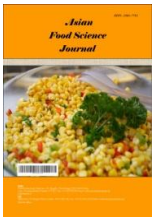

Asian Food Science Journal

16(3): 32-42, 2020; Article no.AFSJ.51461
ISSN: 2581-7752

Production of Nigerian *Yoghurt* Using Lactic Acid Bacteria as Starter Cultures

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Authors' contributions

This work was carried out in collaboration among all authors. Author SA designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors SMW and AAO managed the literature searches and gave technical support. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2020/v16i330173
Editor(s):
(1) Dr. Surapong Pinitglang, Assistant Professor, Dean, School of Science and Technology, University of the Thai Chamber of Commerce, Bangkok, Thailand.
Reviewers:
(1) Mouafo Tene Hippolyte, Institute of Medical Research and Medicinal Plants Studies, Cameroon.
(2) Mustapha Umar, Nigerian Institute of Leather and Science Technology, Nigeria.
(3) Merih Kivanç, Eskisehir Technical University, Turkey.
Complete Peer review History: http://www.sdiarticle4.com/review-history/51461

Original Research Article

Received September 2019
Accepted 29 November 2019
Published 30 June 2020

ABSTRACT

Aim: This work was carried out to investigate the influence of Lactic Acid Bacteria (LAB) on organoleptic quality and proximate composition of yoghurt, and viability of starter cultures in yoghurt.
Methods: The LAB starter cultures were selected based on their ability to produce diacetyl and lactic acid.
Results: *Lactobacillus casei*N1 produced the highest quantity (2.72 g/L) of diacetyl at 48 hrs of incubation while *Pediococcus acidilactici*G1 had the lowest amount (0.50 g/L). The pH of produced *yoghurt* ranged between 4.40 and 5.58 while the corresponding lactic acid contents ranged between 0.70 and 0.96 g/L. *Yoghurt* produced with cow milk inoculated with *L. Plantarum*N24 and *L. Brevis*N10 had the lowest pH (4.40) at significant level of P≤0.05. *Yoghurt* with mixed culture of *L. Plantarum*N24 and *L. Plantarum*N17 had the highest protein content (5.13%) while spontaneous fermentation (control) produced the least (0.48%). *Yoghurt* produced from cow milk inoculated with *L. Plantarum*N24 and *L. Plantarum*N17 was rated best with overall acceptability (9.0) during first day of storage while the commercial *yoghurt* (5.8) and spontaneous fermentation (6.8) had least overall acceptability at P≤0.05.

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Conclusion: *Yoghurt* samples stored in refrigerator had more viable LAB counts for a period of 21 days while the samples stored at room temperature had a day count except for *yoghurt* produced with cow milk inoculated with *L. plantarum*N24 which retained its viability at the second day. The *yoghurt* produced with selected LAB starters are better than commercial *yoghurt* in terms of sensory properties, proximate composition, pH and viability.

Keywords: Lactic Acid Bacteria (LAB); yoghurt; starter cultures.

1. INTRODUCTION

Yoghurt is a food produced by bacterial fermentation of milk [1]. The bacteria used to make *yoghurt* are known as *yoghurt* cultures consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* but other lactic acid bacteria are also utilized. The fermentation of lactose by these bacteria are able to produce lactic acid, which acts on milk protein to give *yoghurt* its texture and characteristic tart flavor. *Yoghurt* consist of water, fat, protein, sugar and minerals (ash), hence could be helpful in enhancing the microflora of the gut. It can be produced from different milk such as goat, cow, sheep, horse, water buffaloes, skimmed milk, non fat milk or low fat milk including milk from plant origin such as soymilk.

Lactic acid bacteria are known in the food industries, and mostly used organisms for making *yoghurt*. They are positive to Gram reaction, rod to cocci shaped, acid tolerant, do not produce spore but have the ability to produce lactic acid. One of the bacteria for making *yoghurt* is *Lactobacillus bulgaricus*, which can grow at 45°C, but some strains cannot survive longer time in *yoghurt* which reduces the organoleptic characteristics and probiotic effect [2,3]. However, LAB such as *L. amylovorous, L. helveticus*, *L. amylophiius*, *L. casei, L. brevis and L. plantarum* could also be used for *yoghurt* production [4].

Moreover*, Lactobacillus plantarum and Lactobacillus casei can* be presented as starters and probiotic candidates, for the production of *yoghurt* or sometimes cheese [5,6]. They are normal resident of the gastrointestinal tract, and also dairy foods [4,7,8]. These organisms have the tendency to produce antimicrobials such as lactic acid and dicetyl which inhibit pathogens, produce desirable characteristics flavor and also increase the organoleptic quality of *yoghurt*. Therefore, this study was to isolate lactic acid bacteria from dairy products and select the LAB with highest quantity of diacetyl for *yoghurt* production.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Two samples of each raw milk from cow, goat and Nigerian locally fermented milk product (*nono*) were randomly collected purposively from Bodija market in Ibadan, Nigeria. They were brought to the Microbial Physiology and Biotechnology Unit laboratory, at Department of Microbiology, University of Ibadan in sterile bottles for microbiological assessment.

2.2 Isolation and Characterisation of Isolates

Lactic acid bacteria (LAB) were isolated from samples of raw milk from goat, cow, and *nono* using pour plate technique and phenotypically identified by reference to Bergey's Manual of Systematic Bacteriology and an Approach to the Classification of Lactobacilli [1].

2.3 Selection of Starter Cultures

The identified LAB were screened and selected based on diacetyl and lactic acid production.

2.4 Determination of Lactic Acid Production

One loopful of 24 hrs old culture of the LAB isolates containing 10^6 CFU/mL were inoculated into 20 mL of MRS broth, and incubated at 24,48,72 and 96 hrs. The production of lactic acid was determined by titrating 20 mL of MRS broth containing LAB isolates at different incubation periods of 24, 48, 72, and 96 hrs with 0.1M of NaOH and l mL of phenolphthalein indicator (0.5% in 50% alcohol). The titratable acidity was calculated as lactic acid (% v/v). The milliliter of IN NaOH can be estimated as 90.08 mg of lactic acid. The lactic acid was calculated according to AOAC [9].

Lactic acid contents =

$$\frac{\text{M1 NaOH x N NaOH x M.E. x100}}{\text{Volume of sample}}$$

Where

Ml NaOH = Volume of NaOH used,
N NaOH = Normality of NaOH solution
M.E. = Equivalence Factor

2.5 Determination of Diacetyl Production

One loopful of 24 hrs old culture of the LAB isolates were inoculated into 25 mL of MRS broth, and incubated at 24,48,72 and 96 hrs. Diacetyl production was determined by transferring 25 mL of MRS broth containing LAB isolates at different incubation time of 24, 48, 72, and 96 hrs into 100 mL of conical flasks. Both flasks were titrated with 0.IN HCl to a greenish yellow end point using bromophenol blue as indicator [9]. Hydroxylamine was used for residual titration.

$$AK = \frac{(b-s)\,(100E)}{W}$$

K = Percentage of diacetyl
B = No of mL of 0.IN HCl consumed in titration of sample;
E = Equivalence factor
W = Volume of sample
S = No of mL of 0.IN HCl consumed in titration of samples.

2.6 Production of *Yoghurt* with Selected Starter Cultures

The *yoghurt* samples were prepared according to the method of Rahmann et al .(1999) with a slight modification. The inoculum size (10^6 CFU/mL) of the selected LAB starter cultures were obtained using Mcfarland standard 0.5. However, sterile glass bottles containing 100 mL of raw milk samples from cow were pasteurized at 85°C for 30 minutes with the use of a waterbath, and cooled to 37°C. The "pasteurized raw cow milk samples" were inoculated with 1.0 mL of selected starter cultures containing inoculums size of 10^6 CFU/mL. For mixed cultures, each 100 mL of pasteurized raw milk were inoculated with selected starter cultures of inoculums size of 10^6 CFU/mL at equal proportion of 1:1. After inoculation, the contents were thoroughly mixed, and incubated at 42°C for 4-6 hrs using a thermostatically controlled waterbath, and cooled to 4°C. However, the yoghurt samples were stored at 4°C (cold storage). *Yoghurt* produced with spontaneous fermentation was used as control, and a commercial *yoghurt* was also used as treatment during the experiment.

2.7 pH and Lactic Acid Contents of *Yoghurt* Produced by Starter Culture Using Cow Milk

Yoghurt produced with raw cow milk using different LAB starters, *yoghurt* produced with spontaneous fermentation, and commercial *yoghurt* were tested for pH using pHmeter. Lactic acid contents was also examined as previously determined [9].

2.8 Proximate Analysis

Proximate contents such as protein, moisture, ash, carbohydrate, and crude fat were determined on the *yoghurt* samples using standard procedures as described by AOAC [9].

2.9 Organoleptic Studies

Yoghurt samples were randomly numbered and a panel of 20 judges that are familiar with the consumption of *yoghurt*, conversant with such properties, and their consent sorted were asked to evaluate a day and 2 weeks old starter-*yoghurt* for flavor, body-texture, appearance and overall acceptability. A 9 point hedonic scale of 1 (dislike extremely) and 9 (like extremely) was used for the sensory evaluation.

2.10 Viability of Lactic Acid Bacteria (LAB) Cultures in *Yoghurt* Stored under Refrigeration and Room Temperature

Yoghurt samples were prepared according to the method of Rahmann et al. (1999) with slight modification and samples of *yoghurt* containing the selected starter cultures were stored at 4°C (cold storage) and 28°C (room temperature) to determine their viability in storage over a period of time. 1 mL of appropriate dilution of *yoghurt* samples were plated on MRS agar at 37°C for 48 hrs, and colony forming units per sample was estimated over a period of 21 days using pour plate method [10]. The entire procedure was performed to determine the viability of LAB isolates during cold storage and room temperature.

2.11 Statistical Analysis

The experiments were carried out in duplicates. Data was analysed using descriptive statistics and Analysis of Variance (ANOVA) with Duncan Multiple Range Test for significance at $P \leq 0.05$

according to statistical procedure (Statistical Analysis Systems (2002) SAS Version 9.1. SAS Institute Inc., Cary.). Means and standard deviation were also presented. Data were presented in tables.

3. RESULTS AND DISCUSSION

The phenotypically identified LAB isolates were assessed for diacetyl and lactic acid production. The quantity of lactic acid produced by LAB isolated from raw milk and *nono* samples is shown in Table 1. The quantities of lactic acid produced by the LAB isolates ranged between 0.38 to 1.90 g/L at 24-96 hrs of incubation. Lactic acid quantities increases at 48 hrs of incubation resulting to the highest production and later decreases from 72 to 96 hrs. The highest quantity of lactic acid (1.90±0.01 g/L) was produced by *Lactobacillus casei*N1 at 48 hrs and decreased to 0.83±0.00 g/L at 96 hrs. This was followed by *Lactobacillus plantarum*N24, isolated from *nono* samples which produced 1.42±0.01 g/L at 48 hrs and decreased to 1.25±0.00 g/l and 0.98±0.00 g/L at 72 and 96 hrs respectively. *Lactobacillus plantarum*N17 also increased to 1.60±0.01 g/L at 48 hrs after which it declined to 1.23 g/L at 72 hrs. *Lactobacillus plantarum*N24 had the highest lactic production of 1.25±0.01 g/L at 72 hrs of which *Lactobacillus plantarum*N6 and *Lactobacillus plantarum*N17 had 1.23±0.00 g/L each. The production of lactic acid by LAB isolates at various incubation times had been achieved [11,12,13,14,15]. The maximum quantity of lactic acid produced at 48 hrs were in accordance with findings of Ogunbanwo et al. [15]. The increased quantity of lactic acid (antimicrobial substance) produced by *Lactobacillus plantarum* and *Lactobacillus casei* at 48 hrs could be strain dependent, fermentation time, source of isolates, and changes in metabolism rate of isolates at that particular period.

Table 2 shows the quantity of diacetyl produced by the LAB isolated from raw milk and *nono* samples which ranged between 0.50 to 2.72 g/L during 24 – 96 hrs of incubation. *Lactobacillus casei*N1produced the highest quantity of diacetyl (2.72±0.01 g/L) at 48 hrs, and later reduced to 1.15±0.00 g/L at the end of 96 hrs. This was closely followed by *Lactobacillus plantarum*N17 which produced 1.99±0.00 g/L at 24 hrs, increased to 2.32±0.00 g/L at 48 hrs and decreased to 1.85±0.01 g/l at the end of 96 hrs incubation. The lowest quantity of diacetyl (0.50±0.00 g/L) was produced at 24 hrs by *Pediococcus acidilactici*G1. It increased to 0.98±0.01 g/L at 48 hrs and later decreased to 0.85±0.03 g/l at the end of 96 hrs. The isolates from the LAB such as *Lactobacillus plantarum*N17 and *Lactobacillus brevis*N10 were able to produce high quality of diacetyl at 48 hours of incubation. This can be attributed to the fact that most LAB usually exihibit good growth, viability and production of metabolites at 48 hours of incubation which is intrinsical. There metabolisms are always significant in terms of growth at 48 hours. Most LAB had been found to produced diacetyl, including the maximum quantity at 48 hours as presented by Ogunbanwo et al. [15]. Diacetyl production is the common substance produced by *Lactobacillus* and other lactic acid bacteria. Lactic acid and diacetyl had being known to contribute to flavor, texture, and other organoleptic properties associated with fermented products [11,15].

The LAB isolates with highest quantity of diacetyl and lactic acid production were selected as the starter cultures for *yoghurt* production. They are *L. plantarum*N24, *L. plantarum*N17, *L. casei*N1 and *L. brevis*N10. The codes of *yoghurt* produced from cow milk inoculated with selected starter cultures are shown in Table 3.

The results on pH and lactic acid contents of *yoghurt* are shown in Table 4. The pH values of the *yoghurt* samples ranged between 4.40 to 5.58. Sample AC had the least pH of 4.40±0.01 which was significantly different from sample AB (4.43±0.00) and sample AD (4.4±0.05). The reasons for this could be attributed to the mixed starters used for *yoghurt* production which is better than single starter. The highest pH of 5.58±0.00 was observed by sample k (control) and the least was sample AC (4.40) with the best significance level at (P≤0.05). Wakil and Onilude. [16] also observed a reduction in pH and increased lactic acid contents of fermented product inoculated with starter cultures that were LAB. However, lactic acid content of the *yoghurt* samples ranged between 0.70 to 0.96 g/L which increases due to reduced pH. The highest lactic acid contents was observed by Sample AC (0.96±0.01 g/L), Sample AB (0.95±0.00g/l), Sample AD (0.95±0.01 g/L), Sample CD (0.93±0.03 g/L) and were not significantly different from each other at P≤0.05. The least acidity (0.70±0.01 g/L) was observed by Sample K (control). The study of Achi and Akobor [17] and Nout [18] revealed that lowered pH will result to higher acidity (lactic acid) and better sensory qualities especially flavor. The development of

acid and reduced pH could be responsible for absence of pathogenic organisms in the *yoghurt*. The presence of lactic acid during fermentation is essential for a well balanced sensory qualities such as flavor, texture and appearance of *yoghurt* including inhibition of pathogens [15].

The proximate analysis of *yoghurt* are shown in Table 5. The moisture contents ranged between 82.93% to 95.73%. Samples K (control) had the highest moisture content of 95.73±1.80 %, while the least value (82.93%) was observed by sample Y. The fat content of the *yoghurt* ranged between 0.94 to 4.94%. Sample B has the highest fat content (4.94±0.21%) at significant level (P≤0.05) with a slight significance difference from Sample C (3.50±1.41%), Sample D (4.10±0.4%), and Sample BC (3.00±0.03%). The least fat contents were observed by Sample K (0.94±0.01%). The protein contents ranged between 0.48 to 5.13%. Sample AB had the highest protein content of 5.13±0.72%, while Samples Y and K were the least with protein content of 0.60±0.00 % and 0.48±0.01 % respectively. The ash contents of the *yoghurt* also ranged between 0.33 to 0.92%. All the samples had good ash contents with no significance difference except for Sample K (control) and Sample Y with the least values of 0.33±0.12% and 0.33±0.08%, respectively. It was observed in this study that milk fermented with LAB starters had better protein contents. This statement had been reported by Ibeawuchi and Dalyop (1995) who revealed that pasteurized milk inoculated with lactic acid bacteria starters could have better crude protein and fat contents.

Table 1. Quantities of lactic acid (g/L) produced by LAB isolated from raw milk and *nono* samples

LAB Isolates	Incubation times (hours)			
	24	**48**	**72**	**96**
*Pediococcus acidilactici*G1	*0.44±0.01	0.78±0.01	0.73±0.01	0.50±0.00
*Pediococcus acidilactici*G2	0.45±0.01	0.73±0.01	0.73±0.01	0.42±0.00
*Pediococcus acidilactici*G3	0.39±0.01	0.73±0.02	0.72±0.01	0.50±0.00
*Pediococcus acidilactici*G4	0.38±0.01	0.72±0.01	0.70±0.00	0.66±0.03
*Pediococcus acidilactici*G5	0.68±0.00	0.86±0.01	0.85±0.01	0.60±0.01
*Pediococcus acidilactici*G6	0.63±0.00	0.83±0.01	0.79±0.01	0.60±0.00
*Lactobacillus plantarum*G7	0.80±0.01	1.10±0.00	0.95±0.01	0.70±0.01
*Pediococcus acidilactici*G8	0.53±0.01	0.70±0.00	0.48±0.01	0.45±0.01
*Pediococcus acidilactici*G9	0.54±0.01	0.80±0.01	0.50±0.01	0.47±0.01
*Pediococcus acidilactici*G10	0.66±0.01	0.85±0.01	0.72±0.00	0.50±0.00
*Pediococcus acidilactici*G11	0.50±0.01	0.82±0.01	0.68±0.00	0.55±0.01
*Lactobacillus plantarum*G12	0.61±0.00	0.90±0.01	0.72±0.01	0.50±0.01
*Pediococcus acidilactici*C1	0.56±0.00	0.76±0.01	0.68±0.03	0.45±0.01
*Pediococcus acidilactici*C2	0.54±0.01	0.72±0.01	0.65±0.01	0.58±0.00
*Lactobacillus plantarum*C3	0.90±0.00	0.94±0.00	0.72±0.01	0.52±0.01
*Lactobacillus plantarum*C4	0.90±0.00	0.95±0.01	0.83±0.00	0.70±0.01
*Lactobacillus plantarum*C5	0.69±0.01	0.95±0.01	0.78±0.01	0.50±0.00
*Lactobacillus plantarum*C6	0.66±0.00	0.98±0.01	0.96±0.01	0.49±0.01
*Lactobacillus plantarum*C7	0.72±0.01	0.99±0.00	0.83±0.01	0.52±0.03
*Lactobacillus plantarum*C8	0.77±0.01	0.90±0.01	0.83±0.01	0.52±0.01
*Lactobacillus plantarum*C9	0.90±0.01	0.98±0.00	0.75±0.01	0.49±0.01
*Lactobacillus plantarum*C10	0.81±0.00	0.96±0.01	0.68±0.01	0.56±0.00
*Pediococcus acidilactici*C11	0.65±0.01	0.72±0.01	0.72±0.01	0.68±0.01
*Lactobacillus plantarum*C12	0.72±0.00	0.80±0.01	0.81±0.00	0.52±0.01
*Lactobacillus plantarum*C13	0.95±0.01	1.08±0.00	0.87±0.01	0.66±0.01
*Lactobacillus plantarum*C14	0.60±0.01	0.81±0.01	0.62±0.01	0.57±0.01
*Lactobacillus plantarum*C15	0.95±0.00	1.00±0.00	0.88±0.01	0.65±0.00
*Pediococcus acidilactici*C16	0.38±0.00	0.70±0.01	0.68±0.00	0.45±0.00
*Pediococcus acidilactici*C17	0.50±0.01	0.70±0.00	0.65±0.00	0.49±0.00
*Lactobacillus casei*N1	0.99±0.00	1.90±0.01**	1.00±0.00	0.83±0.01
*Lactobacillus plantarum*N2	1.10±0.00	1.05±0.00	1.14±0.00	0.85±0.00
*Lactobacillus plantarum*N3	0.81±0.01	1.05±0.00	1.00±0.00	0.83±0.01
*Lactobacillus plantarum*N4	0.83±0.01	0.90±0.01	0.99±0.00	0.53±0.01
*Lactobacillus fermentum*N5	0.98±0.01	1.25±0.03	1.16±0.01	0.99±0.01
*Lactobacillus plantarum*N6	1.12±0.01**	1.28±0.00	1.23±0.00**	0.95±0.01

LAB Isolates	Incubation times (hours)			
	24	48	72	96
*Lactobacillus plantarum*N7	0.96±0.00	1.20±0.01	1.00±0.00	0.92±0.00
*Lactobacillus brevis*N8	0.95±0.01	1.10±0.00	0.99±0.00	0.85±0.00
*Lactobacillus casei*N9	0.90±0.01	1.00±0.00	0.85±0.00	0.72±0.01
*Lactobacillus brevis*N10	0.90±0.00	1.10±0.00	0.91±0.00	0.70±0.01
*Lactobacillus plantarum*N11	0.85±0.03	1.10±0.00	0.80±0.01	0.72±0.01
*Lactobacillus brevis*N12	0.83±0.01	1.00±0.00	0.75±0.01	0.62±0.00
*Lactobacillus fermentum*N13	0.72±0.01	1.20±0.01	0.72±0.01	0.49±0.03
*Lactobacillus plantarum*N14	0.77±0.01	1.25±0.01	0.72±0.00	0.49±0.00
*Lactobacillus casei*N15	0.75±0.00	1.16±0.01	0.72±0.00	0.46±0.01
*Lactobacillus plantarum*N16	0.72±0.01	0.81±0.01	0.76±0.01	0.60±0.01
*Lactobacillus plantarum*N17	1.12±0.01**	1.60±0.01	1.23±0.00**	0.99±0.00
*Lactobacillus fermentum*N18	0.80±0.01	1.00±0.00	1.00±0.00	0.98±0.01
*Lactobacillus plantarum*N19	0.89±0.01	1.05±0.00	0.83±0.01	0.69±0.01
*Lactobacillus plantarum*N20	0.92±0.01	1.10±0.00	0.85±0.03	0.73±0.01
*Lactobacillus brevis*N21	0.75±0.00	0.91±0.00	0.65±0.01	0.52±0.00
*Lactobacillus casei*N22	0.70±0.00	0.85±0.01	0.80±0.00	0.49±0.01
*Lactobacillus plantarum*N23	0.80±0.01	0.85±0.01	0.60±0.00	0.49±0.01
*Lactobacillus plantarum*N24	0.98±0.00	1.42±0.01**	1.25±0.01**	0.98±0.00
*Lactobacillus plantarum*N25	0.72±0.01	0.99±0.01	0.68±0.01	0.50±0.01
Lactobacillus plantarumN26	0.69±0.01	0.98±0.00	0.63±0.01	0.58±0.01

**Values are Means of duplicates± Standard Deviation (SD), **Statistically significant at a defined time interval*

Keys:

- *G =Isolates from Goat milk*
- *C= Isolates from Cow milk*
- *N =Isolates from Nono samples*

Table 2. Quantities of diacetyl (g/L) produced by LAB isolated from raw milk and *nono* samples

LAB Isolates	Incubation times (hours)			
	24	48	72	96
*Pediococcus acidilactici*G1	*0.50±0.00	0.98±0.01	0.99±0.01	0.85±0.03
*Pediococcus acidilactici*G2	0.55±0.01	1.18±0.00	0.95±0.01	0.90±0.01
*Pediococcus acidilactici*G3	0.85±0.01	0.99±0.00	0.93±0.01	0.87±0.01
*Pediococcus acidilactici*G4	0.60±0.01	1.08±0.00	1.00±0.00	0.95±0.00
*Pediococcus acidilactici*G5	0.75±0.00	1.36±0.00	1.08±0.00	0.98±0.01
*Pediococcus acidilactici*G6	0.80±0.01	0.92±0.01	0.90±0.03	0.93±0.03
*Lactobacillus plantarum*G7	1.32±0.03	1.54±0.01	1.20±0.00	1.00±0.00
*Pediococcus acidilactici*G8	0.54±0.00	1.00±0.00	1.10±0.00	0.85±0.00
*Pediococcus acidilactici*G9	0.70±0.01	1.05±0.00	1.00±0.00	0.96±0.01
*Pediococcus acidilactici*G10	0.80±0.00	1.22±0.00	1.15±0.03	0.93±0.01
*Pediococcus acidilactici*G11	0.76±0.00	0.98±0.00	1.00±0.00	0.90±0.01
*Lactobacillus plantarum*G12	1.51±0.01	1.64±0.01	1.50±0.01	1.00±0.00
*Pediococcus acidilactici*C1	0.89±0.00	1.23±0.00	1.01±0.00	0.98±0.00
*Pediococcus acidilactici*C2	0.90±0.00	0.95±0.02	0.97±0.01	0.90±0.01
*Lactobacillus plantarum*C3	1.62±0.02	2.13±0.00	1.72±0.01	1.50±0.01
*Lactobacillus plantarum*C4	1.58±0.00	2.06±0.00	2.00±0.00	1.42±0.01
*Lactobacillus plantarum*C5	1.35±0.00	1.92±0.01	1.90±0.01	1.00±0.00
*Lactobacillus plantarum*C6	1.30±0.01	2.00±0.00	1.75±0.03	1.28±0.03
*Lactobacillus plantarum*C7	1.52±0.02	2.15±0.01	2.00±0.00	1.35±0.03
*Lactobacillus plantarum*C8	1.30±0.00	1.92±0.01	1.92±0.01	1.05±0.00
*Lactobacillus plantarum*C9	1.06±0.00	2.20±0.00	2.12±0.01**	1.22±0.01
*Lactobacillus plantarum*C10	1.20±0.00	1.59±0.00	1.50±0.01	1.00±0.00
*Pediococcus acidilactici*C11	0.95±0.00	1.99±0.00	0.89±0.01	0.90±0.00
*Lactobacillus plantarum*C12	1.12±0.00	1.88±0.03	1.82±0.03	1.00±0.00
*Lactobacillus plantarum*C13	1.00±0.00	1.52±0.00	1.37±0.01	0.98±0.01
*Lactobacillus plantarum*C14	0.95±0.01	1.00±0.00	1.00±0.00	0.90±0.01
*Lactobacillus plantarum*C15	1.20±0.00	1.48±0.01	1.31±0.01	1.05±0.00
*Pediococcus acidilactici*C16	0.98±0.00	1.25±0.00	1.00±0.00	0.95±0.01

LAB Isolates	Incubation times (hours)			
	24	48	72	96
*Pediococcus acidilactici*C17	0.95±0.02	1.15±0.00	1.00±0.00	0.92±0.01
*Lactobacillus casei*N1	1.30±0.00	2.72±0.01**	1.80±0.01	1.15±0.00
*Lactobacillus plantarum*N2	1.60±0.00	1.65±0.01	1.62±0.01	1.20±0.01
*Lactobacillus plantarum*N3	1.72±0.00	2.00±0.00	1.86±0.03	1.53±0.00
*Lactobacillus plantarum*N4	1.35±0.00	2.00±0.00	1.75±0.00	1.17±0.00
*Lactobacillus fermentum*N5	1.92±0.00	2.16±0.00	2.04±0.00	1.85±0.01
*Lactobacillus plantarum*N6	1.90±0.00	2.00±0.00	1.95±0.03	1.86±0.01**
*Lactobacillus plantarum*N7	1.51±0.01	2.08±0.00	1.82±0.01	1.00±0.00
*Lactobacillus brevis*N8	1.60±0.01	1.85±0.01	1.92±0.01	1.51±0.02
*Lactobacillus casei*N9	1.52±0.01	2.15±0.03	2.06±0.00	1.26±0.01
*Lactobacillus brevis*N10	1.65±0.01	2.08±0.00	1.92±0.01	1.51±0.00
*Lactobacillus plantarum*N11	1.90±0.01	2.20±0.03	2.05±0.01	1.83±0.00
*Lactobacillus brevis*N12	1.83±0.00	2.00±0.00	1.85±0.01	1.72±0.00
*Lactobacillus fermentum*N13	1.76±0.00	2.00±0.00	1.95±0.01	1.80±0.01
*Lactobacillus plantarum*N14	1.85±0.01	2.15±0.00	2.07±0.00	1.53±0.01
*Lactobacillus casei*N15	1.63±0.00	2.18±0.01	1.82±0.01	1.42±0.01
*Lactobacillus plantarum*N16	1.52±0.01	1.92±0.03	1.72±0.01	1.30±0.01
*Lactobacillus plantarum*N17	1.99±0.00**	2.32±0.00	2.10±0.00	1.85±0.01
*Lactobacillus fermentum*N18	1.72±0.00	2.00±0.00	1.85±0.01	1.53±0.00
*Lactobacillus plantarum*N19	1.51±0.02	1.85±0.01	1.00±0.00	1.12±0.01
Lactobacillus plantarum 20	1.55±0.00	2.08±0.00	2.00±0.00	1.32±0.01
*Lactobacillus brevis*N21	1.60±0.01	2.15±0.01	2.00±0.00	1.45±0.00
*Lactobacillus casei*N22	1.66±0.00	1.95±0.03	1.72±0.01	1.50±0.00
*Lactobacillus plantarum*N23	1.57±0.00	1.96±0.00	1.83±0.01	1.12±0.01
*Lactobacillus plantarum*N24	1.98±0.00**	2.25±0.00	2.08±0.00	1.86±0.01**
*Lactobacillus plantarum*N25	1.90±0.00	2.08±0.00	1.95±0.01	1.82±0.00
Lactobacillus plantarumN26	1.65±0.01	1.90±0.01	1.92±0.01	1.52±0.01

*Keys: *Values are means of duplicates± Standard Deviation (SD). **Statistically significant at a define time interval.*

- *G = Isolates from goat milk*
- *C= Isolates from cow milk*
- *N = Isolates from nono sample*

Table 3. Codes for selected starters for *yoghurt* production

LAB (single and combination)	Code for starter- *yoghurt*
*Lactobacillus plantarum*N24	A
*Lactobacillus plantarum*N17	B
*Lactobacillus brevis*N10	C
*Lactobacillus casei*N1	D
*Lactobacillus plantarum*N24 *& Lactobacillus plantarum*N17	AB
*Lactobacillus plantarum*N24 *& Lactobacillus brevis*N10	AC
*Lactobacillus plantarum*N24 & *Lactobacillus casei*N1	AD
*Lactobacillus plantarum*N17 *& Lactobacillus brevis*N10	BC
*Lactobacillus plantarum*N17 *& Lactobacillus casei*N1	BD
*Lactobacillus brevis*N10 *& Lactobacillus casei*N1	CD
Spontaneous fermentation (control)	K
Commercial *yoghurt*	Y

Keys: A-Yoghurt made from cow milk inoculated with Lactobacillus plantarumN24
B - Yoghurt made from cow milk inoculated with Lactobacillus plantarumN17
C - Yoghurt made from milk inoculated with Lactobacillus brevisN10
D - Yoghurt made from cow milk inoculated with Lactobacillus caseiN1
AB - Yoghurt made from cow milk inoculated with Lactobacillus plantarumN24 &Lactobacillus plantarumN17
AC- Yoghurt made from cow milk inoculated with Lactobacillus plantarumN24& Lactobacillus brevisN10
AD- Yoghurt made from cow milk inoculated with Lactobacillus plantarumN24 &Lactobacillus caseiN1
BC -Yoghurt made from cow milk inoculated with Lactobacillus plantarumN24 &Lactobacillus brevisN10
BD- Yoghurt made from cow milk inoculated with Lactobacillus plantarumN17 &Lactobacillus caseiN1
CD- Yoghurt made from cow milk inoculated with Lactobacillus brevisN10&Lactobacillus caseiN1,
K = Control (spontaneous fermentation); Y = commercial yoghurt

Table 4. pH and lactic acid contents (g/L) of starter produced *yoghurt* using cow milk

Samples	pH	Lactic acid contents (g/L)
A	4.58±0.01^{bc*}	0.84±0.00^{c}
B	4.55±0.00^{cd}	0.85±0.01^{bcd}
C	4.60±0.01^{b}	0.80±0.07^{d}
D	4.53±0.01^{d}	0.84±0.06^{c}
AB	4.43±0.00^{efg}	0.95±0.00^{a}
AC	4.40±0.01^{g}	0.96±0.01^{a}
AD	4.44±0.05^{efg}	0.95±0.01^{a}
BC	4.46±0.03^{ef}	0.90±0.01^{abc}
BD	4.47±0.01^{e}	0.92±0.01^{ab}
CD	4.47±0.00^{e}	0.93±0.03^{a}
K (Control)	5.58±0.00^{a}	0.70±0.01^{e}
Y	4.42±0.00^{fg}	0.89±0.00^{abc}

**Means with the same alphabets within a column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means of duplicates ± Standard Deviation*
Keys: as in Table 3

Table 5. Proximate analysis of starter-produced *yoghurt using* cow milk

	Proximate contents (%)				
Sample	**Moisture**	**Fat**	**Protein**	**Ash**	**Carbohydrate**
A	86.52±0.82^{b*}	3.61±0.21^{b}	4.13±0.64^{a}	0.84±0.71^{a}	4.90±0.31^{bc}
B	86.68±1.61^{b}	4.94±0.21^{a}	4.00±0.45^{ab}	0.83±0.10^{a}	4.55±0.02^{c}
C	86.80±1.10^{b}	3.50±1.41^{b}	3.50±0.07^{b}	0.85±0.10^{a}	5.35±0.56^{b}
D	85.25±0.80^{b}	4.10±0.41^{ab}	4.10±1.56^{ab}	0.89±0.05^{a}	5.56±0.14^{b}
AB	87.70±1.60^{b}	3.77±0.62^{b}	5.13±0.72^{a}	0.90±0.09^{a}	2.50±0.43^{d}
AC	87.50±0.35^{b}	2.08±0.08^{c}	4.50±0.20^{a}	0.92±0.06^{a}	5.00±0.46^{d}
AD	88.55±3.00^{b}	2.13±1.44^{c}	4.10±0.71^{ab}	0.92±0.07^{a}	4.30±0.31^{bc}
BC	87.70±1.70^{b}	3.00±0.03^{b}	4.00±0.57^{ab}	0.90±0.10^{a}	4.40±0.14^{c}
BD	88.10±0.21^{b}	2.39±0.08^{c}	4.60±0.11^{a}	0.91±0.06^{a}	4.00±0.21^{c}
CD	88.25±1.00^{b}	2.40±0.07^{c}	4.15±0.16^{ab}	0.90±0.06^{a}	4.30±0.36^{c}
Y	82.93±1.51^{b}	2.30±1.00^{c}	0.60±0.00^{c}	0.33±0.08^{c}	13.13±2.00^{a}
K(Control)	95.73±1.80^{a}	0.94±0.01^{d}	0.48±0.01^{c}	0.33±0.12^{c}	2.52±0.14^{d}

**Means with the same alphabets within a column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means of duplicates ± Standard Deviation (SD)*

The organoleptic studies of a day old *yoghurt* is shown in Table 6. Sample AB was rated the best and highest for overall acceptability (9.0±0.00), followed by AC 8.6±0.55, the least was observed by sample K and Y of about 5.8±0.04 and 6.2±0.84, respectively.

At two weeks storage as shown in Table 7, samples AB, AC, AD, BC, BD were rated the best for overall acceptability of which sample AD had the highest score (6.4) but not significantly different at P≤0.05 from the best rated samples. Sample k was rated the least (5.0) for overall acceptability. The organoleptic studies of quality and acceptability of *yoghurt* revealed that *yoghurt* produced with starter cultures were much more better during rating. This indicated that pasteurization and fermentation with starter cultures could improve flavor, texture and appearance of fermented milk product like *yoghurt* [19,20].

Moreover, the significant improvement found with the use of selected starters is similar to the search of Wakil and Kazeem [21] who reported that use of *Lactobacillus plantarum* could improve flavor of fermented product. This study also revealed that the milk that was fermented with selected starters were superior in terms of overall acceptability.

The results of viability of starter cultures in *yoghurt* samples stored at refrigeration (4°C) and room temperature (28°C) are shown in Tables 6 and 7. The viability of *yoghurt* samples produced with selected starter cultures indicated that *yoghurt* stored at refrigerated temperature (4°C) had longer preservatives days with viable cells

Table 6. Organoleptic studies of starter produced *yoghurt* (a day storage) using cow milk

Sample	Flavor	Body –texture	Appearance	Overall acceptability
A	8.4±0.55^{ab*}	8.0±0.71^{ab}	8.0±1.23^{ab}	8.2±0.84^{abc}
B	8.2±0.45^{abc}	7.6±0.89^{bc}	7.6±0.89^{abc}	8.0±0.71^{bc}
C	7.6±0.55^{cd}	7.4±1.14^{bc}	7.8±0.45^{abc}	7.6±0.55^{c}
D	7.8±0.84^{bc}	7.8±0.84^{abc}	8.0±0.23^{ab}	8.0±1.00^{bc}
AB	8.8±0.45^{a}	8.8±0.45^{a}	8.4±0.55^{a}	9.0±0.00^{a}
AC	8.6±0.55^{a}	8.0±1.00^{ab}	8.6±0.55^{a}	8.6±0.55^{ab}
AD	8.2±0.45^{abc}	8.0±1.00^{ab}	8.4±0.89^{a}	7.8±0.84^{bc}
BC	8.8±0.45^{a}	8.0±0.71^{ab}	8.4±0.89^{a}	7.6±0.89^{c}
BD	8.4±0.55^{ab}	8.2±0.45^{ab}	8.4±0.55^{a}	8.2±0.45^{abc}
CD	8.6±0.55^{a}	8.2±0.84^{ab}	8.4±0.55^{a}	8.0±1.00^{bc}
K (Control)	6.8±0.84^{e}	6.8±0.45^{c}	6.8±0.84^{c}	5.8±0.84^{d}
Y	7.0±0.00^{de}	6.8±0.84^{c}	7.0±0.71^{bc}	6.2±0.84^{d}

**Means with the same alphabets within a column are not significantly different at p≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± Standard Deviation (SD)"*

Table 7. Organoleptic studies of starter-produced *yoghurt* (2 weeks storage) using cow milk

Samples	Flavor	Body –texture	Appearance	Overall acceptability
A	8.0±0.00^{ab*}	6.0±0.71^{a}	6.2±0.84^{ab}	6.0±0.00^{ab}
B	7.4±0.55^{c}	6.0±0.71^{a}	6.0±0.00^{ab}	5.8±0.45^{b}
C	7.8±0.44^{ab}	5.8±0.44^{a}	5.6±0.55^{b}	5.4±0.55^{bc}
D	7.0±0.00^{c}	5.6±0.54^{a}	5.6±0.60^{b}	5.4±0.55^{bc}
AB	7.8±0.44^{ab}	6.6±0.55^{a}	6.8±0.45^{a}	6.4±0.60^{a}
AC	7.8±0.45^{ab}	6.4±0.55^{a}	6.2±0.45^{ab}	6.2±0.45^{a}
AD	8.0±0.00^{a}	6.2±0.45^{a}	6.6±0.60^{a}	6.4±0.50^{a}
SBC	8.0±0.45^{a}	6.2±0.45^{a}	6.4±0.55^{a}	6.2±0.50^{a}
BD	8.0±0.00^{a}	6.2±0.50^{a}	6.2±0.45^{ab}	6.2±0.45^{a}
CD	7.6±0.54^{bc}	6.0±0.00^{a}	6.2±0.45^{ab}	6.0±0.00^{ab}
K (Control)	6.0±0.00^{d}	5.2±0.50^{a}	5.2±0.50^{b}	5.0±0.00^{c}
Y	6.2±0.84^{d}	5.8±0.83^{a}	5.8±0.45^{b}	5.4±0.55^{bc}

**Means with the same alphabets within a column are not significantly different at p≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± Standard Deviation (SD)".*
Keys: as in Table 3

Table 8. Viability (X10^6 CFU/mL) of starter cultures in produced *yoghurt* stored under refrigeration temperature (4°C)

	Storage Time (Days)			
Samples	**1**	**7**	**14**	**21**
A	1.5±0.42^{c*}	1.7±0.99^{a}	2.2±0.70^{bc}	1.1±0.14^{a}
B	1.8±0.56^{c}	2.4±0.9^{a}	2.4±0.56^{bc}	1.4±0.42^{a}
C	1.8±0.28^{c}	1.6±0.56^{a}	1.8±0.84^{c}	1.0±0.00^{a}
D	1.4±0.14^{c}	2.0±0.70^{a}	2.5±0.56^{bc}	1.3±0.42^{a}
AB	2.3±0.42^{c}	1.7±0.42^{a}	4.0±0.70^{b}	1.4±0.56^{a}
AC	2.23±0.32^{c}	1.6±0.42^{a}	1.6±0.42^{c}	1.2±0.14^{a}
AD	30.0±4.24^{b}	1.8±0.56^{a}	3.0±0.99^{bc}	1.2±0.02^{a}
BC	2.5±0.14^{c}	1.3±0.28^{a}	11.0±1.41^{a}	1.0±0.00^{a}
BD	2.3±1.27^{c}	1.3±0.42^{a}	2.8±0.84^{bc}	1.3±0.28^{a}
CD	42.0±12.72^{a}	1.2±0.28^{a}	2.0±0.84^{bc}	1.5±0.02^{a}
K (Control)	0.02±0.00^{d}	-	-	-
Y	-	-	-	-

**Means with the same alphabets within a column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means of duplicates ± Standard Deviation (SD)"*

than that of room temperature, which had shelf - life of two days but the highest cultures count was observed at the first day of storage [22]. The findings of Oyawoye et al. (1997) documented

that fermented foods inoculated with starter cultures could have viable organisms when refrigerated at 4°C.

Table 9. Viability (X10^6 CFU/mL) of starter cultures in produced *yoghurt* stored under room temperature (28°C)

	Storage Time (Days)	
Samples	**1**	**2**
A	2.5±0.56^{d*}	16.0±1.41^{a}
B	3.0±0.84^{d}	-
C	51.0±9.89^{b}	-
D	3.5±0.14^{d}	-
AB	2.8±0.28^{d}	-
AC	30.0±11.31^{c}	-
AD	55.0±5.65^{b}	-
BC	70.0±15.55^{a}	-
BD	50.0±5.65^{b}	-
CD	51.0±9.89^{b}	-
K (Control)	0.06±0.00^{e}	-
Y	-	-

**Means with the same alphabets within a column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means of duplicates ± Standard Deviation (SD)", - = no growth*

Keys: as in Table 3

4. CONCLUSIONS

Some lactic acid bacteria isolated from raw cow milk, raw goat milk and *nono* samples are able to produce lactic acid and diacetyl which are important for *yoghurt* production. These strains tested alone and in combination for *yoghurt* production permitted to obtained product with sensory properties higher than commercial one.

Yoghurt produced with mixed starter cultures were better in terms of organoleptic acceptability, proximate composition and viability compared to the commercial *yoghurt*. However, *yoghurt* produced with selected starter cultures exhibited better LAB counts. Therefore, lactic acid bacteria that possessed antimicrobial properties in terms of diacetyl and lactic production will not only improve the flavor, proximate contents, but would retain the viability of the cultures in the product when stored at refrigeration temperature. The occurrence of pathogens in the *yoghurt* and molecular identification of the selected LAB starters should be recommended.

CONSENT

As per international standard informed and written participant consent has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

My sincere appreciation goes to Professor A. A. Onilude, Department of Microbiology, University of Ibadan, Nigeria for his valuable supervision and guidance. I also have to thank Dr.Wakil for her technical support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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