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MICROBIOLOGICAL QUALITY ASSESSMENT OF FERMENTED SOY DRINK FROM TAMARIND AND NONO

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ABSTRACT

Microbiological quality of fermented soy drink with isolated lactic acid bacteria (LAB) from tamarind was evaluated. Soymilk was extracted from milled whole soybean grains, sieved and allowed to stay before subjecting it to pasteurization at76°C for 30 minutes and were thereafter divided into two: one portion inoculated with tamarind pulp juice containing 5.3×10³

INTRODUCTION

Soymilk is an aqueous extract of soy beans (*Glycine max*) (Agure-Dam, 1997). It is quite similar in appearance to cow milk. It is commonly characterized as having a beany, grassy or soy flavour, which reportedly can be improved by lactic acid fermentation, as in yoghurt-like products (Jimoh and Kolapo, 2007). However, there are two basic types of soy yoghurt: fermented and non-fermented. The fermented type is usually made from soymilk in the same way as typical dairy yoghurt with inoculation of yoghurt starter to soymilk (Rezvan *et al.,* 2012). Microorganisms possess endogenous β-glucosidases which can be utilized to hydrolyze predominant isoflavone-glucosides in soymilk to improve its biological properties (Bordignon *et al*., 2004; Chien *et al*., 2006).

Due to the worldwide shortage of food, attempts have been made to find alternative sources of protein, particularly for the developing countries, where

cfu/mL and the other with nono containing 11.6×10³ cfu/mL. They were incubated at 42°C for 12 hours, and thereafter the products obtained were subjected to microbial analysis using the standard method. The Lactic Acid Bacteria isolated was characterized and identified using molecular techniques. Second generation fermented soy drink (A2) was produced using the back slopping method from the first generation fermented soy drink (A).The total viable count in fermented soy drink with tamarind (A), fermented soy drink with nono bought from Fulani woman hawking (B) and commercial yoghurt (C) ranged from 2.67×10³ – 8.7×10³ cfu/mL while coliform and fungal counts for B were 1.0×10³ cfu/mL and 3.7×10³ cfu/mL respectively. Neither coliforms nor fungal species were isolated in A and C. Sample A2 had lower bacterial count of 2.0×10³ cfu/mL with no coliform and fungal growth. Species of Lactobacillus, Bacillus, Staphylococcus and Pseudomonas were isolated from the samples. Molecular identification of the isolates revealed coded strains of Lactobacillus. plantarumVJC38 16S, Lactobacillus plantarumM10, Lactobacillus pentosusZU 22 and PediococcuspentosaceusJN 1. Appropriate aseptic and HACCP techniques could significantly improve the microbiological quality of the fermented drink produced using isolates from tamarind.

Keywords: Bacteria, Fermentation, Lactobacillus, Soy, Tamarind.

malnutrition exists (Raja *et al*., 2014). As a result, shifts from animal to vegetable sources of protein have increased significantly in which soy beans is a potential candidate (Abd El- Gawad *et al*., 2015). Several reports indicate that some probiotic bacteria could compete with yoghurt cultures in a soy-based substrate (Tzortzis *et al*., 2004). Soy has been examined as a substrate for the *Lactobacillus* species: *Lactobacillus casei, Lactobacillus helveticus, Lactobacillus fermenti, Lactobacillus fermentum*and *Lactobacillus reuteri* (Garro *et al*., 2004). Documented information indicates that soymilk has a significant amount of raffinose and stachyose but does not contain lactose and that lactic acid bacteria (LAB) from different sources are quite different in their efficiencies in soy yoghurt fermentation (Tuitemwong and Tuitemwong, 2003).

The inefficiency of lactic acid bacteria (LAB) to ferment soy milk highlights the need for research on new LAB species as starter culture from indigenous fruits

that have less degradation effect on milk-derived peptides for the production of soy yoghurt at lower cost and that will also mask the beany flavour of soymilk. Tamarind (*Tamarindusindica*) is a leguminous tree in the family Fabaceae and is indigenous to tropical Africa. The genus *Tamarindus* is a monotypic taxon, having only a single species. Tamarind is better known for the pod pulp (40%) which has a sweet-sour flavor. The pods are fed to livestock, and the pulp within the pods is used to make beverages, curries, chutneys and sauces. It is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008), either nutritional or medicinal (Nyadoi and Abdullah, 2004;Abubakar *et al.*, 2008). Soy milk, like dairy milk, is susceptible to microbial spoilage, with short shelf life and it is not acceptable by many individuals due to its beany flavour. It is perceived that tamarind may mask the undesirable flavour of fermented soy drink which reduces its acceptability among people and may prevent degradation of useful nutrients in fermented soy drinks. Therefore, this research focused on the assessment of the microbiological quality of fermented soy drinks produced from tamarind.

MATERIALS AND METHODS

Collection of raw materials

Soybean seeds, commercially available starter cultures (*nono*) and tamarind fruits were purchased from Bosso market in Minna, Niger State Nigeria. One kilogram (1kg) of Soybean seeds and 50kg of Tamarind were transported in sterile sampling bags and *nono* was collected in sterile sampling bottles and immediately transported in ice packed box at temperature of 10°C to the laboratory of the Department of Microbiology, Federal University of Technology, Minna, Nigeria for analysis.

Microbiological examination of tamarind and fermented soy drink

Tamarind pulp and fermented soy drink samples were examined for the viable count of bacteria, enteric bacteria, possible lactic acid bacteria, and fungi using Nutrient agar, MacConkey agar, Lactic acid bacteria agar, De Mann Rogosa Sharpe agar, M17 agar, and Sabouraud Dextrose agar, respectively.The pour plate method of Cheesbrough (2000) was used for the microbial count. Serial dilution to 10^{-3} was carried out on tamarind pulp and fermented soy drink using normal saline and 1 ml of 10^{-3} diluent was transferred unto petri dishes. Nutrient agar and MacConkey

agar were added into separate plates, swirled gently and allowed to solidify and incubated at 37°C for 48 hours. The numbers of colonies were counted on the plates taking into consideration the dilution factor to obtain the total viable count using pour plate method. While yeasts and moulds were determined by inoculating aliquot of 1ml of the sample on Sabouraud Dextrose agar, the plates were incubated at 25°C for 72 hours. The number of colonies were counted and expressed as colony forming units per gram (cfu/g) for tamarind pulp and colony forming unit per milliliter (cfu/mL) for fermented soy drink.

Isolation of lactic acid bacteria

Lactic acid bacteria were isolated from tamarind and fermented soy drink. One gram (1g) of powdered tamarind was dissolved in 10mLdistilled water and filtered to obtain a clear solution. An aliquot of 1mL of the tamarind solution was taken as stock for serial dilution to obtain diluents. An aliquot of 1mL from 10^{-3} diluent was dispensed into petri dishes for pour plating. Selective media such as lactic acid bacteria agar (LABA), De Mann Rogosa Sharpe (MRS) agar and M17 agar were added into the plates separately, swirled, allowed to solidify and incubated at 37°C for 48 hours, MRS plates were incubated anaerobically. The isolated bacteria were sub cultured on media repeatedly after which it was used for primary isolation.

Characterization and Identification of Microbial Isolates

The bacteria isolates were characterized using colonial morphology, Gram staining and biochemical tests. The biochemical tests conducted include catalase, citrate, spore forming and sugar fermentation profiles. The isolates were identified by comparing their characteristics with those of known taxa using Bergey's Manual of Determinative Bacteriology (Whitman *et al.,* 2012).

Molecular identification and characterization of LAB isolated from fermented soy drink and tamarind

DNA extraction

DNA was extracted using the protocol stated by Trindade*et al*, (2007). Single colonies grown on the medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 hours at 28 °C. Thereafter, cultures were centrifuged at 4600x g for 5 minutes. The resulting pellets were re-suspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20%

SDS and 3 µl of Proteinase K (20 mg/ml) were added. The mixture was incubated for 1 hour at 37 °C, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and mixed. The suspension was incubated for 10 minutes at 65 °C and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugation at 7200 x g for 20 minutes. The aqueous phase was transferred to a new tube, isopropanol (1: 0.6) was added and DNA was precipitated at –20 °C for 16 hour. DNA was collected by centrifugation at 7200 x g for 10 minutes, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

16S Ribosomal (rRNA) gene amplification

Polymerase chain reaction cocktail consisted of 10 µL of 5x GoTaqcolourless reaction, 3 µL of $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F (3' AGAGTTTGATCCTGGCTAG 5') and - 1525R, (3' AGAAAGGAGGTGATCCAGCC 5') Primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8µL DNA template. Polymerase chain reaction carried out in a GeneAmp 9700 PCR System Thermocycler (Applied Biosystem Inc., USA) at adjusted temperatures for different time intervals showed an initial denaturation at 94 °C for about 5 minutes (which is 30 cycles at 94 °C for 30 seconds), then adjusted to 50 °C for 60 seconds and 72 °C for 1 minute 30 seconds. The time was finally extended to 10 minutes at 72 °C, it was then chilled at 4 °C in a GEL(Wawrik*et al.*, 2005).

Amplification

The integrity of the amplified (about 1.5Mb) gene fragment was checked on a 1% Agarose gel ran to confirm amplification. This was done by mixing 8µl of amplified product to 4µl of loading dye and ran on the solidified Agarose gel at 110V for about 1 hour. The picture was taken under UV light. Also the amplified product was checked on a nanodrop of model 2000 from thermo scientific to quantify the concentration of the amplified product.

Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were

added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, thoroughly mixed by vortexing and kept at -20°C for at least 30 minutes. It was then centrifuged for 10 minutes at 13000 rpm and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet was washed by adding 150 µl of 70% ethanol and mixed then centrifuged for 15 minutes at 7500 rpm and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on tissue paper and let it dry in the fume hood at room temperature for 10-15 minutes. Then it was re-suspended with 20 µl of sterile distilled water and kept at -20°C before sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1 hour as previous, to confirm the presence of the purified product.

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 was used for all the genetic analysis.

Statistical Analysis

Data obtained in this study were analyzed using statistical package for social science (SAS) version 9.4 and presented as means ± standard error of the mean. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to determine the significant differences between the fermented soy drink samples.

RESULTS

Comparative analysis of microbiology of tamarind pulp, fermented soy drink and commercial yoghurt

Table 1 shows the microbial count of tamarind, soy drink fermented with tamarind, A2 and *nono.* There was no coliform count for tamarind pulp but the bacterial count was 8×10^3cfu/g and fungal count 10×10^3cfu/g. The soy yoghurt sample A had a higher bacterial count of 8.7×10^3cfu/mL than sample B 4.3×10^3cfu/mL while sample C had the least bacterial count of 2.7×10^3 cfu/mL. There were no coliform and fungal counts in samples A and C, however, sample B had coliform count (1.0×10^3cfu/mL) and fungal count (3.7×10^3cfu/mL). The bacterial count 8.7×10^3

cfu/mL in sample A was higher than A2 (2.0×10^3 cfu/mL). Although, there were no coliform and fungal growth on A, A2 and sample C

Table 1: Total microbial count for tamarind pulp, fermented soy drink and commercial yoghurt

Sample	Microbial count (cfu/mL)		
	Bacteria	Coliform	Fungal
A	8.7×10^3	0.0×10^3	0.0×10^3
A2	2.0×10^3	0.0×10^3	0.0×10^3
B	4.3×10^3	1.0×10^3	3.7×10^3
C	2.7×10^3	0.0×10^3	0.0×10^3
D	8.0×10^3	0.0×10^3	10×10^3

A: fermented soy drink with tamarind, A2 second generation fermented soy drink with tamarind, B: fermented soy drink with *nono;* C- Commercial yoghurt, D: Tamarind Pulp

Biochemical identity of bacterial isolates from tamarind pulp, fermented soy drinkand *nono*

The results of microscopic and biochemical identification of bacterial isolates from fermented soy drink and tamarind are shown in Table 2. Five genera of bacteria which are *Bacillus, Lactobacillus, Pediococcus, Pseudomonas* and *Staphylococcus* were identified fromfermented soy drink, *nono* and tamarind pulp.

Table 2: Identity of lactic acid bacteria from fermented soy drink and tamarind

S/N	Grmrxn	Shp	Cat	Oxi	Cit	VP	Mot	Ind	MR	S/F	Glu	Lac	Suc	Fru	Ara	Man	Sor	Suspected organism
1	+	R	+	-	+	-	+	-	-	+	AG	AG	AG	AG	G	AG	AG	*Bacillus* spp
2	-	R	+	+	+	+	+	-	-	-	AG	AG	AG	AG	G	AG	AG	*Psuedomonas*spp
3	+	C	+	-	+	+	+	-	+	-	AG	G	ND	ND	ND	ND	ND	*Staphylococcus* spp
4	+	R	+	-	-	-	-	-	-	-	AG	G	AG	AG	G	AG	G	*Lactobacillus* spp

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5	+	T	+	-	-	-	-	-	-	-	AG	G	AG	AG	G	AG	AG	*Pediococcus*spp
6	+	R	+	-	-	-	-	-	-	-	AG	G	AG	A	G	AG	AG	*Lactobacillus* spp
7	+	R	+	-	-	-	-	-	-	-	AG	AG	AG	AG	G	AG	AG	*Lactobacillus* spp

Grmrxn- Gram reaction Shp- shape Cat- catalase Oxi- oxidase Cit- citrateVP- VogesProskauerMot- Motility Ind- IndoleMR- Methyl red SF- spore formation Glu- glucose Lac- lactose Suc- sucrose Fru- fructose Ara- arabinose Man- mannose Sor- sorbitol AG- acid and gas production ND- not determined
R- rod C- Cocci T- Tetrad

Molecular characteristics of the lactic acid bacteria isolated from fermented soy drink and the tamarind fruit pulp.

The result (S- T) show the sequenced amplicons of LAB isolated from fermented soy drink with tamarind and tamarind fruit pulp as the resulting alignment of the series of nucleotide (from 5' 3' and 3' 5') with other known sequences previously blasted in Ibadan Institute of Training on Agriculture (IITA) data base. The gel documented images of the isolated bacterial DNA after electrophoresis appeared at 1500kb which indicated pure isolates. Lane Mk represents molecular marker (ladder), lane A1 to S3 (T1) represents DNA extracted from soy drink fermented with tamarind and lane T5 DNA extracted from isolate of tamarind pulp as shown in Plate I.

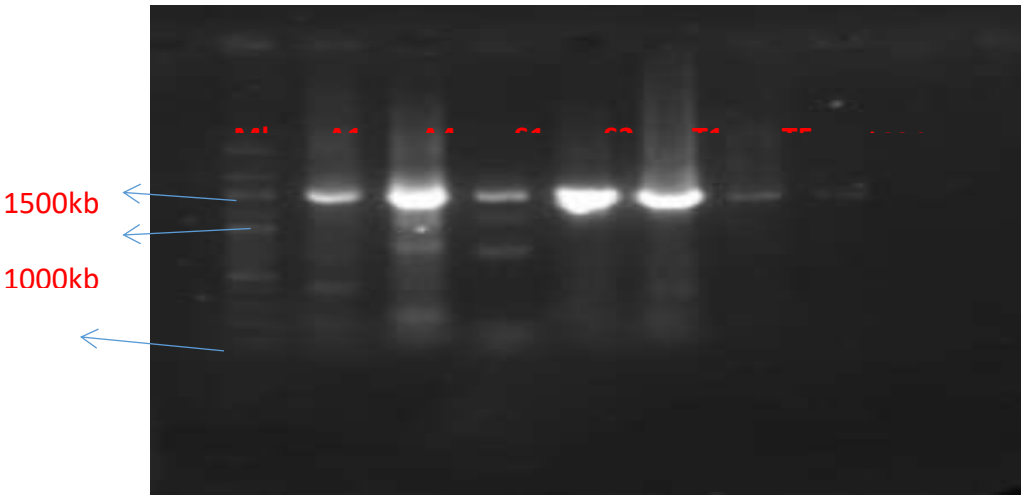

Plate I: Amplified image of the LAB isolates from fermented soy drink and the tamarind pulp

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A4/ S1: *Lactobacillus pentosus* strain: ZU 22, S2: *Pediococcuspentosaceus* strain JN1, S3: *Lactobacillus plantarum* strain M10 T5/A1: *Lactobacillus plantarum* strain VJC38

DISCUSSION

In the present study, a variety of microorganisms were isolated from the tamarind and fermented soy drink samples which are pathogenic as well as non-pathogenic in nature. Tamarind and fermented soy drink were examined for the presence of microorganisms.

The total bacterial count obtained from the samples were 8×10^3cfu/g, 8.7×10^3cfu/mL, 4.3×10^3cfu/mL, 2.0×10^3 cfu/mL and 2.7×10^3 cfu/mL, for tamarind (*Tamarindus Indica*), fermented soy drink tamarind, second generation fermented soy drink with tamarind and fermented soy drink with *nono* respectively. The total bacterial count (10^3cfu/mL/g) for all the samples were within the acceptable limit (4.0×10^3- 5.0×10^4cfu/g) recommended by International Commission on Microbiological Specifications for Food ICMSF(1986). This count was also lower than the total bacterial count of (10^5- 10^6cfu/mL) observed in cow-camel milk yoghurt by Shori (2013). The bacterial load in tamarind and *nono* observed in this study might be related to contamination from the soil, open air, uncleaned utensils or handlers. These microorganisms could have been introduced either in the pre or post processing stages (Omotosho *et al.,* 2013). There was no coliform count observed for fermented soy drink with tamarind and the commercial yoghurt when compared with 1.0×10^2 cfu/mL standard recommended by NAFDAC (2009) for yoghurt. Shehu and Adesiyun (1990) obtained coliform count of (3.0×10^4 cfu/mL)for *fura* da *nono* higher than the result obtained in this study.

The coliform count obtained for *nono* in this study (1.0×10^3cfu/mL) was lower when compared to the result obtained by Abdulkadir and Mugadi (2012) (4.0 x 10^4cfu/mL) for *fura* da *nono*. Okonkwo (2011) for *nono*. (2.21×10^4 cfu/mL) and Okeke *et al*. (2012) coliform count of (6.0×10^4 cfu/mL). The presence of coliform in *nono* might be from unclean water or improper hygienic practices. Exposure to unhygienic sanitary conditions during and after processing and also handling techniques usually associated with milking may be possible sources of contamination (Abdulkadir and Mugadi, 2012).

The fungal count (3.7×10^3 cfu/mL) obtained for *nono* was high in the current study when compared with the result obtained by Omotosho *et al.* (2013) (2.0×10^3 cfu/mL) and (5.1×10^4 cfu/mL) obtained by Okeke *et al*. (2012). The high fungal count

obtained for *nono* could be related to the milking. This is not unexpected as the milking is done in a dusty environment contaminated through the spores in the dust and air (Omotosho *et al.,* 2013).

In the present study, pathogenic and non -pathogenic bacteria were isolated in the samples. Pathogenic bacteria contribute to globally important diseases such as food borne illness which can be caused by species of *Brucella, Listeria* and *Salmonella* (Ijah *et al*., 2002). Bacterial species such as *Bacillus* spp and *Pseudomonas* spp are often introduced from the soil and are not completely inactivated despite the heat applied and thus could cause spoilage or health risk if favourable condition is restored for their germination and growth (Ruangwittayanusorn *et al.,* 2016). *Bacillus* spp are Gram positive spore formers and most members of the genus are saprophytic prevalent in air and on vegetation while genus *Pseudomonas* belongs to a group of Gram negative non spore formers and are predominantly found in various natural environments, such as on plants and animals (Ijah *et al*., 2002; Nebedum and Obiakor, 2007).

Staphylococcus spp is pathogenic, poisonous microorganisms and have been isolated from *nono*. They are also part of the normal human flora, usually found on the skin, nose and throat (Pelczar *et al.,* 2003). The presence of *Staphylococcus* spp in *nono* indicates contamination from handlers. According to Omotosho *et al*. (2013), the organism can be passed on to food during processing. Its high occurrence in *nono* may be due to poor hygiene practices of the food handlers during milking. Furthermore, lack of proper handling and processing of fruits particularly banana in flavouring of the fermented drink may contaminate the drink with species of *Staphylococcus* (Farinde *et al.* 2010).

In the present study, Lactic acid bacteria such as genus of *Lactobacillus* and *Pediococcus* were identified in tamarind and fermented soy drink. These microorganisms are considered as commercial probiotics for the fermentation of soy milk to fermented soy drink. Parvez *et al.* (2006); Carol and Leon, (2010); Khalid (2011); Lee *et al*. (2015) reported similar observation. According to Carol and Leon (2010); and Khalid (2011), these groups of microorganisms are Gram-positive, non sporulating, non-respiring cocci or rods, which ferment of carbohydrates, thus producing lactic acid as their major end product. Lactic acid bacteria present in various dairy products are involved in many diverse processes such as milk protein coagulation, acceleration of acidification, production of proteinase,

exopolysaccharides, aroma, and providing health-promoting properties (Lee *et al*., 2015).

In the current research, molecular characterisation using 16s rRNA confirmed the isolates as coded strains of *Lactobacillus pentosus*: ZU 22, *Pediococcuspentosaceus* strain JN1, *Lactobacillus plantarum* strain M10 and *Lactobacillus plantarum* VJC38 16S respectively. This result is similar to the result obtained by Rezvan *et al*. (2012) for the identification of *Bifidobacteriumlactis*B-12, *Bifidobacteriumlactis*B-94. The direction of bands migrated from negative to positive electrodes due to the natural occurrence of negative charge carried by their sugar-phosphate backbone (Frank *et al*., 2008).

Conclusion

The inoculum size (5.3×10^3cfu/mL) of lactic acid bacteria in tamarind pulp fermented soy milk to soy drink. Bacterial isolates responsible for the fermentation of the soy milk to soy drink were strains of *L. plantarum*VJC38 16S, *L. plantarum*M10, *L. pentosus*ZU 22and*Pediococcus. pentosaceus*JN 1. These strains grew and fermented soymilk by utilizing the available sugar in the absence of lactose in the soy milk.

S1 99% identical to *Lactobacillus pentosus* strain: ZU 22

CAGGCACAGGACGAACGTGCGGCAGCTATAATGCAAGTCGACGAACTCTGGTATTGAT
TGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGG
AAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAA
CTTGGACCGCATGGTCCGAGTTTGAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGC
GGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCT
GAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAA
GAAGGGTTTCGGCTCGTTAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGT
TCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG
TAATACGTAGGTGGCAAGCGTTGTCCGGATTTTATTGGGCGTAAAACGAGCGCAGGCG
GTTTTTTAAGTTTGATGTGAAAGCCTTCGGCTCAACCCGAAGAAGTGCATCGGAAACTG
GGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGGAAATGCGTAG
ATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGG
TCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATT
CCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCAC

AAGCGGTGGAGCATGTGGTTTAATTTGAAGCACGCGAAGAACCTTACCAGGTCTTGACA
TACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCA
TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
ATATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGT
GCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAG
CCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTA
ATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCATGAGAGTTTGTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAGCCGCC
TAAGTGAGCCGTTGATTAGGGTGAAGTCGTAACAAAGTCACCT

S2 99% IDENTICAL TO *Pediococcus pentosaceus* strain JN1
TTAGGGGTCTACGTATCGACAGCTACACTGCAGTCGTACGAACTTCCGTGTAATTGATT
ATGACGTACTTGTACTGATTGAGATTTTATCACGAAGTGAGTGGCGAACGGGTGAGTAA
CACGTGGGTAACCTGCCCAGAAGTAGGGGATAACACCTGGAAACAGATGCTAATACCG
TATAACAGAGAAAACCGCATGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTTCTGGAT
GGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCAGTGATACGT
AGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGC
GTGAGTGAAGAAGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTAAG
AGTAACTGTTTACCCAGTGACGGTTTAAACCAGAAAGCCACGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGC
GCAGGCGGTCTTTTAAGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGG
AAACTGGGAGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAAT
GCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACG
CTGAGGCTCGAAAAGCAGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT
AAACGATGATTACTAAGTGTTGGAGGGTTCCGCCCTTCAGTGCTGCAGCTAACGCATTA
AGTAATCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAAGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTATTTCGAAGCTACGCGAAGAACCTTACCAGGT
CTTGACATCTTCTGACAGTCTAAGAGATTAGAGGTTCCCTTCGGGGACAGAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGA
CAAACCGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGGGCTA
CACACGTGCTACAATGGATGGTACAACGAGTCGCGAAACCGCGAGGTTAAGCTAATCT
CTTAAAACCATTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATC

GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGGGGTAACCTGTAGGAGCTA
GCCGTCTAAAGGTGGACCCGG

SAMPLE T1 *Lactobacillus plantarum* strain M10
GGGTGCTAGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGG
GAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACA
ACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCC
GCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGA
CCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGG
CAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGT
GAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAA
CTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAG
GCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAAC
TGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGT
AGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGA
GGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAAC
GATGAATGC
TAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTG
GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTA
TGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATT
ATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTAC
AATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTC
TCAGTTCGGATTGTAGGCTGAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGG
ATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATG
AGAGTTTGTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAACCCGAT

T5 99% IDENTICAL TO Lactobacillus plantarum strain VJC38 16S ribosomal RNA gene, partial sequence
GGCCCAAGACGAACGCTGGCCGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTA
TTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACAC

GTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCAT
AACAACTTGGACCGCATGGTCCGGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGG
TCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC
CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGAGCAACGCCGCGTGA
GTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGT
AACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGC
AGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAA
ACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCG
TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTG
AGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAA
CGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAG
CATTCCGCCTGGGCGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGT
CTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATTATCAGTTGCCAGCATTAGTTGGGCACTCTGGTGAGACTGCCGGTGAC
AAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTAC
ACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCT
TAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGC
TAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACACCATGAGAGTTTGTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAG
CCGCCTAAGGTGGGACAGATGATTAGGGGAAGTCGTAACAAGGTAGTAAAC

References

Abdulkadir, M., &Mugadi, A. G. (2012). Bacteriological examination of 'fura da nono' (fermented milk; cereals mix) sold in some selected areas of BirninKebbi metropolis. *Journal of Science and Technology,* 2, 2225-7217.

Abukakar, M. G., Ukwuani, A. N., and Shehu, R. A. (2008). Phytochemical screening and antibacterial activity of *Tamarindusindica* pulp extract. *Asian Journal of Biochemistry*, 3, 134-138.

Agure-Dam, C. (1997). Soy bean production and use in Benue State, Nigeria. A publication of the Ministry of Agriculture and Natural Resources. Makurdi, Benue State, Nigeria.

Bordignon, J. R., Nakahara, K., Yoshihashi, T., and Nikkuni, S. (2004). Hydrolysis of isoflavone and consumption oligosaccharides during lactic acid fermentation of soybean. Food and Technology Division, *Japan International Research Centre for Agricultural Science,* Tsukuba, Ibaraki 305-8686, Japan.

Carol, A. R., & Leon, M.T. D. (2010). Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. *Archives of Microbiology*, 193(3), 157-168.

Cheesbrough, M. (2000). District Laboratory Practice In Tropical Countries, (2nd edition). The Press Syndicate of the University of Cambridge, pp.64-75.

Chien, H. L., Huang, H. Y., and Chou, C. C (2006). Transformation of isoflavone phytoestrogens during the fermentation of soymilk with lactic acid bacteria and bifidobacteria. *Journal of Food Microbiology*, 23, 772-778.

Farinde, E.O., Obatolu, V. A., Oyarekua, M. A., Adeniran, H. A., Ejoh, S. I., and Olanipekun, O.T. (2010). Physical and microbial properties of fruit flavoured fermented cow milk and soy milk (yoghurt-like) under different temperature of storage. *African Journal of Food Science and Technology,* 1, (5), 120-127.

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., and Disen, G. J. (2008). Critical Evaluation of two primers commonly used for Amplification of Bacterial 16s rRNAGenes. *Applied and Environmental Microbiology*, 74, 2461- 2470.

Garro, M. S., De Valdez, G. F., & De Giori, G. S (2004). Temperature effect on the biological activity of Bifidobacteriumlongum CRL 849 and *Lactobacillus fermentum* CRL 251 in pure and mixed cultures grown in soymilk. *Journal ofFood Microbiology*, 21, 511–518.

ICMSF (1986). The International Commission on Microbiological Specifications for Food sampling plans and recommended microbiological limits for vegetables, fruits, nuts and yeast 3. In: Microorganisms in Foods 2, Sampling for Microbial Analysis: Principles and Specific Applications, 2nd ed. Palo Alto, CA: ICMSF Blackwell Scientific Publications. Pp.197–202

Ijah, J. J., Odagboyi, G., &Uwabujo, A. E. (2002). Microbiological screening of *furan da nono*. *Nigerian Journal of Science Technology and Math Education,* 5(1), 9-16.

Jimoh, K.O., &Kolapo, A.L. (2007). Effect of different stabilizers on acceptability and shelf-stability of soy-yoghurt. *African Journal of Biotechnology*, 6(8), 1000-1003.

Khalid, K. (2011). An overview of lactic acid bacteria, Review paper *International Journal of Biosciences* (IJB), 1, 3, 1-13.

Kumar, C.S., & Bhattacharya, S. (2008). Tamarind Seed: Properties, Processing and Utilization. Critical Reviews in *Journal of Food Science and Nutrition*, 48, 1-20.

Lee, N., Mok1, B. R., Jeewanthi, R. K. C., Yoon, Y.C., and Paik1, H. (2015). Physicochemical and Microbiological Properties of Yogurt-cheese Manufactured with Ultrafiltrated Cow's Milk and Soy Milk Blends. *Korean Annual Journal of Food Science,* 35, 2, 205-210.

Nebedum, J. O., & Obiakor, T. (2007). The effects of different preservation methods on the quality of nunu: A locally fermented Nigerian Dairy product. *African Journal of Biotechnology,* 6, 454-458.

Nyadoi, P., & K. Abdullah, (2004). Tamarind growth in Tharaka, Eastern Kenya. Prunus tribune. World Agroforestry Centre (ICRAF), 4-12.

Okeke, K. S., Abdullahi I. O., Makun, H. O., and Mailafiya, S. C. (2012). A Preliminary survey of Aflatoxin M1 in Dairy cattle products in Bida, Niger State, Nigeria. *Afrcan Journal of Food Science and Technology,* 3(10), 273-276.

Okonkwo, O.I. (2011). Microbiological Analyses and Safety Evaluation of Nono: A fermented milk product consumed in Northern part of Nigeria. *International Journal of Dairy Science,* 6(3), 181-189.

Omotosho, A. O., Abdullahi, I. O., and Damisa, D. (2013) Microbiological quality and HACCP concept in the production of "*Nono*" in a farm settlement in Minna, Niger state, Nigeria. *African Journal of Microbiology Research*, 7(25), 3234-3239.

Parvez, S., Malik, K. A., Kang, S. A., and Kim, M. (2006) Probiotics And Their Fermented Food Products Are Beneficial For Health. *Journal of Applied Microbiology,* 100, 1171-1185.

Pelczar, M. J., Chan, E. C. S., and Krieg, W. R. (2003). *Microbiology: Concepts and Applications.* McGraw – Hill Inc, New York. pp. 680–708.

Raja, S., Picard, D., Baek, S., and Delgado, C. (2014). Rustbelt radicalism: A decade of food systems planning in Buffalo, New York. *Journal of Agriculture, Food Systems, and Community Development*, 4(4), 1 73-189.

Rezvan, P., Atefeh, G., Masoud, F., and Mahnaz, M. A. (2012). Production of probiotic soy yoghurt. *Annals of Biological Research,* 3 (6), 2750-2754.

Shehu, L. M., & Adesiyun, A. A. (1990). Characteristics of strains of *Escherinchia coli* isolated from locally fermented milk (*nono*) in Zaria. *Journal of Food Protection*, 53, 574-577.

Shori, A.B. (2013). Phytochemical analysis and antimicrobial activity of some medicinal plants.*Asian Pacific Journal of Tropical Disease*, 6, (1) 15-20.

Trindade, W. C., Marques, E., Lopes, D. B., and Ferreira, M. A. S. V. (2007). Development of a molecular method for dictation and identification of *Xanthomonascampestris*pv. *viticola summa phytopathologica*, 33(1), 16- 23.

Tuitemwong, P., & Tuitemwong, K. (2003). Development of flatulent-free and high quality soy yoghurt and frozen soy yoghurt with Bifidobacteria [Online] Available: http://agriqua.doae.go.th/worldfermenredfood/P9_Tuitemwong.pdf, Accessed July 28th 2016.

Tzortzis, G., Goulas, A. K., Baillon, M. A., Gibson, G. R., and Rastall, R.A (2004). In vitro evaluation of the fermentation properties of galacto-oligosaccharides synthesised by a-galactosidase from Lactobacillus reuteri. *Applied Microbiology and Biotechnology*, 64, 106–111.

Wawrik, B., Kerkhof, L., Zytstra, G. J., and Kukor, J. J. (2005). Identification of unique Type II polyketide synthase Genes in soil. *Applied Environmental Microbiology,* 71(5), 2232- 2238.

Whitman, W. B., Goodfellow, M., Kämpfer, P., Busse, H. J., Trujillo, M. E., Ludwig, W., and Suzuki, K.-i. (eds., 2012). Bergey's Manual of Systematic Bacteriology, 2nd ed., vol. 5, parts A and B, New York, NY, Springer-Verlag.