



MICROBIOLOGICAL EVALUATION OF YOGHURT PRODUCED BY BLENDING COW MILK AND TIGER – NUT MILK IN ADO EKITI, EKITI STATE NIGERIA

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ABSTRACT

Yoghurt is one of the fermented milk products produced by bacteria fermentation and it is consumed worldwide. This study evaluated microbial analysis and sensory attributes of yoghurt produced by blending cow milk and tiger nut milk in Ado Ekiti, Ekiti State Nigeria. Both the cow milk and the tiger nut milk were blended in ratio (90:10) = A, (80:20) = B, (70:30) = C, (60:40) = D, (50:50) = E, and 100% cow milk = F which will serve as control. The starter culture used are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Microbial count of the blended yoghurt showed that the count increases as the volume of tiger nut milk increases. Under Heterotrophic count, sample A had count of 2.1×10^5 cfu/ml for 24 hrs and 2.4×10^5 cfu/ml for 48 hrs, sample B had 2.3×10^5 cfu/ml for 24 hrs and 2.7×10^5 cfu/ml for 48hrs, sample C had 2.6×10^5 cfu/ml for 24 hrs and 2.8×10^5 cfu/ml for 48 hrs, sample D had 2.9×10^5 cfu/ml for 24 hrs and 3.0×10^5 cfu/ml for 48 hrs, sample E had 3.1×10^5 cfu/ml for 24 hrs. and 3.2×10^5 sample F had 1.2×10^5 cfu/ml for 24 hrs. and 1.6×10^5 cfu/ml for 48 hrs. Total fungal count (TFC) showed the same pattern of bacterial count, that is sample A had 2.3×10^5 cfu/ml for 24 hrs. and 2.6×10^5 cfu/ml for 48 hrs., sample B had 5.0×10^5 , sample C had 3.5×10^5 , sample D had 8.2×10^5 , sample E had 6.7×10^5 and sample F had 2.3×10^5 . The sensory attribute of the blended yoghurt sample showed there were significant different ($p \leq 0.05$) in all the samples analyzed. The suspected bacterial isolates include *E. coli*, *Lactobacillus* sp. *Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas* sp., *Shigella* sp., Fungal isolates include *Aspergillus* sp., *Saccharomyces cerevisiae*. *Mucor* sp.

Keywords: Yoghurt, Tiger, nut, Milk, Microbial, Cow

INTRODUCTION

Yoghurt is a fermented food in the form of a thick, slightly sour liquid made by adding bacteria to milk (cow milk, or cow milk and tiger-nut milk) and yoghurt is regarded as a probiotic carrier (Serra *et al.*, 2009 ; De *et al.*, 2014). Yoghurt is nutritionally rich in protein, vitamin B2 and vitamin B12, calcium, potassium, magnesium, fat and milk (Staffolo *et al.*, 2004; Saint-Eve *et al.*, 2006). Yoghurt production from blending tiger-nut milk and cow milk was subjected to proximate, sensory and physiochemical analysis. Yoghurt is assumed to be the oldest fermented milk product that is consumed globally (Dryden, 1999 and

Willey *et al.*, 2008). It has nutritional benefits more than milk, in-fact, energy from yoghurt is more than energy from milk because of this people who are slightly lactose intolerant can enjoy taking yoghurt without fear, since most of the lactose in milk have been converted to lactic acid by the starter culture (Bhattarai and Das, 2016). The nutrients in yoghurt are complete and balance, this made yoghurt to be a balance food and it is believed that yoghurt has valuable curing ability for gastrointestinal problems and disorders because of probiotic characteristics (Olugbuyiro, 2011). The consumption of yoghurt has been on increase in Nigeria especially in



big cities like Lagos, Abuja, Enugu, Kano, Ado Ekiti, Kaduna Ibadan, Owerri, Onitsha etc (Nwamaka and Chike, 2010). It is also believed that yoghurt can promote good health and encourage the absorption of calcium, thereby preventing osteoporosis, possibly because of probiotic effect of lactic acid in yoghurt (Kerry *et al.*, 2001). The lactic acid produced is responsible for the characteristic flavor and aroma of yoghurt, also the souring nature of yoghurt is as a result of *Streptococcus thermophilus* (Saint *et al.*, 2006). Yoghurt is produced when the lactose component of cow milk is acted upon by bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to break down the glucose and galactose under anaerobic conditions (Farnworth *et al.*, 2007; Sanful, 2009). Cow milk is available worldwide and this makes it the milk of choice for yoghurt production, at the same time, tiger-nut milk is available and accessible for the production of Yoghurt. Yoghurt is very susceptible to microbial attack and hence it is easily degraded (Martin *et al.*, 2018). In this study, microbiological assessments of yoghurt was carried out, in order to know its associated microorganisms so as to create public awareness on the hazard or risk that arises from taking unhygienic yoghurt. Microbiological assessment of this product can help to reduce economic losses by producing, packaging and preservation, all under aseptic environments (Martin *et al.*, 2018). There have been reports of food infections and intoxication due to poor handling during processing of such foods as yoghurt. However, some bacteria have been isolated which includes *Staphylococcus aureus*, *Escherichia coli*, and Fungi. These microorganisms have been ascribed to cause yoghurt borne infections (Okonkwo, 2011; Ifeanyi *et al.*, 2013).

The aim of this research work is to carryout microbial assessment on yoghurt produced with blended cow milk and tiger-nut milk.

MATERIALS AND METHODS

Materials:

The materials that were used in this research work include Tiger-nuts, Cow milk, distilled water, Autoclave, Hot –air oven Kenwood blender, sieve and some glass-ware.

Methods:

Tiger-nuts were purchased from the hawkers in Ado Ekiti town along oja Irona. The tiger-nuts were taken to the food laboratory in Federal Polytechnic Ado Ekiti for analysis. The tiger-nuts were picked and washed with clean water, the water was added a small quantity of salt (NaCl). After the sorting and washing, the tiger-nuts were crushed by milling with a kenwood blender. Enough water was added while milling was going on, after milling the milled nuts paste was sieved making use of clean filter mesh to separate the liquid (milk) from the residue or chaff. The milk was sterilized by pasteurization at 95 °C for 15 minutes and later cooled down to 40°C. (Abdul *et al.*, 2018).

Preparation of the Blends:

The cow milk and tiger-nut milk were mixed or blended in the ratio of 90:10, 80:20, 70:30, 60:40, and 50:50 respectively. A litre of each ratio was poured into bowl for fermentation. At a temperature of 43 °C the blended milk of cow and tiger-nut was inoculated with 4% v/v of starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The containers were covered and incubated for 4hours at 42°C, for it to coagulate into yoghurt, other ingredients (sugar and flavor) were added into it. The final product was packaged into bottles and sachets, these packs were refrigerated (Abdul *et al.*, 2018).

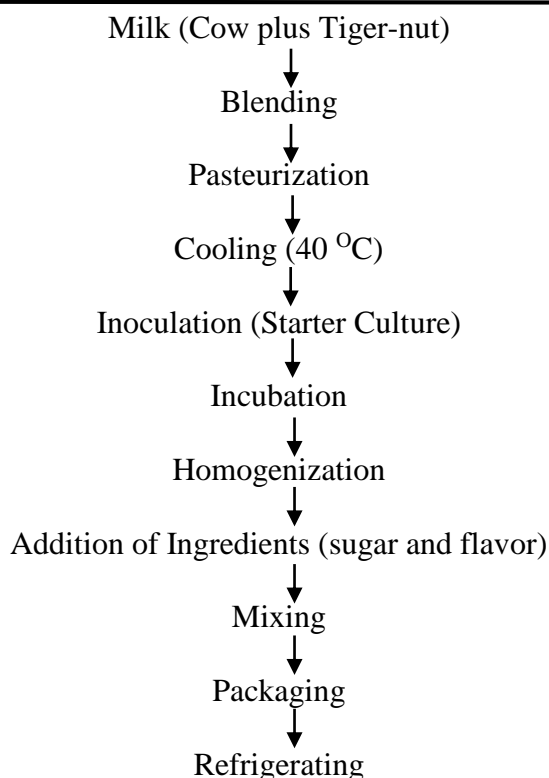


Fig. 1 Flow chart for the production of yoghurt blende with Tiger-nut milk.

Microbial Analysis:

Microbial analysis was carried out on the produced blended tiger-nut yoghurt by weighing a given quantity of Mac Conkey Agar, distribute into a given volume of distilled water and autoclaved at 121 °C for 15 minutes. All the glass -wares were sterilized in the hot-air oven at 160 °C. Serial dilution of the yoghurt was performed and the least diluted was introduced into sterile two petri dishes and about 10ml of molten sterilized Mac Conkey agar was poured into the petri dishes that have 1ml of the diluted yoghurt sample. The plates were rocked gently for the content to mix thoroughly, the agar inside the petri dishes was allowed to solidify, after which the plates were put inside the incubator that was set at 37 °C for 24 hours. The petri dishes were placed in inverted form to avoid condensation. After incubation, the plates were observed for developed colony formation and these colonies were counted to know the

microbial load of the yoghurt and slides were prepared for morphological identification of the isolates (Banerjee and Sarkar, 2003).

Characterization of Bacterial isolates:

Gram staining:

This is a differential test that allows separation of microorganisms based on their grams reaction whether gram positive or gram negative. In gram staining, glass slides were cleaned and a drop of distilled water was placed on them, then an inoculating loop was sterilized on a Bunsen burner flame was used to pick an inoculum from young a cultured plate and smeared with distilled water on the clean glass slides. After the smear has dried, drops of crystal violet were placed on the smear and allowed to stay for about a minute, after which drops of grams iodine were added and allowed to stay for a minute then drops of ethanol were sprinkled on the smear and



allowed to stay for only 30 seconds swift running water was used to rinse the excess of ethanol, then safranin the counter stain was put on the smear and allowed to stay for a minute. The slides were allowed to dry and the slides were viewed with the microscope using x100 objective.

Catalase Test:

Many bacteria which that grow aerobically produce peroxide as a product of their metabolism. $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$ The hydrogen peroxide is toxic and is decomposed by the enzyme catalase as soon as it is formed. Note, a few bacteria which do not produce cvatalase are insensitive to hydrogen peroxide. The method involves preparing a slide by putting a drop of Hydrogen peroxide on it, use a sterile inoculating loop collect an inoculum from a cultured plate and put the inoculums on the drop of the Hydrogen peroxide and you will see bubbles which shows that catalase is present. No bubbles shows there is no catalase (Martin *et al.*, 2018).

Oxidase Test:

This test was performed to differentiate between *Psuedomonas* and other gram negative organisms or enteric bacteria. Oxidase positive colonies develop a pink color which becomes dark-red, purple and black within 10 to 30 seconds. The method involves pouring the reagent (1% aqueous solution of tetramethyl-p-phenylenediamine hydrochloride on the surface of the agar growth in the petri dish. Oxidase positive colonies develop a pink colour which becomes successively dark-red, purple and black in 10 to 30 seconds (Martin *et al.*, 2018).

Coagulase Test:

This test was performed to differentiate pathogenic *Staphylococcus pyogens* or *S.aureus* from non-pathogenic

Staphylococci. Coagulase is an enzyme capable of coagulating certain blood plasma, especially human and rabbit plasma. The test was performed on cultures of 18 to 24 hours, the reagents were Human and rabbit plasma. Procedure involved preparing a slide by marking it into two parts with a grease pencil. A loopful of normal saline (0.85% NaCl in aqueous solution) was placed on each section and emulsified a small amount of 18-24 hrs. Culture in each drop until a homogenous suspension formed. A drop of human or rabbit plasma was added to one of the suspensions and stirred for 5 seconds. Positive result of coagulase production was indicated by clumping that will not re-emulsify. The second suspension serves as control (Cheesbrough, 2006).

Carbohydrate Fermentation Test:

Fermentation test was carried out, using peptone water sugars (glucose, lactose, sucrose, maltose). These carbohydrates were carefully inoculated with the isolated microorganisms. The inoculated test tubes were incubated in the incubator for 24 hours at 37 °C. At the end of incubation, the test tubes were observed for results. Gas in the durham tubes showed that gas was produced during the reaction between the organisms and the carbohydrates, and change in colour showed that acid was produced in the reaction of organisms and carbohydrates (Cheesbrough, 2006).

Motility Test: This test is used to identify motile microorganisms, it is carried out by making a hanging drop slide with the test organisms and viewed under the microscope. The motile objects will be seen clearly on the field. Motile organisms will be marked positive +, and non-motile ones will be marked negative – (Cheesbrough, 2006 and Martins *et al.*, 2018).



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Citrate Test: This test is used for the identification of microorganisms that utilize citrate as the sole source of carbon. Coliform organisms can be differentiated from other microorganisms by their ability to utilize citrate as a sole source of carbon. The citrate medium is inoculated with a young culture of test organisms, the inoculated plate is incubated at the optimum growth temperature for about 2 – 5 days and examine change in colour of the indicator (Martins *et al.*, 2018)

Sensory Evaluation:

The sensory assessment of the blended yoghurt of Tiger-nut milk and cow milk was carried out using Ten member panelists. The samples were assessed based on the following parameters colour, taste, texture, appearance, aroma, favour ad general acceptability. These parameters were evaluated using a scoring scale like; very much for 9 points and dislike very much for 1 point.

RESULTS AND DISCUSSION

Results:

Table 1: This shows the total viable bacterial counts (TVBC) from different samples of the blended tiger nut yoghurt under 24 hours and 48 hours of incubation and pH.

Samples	24 hours incubation cfu/ml	48 hours incubation cfu/ml	pH
A	2.1×10^5	2.4×10^5	4.05
B	2.3×10^5	2.7×10^5	3.50
C	2.6×10^5	2.8×10^5	3.18
D	2.9×10^5	3.0×10^5	4.50
E	3.1×10^5	3.2×10^5	4.25
F	1.2×10^5	1.6×10^5	4.07

Key: A = 90% cow milk plus 10% tiger-nut milk, B= 80% cow milk plus 20% tiger-nut milk

C = 70% cow milk plus 30% tiger-nut milk, D = 60% cow milk plus 40% tiger-nut milk,

E = 50% cow milk plus 50% tiger-nut milk and F = 100% cow milk as the CONTROL.

Table 2: Total fungal count (TFC) from different samples of blended tiger-nut yoghurt under 48 hours and 72 hours incubation and pH.

Samples	48 hours incubation	72 hours incubation	pH
A	2.3×10^5	2.6×10^5	4.05
B	3.0×10^5	3.6×10^5	3.50
C	3.4×10^5	3.9×10^5	3.18
D	3.7×10^5	4.1×10^5	4.50
E	4.1×10^5	4.6×10^5	4.25
F	1.8×10^5	2.2×10^5	4.07

Table 3: This shows the results of biochemical and motility tests carried out on the bacterial isolates from the samples of yoghurt blended with tiger nuts.

Isolates	Grams rxn.	Catalase	Coagulase	Citrate	Fermentatio	Motility	Oxidase
E coli	-ve	+ve	-ve	-ve	+ve	+ve	-ve
Salmonella sp.	-ve	+ve	-ve	+ve	+ve	+ve	-ve



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Shigella sp.	-ve	D	-ve	-ve	-ve	-ve	-ve
Pseudomonas	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Lactobacillus	+ve	-ve	-ve	-ve	+ve	+ve	+ve
Staphylococcus	+ve	+ve	+ve	-ve	-ve	-ve	-ve

Key: + ve = Positive

-ve = Negative

Table 4: This shows Frequency of Occurrence and Percentage Frequency

Isolates	Frequency	Percentage frequency
E. coli	3	15
Salmonella	4	20
Shigella	2	10
Staphylococcus	3	15
Pseudomonas	3	15
Lactobacillus	5	25

Table 5: The result of sensory evaluation

Sample	Colour	Taste	Flavour	Texture	Appearance	Overall
A	6.5	6.5	6.0	6.0	Better	Better
B	6.5	5.5	6.0	5.5	Good	Better
C	6.0	5.0	5.0	5.5	Good	Better
D	6.0	4.0	4.5	5.0	Good	Better
E	5.5	3.0	3.5	4.0	Good	Good
F	7.0	6.5	7.0	6.0	Excellent	Excellent

Keys are as follows: Excellent= 8, Better = 7, Good = 6, Fairly good= 5, Fair 4

DISCUSSION

The high bacterial and fungal count of yoghurt can be attributed to the presence of the tiger nut (*Cyperus esculentus* L.) used as the enrichment of the yoghurt or due to unhygienic measure applied during production of the yoghurt, which may arise from the utensils, raw materials and the handlers. Almost all the yoghurt samples formulated by blending with the tiger-nut recorded counts from 2.1×10^5 - 3.2×10^5 cfu/ml. The pH of the samples showed that all of the samples are acidic with the highest

of 3.18 and the least to be 4.5. The contamination of these yoghurt samples irrespective of acid content may be from the handlers or processors, droplets from the skin of the handlers or even cough during production can cause contamination. During packaging, droplets such as *Staphylococcus* sp. within the environment can also bring about contamination (Park *et al.*, 2012).

The presence of *E. coli* in yoghurt can pose great danger to the consumers and this can



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be seen as unhygienic and unhealthy handling by the processors or vendors of the yoghurts (Kawo *et al.*, 2006). *Coliform* organisms as enteric flora of human and animal origin, their presence in yoghurt reveal direct fecal contamination of the product (Osundahunsi *et al.*, 2007; Agu *et al.*, 2014).

Table 2 of result showcased the result of the fungal isolates of the yoghurt samples, the fungal counts are from $2.3 \times 10^5 - 4.6 \times 10^5$ cfu/ml. The fungi organism isolated include *Mucor sp.* *Rhizopus sp.* and *Aspergillus sp.* and *Penicillium sp.* (Taiwo *et al.*, 2018).

Table 3 shows result of biochemical and suspected isolates from Tiger – nut blended yoghurt, frequency of occurrence and percentage frequency of occurrence of the isolates are shown on table 4. Like earlier stated, the spread of contamination on the yoghurt samples can be from the handlers. As previously reported by Osundahunsi *et al.*, 2007.

The sensory evaluation on Table 5, showed that sample F had the best of all the parameters tested, because it was whole cow milk not blended with tiger – nut. Sample A is closer in the sensory evaluation because it was made in the ratio of 90% cow milk and 10% tiger-nut milk. Other samples were still acceptable since there was no much deviation in the values.

CONCLUSION:

The result of this work showed that the addition of Tiger-nut to the plain yoghurt gave it a palatable taste, it can also increase the vitamin and the mineral contents of the yoghurt.

RECOMMENDATION: Due to high count of microorganisms in some of the yoghurt samples in this work, I recommend

that the producers and all stake holders should do more on their good manufacturing practices to reduce the rate of contamination of yoghurt. More training should be organized for the vendors and marketers on the distribution channels. More work should be done on tiger – nut yoghurt blend to achieve best of the product.

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