

Probiotics Screening from selected Nigerian Prebiotics: Alternative Starter Culture Sources for Yoghurt Production

**Egwim, E. C.^{1,2}, Nebechukwu, P, C.² Ojo, O. O.², Akpakpan, F. U.^{*3},
Oloninefa, S. D.⁴, Akan, O. D.⁵**

Centre for Genetic Engineering and Biotechnology, Federal University of Technology, PMB 65,
Minna, Niger State, Nigeria.

Department of Chemical Science, Anchor University Lagos, PMB 001, Iyana-Ipaja Ayobo Road,
Lagos, Nigeria.

Department of Biochemistry, PAMO University of Medical Science, PH-Aba Express, Rivers State,
Nigeria.

Department of Quality Control, Jesil Pharmaceutical Industries Limited, Minna, Niger State,
Nigeria.

College of Food Science and Engineering, Central South University of Forestry and Technology,
Hunan, China.

Microbiology Department, Akwa-Ibom State University, Akwa-Ibom State, Nigeria.

Abstract

The rise of functional foods has expanded food production, offering not just essential nutrition but also potential health and longevity benefits. This study aimed to identify probiotics from common Nigerian fermented foods as potential starter cultures for yogurt production. Ten local fermented food were screened for suitable starter culture (probiotics) for yogurt production, including fresh milk from cow (CM), sheep (SM), and goat (GM), as well as pap made from maize, millet, sorghum, cassava products (garri, fufu, and lafun), and Kunuzaki. Probiotic isolates with desired characteristics underwent tests for tolerance to acidic pH and bile conditions; antioxidative properties. Test yogurt was produced using *Lactobacillus* (LAB) isolates and physicochemical and organoleptic scores were determined for the yogurt samples. LAB species from CM, SM, and Lafun samples showed tolerance to both acidic pH (pH 3.0) and 0.5 percent bile. CM isolates exhibited strong antioxidant effects comparable to vitamin C. Yogurt samples had pH ranging from 4.77 to 5.45 and titratable acidity from 5.56 to 8.06. Sensory characteristics of the test yogurt did not significantly differ from commercial yogurt ($p \leq 0.05$). Thus, probiotics (LAB) from common Nigerian fermented foods like CM, SM, and lafun could serve as cost-effective, nutrient-rich alternatives for yogurt starter cultures.

Keywords: Probiotics, *Lactobacillus*, Yoghurt, Nigerian fermented foods, Antioxidant.

Corresponding AuthorEmail: ekam.akpakpan@gmail.com

Introduction

Fermented foods are a significant part of the traditional African staple food. Fermentation processes not only improves the shelf-life, nutrient content, aroma, taste, and texture of the end-products; it also improves their anti-pathogenic status and serves as a possible source of safe fermenting bacteria (Guan *et al.* 2017; Mulaw *et al.* 2019; Okeke *et al.* 2021;). Fermented foods have active microorganisms

that degrade food components into more useful or health-promoting metabolites. In hosts, certain microbes that line the intestinal tracts act as biological barriers, inhibit pathogenic bacteria, and regulate gut microbiota mediated mechanisms, also ferment ingested food substances (Guan *et al.* 2017; Mulaw *et al.* 2019). According to Food and Agricultural Organization/World Health Organization (FAO/WHO) (2006), microorganisms that can affect host's health

include those that can tolerate the severe gastrointestinal tract (GIT) conditions, such as adhering to epithelial tissues and withstanding bile salt and the acidic pH of gastric juice. These probiotics microbes possess high antioxidative capabilities (Egwim *et al.* 2013; Fijan, 2014; Somashekaraiah *et al.* 2019); however, the mode of pre-fermentation processes and raw materials/products influence the survival and type of microbes inherent in the end-product.

Lactic acid bacteria (LAB) are the prevalent bacteria type that make up the majority of probiotics. LAB have been used as food supplements due to their favourable properties and are quite helpful (Chen *et al.* 2014). Although numerous probiotic strains are already known and utilized in the production of commercially available prebiotic fermented milk products all over the world, there is increased market demand for varied functional dairy products in general and prebiotic dairy products in particular (Tamime *et al.* 2005; Edith *et al.* 2018). Also, there is an urgent need to find new sources of diversity of LAB strains, particularly from some under-utilized traditionally fermented foods in Nigeria. Traditionally fermented foods have numerous microbes that are potential starter cultures; screening and sourcing for capable starter cultures from the large array of locally available traditionally fermented foods will expand the database sources for food and fermentation industries.

There are numerous successful instances where isolates from indigenous fermented foods are utilized to upscale the production of other fermented foods (Mulaw *et al.* 2019). In order to make soy yoghurt, Opara *et al.* (2018) used starter culture extracted from fermented African oil bean (Ugba). The technological potential of LAB isolated from fermented Nunu was assessed by Akabanda *et al.* (2014) for their potential starter culture capability for Nunu production. In addition, Osuntoki and Korie (2010) reported high antioxidant properties in whey from LAB fermented milk.

Cassava (*Manihot esculenta*) tubers are a very important crop that can be processed (fermented) differently to produce end-products like garri, fufu, and lafun. These different end-products contain a lot of carbs. Garri is a creamy-white, granular flour made from raw cassava tubers that have been fermented and gelatinized. It has a mildly sour taste and is a well-known and popular foodstuff in Nigeria and other West African

nations. Another Cassava fermentation by-product is fufu, a white popular gel/paste indigenous of the Southern part of Nigeria. It is made by reducing the potentially hazardous cyanogenic chemical by steeping whole or sliced peeled cassava roots in water for up to three days. Fermentation during steeping reduces pH, softens roots, and helps to reduce the amount of cyanogenic compounds, which may be poisonous (Agbor-Egbe and LapeMbome 2006). Similar to fufu is a fibrous lafun, a powdery form of cassava. Lafun is fresh cassava roots sliced into chucks and are steeped for three to four days, or until mushy. They are sun dried after being peeled on mats or flat rocks, split up into small pieces, and peeled again. The dried particles are then used to make flour (Egwim *et al.* 2013).

Pap (gruel) consumers enjoy the fermentation product made from either maize (*Zea mays*), millet (*Peninselum americanum*), or sorghum (*Sorghum bicolor*). Additionally, some consumers also add spices just as ginger, cloves, and pepper to garnish their pap- like the Kunuzaki drink made from milled and fermented millet. This nutrient-rich drink is well consumed both in northern and southern Nigeria (Egwim *et al.* 2013). All the aforementioned fermented foods are well consumed staples in Nigeria. Therefore, this study aimed to screen, isolate, and use probiotics (*Lactobacillus sp*) from some nutritionally rich Nigerian fermented foods, evaluate their potentials as starter cultures for yoghurt production, and suggest these foods as cheap and safe sources of LAB strains or probiotics.

Materials and Methods

Materials

The De Mann Rogosa Sharpe (MRS) media and MRS broth were quality grade and purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and peptone water were procured from Sigma Chemical Co. in St. Louis, Missouri, and Difco Laboratories in Sparks, Maryland, respectively. Other reagents utilized in this research were all of analytical grade.

Sample collection and preparation

Suitable starter cultures for yoghurt production were screened from ten (10) local fermented food items. Fresh milk (from cow, sheep and goat); pap made from maize, millet sorghum; cassava products (garri, fufu, and lafun) and

Kunuzaki were purchased from a rural market in Minna, Niger State of Nigeria. These fermented foods are usually processed differently as reported by Egwim *et al.* (2013). The fresh milk samples were kept at room temperature (25 °C) and allowed to ferment for 24 hours to improve on the fermenting organism.

Isolation and identification of lactic acid bacteria

2.3.1 Preparation of food homogenate

Food homogenate was made using the Food Safety and Standard Authority of India (FSSAI, 2012) technique. Five (5) grams of each sample were aseptically transferred into 50 ml of sterilized buffered peptone water (BPW). Each mixture was thoroughly stirred with a sterile spatula. With the aid of a sterile needle and syringe, 2 ml was withdrawn from the food homogenate (1:10) and transferred aseptically into 18 ml of buffered peptone water contained in sterile McCartney bottles. A serial dilution was done by taking 2 ml from the first bottle (1:10) into 18 ml of buffered peptone water to obtain a dilution of 1:100 while a dilution of 1:1000 was obtained from 1:100 following the same procedure.

Enumeration of total anaerobic bacteria

The enumeration of total anaerobic bacteria was done using pour plate method as described by FSSAI (2012). One millilitre (1 ml) from a well-stirred food homogenate was delivered into already labelled sterile petri dishes. This was followed by the addition of 10 ml of pre-cooled (45°C) sterilized De Man-Rogosa-Sharpe (MRS) agar. The plates were allowed to set after both the sample and the MRS were mixed thoroughly and then incubated in an inverted position at 37°C in anaerobic jars. The analyses were done in triplicates. At the end of the incubation period, distinct and discrete colonies were counted and sub-cultured on slants for microscopic view of the purified isolates (Aarti *et al.*, 2012; Cheesbrough, 2010). Further analyses were carried out only in fermented foods that produced LAB species including cow milk, sheep milk and lafun.

Tolerance to acidic pH values.

With a few minor alterations, the acid tolerance test was conducted as Bin *et al.* (2018) stated. At 37°C for 24 hours, all bacterial strains were cultured in MRS broth. Active cultures were divided into precisely 0.1

ml aliquots, adjusted to pH 3.0 with 5 N HCl, and incubated for 3 hours each at 37°C. Every hour, three samples were collected, and the vitality of the bacteria was assessed by pour plate counts with 10-fold serial dilutions made in peptone water with a 0.1 percent concentration.

Tolerance to bile condition

A few minor alterations were made to Bin *et al.* (2018) method for the bile tolerance test. A saturated bile solution was made separately by dissolving powdered bile extract after strains were cultured in MRS broth at 37 °C for 24 hours (Oxoid). The bacterial cells were then cultured in 0.5 percent w/v bile salt for 3 hours after bile solution had been sterilized and filtered through a 4-micron filter. By employing 10-fold serial dilutions of all samples made in 0.1 percent peptone water, pour plate counts were used to estimate the bacterial viability.

Determination of antioxidant property of lactic acid bacteria

Only fermented foods that produced LAB species, such as cow milk, sheep milk, and lafun, were used to determine antioxidant properties using the Ferric Reducing Antioxidant Power assay (FRAP), scavenging activity against the 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical, and inhibition of lipid peroxidation.

Ferric reducing antioxidant power assay (FRAP)

The ferric reducing potentials of bacterial strains were ascertained using the ferricyanide-ferric chloride technique (Tundis *et al.* 2013). One millilitre of the dilute samples was added to three millilitres of the phosphate buffer (0.2 M, pH 6.6) in separate test tubes and thoroughly mixed. The test tube was filled with precisely 2.1 ml of potassium ferric cyanide (1%) ($K_3Fe(CN)_6$), and it was gently shook before being incubated for 20 minutes at 50°C. Exactly 2.5 ml of 10 % trichloroacetic acid (TCA) was added after incubation, and the resulting mixture was centrifuged for 10 minutes at 300 rpm. Exactly 2.5 ml of the solution was placed into a new test tube, along with 2.5 ml of distilled water and 0.5 ml of ferric chloride that was 0.1 percent ($FeCl_3$). Ascorbic acid (Vitamin C) was employed as the standard as the absorbance was measured at 700 nm against the blank. Absorbance value and

sample reducing power were associated. Equation 1 was used to calculate percentage inhibition (E1).

$$\% \text{ inhibition} = \frac{\text{absorbance of sample} - \text{absorbance of blank}}{\text{absorbance of sample}} \times 100 \quad (\text{E1})$$

Scavenging activity against 1,1-diphenyl-2-picryl hydrazyl radical (DPPH)

In this work, the DPPH radical scavenging activity of samples was assessed using the method proposed by Sopheak and Betty (2002). In 95 percent ethanol, a stock solution of the DPPH radical (0.004 percent, w/v) was made. Each sample was given 2 ml of DPPH, which was then added, mixed, and allowed to sit for 30 mins at room temperature (25 °C) in a darkened space. A UV-visible spectrophotometer was used to assess each sample's absorbance at 517 nm (Varian Carry 50 Conc.). A control was ascorbic acid (Sigma, Germany; concentration of 0.02 mg/ml). According to equation 2, the antioxidant activity was determined as a percentage of DPPH activity (E2).

$$\% \text{ inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (\text{E2})$$

Inhibition of lipid peroxidation

The method of Lin and Chang (2000) was used to perform the inhibition of the lipid peroxidation experiment. Precisely 0.1 ml of each sample was pipetted into a test tube, which was then filled to the top with distilled water and treated with 0.05 ml of ferrous sulphate (FeSO₄) (0.07 M). The combination was then incubated for 30 mins. The liquid was vortexed, heated to 95°C for 60 minutes, and 1.5 ml of 0.8 percent (w/v) thiobarbituric acid, 1.1% sodium dodecyl sulfate, and 20% trichloroacetic acid were added to it. After cooling, 5 ml of butan-1-ol was added, and 10 minutes were spent centrifuging at 3000 rpm. At 532 nm, the absorbance was measured. As shown in Equation 3, the percentage inhibition of lipid peroxidation was computed (E3).

$$\% \text{ inhibition of lipid peroxide formed by the sample} = 1 - \frac{E}{C} \times 100 \quad (\text{E3})$$

Where:

C= absorbance of fully oxidized control

E= absorbance in the presence of extract

The physicochemical parameters of laboratory-produced yoghurts

At prevent scorches, a specific amount of milk was heated to a temperature between 76 – 82 °C and frequently stirred. The milk was allowed to cool to room temperature (29°C) after reaching the predetermined temperature. After cooling, probiotic bacteria were added to plain yoghurt or starter. To achieve a uniform spread, the solution was carefully mixed. After that, the cultured milk was placed into containers and kept in a refrigerator with hot water pitchers. Throughout the procedure, the heat from the containers was maintained consistently. After a 6–8 hour fermentation period, the samples of yoghurt were examined to see how solid they were. Confirmed samples were refrigerated- ready for consumption.

pH and titratable acidity

Using a digital pH meter (Martini, Italy), the pH of each yoghurt sample was tested. The method outlined by Tomovska *et al.* (2016) was used to measure the titratable acidity. An Erlenmeyer flask was filled with 20 milliliters of the yogurt sample before 1 milliliter of a 2 percent w/v solution of phenolphthalein was added. After that, the substance was titrated with 0.1 M NaOH solution to produce a faint but permanent pink appearance. Triplicate analyses were carried out. Using equation 4, the titratable acidity percentage was obtained (E4).

$$\text{Titratable acidity (\%)} = \frac{\text{ml} \times \text{N} \times 90 \times 100}{\text{V} \times 1000} \quad (\text{E4})$$

Where; ml= ml of NaOH used, N= Normality of 0.1 N NaOH and V= volume of milk solution used.

Organoleptic score

Ten (10) panelists—six men and four women—evaluated how well the generated yoghurts were received by consumers. Each panelist received three test samples with random labels. A 9-point hedonic scale was used to grade the yoghurt products' appearance, color, sourness, aroma, taste, and general acceptability (9- extremely like, 8- very much like, 7- moderately like, 6- slightly like, 5- neither like nor dislike, 4- slightly dislike, 3- moderately dislike, 2- very much dislike, and 1- extremely dislike).

Statistical analysis

Data were collected in triplicate and the mean and standard deviation were calculated. One-way analysis of variance (ANOVA) was used to analyse the significant difference, and $p \leq 0.05$ was used to determine its significance.

Results

Isolation and characterization of probiotics from selected Nigerian fermented foods.

Total anaerobic bacterial count from fermented food samples is presented in Table 1. Sorghum and non-refrigerated goat milk samples had the highest bacterial counts (9.7 and 9.1 $\times 10^4$ cfu/ml respectively) while fermented cassava (fufu) recorded the lowest counts (7.0 $\times 10^3$ cfu/ml).

Table 1: Bacterial count for locally sourced fermented samples ($\times 10^3$) colony-forming unit per millilitres (cfu/ml)

Samples/Counts	1	2	3	Average
RCM	47	45	47	4.6 $\times 10^4 \pm 1.15$
NRCM	79	82	77	7.9 $\times 10^4 \pm 2.52$
RGM	70	75	72	7.2 $\times 10^4 \pm 2.52$
NRGM	95	90	89	9.1 $\times 10^4 \pm 3.21$
RSM	70	61	68	6.6 $\times 10^4 \pm 4.72$
NRSM	86	84	83	8.3 $\times 10^4 \pm 1.53$
Fufu	8	10	7	7.0 $\times 10^3 \pm 1.53$
Lafun	7	12	11	1.0 $\times 10^4 \pm 2.65$
Pap (Millet)	65	60	62	6.2 $\times 10^4 \pm 2.52$
Pap (Maize)	50	50	51	5.0 $\times 10^4 \pm 0.58$
Garri Water	90	92	89	9.0 $\times 10^4 \pm 1.53$
Kunnu	70	65	68	6.7 $\times 10^4 \pm 2.52$
Pap (Sorghum)	100	95	98	9.7 $\times 10^4 \pm 2.52$

Key: RCM-refrigerated cow milk, NRCM- non-refrigerated cow milk, RGM- refrigerated goat milk, NRGM- non-refrigerated goat milk, RSM- refrigerated sheep milk, and NRSM- non-refrigerated sheep milk

Figure 1 shows the microscopic view of the purified bacterial strains. This includes: *Streptococcus thermophilus*, LAB, *Streptococcus faecalis* and Yeast cells obtained from four (4) food samples including

fermented cow milk, fermented sheep milk, lafun and garri water. However, only lafun, fermented cow milk and sheep milk produced LAB species.

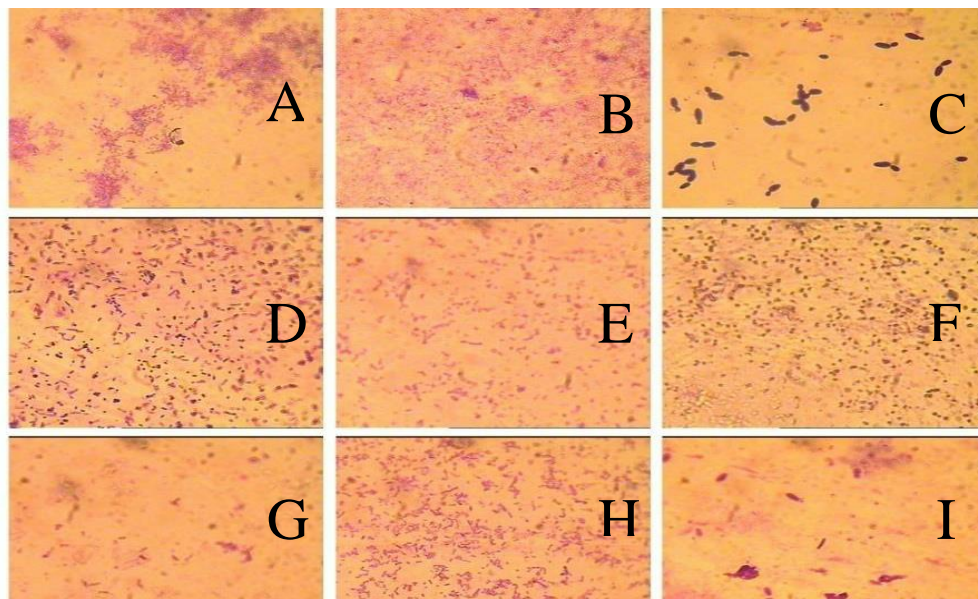


Fig 1: Microscopic view of purified bacterial and yeast strains.

Key:

- (A) Yeast cells and *Streptococcus thermophiles*
- (B) *Streptococcus thermophiles*
- (C) *Lactobacillus* species and *Streptococcus thermophiles*
- (D) *Streptococcus thermophiles* and *Streptococcus feacalis*
- (E) *Streptococcus thermophiles*
- (F) *Streptococcus thermophiles* and *Streptococcus feacalis*
- (G) *Lactobacillus* species and Yeast cells
- (H) *Streptococcus thermophillus*
- (I) *Lactobacillus* species and Yeast cells.

Acid and bile tolerance test for probiotics isolated from a Nigerian fermented food.

Figures 2 and 3 show results from acid and bile tolerance tests. Data from Figure 2 demonstrate that at a pH of 3.0, CM probiotics

had the highest percentage of survival, followed by lafun and fermented sheep milk. Similar to Figure 2, isolates survived the most in fermented cow milk under 0.5 % bile salt conditions, followed by fermented sheep milk and lafun (Figure 3). However, there were no differences in survival rates across the samples that were significant ($p \leq 0.05$).

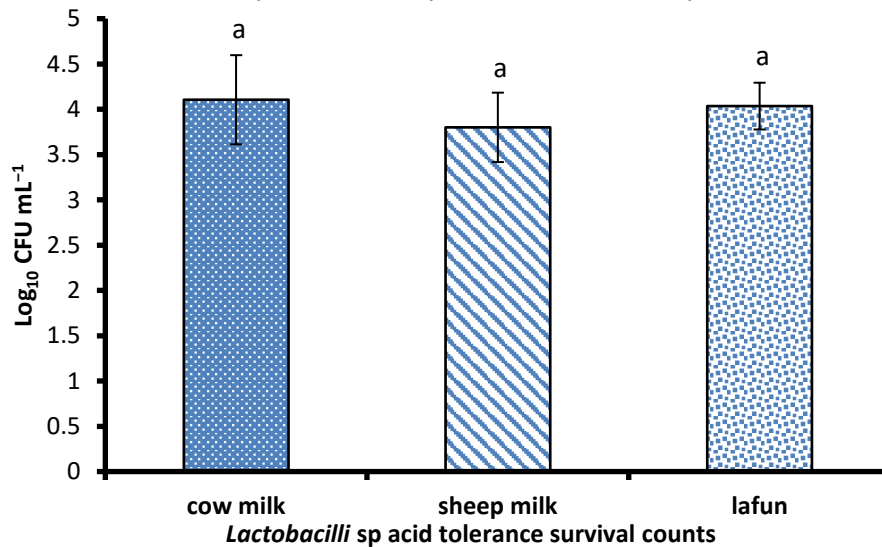


Fig 2: Probiotics survival acidic pH condition of 3. Values are expressed as the mean ± SD. Value with letter a is significantly different (sig. diff.) at $p \leq 0.05$.

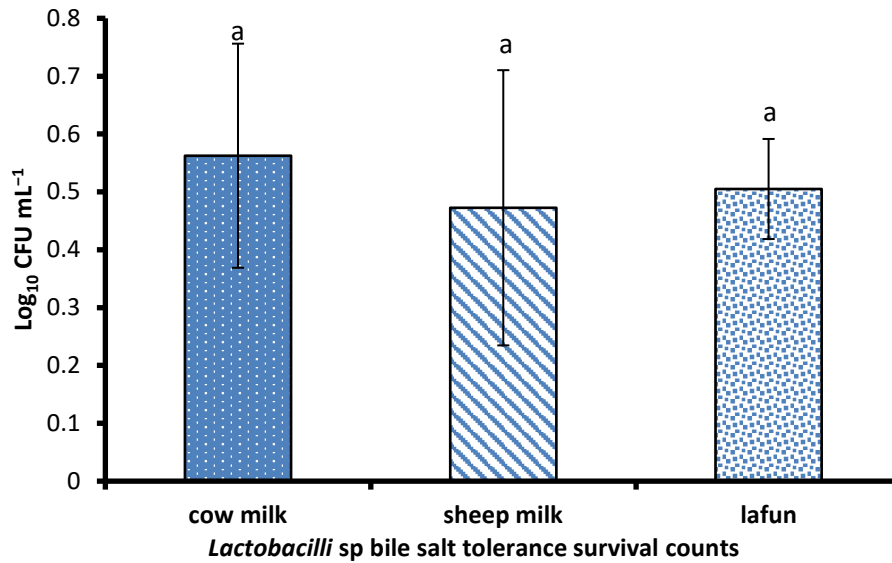


Fig 3: Probiotics survival under 0.5% bile salt conditions. Values are expressed as the mean ± SD. Value with letter a is significantly different (sig. diff.) at $p \leq 0.05$.

In vitro antioxidant activity of probiotics isolated from selected foods.

Table 2 shows the antioxidant activity of isolated probiotics from the selected foods. In varied degrees, the samples elicited strong antioxidant potentials. The ability of the three samples to scavenge DPPH radicals was not statistically different ($p < 0.05$); while vitamin C, the control sample had much stronger antioxidant activity than the samples of fermented cow milk, lafun, and fermented sheep milk. Comparing the reference (vitamin

C), fermented cow milk, and Lafun samples to the isolates from the fermented sheep milk sample, it was found that they had a considerably higher ($p < 0.05$) ferric reducing potential. Compared to fermented sheep milk and lafun, isolated fermented cow milk effectively decreased lipid peroxidation. However, compared to other samples, the control sample (vitamin C) exhibited considerably higher ($p < 0.05$) inhibitory potentials.

Table 2: Antioxidant activity of probiotics isolated from selected Nigerian fermented foods.

Sample	DPPH (%)	FRAP (abs)	Inhibition of Lipid Peroxide (%)
Vitamin C	92.97 ± 0.12 ^a	44.96 ± 0.32	71.17 ± 0.03 ^a
Cow milk	40.36 ± 0.32	43.86 ± 0.23	49.41 ± 0.06 ^b
Lafun	37.23 ± 0.06	47.70 ± 0.22	31.76 ± 0.09
Sheep milk	37.76 ± 0.50	52.70 ± 0.02 ^a	27.49 ± 0.12

Values are expressed as the mean ± SD. Values with different letters (a, b, c, d) in the same column are significantly different at $p \leq 0.05$

Laboratory yoghurt's pH, titratable acidity and organoleptic scores.

The pH and titratable acidity fluctuations of prepared yoghurt samples are depicted in Figure 4. The pH of fermented sheep milk was highest, followed by Lafun, then fermented

cow milk, and finally the control. The pH range for all yoghurt samples was 4.77 to 5.45. The pH of prepared yogurt samples was considerably higher ($p \leq 0.05$) than the control (D) sample (commercial yoghurt).

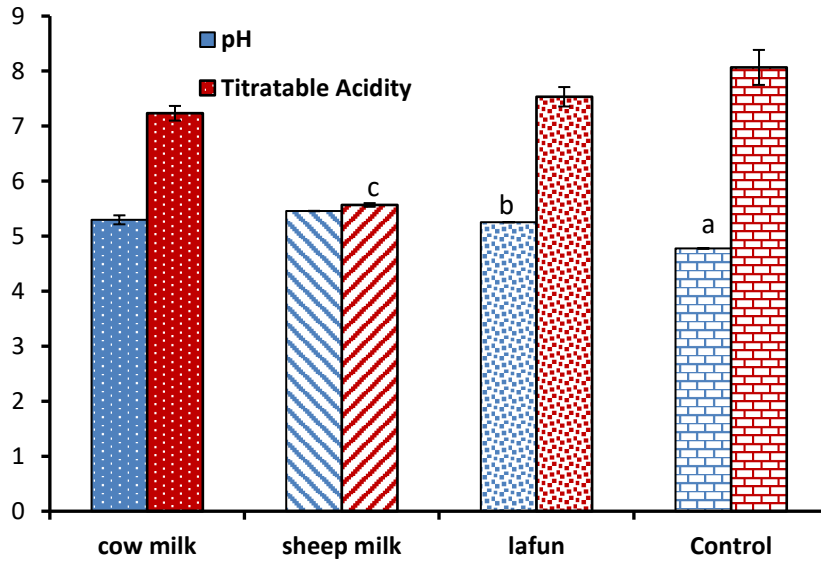


Fig 4: Changes in pH and titratable acidity of yoghurt samples produced with probiotics isolated from different fermented foods. Values are expressed as mean ± SD. Values with different letters (a, b, c,) are significantly different (sig. diff.) at $p \leq 0.05$. Blue: a = sig. diff from CM, SM and Lafun, b= sig. diff. from SM. Red: c = sig. diff. from CM, Lafun and control.

Table 3: Sensory characteristics of yoghurt samples produced from different fermented food samples.

Sensory characteristics	Cow milk	Sheep milk	Lafun	Commercial yoghurt
Appearance	3.85±0.88	3.80±1.06	3.95±1.15	3.85±1.18
Colour	4.15±0.88	3.70±1.22	4.25±0.64	4.10±0.85
Aroma	3.95±0.89	4.05±0.95	4.15±0.75	3.60±1.19
Taste	3.45±1.15	4.20±0.95	3.95±0.83	3.80±1.20
Smoothness	4.35±0.88	4.20±0.95	4.00±1.26	4.15±0.99
Sourness	3.05±1.05	3.55±0.89	3.80±1.09	3.85±1.20
General acceptance	3.85±1.09	4.05±0.83	4.00±0.80	3.80 ± 0.89

Values are expressed as the mean ± SD. Values with different letters (a,b,c,d,e,f,g) are significantly different at $p \leq 0.05$

Titratable acidity from the samples ranged from 5.56 - 8.06 %. All the yoghurt samples had significantly lower ($p \leq 0.05$) titratable acidity than the control (D) sample. Titratable acidity decreased in the order of control > Lafun > fermented cow milk > fermented sheep milk.

Discussion

In this study, we screened and isolated probiotics (*Lactobacillus sp*) from some nutritionally rich Nigerian fermented foods; evaluated their potentials as starter cultures for yoghurt production; and suggested these foods as cheap and safe sources of LAB strains or probiotics.

Total anaerobic bacterial counts from food samples are presented in Table 1. Sorghum and non-refrigerated goat milk samples had the highest two bacterial counts (9.7 and 9.1

$\times 10^4$ cfu/ml respectively) while fermented cassava (fufu) recorded the lowest counts (7.0×10^3 cfu/ml). The variable bacterial counts indicate the type of processing the samples were subjected to; however, persistent microorganisms survived. Several studies have isolated LAB species and applied the same for fermented food products like yoghurts (Lorenzo *et al.* 2018, Opara *et al.* 2014; Lorenzo *et al.* 2018).

Figure 1 showed that only lafun, fermented cow milk and sheep milk produced LAB

species- the species of interest in this study. These LAB isolates were confirmed through their morphological and biochemical characteristics after inoculation on MRS agar plates: Gram-positive rods, catalase, indole and oxidase-negative fermentative bacteria. Karami *et al.* (2017) also isolated LAB species from dairy samples with similar characteristics. This study underscores that fermented foods harbour functionally diverse and active microorganisms whose bio-transformative actions have industrial capabilities. During fermentation, microbes elicit anti-pathogenic abilities and improve raw food's nutritional, organoleptic and health-promoting statuses (Okeke *et al.* 2021). Local Nigerian fermented foods are very rich in essential and non-essential nutrients with which they support a host of self-perpetuating microbes. Companies or families that produce fermented food items could source these active mercenaries locally, multiply and apply them to work.

The ability of strains to survive in low pH stomach settings was investigated by acid and bile tolerance testing. It is an *in vitro* assessment of the isolates' small intestine colonization and metabolic activity. Figure 2 demonstrates that at a pH of 3.0, isolates from CM had the highest survival rate, followed by lafun, and fermented sheep milk. Several LAB species retain their viability after exposure to a pH range of 2.5 - 4.0; however, Samuel *et al.* (2019) recorded reduced LAB viability at lower pH values. The survival patterns for isolates over 3 hours indicate that isolates survived well at low acidic conditions. Our findings support those of Berebon *et al.* (2019), who found that LAB species rapidly increased between pH values of 1.5 and 3.5. The study of Succi *et al.* (2017) also supports this finding. The survival of LAB species in reported pH conditions could be due to their possessing enzymes such as glutamate decarboxylase, arginine deaminase and H-ATPase proton pump- these enzymes control homeostasis (De Angelis and Gobbetti 2011). Isolating and identifying probiotics capable of withstanding gastrointestinal conditions can be applied to manage gastrointestinal tract (GIT) infections, e.g., salmonellosis, ulcer, diarrhoea, etc. (Sahadeva *et al.* 2011; Vantsawa *et al.* 2017). The apparent acidic resistance and potential anti-pathogenic functions make using LAB species in yoghurt production desirable (Schubert and Kaunitz, 2021; Vincent, 2023).

Additionally, on the MRS agar, all test species were bile resistant. The maximum level of tolerance was found in LAB species gotten from fermented cow milk, whereas the lowest level was found in LAB species isolated from fermented sheep milk (Figure 3). The survivability of probiotic species in the gut is essential for them to be effective. The intestinal bile contains deleterious salts capable of negatively affecting non-resistant species (Samuel *et al.* 2019; Lorena *et al.* 2013). According to our findings, as shown in Figure 3, a bile salt concentration of 0.5 percent was unable to stop lactic acid bacteria from growing. LAB species possess proteins that help pump out bile salts or protons - preventing misfolding (Lorena *et al.* 2013).

The antioxidative potentials of the isolates were evaluated with the DPPH, FRAP, and inhibition of lipid peroxidation tests. In varied degrees, the samples elicited strong antioxidant potentials. In ethanol, DPPH absorbance was steady at 517 nm, but when it came into contact with an antioxidant, the radical was neutralized, and the absorbance was decreased (Abubakar *et al.* 2012). According to a literature (Ayyash *et al.* 2017), LAB's proteolytic activity during fermentation may have an impact on the peptides that are released from milk protein and, as a result, may have an impact on the FRAP values of fermented milk. Peptides in fermented foods have potent antioxidants and can inhibit DPPH radicals, peroxides; ultimately resulting in increased antioxidant capacity. Their free radical scavenging activities suggest that they could be used as natural antioxidant supplements for improving human health. Therefore, isolates from these fermented food samples are suitable for yoghurt production due to their antioxidant qualities. Results from this study correlate with that of Osuntoki and Korie (2010), who reported a high DPPH scavenging activity from LAB species isolated from two fermented Nigerian foods (*Wara* and *Ugba*) inoculated in whey fractions. Additionally, Al-Dhabi *et al.* (2020) noted that LAB species obtained from pineapple puree displayed, DPPH, superoxide, ferric reducing power acid and bile tolerance, hydroxyl radical scavenging potentials and anti-pathogenic activity.

Two methods were adopted in quantifying the acid content of yoghurt- pH and titratable acidity (Bamise and Bamise, 2008). The pH

and titratable acidity fluctuations of the yoghurt samples are depicted in Figure 4. The pH of yogurt samples decreased in this order; fermented sheep milk > Lafun, > fermented cow milk > control, with yoghurt samples having a pH range of 4.77 to 5.45. All yogurt samples, including the control (commercial yoghurt), were higher than the FDA standard of 4.6 (The U.S. Food and Drug Administration 2021). Titratable acidity from yogurt samples ranged from 5.56 - 8.06 % and were within the FDA standard of ≥ 0.7 % (U.S. Food and Drug Administration 2021). This may be due to process variables, fermentation time, as well as raw materials sourcing; however, it is worthy to note that they are all within their safe limits.

In food analysis, pH and titratable acidity are interlinked because they give insights into the quality of foods. Microbes thrive in different environments at a certain pH range and the quality of a food can be predicted by its flavour and organic acid contents- which are reflected in their titratable acidity measurements (Tyl and Saddler 2017; Bamise and Bamise, 2008). The isolates' acidity is a result of LAB metabolism; factors that may have affected it include: how long the samples naturally fermented, how many organic acids there were, and how much protein was broken down (Papadimitriou *et al.* 2007; Al-Sheraji, 2013). Additionally, no connection between the pH levels and titratable acidity was seen (Olugbuyiro, 2011). Isolates were adjudged appropriate as starting cultures.

There were no significant variation amongst the yogurt samples in their organoleptic score means ($p > 0.05$), demonstrating homogeneity between parameters (Table 3). Hence, prepared yoghurt samples could pass for commercial yoghurts in quality.

5.0 Conclusion

This study shows that fermented foods harbour active microbes that can also be used effectively as starter cultures for similar fermented products. Probiotics from our fermented food samples (fermented sheep milk, fermented cow milk, and lafun) survived low pH, withstood bile salt conditions, and elicited significant antioxidant activities.

Interestingly, the prepared yogurts had good sensory and quality characteristics. Therefore, using isolated probiotics from fermented foods to produce similar healthy foods is safe and beneficial. Food processing influence the survival of inherent microbial species; whether or not these surviving species can affect food

transformation, depends on the species. Isolates from fermented cow milk samples survived pH and bile acid tests more and fewer from sheep milk; however, there was no appreciable differences in organoleptic characteristics between the three fermented food (CM, Lafun, and SM) samples.

Results for antioxidative and lipid peroxidation suggests that our prepared yogurts and their inherent microbes are beneficial for treatment and prevention of inflammation-induced ailments as experts believe that inflammation is foundational to many ailments- results from cell stress and the production and accumulation of reactive species. The titratable acidity values of the products were all within the USFDA recommended standards (≥ 0.7 % - the U.S. Food and Drug Administration, 2021). These combined results suggests that certain Nigerian fermented foods could serve as a cheap, safe, efficient, and healthy source of starter culture for the production of similar fermented food products. Further studies should explore the molecular characterization of the probiotics to enable their molecular manipulations for commercial scale-up productions- seeing that these prebiotics contain good source of antioxidants which are anti-aging and chronic diseases inhibitor.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

We acknowledge Mr. Utibe Evans for his intellectual contributions and the Management of Jesil Pharmaceutical Industries Limited, Minna, Niger State, Nigeria, who made the Microbiology Laboratory available for use throughout the research work.

References

1. Aarti, B., Puniya, M., Sangu, K. P. S., Kumar, S. and Dhewa, T. (2012). Isolation and Biochemical Characterization of Lactobacillus species Isolated from Dahi. *A Journal of Dairy Science and Technology*, 1 (2): 18-31.
2. Abubakar, M. A., Hassan, Z. and Imdakim, M. M. (2012). Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as

- determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA). *Afr. J. Microbiol. Res.* 6(34):6358-6364. DOI: 10.5897/AJMR12.702
3. Agbor-Egbe, T. and Mbome, I. L. (2006). The effects of processing techniques in reducing cyanogen levels during the production of some Cameroonian cassava foods. *J. Food Compos. Anal.*, 19(4), 354-363. <https://doi.org/10.1016/j.jfca.2005.02.004>
 4. Akabanda, F., Owusu-Kwarteng, J., Tano-Debrah, K., Parkouda, C. and Jespersen, L. (2014). The use of lactic acid bacteria starter culture in the production of Nunu, a spontaneously fermented milk product in Ghana. *Int. J. Food Sci.* 2014. <https://doi.org/10.1155/2014/721067>
 5. Al-Dhabi, N. A., ValanArasu, M., Vijayaraghavan, P., Esmail, G. A., Duraipandiyan, V., Kim, Y. O. and Kim, H. J. (2020). Probiotic and Antioxidant Potential of *Lactobacillus reuteri* LR12 and *Lactobacillus lactis* LL10 Isolated from Pineapple Puree and Quality Analysis of Pineapple-Flavored Goat Milk Yoghurt during Storage. *Microorganism.* 8(10):1461. <https://doi.org/10.3390/microorganisms8101461>
 6. Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M. and Hassan, F. A. (2013). Prebiotics as Functional Foods: A Review. *J. Funct. Foods.* 5:1542-1553. <https://doi.org/10.1016/j.jff.2013.08.00>
 7. Ayyash, M., Al-Nuaimi, A. K., Al-Mahadin, S. and Liu, S. Q. (2017). In Vitro Investigation of Anticancer and ACE-Inhibiting Activity, α -Amylase and α -Glucosidase Inhibition, and Antioxidant Activity of Camel Milk Fermented with Camel Milk Probiotic: A Comparative Study with Fermented Bovine Milk. *Food Chem.* 239:588-597. <https://doi.org/10.1016/j.foodchem.2017.06.149>
 8. Bamise, C. T., and Bamise, O. F. (2008). Quantifying the acidic content of commercial yoghurt drinks in Nigeria. *The Internet Journal of Dental Science* 6(1):8401. DOI:10.5580/14ff
 9. Berebon, D. P., Ofokansi, K. C., Attama, A. A., Eze, C. O., Onwusoba, R. C., Ugwu, C. T., and Evurani, S. A. (2019). Evaluation of *Lactobacillus* spp. isolated from locally consumed probiotic food in Nsukka, Enugu State, Nigeria for antimicrobial activity utilizing agar well diffusion and pH tolerance tests. *Afr. J. Biotechnol.* 18(32):1091-1097. DOI: 10.5897/AJB2019.16924
 10. Bin Masalam, M. S., Bahieldin, A., Alharbi, M. G., Al-Masaudi, S., Al-Jaouni, S. K., Harakeh, S. M. and Al-Hindi, R. R. (2018). Isolation, molecular characterization and probiotic potential of lactic acid bacteria in Saudi raw and fermented milk. *Evid.-based Complement. Alter.* 2018. doi: 10.1155/2018/7970463
 11. Chen P, Zhang Q, Dang H, Liu X, Tian F, Chen Y, Zhang H, Chen W. (2014). Screening for potential new probiotic based on probiotic properties and α -glucosidase inhibitory activity. *Food Control.* 35:65–72. <https://doi.org/10.1016/j.foodcont.2013.06.027>
 12. Cheesbrough, M. (2010). Laboratory Manual. In: *District Laboratory Practice in Tropical Countries*. United Kingdom: Cambridge University Press, pp.146-157
 13. De Angelis, M. and Gobbetti, M. (2011). Stress responses of lactobacilli. In: *Stress responses of lactic acid bacteria* Springer, Boston, MA. pp. 219-249.
 14. Edith Marius, F. K., François, Z. N., Pierre, M. K., Rui, Y. W., Taicheng, Z. and Li, Y. (2018). Screening and characterization of *Lactobacillus* sp. from the water of cassava's fermentation for selection as probiotics. *Food Biotechnol.* 32(1):15-34. <https://doi.org/10.1080/08905436.2017.141398>
 15. Egwim, E. C., Gana, M. N., Adefolalu, F. S., Oyelade, D. O., Evans, P. O., Oyewole, O. A. and Okoliegbe, I. N. (2013). Optimization of malt drink production from acha and rice blends. *BTAIJ*, 7(1):11-15. https://www.researchgate.net/publication/289322957_Optimization_of_malt_drink_production_from_acha_and_rice_blends
 16. Egwim, E., Amanabo, M., Yahaya, A. and Mainuna, B. (2013). Nigerian indigenous fermented foods: processes and prospects. *Mycotoxin and Food Safety in Developing Countries.* 153, pp.153-180. DOI:10.5772/52877
 17. Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. *Int. J. Environ. Res. Public Health.* 11(5): 4745-4767. <https://doi.org/10.3390/ijerph110504745>
 18. Food and Agriculture Organization & World Health Organization (2006). *Probiotics in food: Health and Nutritional Properties and Guidelines for Evaluation*. FAO.
 19. Food Safety and Standards Authority of India, FSSAI (2012). *Manual of analysis of methods*

- of foods, milk and milk products. Food Safety and Standards Authority of India, pp.12.
20. Guan, X., Xu, Q., Zheng, Y., Qian, L. and Lin, B. (2017). Screening and characterization of lactic acid bacterial strains that produce fermented milk and reduce cholesterol levels. *Braz. J. Microbiol.* 48:730–739. <http://dx.doi.org/10.1016/j.bjm.2017.02.011>.
 21. Karami, S., Roayaei, M., Hamzavi, H., Bahmani, M., Hassanzad-Azar, H., Leila, M. and Rafieian-Kopaei, M. (2017). Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. *Int. J. Pharm. Investig.* 7:137-141. https://doi.org/10.4103/jphi.JPHI_8_17.
 22. Lin, M. Y., Chang, F. J. (2000). Antioxidant effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. *Dig. Dis. Sci.* 45:1617-1622. DOI: [10.1023/a:1005577330695](https://doi.org/10.1023/a:1005577330695)
 23. Lorenzo, J. M., Munekeata, P. E., Dominguez, R., Pateiro, M., Saraiva, J. A. and Franco, D. (2018). Main Groups of Microorganisms of Relevance for Food Safety and Stability: General Aspects and Overall Description. *Innov Food Sci Emerg Technol.* 53:107. <https://doi.org/10.1016/B978-0-12-811031-7.00003-0>.
 24. Mulaw, G., Tessema, T. S., Muleta, D. and Tesfaye, A. (2019). *In Vitro* Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. *Int. J. Microbiol.* 27179514:1-11. <https://doi.org/10.1155/2019/7179514>.
 25. Okeke, E. S., Ita, R. E., Egong, E. J., Udofia, L. E., Mgbekhidinma, C. L. and Akan, O. D. (2021). Metaproteomic Insights into Fermented Fish and Vegetable Products and Associated Microbes. *Food Chem.: Mol. Sci.* 3:100045. <https://doi.org/10.1016/j.fochms.2021.100045>
 26. Olugbuyiro, J. A. (2011). Physico-chemical and sensory evaluation of market yoghurt in Nigeria. *Pak J Nutr.* 10(10):914-918. DOI: [10.3923/pjn.2011.914.918](https://doi.org/10.3923/pjn.2011.914.918)
 27. Opara, C. C., Kuru, T. and Ezenwaka, I. B. (2012). Production of Soy yoghurt from *Lactobacillus* isolated from fermented African oil bean seed (Ugba). *Greener J. Agric. Sci.* 3:110-119.
 28. Osuntoki, A. and Korie, I. (2010). Antioxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. *Food Technol. Biotechnol.* 48(4):505-511. <https://link.gale.com/apps/doc/A248659710/AONE?u=anon~b118558b&sid=googleScholar&xid=bbf795b6>
 29. Papadimitriou, C. G., Mastrojiannaki, A. V., Silva, A. V., Gomes, A. M., Malcata, F. X. and Alichanidis, E. (2007). Identification of Peptides in Traditional and Probiotic Sheep Milk Yoghurt with Angiotensin I-Converting Enzyme (ACE)-Inhibitory Activity. *Food Chem.* 105:647-656. <https://doi.org/10.1016/j.foodchem.2007.04.028>
 30. Sahadeva, R. P. K., Leong, S. F., Chua, K. H., Tan, C. H., Chan, H. Y., Tong, E. V. and Chan, H. K. (2011). Survival of commercial probiotic strains to pH and bile. *International Food Res.* 18(4).
 31. Samuel, O., Mavis, O. and Frederick, O. (2019). *In Vitro* Studies of the Probiotic Properties of Lactic Acid Bacteria Isolated from Akamu–A Nigerian Weaning Food. *Immunol and Infect. Dis.* 7(2):13-20. DOI: [10.13189/iid.2019.070201](https://doi.org/10.13189/iid.2019.070201)
 32. Schubert, M. L., Kaunitz, J. D. (2021). Gastric secretion. In: Feldman, M., Friedman, L. S., Brandt, L. J. 2021. eds. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 11th ed. Philadelphia, PA: Elsevier, pp. 51.
 33. Sopheak, S. and Betty, A. (2002). Free radical scavenging and antioxidant activity of caffeic acid amide and ester analogues: structure-activity relationship. *J. Agric. Food Chem.* 50:468-72. doi: 10.1021/jf010830b.
 34. Succi, M., Tremonte, P., Pannella, G., Tipaldi, L., Cozzolino, A., Coppola, R. and Sorrentino, E. (2017). Survival of commercial probiotic strains in dark chocolate with high cocoa and phenols content during the storage and in a static in vitro digestion model. *J. Funct. Foods* 35:60-67. <https://doi.org/10.1016/j.jff.2017.05.019>
 35. Tamime, A., Saarela, M., Korslund, S. and Mistry V. (2005). Production and maintenance of viability of probiotic microorganisms in dairy products. In Tamime, A.Y., Ed. *Probiotic Dairy Products.* Oxford: Blackwell Publishing Ltd., pp. 39–72
 36. Tomovska, J., Gjorgievski, N., and Makarijoski, B. (2016). Examination of pH, titratable acidity and antioxidant activity in fermented milk. *J. Mater. Sci.* 6(11-12):326-333. DOI: [10.17265/2161-6213/2016.11-12.006](https://doi.org/10.17265/2161-6213/2016.11-12.006)
 37. Tundis, R., Menichini, F., Bonesi, M., Conforti, F., Statti, G., Menichini, F. and Loizzo, M. R. (2013). Antioxidant and hypoglycaemic

- activities and their relationship to phytochemicals in *Capsicum annuum* cultivars during fruit development. *LWT- Food Sci. Technol.* 53(1):370-377. DOI:[10.1016/j.lwt.2013.02.013](https://doi.org/10.1016/j.lwt.2013.02.013)
38. Tyl, C. and Sadler, G. D. (2017). pH and titratable acidity. In *Food Analysis*. Springer, Cham. pp. 389-406.
39. U.S. Food and Drug Administration (2021). *Food and Drugs. Code of Federal Regulations 21: 2-21CFR131.200*
40. Vantsawa, P. A., Umar, T. and Bulus, T. (2017). Effects of probiotic *Lactobacillus acidophilus* on performance of broiler chickens. *Direct Res. J. Agric. and Food Sci.* 5(8):302-6. DOI: <https://doi.org/10.26765/DRJAFS.2017.DRJA16284589>
41. Vincent, K. (2023). Gastritis and peptic ulcer disease. In: Kellerman, R. D, Rakel, D. P, Heidelbaugh J. J. and Lee EM, eds. *Conn's Current Therapy*. Philadelphia, PA: Elsevier, pp. 216-219