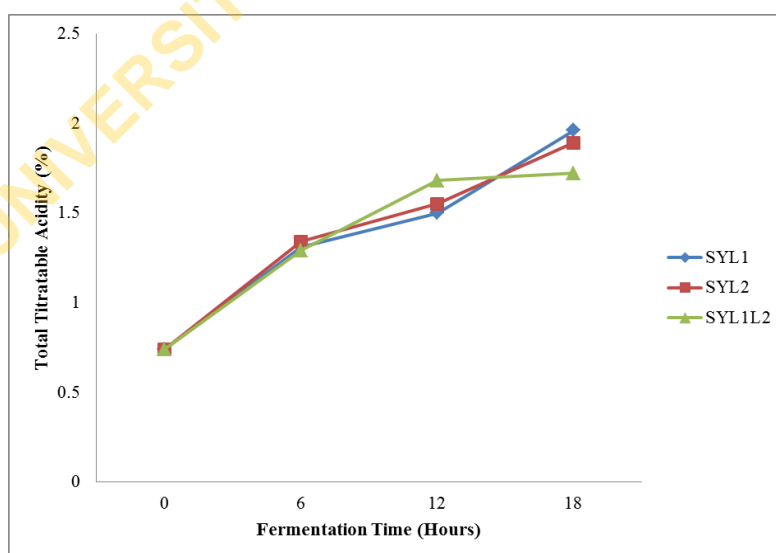


Figure 2. Change in titratable acidity of fermenting soymilk inoculated with single and combined starter of LAB species

SAMPLE CODE

SYL₁- Soymilk inoculated with single starter of *Lactobacillus casei* SO36

SYL₂- Soymilk inoculated with single starter of *Lactococcus lactis* SO51

SYL₁L₂- Soymilk inoculated with combined starters of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51

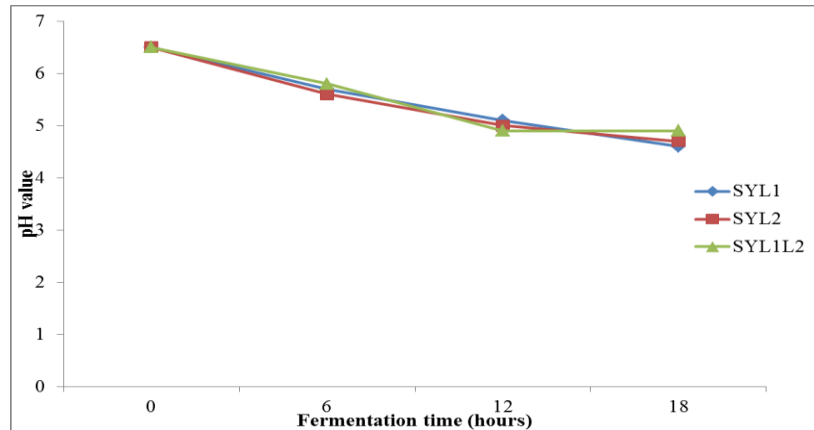


Figure 3. Change in pH of fermenting soymilk inoculated with single and combined starter of LAB species

SAMPLE CODE
 SYL₁- Soymilk inoculated with single starter of *Lactobacillus casei* SO36
 SYL₂- Soymilk inoculated with single starter of *Lactococcus lactis* SO51
 SYL₁L₂- Soymilk inoculated with combined starters of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51

Table 1. Anti-nutritional content of Fermented and Traditional Tofu

Sample Code	%Phytate Content	Lectin content (HU/mg)
BFTL ₁	*0.08±0.01 ^{b+}	22.17±0.02 ^d
BFTL ₂	0.10±0.00 ^{ab}	25.20±0.02 ^b
BFTL ₁ L ₂	0.11±0.00 ^a	29.77±0.02 ^a
BUT	0.09±0.09 ^b	23.67±0.04 ^c
FFTL ₁	0.03±0.00 ^e	11.24±0.02 ^h
FFTL ₂	0.07±0.00 ^c	20.74±0.02 ^e
FFTL ₁ L ₂	0.05±0.00 ^d	15.46±0.02 ^g
FUT	0.06±0.00 ^c	19.85±0.12 ^f

*Values are in means of determined parameters± standard deviation

+Means with different superscripts along the same column are significantly different at P≤0.05 using Duncan's Multiple Range Test

SAMPLE CODE

BFTL₁- Boiled Tofu fermented with single starter of *Lactobacillus casei* SO36
 BFTL₂- Boiled Tofu fermented with single starter of *Lactococcus lactis* SO51
 BFTL₁L₂- Boiled Tofu Fermented with combined starter of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51
 BUT- Boiled Unfermented/ Traditional Tofu
 FFTL₁- Fried Tofu fermented with single starter of *Lactobacillus casei* SO36
 FFTL₂- Fried Tofu fermented with single starter of *Lactococcus lactis* SO51
 FFTL₁L₂- Fried Tofu Fermented with combined starter of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51
 FUT- Fried Unfermented /Traditional Tofu

Table 1 shows the anti-nutritional content of fermented and traditional tofu. The tofu samples were analyzed for phytate and lectin (Haemagglutinin) content present in them. The table revealed that the phytate content of the fried samples were lower than that of the boiled samples. FFTL₁ had the lowest phytate content with a mean value of 0.03% and FFTL₂ had the highest phytate content having a mean value of 0.07% among the fried samples. For the boiled samples, BFTL₁ had the lowest phytate content

with 0.08% mean value while BFTL₁L₂ had the highest phytate content with a mean value of 0.11%.

The table also revealed the fried samples had lower lectin content compared to that of the boiled samples. The highest lectin content among the boiled samples was found with BFTL₁L₂ with a mean value of 29.77 HU/mg and the lowest was that of BFTL₁ with a mean value of 22.17HU/mg while among the fried samples the lowest was found with FFTL₁ with

a mean value of 11.24HU/mg and the highest was FF_{TL}₂ with a mean value of 20.74HU/mg. Table 2 shows the proximate analysis fermented Tofu and traditional tofu. The food sample was done in two different categories and these include the boiled category and then the fried category. Within the boiled group, fermented tofu using combined starter *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51 (BF_{TL}_{1L}₂) had the highest moisture content with the mean value of 73.99% while the boiled fermented tofu using *Lactobacillus casei* SO36 (BF_{TL}₁) had the lowest moisture content with the mean value of 71.87%. Within the fried group, fried fermented tofu using combined starter (FF_{TL}_{1L}₂) had the least moisture content with the mean value of 52.74% while that fermented with *Lactococcus lactis* SO51 (FF_{TL}₂) had the highest moisture content with the mean value of 72.99%.

For crude protein, within the boiled group, BF_{TL}₁ had the highest crude protein content with the mean value of 19.33% while the lowest was that of unfermented/traditional tofu (BUT) with the mean value of 17.16%. For the fried groups, FF_{TL}_{1L}₂ had the highest crude protein content with the mean value of 24.81% while FF_{TL}₁ had the lowest moisture content

having mean value of 23.06%. The fried samples had higher protein content than the boiled samples.

The crude fat results showed that the fried samples had higher fat contents than the boiled samples. Within the boiled group, BF_{TL}₁ had the highest fat content having a mean value of 2.30% while BUT had the lowest with a mean value of 0.88%, while within the fried group, FF_{TL}₂ had the highest fat content with a mean value of 11.02% and FUT had the lowest with a mean value of 7.91%

The table also showed that the ash content of the boiled traditional tofu (BUT) was the highest within the boiled group with a mean value of 1.13% while that of BF_{TL}₁ was the lowest with a mean value of 0.32%.

Among the fried samples, FUT and FF_{TL}_{1L}₂ had the same ash content being the highest with a mean value of 1.58% while FF_{TL}₁ had the lowest ash content with the mean value of 0.63%. For the carbohydrate content, BUT had the highest carbohydrate content with a mean value of 6.85% and BF_{TL}₂ had the lowest carbohydrate content with a mean value of 4.63% while FUT had the highest carbohydrate content among the fried samples with the mean value of 11.66% and the least which was FF_{TL}₂ had no carbohydrate content at all.

Table 2. Proximate Analysis of Fermented and Traditional Tofu

Sample Code	%Moisture Content	%Crude Protein	%Crude Fat	%Ash Content	%CHO Content	Energy Density(Kcal)
BF _{TL} ₁	*71.87±0.30 ^{c+}	19.33±0.13 ^c	2.30±0.02 ^e	0.32±0.01 ^g	6.17±0.10 ^c	122.76±0.12 ^e
BF _{TL} ₂	73.84±0.13 ^a	19.29±0.05 ^e	1.82±0.04 ^f	0.42±0.01 ^f	4.63±0.18 ^f	112.05±0.48 ^f
BF _{TL} _{1L} ₂	73.99±0.11 ^a	17.58±0.13 ^f	1.32±0.03 ^g	0.93±0.04 ^c	6.18±0.15 ^e	106.90±0.43 ^g
BUT	73.97±0.09 ^a	17.16±0.08 ^g	0.88±0.02 ^h	1.13±0.01 ^b	6.85±0.15 ^d	103.97±0.27 ^h
FF _{TL} ₁	60.75±0.12 ^d	23.06±0.12 ^d	7.91±0.02 ^d	0.63±0.01 ^e	7.65±0.13 ^c	194.02±0.46 ^c
FF _{TL} ₂	72.29±0.05 ^b	23.57±0.13 ^b	9.32±0.02 ^c	0.78±0.03 ^d	0.00±0.00 ^g	178.12±0.33 ^d
FF _{TL} _{1L} ₂	52.74±0.08 ^f	24.81±0.13 ^a	11.02±0.05 ^a	1.58±0.01 ^a	9.84±0.22 ^b	237.81±0.44 ^a
FUT	53.27±0.06 ^e	23.27±0.13 ^c	10.22±0.03 ^b	1.58±0.03 ^a	11.66±0.14 ^a	231.70±0.23 ^b

*Values are in means of determined parameters± standard deviation

+Means with different superscripts along the same column are significantly different at P≤0.05 using Duncan's Multiple Range Test

SAMPLE CODE

BF_{TL}₁- Boiled Tofu fermented with single starter of *Lactobacillus casei* SO36

BF_{TL}₂- Boiled Tofu fermented with single starter of *Lactococcus lactis* SO51

BF_{TL}_{1L}₂- Boiled Tofu Fermented with combined starter of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51

BUT- Boiled Unfermented/ Traditional Tofu

FF_{TL}₁- Fried Tofu fermented with single starter of *Lactobacillus casei* SO36

FF_{TL}₂- Fried Tofu fermented with single starter of *Lactococcus lactis* SO51

FF_{TL}_{1L}₂- Fried Tofu Fermented with combined starter of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51

FUT- Fried Unfermented /Traditional Tofu

The gross energy density among the boiled group showed that BFTL₁ had the highest mean value of 122.76Kcal while BUT was least with mean value of 103.97Kcal. Among the fried samples, FF₁L₂ had the highest gross energy density with a mean value of 237.81Kcal and the least was recorded with FF₂L₂ having a mean value of 178.12Kcal.

The protein content of the fermented and unfermented soymilk to be used in the production of improved tofu was in contrast to the reports of Amaze and Amaze (2011) as well as that of Odu *et al.* (2012). The protein content in this study ranged from 2.67-4.06% for the four treatments used while those of the findings of the researchers ranged from 2.02-2.81% respectively. The differences in the protein content could probably be attributed to the processing treatments used.

Scientific studies have reported that during fermentation, carbohydrates are mobilized to synthesize amino acids for the proliferation of microbes and this could have accounted for the increased protein content and reduced carbohydrate content in the fermented soymilk. However, the moisture content alongside with crude fat and ash contents could be favourably compared to other studies.

The anti-nutritional contents of the eight tofu samples in this study were very low and this may not be far-fetched from the processing variables used. It is evident that anti-nutrients could have adverse and beneficial effects in humans (Soladoye and chukwuma, 2012). Phytate represents a complex class of naturally occurring phosphorus compounds that can significantly influence the functional and nutritional properties of foods (Haug and Lantzch, 1983).

Lectins are glycoproteins noted for their capability to agglutinate erythrocytes and bind sugar components. The results showed that the levels of phytate and lectins were relatively lower than standard ranges of soy finished products. This also relatively indicates that the heat treatment given to the tofu samples was very effective. The proximate components of the improved tofu produced under controlled fermentation showed significant improvement

when compared to the control products (BUT and FUT) in terms of protein contents. Reasons for this might have been as a result of the usage of defined starter cultures. It was also observed that the fried samples (FF₁L₂) with combined starter (*Lactobacillus casei* SO36 and *Lactococcus lactis* SO51) had the highest proximate content in terms of crude protein and crude fat. Although frying has little or no impact on the protein or mineral content of foods (Fillion and Henry, 1998), Udofia and Obizoba (2005) reported that the lower the moisture contents of a food, the higher the nutrient density. This probably explains the higher values of the crude protein and fat content as the fried samples had lesser moisture content when compared to the boiled samples.

Table 3 shows the sensory evaluation of both fermented and unfermented Tofu.

From the table, the boiled unfermented tofu (BUT) was rated least in terms of colour (6.07), Aroma (4.93), Taste (4.73) and mouth feel (4.80) while BFTL₁L₂ was rated least in terms of the overall acceptability (4.93).

The fried unfermented tofu (FUT) was rated the best in terms of all the parameters given. Among the boiled samples, BFTL₁ was rated the best in all the parameter used also. For the sensory evaluation, there were significant differences amongst the tofu samples in terms of colour, aroma, taste, mouthfeel and overall acceptability indicating the products' wholesomeness. The boiled unfermented tofu sample (BUT) had the least mean score for all the organoleptic parameters assessed.

The fried unfermented (FUT) tofu recorded the highest mean score for all the parameters and this was closely followed by the fried fermented tofu (FF₁L₂) with combined starters. The frying treatment could probably have enhanced the sensory quality attributes of the control sample which made it to be more preferable to the rest of the tofu products.

The pH of the soymilk used in the production of the tofu might also have affected the gustatory neurons in the taste buds (of the participants) as suggested by Yangang and Wenyii (2009).

Table 3. Sensory evaluation of fermented and Traditional Tofu

Sample	Colour	Aroma	Taste	Mouth feel	Overall acceptability
BF_{TL1}	*6.87 ± 0.99 ^{bc+}	6.33 ± 1.11 ^{bc}	7.13 ± 0.92 ^{ab}	6.33 ± 1.18 ^{bc}	6.73 ± 0.88 ^b
BF_{TL2}	6.20 ± 1.08 ^{cd}	5.73 ± 1.10 ^{cd}	5.47 ± 1.19 ^{cd}	5.60 ± 1.06 ^{cd}	5.53 ± 0.92 ^c
BF_{TL1L2}	6.80 ± 0.94 ^{cd}	5.87 ± 1.13 ^{cd}	6.33 ± 1.23 ^{bc}	6.20 ± 1.37 ^{bc}	4.93 ± 1.67 ^c
BUT	6.07 ± 1.99 ^d	4.93 ± 1.94 ^d	4.73 ± 1.53 ^d	4.80 ± 1.66 ^d	6.33 ± 1.23 ^b
FF_{TL1}	6.47 ± 1.06 ^c	5.93 ± 1.22 ^c	6.27 ± 1.34 ^{bc}	6.20 ± 1.32 ^{bc}	6.67 ± 1.18 ^b
FF_{TL2}	7.27 ± 0.88 ^{abc}	6.67 ± 1.11 ^{abc}	6.47 ± 1.41 ^b	6.53 ± 1.41 ^{bc}	6.93 ± 0.88 ^b
FF_{TL1L2}	7.33 ± 0.98 ^{ab}	7.00 ± 0.85 ^{ab}	6.53 ± 1.19 ^b	6.93 ± 1.03 ^{ab}	6.93 ± 0.88 ^b
FUT	7.73 ± 0.8 ^a	7.60 ± 1.12 ^a	7.73 ± 0.96 ^a	7.80 ± 0.86 ^a	7.93 ± 0.88 ^a

*Values are in means of determined parameters ± standard deviation

+Means with different superscripts along the same column are significantly different at P≤0.05 using Duncan's Multiple Range Test

SAMPLE CODE

BF_{TL1}- Boiled Tofu fermented with single starter of *Lactobacillus casei* SO36

BF_{TL2}- Boiled Tofu fermented with single starter of *Lactococcus lactis* SO51

BF_{TL1L2}- Boiled Tofu Fermented with combined starter of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51

BUT- Boiled Unfermented/ Traditional Tofu

FF_{TL1}- Fried Tofu fermented with single starter of *Lactobacillus casei* SO36

FF_{TL2}- Fried Tofu fermented with single starter of *Lactococcus lactis* SO51

FF_{TL1L2}- Fried Tofu Fermented with combined starter of *Lactobacillus casei* SO36 and *Lactococcus lactis* SO51

FUT- Fried Unfermented /Traditional Tofu

4. CONCLUSIONS

In conclusion, fermentation processes has been known to confer advantageous effect on food and this was revealed in this study. This study provided a new variety of food through fermentation that has higher nutritional quality and an increased longevity. Lactic acid bacteria used as Starters for the fermented food (tofu) were able to enhance the foods quality and consistency. However, there is a need to still explore other Lactic Acid Bacteria with higher potential that are highly compatible for usage as starter culture. Also, optimization of the production process should also be looked into in order to reduce nutrient loss through processing.

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