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ORIGINAL ARTICLE

Effect of Gum Producing Lactic Acid Bacteria from Palm Sap on the Quality Attributes of Yoghurt

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Abstract

The effect of gum-producing lactic acid bacteria (GP-LAB) from palm sap and their exopolysaccharides (EPSs) on the quality attributes of yoghurt were investigated. GP-LAB such as *Lactobacillus plantarum, Leuconostoc lactis* were used in a mono-or mixed –cultures, and their EPS as stabilizing agent for yoghurt production.Yoghurt (Y) formulations (A, Y+commercial starter culture; B, Y+*lactobacillus plantarum;* C, Y+*leuconostoc lactis*; D, Y+*lactobacillus plantarum* and *leuconostoc lactis*) were produced by reconstitution of whole skimmed milk while reference yoghurt (RY) was obtained from the market and stored at 4^{o}C. Sensory, proximate, textural and rheological analyses of samples were determined. Microbial and physicochemical analysis was carried out every week for four weeks. Formulations RY and D were the most preferred while formulation A, B and C were not significantly different ($p > 0.05$). Formulation D had the least moisture (80.64 ± 0.11%), highest ash (2.19 ± 0.01%) and highest protein (6.48 ± 0.04%). Whereas, RY had the least ash, fat, protein and carbohydrate contents as well as the highest moisture (89.85 ± 0.37). RY and sample D (3.10 ± 0.00; 3.50 ± 0.71) had least % whey separation, highest firmness (20.50 ± 0.71; 21.50 ± 0.71) and cohesiveness (22.96 ± 0.07; 23.22 ± 0.05) respectively. In all shear rates, formulation D and RY had the highest and the least apparent viscosities respectively. Total viable count ranged from 1.3 - 3.8 × 10^7, 3.2 – 4.9 × 10^7, 2.5 – 5.5 × 10^7, 2.3 – 5.6 × $10^7$and 1.3- 6.3 × 10^7cfu/ml on days 1, 7, 14, 21 and 28, respectively. At day 28, formulation D had the highest microbial count (6.3× 10^7cfu/ml) whereas RY had the least microbial population (1.3 × 10^7cfu/ml). The pH and titratable acid (TTA) (mg lactic acid/g) ranged (4.19 – 4.66; 0.88 – 1.41). In conclusion, formulation D competed favourably with the reference sample in all parameters determined. The combination of *Lactobacillus plantarum* and *Leuconostoc lactis* cultures as well as their gums improved the quality attributes of yoghurt.

Practical application

Exopolysaccharide obtained from *lactobacillus plantarum* and *leuconostoc lactic* from palm sap in this study can find practical applications in food industry as food additive: a stabilizing agent (yoghurt), emulsifying agent (mayonnaise), biothickening agent (yoghurt), gluten replacers in cassava-wheat composite bread. EPS can be locally source for the purpose mentioned above and thus, eliminate dependence on importation foreign food additive.

Keywords: Exopolysaccharide, lactic acid bacteria, fermented foods, quality yoghurt, apparent viscosity.

1.Introduction

Yoghurt is known for its unique taste, aroma and a veritable nutritional source of protein, energy, milk-fat, unfermented lactose, vitamins and calcium (Ebringer *et al*., 2008).

The potential benefits derived from consuming dairy products, especially fermented milk, containing probiotic ranged from therapeutic to dietetic (Roberfroid *et al*., 2010). The

consumption of yoghurt containing probiotic has gain popularity globally over the years. Consumers are constantly demanding quality products, with health-claim and desirable sensory attributes including; appearance, texture, colour, smell and taste in addition to whey flow. In most cases, yoghurt products fail to maintain stability during storage and for the duration of the product shelf life due to syneresis (Vital *et al*., 2015; Paulina *et al*., 2091). Interestingly, one of the major setbacks in the dairy industry is the technological defect occasioned by rearrangement of the network of casein micelles in yoghurt. The shrinkage of milk protein gel with the expulsion of water from the network leading to whey separation, and consequently, diminishes yoghurts sensory attractiveness (Rekha *et al*., 2012).

For consistency and self-stability of yoghurt, the inclusion of stabilizing agents that binds water has been advocated. Stabilizing agents interact with the network of casein micelles promoting a protein-stabilizing –agents-complex and thereby stabilizing the structure of yoghurt. In addition, most of these stabilizers currently being used are from plant origin, which is chemically or enzymatically synthesized and/or modified (Tamine & Robinsion, 1999). For applications in food, these chemically synthesized stabilizing agents use are restricted (De Vuyst *et al*., 1998).

Further, good quality yoghurt is firm and smooth consistency with a sweet aroma and pleasant taste. One of the most essential parameters of good quality yoghurt is the texture. The textural, rheological properties and flavour characteristics of fermented milk products are influenced by culture starter type and the activity of microorganisms present in the culture used (Kakisu *et al*., 2011). In general, *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus* lactic acid bacteria are the most widely used starter cultures for acid production in yoghurt. Interestingly, other species belonging to genera of *Lactobacillus* have been used as starter cultures in dairy products and in commercialized probiotic foods (Reid, 2015).

To improve the physical stability of yoghurt, lactic acid bacteria that produce exopolysaccharide are currently used as starter cultures to promote physicochemical properties; stabilization, emulsification, provision of texture and mouth-feel. The use of exopolysaccharide-producing lactic acid bacteria (LAB) as starter cultures in yoghurt to reduce susceptibility to syneresis, improve viscosity and consistency, and eliminate mechanical damage has been reported (Shah, 2002). Consequently, the presence of exopolysaccharide produced by yoghurts' LAB enhances smoothness, thicker texture and higher viscosity. In addition, it prevents gel fracture and wheying off, and enhances texture characteristics.

The EPS excreted by LAB can be regarded as safe biopolymers since LAB are generally considered as safe (GRAS).To this end, several studies have reported health benefits attributed to EPS synthesized by LAB owing to biological activity; antioxidant, antitumour, probiotic and cholesterol-lowering activities, and antibiofilm forming by pathogens and immunomodulation (Sule *et al*., 2018). Consequently, the increasing attention on some strains of gum-producing LAB and their EPSs in the last two decades has been documented (Wang *et al*.; 2010). In addition, the search for LAB strains with the capacity to produce a large amount of EPS with better rheological properties than those already in existence has increased in the last decade (Badel *et al*., 2011). Recent studies reported the isolation of some LAB strains with the capacity

to produce large quantities of EPSs with techno-functional properties (Adamu-governor *et al*., 2018; Adesulu-Dahun*set al*., 2018c). In this study, gum-producing LAB strains isolated from oil (*Eleais guineensis*) and raphia (*Raphia regalis*) palm sap were evaluated for their *in-vitro* starter potential and suitability of their gums as stabilizing agents in yoghurt. This study aimed to identify a suitable starter culture and biopolymer for the improvement of the quality attributes of yoghurt.

2. Materials and Methods

2.1 Materials

Skimmed powdered milk, commercial starter culture (yogourmet®), xanthan gum and granulated sugar were purchased from Bariga market, Bariga, Lagos State, Nigeria. Freshly produced yoghurt sample (reference yoghurt) was purchased from Shop Rite, Lagos. Evaporated whole milk/skimmed solids remain the milk of choice for yoghurt production given the scarcity in the supply of fresh cow milk due to low scale of pastoral farming in Nigeria and most other Africa countries. In addition, evaporated whole milk has already been processed, fortified with nutrients and vitamins (Tamine & Robinsion, 2000) and not prone to immediate spoilage when properly packaged.

2.2.1 Isolation of Gum-producing Bacteria

Gum-producing bacteria were isolated from palm wine according to the method described by Adamu-Governor *et al*. (2018). A loop full of palm sap sample were streaked on 6% sucrose agar and incubated for 24 hr at 35^{o}C. Pure cultures were stored in 6% sucrose agar slants and stored at 4°C after distinct colonies were sub-cultured severally.

2.2.2 DNA extraction

Overnight cultures of the mucoid isolates on Tryptone Soy Broth (TSB) were used for genomic DNA extraction by using the ZR Fungal/Bacterial DNA kit (Zymo Research, California, USA) according to the instructions of the manufacturer.

2.2.3 Amplification of partial 16S rRNA gene, sequencing and analysis

The 165 rRNA gene from genomic DNA was amplified using bacteria universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR amplification was carried out in an Eppendorf Thermocycler (PTC-200, USA), in 25µl reactions containing 12.5µl of 2×PCR Master Mix (Biolabs, New England), 2.0µl of template DNA, 0.2µl of broth forward and reverse primers and 10.1µL of nuclease-free water (Thermo Fisher Scientific,). PCR conditions was an initial denaturation step at 94^{o}C for 5min, followed by 30 cycles of denaturation at 94^{o}C for 30s, hybridization at 55^{o}C for 30s and elongation at 72^{o}C for 1min, followed by a final extension step at 72^{o}C for 10 min. Amplicons were verified by agarose gel electrophoresis. The 16s rRNA gene amplicons were sequenced using the same set of primers used for PCR at Inqaba biotech (South Africa) using the Big Dye Terminator v 3rd cycle sequence kit (Applied Biosystems, UK), purified sequencing PCR products were run on a 3130 Genetic analyze (Applied Biosystems/Hitachi, Japan). All the obtained sequences were screened using the Basic Local Alignment Search Tool (BLAST) for their identity (http://blast.ncbi.nlh.gov/Blast.cgi) (NCBI).

2.2.4 Microbial gum production and quantification of EPS

Cultivation was performed on basal medium (v\v, 6% sucrose, 0.5% peptone, 0.05% K_2HPO_4, 0.025% $MgSO_4$) in 1.5 L flasks with 1 L working volume according to the method described by Adamu-Governor *et al*. (2018). Inoculation of the medium was carried with 3% (v/v) overnight culture of gum-producing LAB and growth was monitored by absorbance measurement at 650 nm using a spectrophotometer (Spectrum lab S23A, Globe Medical, England). Isolation and quantification of EPS were carried out according to the method described by Adamu-Governor *et al*. (2018). Cells were harvested from fermented culture broth by centrifugation at 11,000×g for 30 min in a pre-weighed tube. The suspension was initially stirred with a glass rod and heated at 80^{o}C (heating block) to extract EPS associated to bacteria cell. The quantification of EPS was done using dry weight method.

2.2.5 Production of yoghurt sample

Yoghurts were produced according to Nigeria Industrial Standard (NIS, 2004). Four hundred grams (400g) of whole evaporated skimmed milk was reconstituted in 1000mL distilled water and heated to 80^{o}C for 15 min for homogenization and pasteurization, and then allowed to cool to 42-45^{o}C before inoculation with starter culture. Incubated at 32 ± 2^{o}C for 10-12 hours (overnight) until a pH of about 4.53- 4.66 was attained. For this study, different yoghurt formulations (A-D) were prepared as indicated in Table 1 while yoghurt sample RY was a reference sample. Titratable acidity (TTA), pH, and microbial population of the yoghurt were measured 24 hours after production and weekly during storage for a total of four weeks post-production.

Table 1: Yoghurt recipe and formulation used in production

S/n	Ingredients/Amount	Form A	Form B	Form C	Form D
1	Skimmed milk (g)	400	400	400	400
2	Sugar (%)	10	10	10	10
3	Xanthan gum (%)	0.5	-	-	-
4	EPSLp (%)	-	0.5	-	-
5	EPSL1 (%)	-	-	0.5	-
6	EPSLp +EPSL1(%)	-	-	-	0.5
7	Starter culture (%)	1.25	-	-	-
8	*Leuconostoclactis* (%)	-	1.25	-	-
9	*Lactplantarum* (%)	-	-	1.25	-
10	Leu lac + Lact plan (%)	-	-	-	1.25
11	Water (mL)	1000	1000	1000	1000

Note: All amounts are the percentage of evaporated whole skimmed milk weight in grams (g). EPSLp; *Lactobacillus plantarum* exopolysaccharides, EPSL1; *Leuconostoc lactis* exopolysaccharide. EPSLp+EPSL1, and Leu lac + Lact plan were applied in ratio 1:1.The addition of bacterial starter cultures was 5 g/L (5% v/v) and bacteria inoculums were approximately 7 log10 cfu/mL, while stabilisers; (Xanthan gum, EPSLp and EPSL1) were added at a rate of 0.50% w/v at 70^{o}C.

2.2.6 Organoleptic evaluation

The yoghurt samples were evaluated for sensory characteristics and overall acceptability by a panel of 50 randomly selected judges. For the evaluation, each judge was served coded samples of all yoghurt formulations and asked to rate their taste, aroma, texture, appearance, and overall acceptability on a 9-hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely).

2.2.7 Proximate composition analysis

Proximate composition of yoghurts was determined using the standard method of AOAC (2005). Two grams of the samples were used for each of the parameters determined. Crude protein content was determined using the macro Kjeldahl method as described by AOAC (2005).The percentage carbohydrate content of yoghurt samples was determined by summing up the percentages of moisture, ash, crude protein, fat (ether extract) and subtracting from 100%.

2.2.8 Microbiological analysis of yoghurt during storage

The microbial analysis of the samples for day 1, 7, 14, 21 and 28 were carried out according to the method described by Udeozor & Awonorin (2014). An aliquot portion (0.1ml) of the 10^6 and 10^5 dilutions were spread plated onto freshly prepared, surface-dried nutrient agar (NA), De Man, Rogosa and Sharpe (MRS) agar (Heywood, Lancashire, UK) and 6% sucrose agar (SA) respectively. The anaerobic conditions were achieved in anaerobic jars supplemented with a pad of Oxoid^{TM} (Oxoid, Cambridge, UK) CO_2 Gas Generating kit. Similarly, 0.1ml of the 10^4dilution was spread plated on potato dextrose agar (PDA). Inoculated NA, MRS and 6% SA plates were incubated at 37^{o}C for 24-48h, while PDA plate was incubated at ambient temperature (28± 02^{o}C) for 3-5 days. Total plate counts were done by counting colonies on the cultured NA, MRS and 6% SA plates. For colony count on PDA plates, a hand lens was used to aid the accuracy of the enumeration of moulds. Total colony count was expressed in colony-forming units per millilitre (CFU/ml).

2.2.9 Morphological characterization

Morphological characterization of typical colonies was done by examining colony growth, Gram reaction, cell morphology (cocci or rods) and catalase reaction.

2.2.10 Physicochemical Analysis of yoghurt during storage

2.2.10.1 pH and Total Titratable Acidity (TTA) Determination

The pH of the yoghurts was determined by a pH meter (860032 Spec scientific, Scottsdale). The acidity was determined by the titration method using sodium hydroxide (0.1 mol/L) and 2-3 drops of phenolphthalein as an indicator

2.2.11 Apparent viscosity

Apparent viscosity of the homogenized samples was measured using a Brookfield viscometer (DV-E viscometer, Brookfield engineering Laboratory, Inc II commerce, Middleboro) by the modified method of Izadi *et al.* (2015). With a spindle no.3 and 3 rpm rotation speed (50, 60 and 100) at 25 ± 2 °C. Results recorded in centipoises (cP) after 50 s of shearing.

2.2.12 Whey separation determination (ml %)

Whey separation of yoghurts was measured based on spontaneous movement of whey out of the gel under the force of gravity as described by Alaa (2015). All samples were evaluated in triplicate.

2.2.13 Instrumental Texture Analysis

Instrumental textural attributes of yoghurt samples were measured in duplicate using a 300XPH analyzer (Perten Instruments AB, Hagersten, Sweden), equipped with a 7 kg load cell and a back-extrusion set consisting of a

sample container (50 mm diameter) and a compression plate (40 mm diameter). The sampling distance was 20 mm, the test speed 1 mm/s, and the retraction speed 5 mm/s. TexCalc software (version 4.0.4.67) was used in measuring the textural properties.

2.2.14 Statistical analysis

All statistical analyses were performed using a one-way Analysis of Variance test (ANOVA) with Duncan's Multiple Range post hoc tests to identify significant difference among the means where it exists ($p < 0.01$ and $p < 0.05$) with the aid of SPSS (SPSS Inc. version 20. Chicago, IL. USA).

SA, MRS agar plates and molecular profile respectively. The isolation of gum-producing (GP) bacteria isolates from palm sap was based on the ability of bacteria to form a mucoid colony on 6% SA. All bacteria isolated were Gram-positive, oxidase and catalase negative, and consisted of rod and/or cocci shape, arranged in chains or pair as shown in table 2.The use of microscopic cell shape in the description and classification of bacteria specie has earlier been reported (Cobeen & Jacob-Wagner, 2005). Bacteria isolates from palm sap and reference yoghurt sample fit the classification of lactic acid bacteria as Gram-positive, catalase and oxidase negative (Adamu-Governor *et al.*, 2018).

Table 2: Morphological and microscopic characteristics of bacteria isolates

Sample	Microscopic characteristics	Gram reaction	Media	Colour	Shape	Edge	Elevation
Refer	Cocci in pairs or in chain	+	MRS	Creamy white	irregular	Entire	Convex
-	Small rods in pairs or chain	+		Creamy gray	irregular	Entire	Raised
Form A	Cocci in pairs or in chain	+	MRS	Creamy white	irregular	Entire	Convex
-	Small rods in pairs or chain	+		Creamy gray	irregular	Entire	Raised
FormB	Small rods in pair	+	MRS	Creamy gray	irregular	Entire	Raised
Form C	Small cocci in chain	+	MRS	Creamy	irregular	Entire	Convex
Form D	Small cocci in chain	+	MRS	Creamy	irregular	Entire	Convex
-	Small rods in pairs or chain			Creamy gray	irregular	Entire	Raised

Reference yoghurt (Refer), Formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*), C (Y + *Leu.lactis*), D (Yoghurt + *Lactobacillus plantarum* and *Leuconostoc lactis*).

3. Results and Discussion

3.1Identification of gum-producing LAB

Presented in table 2 and 3 are morphological and microscopic characteristics of bacteria isolates in

All the produced yoghurts (A, B, C, and D) and the reference yoghurt (RY) were not contaminated by Fungi. The absence of fungi contamination in the yoghurt samples in this study may be attributed to the microbial quality

of ingredients used in the production as well as personal hygiene during production and packaging. The GP lactic acid bacteria isolated from palm sap were identified prior to their application in yoghurt production using 16S rRNA gene analysis as *Lactobacillus plantarum* and *Leuconostoc lactis* as shown in table 3. Earlier, Relman *et al*. (1999) reported the use of the 16S rRNA gene analysis for phylogenetic studies as it is highly conserved between different species of bacteria and archaea. Similarly, Okolie *et al*., (2013) reported the use of 16S rDNA gene analysis in the identification of the bacterial community in palm wine and most of the bacteria identified were lactic acid bacteria.

Table 3: Molecular identity of gum producing LAB isolated from palm wine

S/N	EPS Code	Name	Similarity %	Sequence ID No
1	EPS 1	*Lactobacillus plantarum*	99	EU121672
2	EPS 2	*Leuconostoclactis*	100	AB023968

3.2 Sensory evaluation

The mean sensory scores of the organoleptic evaluation and acceptability for the different yoghurt formulations are shown in table 4.

Table 4: Sensory evaluation of yoghurt samples

Sample	Appearance	Taste	Texture	Colour	Aroma	Overall
RY	8.40±0.82^{b}	8.60±0.60^{c}	8.40±0.75^{b}	7.85±1.60^{a}	8.40±0.82^{c}	8.20±1.20^{b}
Sample A	7.45±1.22^{a}	7.32±1.04^{b}	7.05±1.00^{a}	7.91±1.23^{a}	7.23±1.15^{ab}	7.41±1.01^{a}
Sample B	7.38±1.83^{a}	6.76±1.64^{ab}	7.00±1.67^{a}	7.29±1.62^{a}	7.67±1.32^{bc}	7.43±1.36^{a}
Sample C	6.95±1.28^{a}	6.33±1.28^{a}	6.52±1.69^{a}	7.52±1.33^{a}	6.71±1.52^{a}`	7.14±1.24^{a}
Sample D	8.26±1.44^{b}	8.49±1.97^{c}	8.86±1.62^{b}	7.62±1.53^{a}	8.31±1.47^{c}	8.15±1.20^{b}

Values are recorded in triplicate. The mean values in the same column with different superscript are significantly different ($p < 0.05$) from each other. Reference yoghurt (RY), formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*), C (Y + *Leu.Lactis*), D (Yoghurt + Lactobacillus *plantarum* and *Leuconostoclactis*).

Reference yoghurt (RY) was rated highest in appearance, taste, texture, aroma and overall acceptability and was significantly different (p <0.05) from other yoghurt samples. The consumer's acceptability of yoghurt is greatly influenced by the sensory attributes (Adriano *et al*., 2012). Further, the choice of probiotic

yoghurts by consumers are usually influenced by factors such as colour, aroma, health claims, price, brand and nutritional information (Menezes *et al*., 2011). Formulation A was not significantly different ($p > 0.05$) from formulation B, C and D in all aspects except for aroma. Similarly, yoghurt formulated with GP-lab and their EPSs did not differ organoleptically from the formulation containing commercial starter culture with xanthan gum as stabilizing agent in all the sensory quality attributes. This could be attributed to the interactions between the exopolysaccharides produced by EPS-producing LAB with the free water in the gel-like structure and the protein network of the yoghurt (Roua *et al*., 2018).

3.3 Proximate composition and whey separation analysis of yoghurts

The mean values of proximate composition differ significantly ($p < 0.05$) within the parameters (moisture, ash, fat, protein and carbohydrate) across the yoghurt formulations (A, B, C and D) and as well as the reference sample (RY) shown in table 5. The Reference yoghurt (RY) had the highest moisture content (89.85 ± 0.37%) followed by formulation C (82.19 ± 0.02%), B (81.51 ± 0.03%) and D (81.30 ± 0.04%), and formulation A with the least moisture content (80.64 ± 0.11%) and these means were significantly different each other ($p< 0.05$) except formulations B and D. The mean values of the moisture content of all yoghurt samples in this study were marginally above 80% except the reference yoghurt sample with 89% moisture content. Studies have reported 84-87% for moisture content in yoghurt samples made from raw and powdered cow milk (Yu *et al*., 2016). Also, Charles *et al*. (2015) reported moisture content of 88% for cow milk yoghurt.

Table 5: Proximate analysis of yoghurt samples

Sample	MC (%)	SC (%)	Ash (%)	Fat (%)	Protein (%)	CHO (%)	WS(%)
RY	89.85±0.37^{d}	10.15±0.37^{a}	0.27±0.01^{a}	0.76±0.02^{a}	3.41±0.13^{a}	5.73±0.22^{a}	3.10±0.00^{a}
Form A	81.30±0.04^{b}	18.77±0.55^{a}	2.03±0.01^{d}	1.93±0.00^{d}	6.27±0.01^{c}	8.54±0.02^{b}	5.20±0.28^{b}
Form B	81.51±0.03^{b}	18.49±0.03^{a}	1.71±0.01^{c}	1.78±0.00^{c}	6.19±0.00^{c}	8.82±0.01^{c}	5.90±0.14^{b}
Form C	82.19±0.02^{c}	18.90±0.59^{a}	1.47±0.02^{b}	1.69±0.00^{b}	5.98±0.04^{b}	8.69±0.05^{bc}	7.10±0.14^{c}
Form D	80.64±0.11^{a}	19.37±0.11^{a}	2.19±0.01^{e}	2.02±0.01^{c}	6.48±0.04^{d}	8.69±0.06^{bc}	3.50±0.71^{a}

Reference yoghurt (RY), Formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*), C (Y + *Leu.Lactis*), D (Yoghurt + *Lactobacillus plantarum* and *Leuconostoclactis*),whey separation (WS); %, percentage. Values are recorded in triplicate. The mean values in the same column with different superscript are significantly different ($p < 0.05$) from each other.MC; Moisture content, SC; Solid content, CHO; Carbohydrate

The texture and mouth-feel of yoghurt sample are affected by the amount of water content in the yoghurt. Reduction of shelf life of milk sample and microbial growth are the features of high water activity in yoghurt (Ajai *et al*., 2012). The total solids of all yoghurt formulations except the reference yoghurt were higher than the values earlier reported (Muhammed *et al*., 2005; Charles *et al*., 2015). The high total solids observed in this study may be attributed to high

powered milk and/or sugar content. The addition of powdered milk or evaporation during pasteurization milk are mechanisms for controlling moisture and total solids ratio for desired yoghurt (Stringer, 2000).The richness of yoghurt is usually defined by its high total solid content. However, low percentage of total solids in yoghurt can lead to malfunction of the starter culture.

Further, formulation A (2.19 ± 0.01) had the highest mean ash (%) followed by formulation D (2.03 ± 0.01), C (1.71 ± 0.01) and C (1.47 ± 0.02) while reference yoghurt (RY) recorded the least mean Ash (%) (0.27 ± 0.01) and these means differ significantly ($p < 0.05$). The variation in the mean fat (%) showed a similar trend as the Ash (%). Formulation D had the highest mean percentage crude protein (6.48 ± 0.00) while the reference sample had the least mean percentage crude protein (3.41 ± 0.13) and they differ significantly ($p< 0.05$). Also, formulation B had the highest mean carbohydrate (%) (8.82 ± 0.01) and reference sample had the least mean percentage carbohydrate (5.73 ± 0.21) and the means were significantly different ($p< 0.05$).The lowest mean value in ash, fat, carbohydrate and crude protein content of the reference sample may be attributed to lack of fortification of the yoghurt with amino acids (Osundahunsi *et al*., 2007), quality of the milk and/or other material used for the production (Early, 1998). Generally, except for the reference yoghurt, the mean values of ash, fat, carbohydrate and crude protein of all yoghurt formulations were higher compared with values reported by other authors (Alakali *et al*., 2009; Charles *et al*., 2015; Ezeonu *et al*., 2017). The higher mean values in ash, fat, carbohydrate and crude protein recorded in this study may be attributed to the fortification of the skimmed cow milk and/or the nutritional composition skimmed milk used (Early, 1998; Osundahunsi *et al*., 2007). Also, the higher mean values of ash content in all the formulations may be attributed to the mineral composition of the milk (Dandare *et al*., 2014). For bone development, teeth formation and other body function, milk is usually fortified with minerals.

Similarly, the mean percentage whey separation (WS) for all yoghurt formulations were significantly different ($p< 0.05$), as shown in table 5. Formulation C had the highest mean (%) for WS, followed by formulation A and B while formulation D competed favourably with the reference (RY) and the mean (%) were significantly different ($p < 0.05$).The presence of whey separation in drinkable yoghurt has been attributed to an insufficient amount of negatively-charged hydrocolloid to provide repulsion on the positively-charged protein molecules and formation of complexes with the milk proteins. Studies have reported the used of hydrocolloid stabilizers to control whey separation in yoghurt (Kiani *et al*., 2008). Similarly, the presence of EPS either *in-vivo* or *in-vitro* in yoghurt has been shown to decrease syneresis and improve the stability of the product. The ability of EPS to decrease syneresis has been attributed to high water binding capacity property.

3.4 Instrumental texture analysis

As expected, the mean values of the instrumental textural analysis for yoghurt formulations were significantly different ($p< 0.05$) within the yoghurt samples and across the parameters examined except adhesive properties as shown in table 6. Formulation D had the highest mean firmness (g) (21.50 ± 0.71) followed strictly by the reference sample with mean firmness (g)

(20.50 ± 0.71) and both differ significantly from the mean (g) of other formulations A (19.00 ± 0.00), C (18.25 ± 0.35) and B (17.75 ± 0.35) ($p <$ 0.05). The recorded differences in the firmness of yoghurt formulations may be attributed to the protein matrix structure of the gel. Also, increase in the firmness of yoghurt has been attributed to the entrapment of fat within protein network and occasioned by the higher total solid content of the yoghurt (Izadi *et al*., 2015).

Table 6: Instrumental textural analysis of yoghurt samples

Sample	Firmness (g)	Cohesiveness (g/s)	Stickiness (g)	Adhesiveness (g/s)
RY	20.50±0.71^{b}	22.96±0.07^{d}	4.10±0.14^{c}	0.01±0.01^{a}
Form A	19.00±0.00^{a}	17.29±0.30^{b}	3.10±0.14^{b}	0.01±0.01^{a}
Form B	17.75±0.35^{a}	18.57±0.62^{c}	1.50±0.71^{a}	0.01±0.01^{a}
Form C	18.25±0.35^{a}	15.93±0.12^{a}	2.00±0.00^{a}	0.01±0.01^{a}
Form D	21.50±0.71^{b}	23.22±0.05^{d}	2.25±0.35^{ab}	0.01±0.01^{a}

Reference yoghurt (RY), formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*), C (Y + *Leu.Lactis*), D (Yoghurt + *Lactobacillus plantarum* and *Leuconostoclactis*). Values are recorded in triplicates. The mean values in the same column with different superscript are significantly different ($p< 0.05$) from each other.

Consequently, reduction in the firmness of yoghurt has been attributed to reduction in fat content. Similarly, formulation D (23.22 ± 0.05) and the reference sample (22.96 ± 0.07) had the highest mean value for cohesiveness (g/s), followed by formulations B (18.57 ± 0.62) and A (17.29 ± 0.30) respectively while formulation C (15.93 ± 0.12) had the least mean cohesiveness (g/s) and they differ significantly ($p< 0.05$). Furthermore, reference yoghurt had the highest mean stickiness (g) (4.10 ± 0.14), followed by samples A (3.10 ± 0.14), and D (2.25 ± 0.35) and their means was significantly different ($p< 0.05$). As expected, the mean adhesiveness (g/s) of all yoghurt formulations was not significantly different ($p > 0.05$). Less hardness, adhesiveness and higher cohesiveness are characteristics of yoghurt with decreased fat content (Sandoval-Castilla *et al.*, 2004).

3.5 Apparent viscosity analysis

The mean apparent viscosities (centipoise, cP) of all yoghurt formulations increased revolution per minute (rpm) rotation speeds, as shown in Figure 1. Expectedly, 100 rmp had the highest mean apparent viscosity (cP), followed by 60 rmp while 50 rmp had the least mean apparent viscosity (cP) for the reference sample. A similar trend was observed in yoghurt formulations A, B, C and D. In this study, the reference yoghurt demonstrated the least amount of shear-thinning and formulation D the highest in all the rotation speeds respectively. The higher apparent viscosity observed in yoghurt formulation D in this study may be attributed to gum-producing properties of the LAB as starter cultures and the addition of their EPSs as stabilizing agent. The potential of exopolysaccharides in improving the physical and rheological properties of drinkable yoghurt has been reported (Zhang *et al*., 2012). Marshall & Rawson (1999) reported an observable increase in apparent viscosity of the stirred yoghurt with EPS-producing starter cultures and attributed the increase in apparent viscosity to the stretchability of EPS. Similarly, studies have reported an increase in apparent

viscosity of ropy EPS-producing starter cultures in stirred yoghurts (Jaros *et al*, 2002). In addition, the apparent viscosity of yoghurt can be affected by the strength, number of bonds between casein micelles, their structure and spatial distribution. Also, over time, apparent viscosity can increase as a result of protein and protein-protein contacts rearrangement (Sahan *et al*., 2008). Higher viscosity in stirred yoghurt has also been attributed to lower incubation temperature.

viable lactic acid bacteria count observed in this study agree with da Silva *et al*. (2013) who reported viable lactic acid bacteria count of 9.0 x 10^6 to 1.8 x 10^7 for the storage period of goat milk base yoghurt samples. Similarly, Karsheva *et al*. (2013) reported viable lactic acid bacteria account of similar ranged in shelf-life study of yoghurt inoculated with *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* (control), and *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* with the inclusion of *Lactobacillus salivarius.*

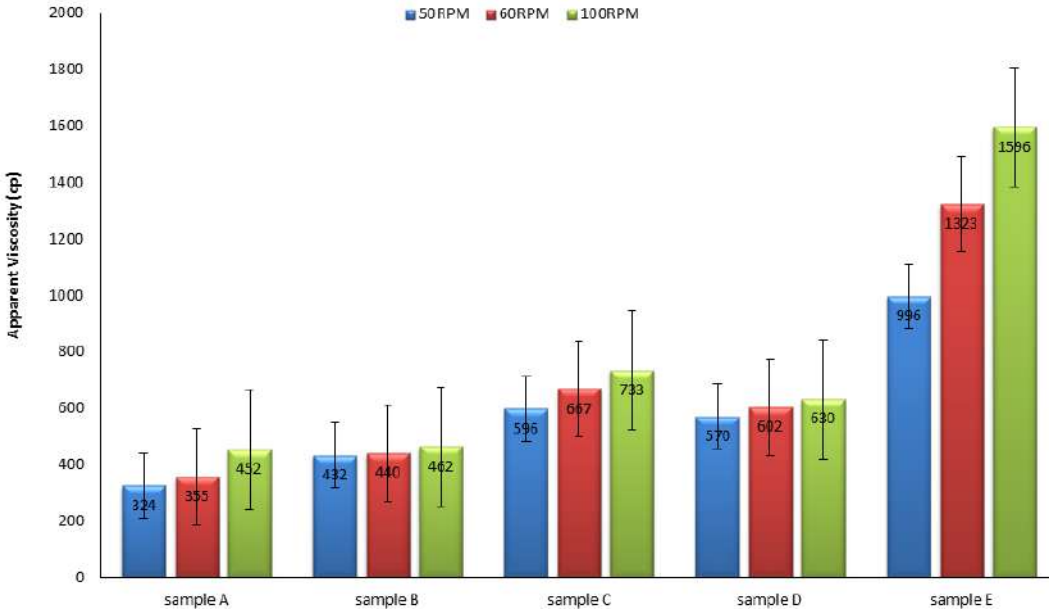


Figure 1: Apparent viscosity profile of yoghurt formulations

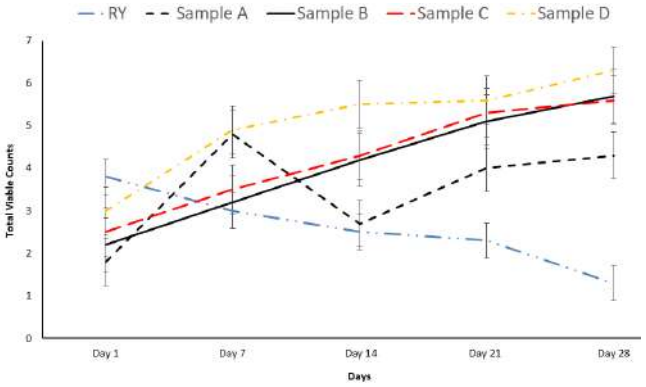
3.5 Microbiological analysis

Total viable lactic acid bacteria count in yoghurt formulations after twenty-eight (28) days storage period at 4^{o}C are presented in figure 2. The lactic acid bacteria colony count varies across the yoghurt samples, storage period and ranges; (1.3 - 3.8 × 10^7cfu/ml) (1.8 - 4.8× 10^7cfu/ml), (2.2 - 5.7 × 10^7cfu/ml), (2.5 -5.6× 10^7cfu/ml) and (3.0 - 6.3× 10^7cfu/ml) for sample RY and formulations; A, B, C and D respectively. The Generally, the lactic acid bacterial colonies increased progressively with the duration of storage in all formulations (A, B, C and D) except sample RY. This stimulation in growth population may be attributed to the reduction of oxygen pressure and the production of substances favourable for growth by LAB in formulations A, B, C and D. Earlier, Saarela *et al*. (2000) reported the stimulation in the

probiotic strain growth population in yoghurt. Similarly, Karsheva *et al*. (2013) reported similar outcome in milk fermented with a commercial starter (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus)* and the same starter in combination with a probiotic *Lactobacillus salivarius*. These authors attributed this stimulation of probiotic growth population to the reduction of oxygen pressure and the production of substances favourable for growth by LAB. Contrarily, other studies have reported decreased in the total viable cell numbers of probiotic yoghurt during the storage period (Da Silva *et al*., 2013). Formulation D had the highest colony-forming unit (CFU/ml) by the starter strain growth population while sample RY had the least colony-forming unit (CFU/ml) at day 28 in this study. The highest colony forming unit observed in yoghurt formulation D may be attributed to the synergetic interactions exhibited by *Lactobacillus plantarum* and *Leuconostoc lactis*. The bacterial metabolic activity in yoghurt was shown to produce pH level that could support the growth of a certain group and limit the growth of others. Overall, the bacterial strains in this study demonstrated ability to adapt very well to low pH values during the whole storage period without being affected (Karsheva *et al*., 2013).

3.6 Physicochemical properties

Physicochemical properties of yoghurt formulations are presented in figures 3a-b. Expectedly, the mean values of pH and TTA (mg lactic acid/g) varied significantly ($p < 0.05$) within the yoghurt formulations across the period of storage. At day 1, formulation A (4.66 ± 0.00) had the highest pH followed by the reference sample (4.52 ± 0.01), formulations B (4.53 ± 0.02), C (4.55 ± 0.01) and D (4.50 ± 0.02) but

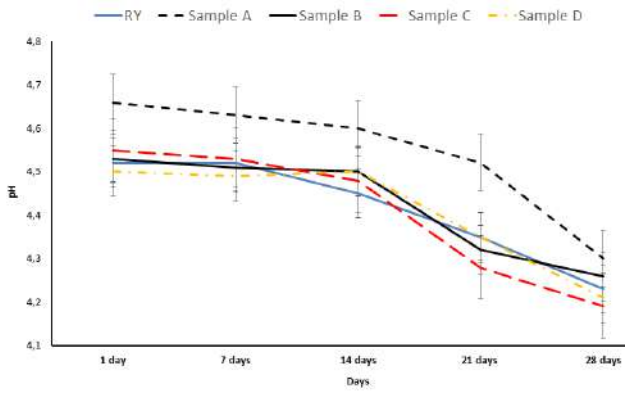


Reference yoghurt (RY), formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*), C (Y + *Leu. lactis*), D (Yoghurt + *Lactobacillus plantarum* and *Leuconostoc lactis*).

Figure 2: Total viable cell count of yoghurt samples at weekly basis

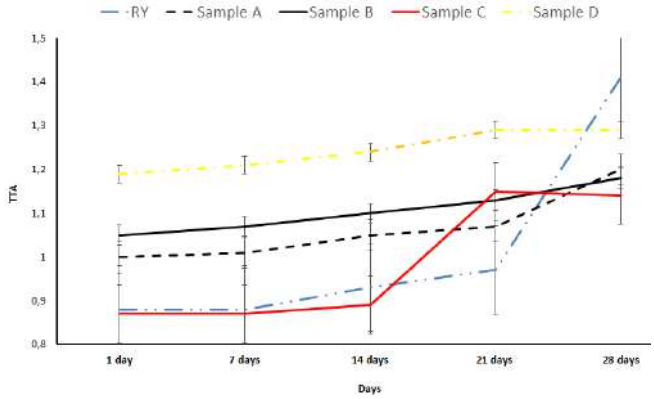
there was no significant difference among them ($p > 0.05$). A similar trend was observed at day 14 and 21 for pH respectively. The decrease in pH and increase in titratable acidity of yoghurt is a desirable quality characteristic of good yoghurt during fermentation. Studies have shown that during fermentation, pH of yoghurt usually decreases while titratable acid increase (Izadi *et al*., 2015). This has been attributed to the availability of more lactose to the fermenting microbes and the presence of higher total solids. Contrarily, Alakali *et al*. (2009) attributed the decreased in pH and increased in titratable acid of yoghurt samples to the percentage of whole milk in the product and the proportional of skinned milk in the blend of 75 percent. However, the mean values of pH for all the yoghurt formulations were not different significantly ($P > 0.05$) at day 28 except for formulation A. This may be attributed to a high concentration of lactose which is an ideal substrate for yoghurt starter cultures. Similarly, Ezeonu *et al*. (2017) reported no significant difference in the pH values of yoghurt samples after 21 days storage at refrigeration temperatures: 2-4^{o}C, and it was attributed to

storage conditions (2-4°C) that inhibited the microorganisms from producing more lactic acid.



Reference yoghurt (RY), formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*), C (Y + *Leu.lactis*), D (Yoghurt + *Lactobacillus plantarum* and *Leuconostoc lactis*).

Figure 3a: pH of yoghurt samples at weekly basis



Reference yoghurt (RY), formulation A (Yoghurt + starter culture), B (Y + *L. plantarum*),C (Y + *Leu. lactis*), D (Yoghurt + *Lactobacillus plantarum* and *Leuconostoc lactis*).

Figure 3b: Titratable acid of yoghurt samples at weekly basis

However, the mean titratable acid (TTA) at day 1 followed a reversed trend. Formulation D had the highest mean (1.19 ± 0.02) followed by B (1.05 ± 0.21) and A (1.00 ± 0.01). As expected, a similar trend was observed at day 14. These differences may be attributed to the viscosity property of the yoghurt samples that may increase diffusion resistance, reduced reactants mobility and consequently reduce the rate/extent of fermentation. At day 21 and 28, formulation D (1.29 ± 0.00) and reference sample (RY) (1.41 ± 0.01) had the highest mean TTA while formulation C (1.14 ± 0.01) had the least mean TTA at day 28. Alakali *et al*. (2009) reported TTA values of yoghurt samples after 24 hours storage period ranged from 1.14 – 1.30 and attributed the high values of TTA to thermization temperature. Also, the TTA mean values obtained at the end of storage period were within the ranged of TTA values reported in other studies (Karsheva *et al*., 2013; Izadi *et al*., 2015). Generally, the TTA mean values obtained in this study were within the acceptable limits for yoghurt.

4. Conclusion

Based on the results of this study, the use of EPS-producing starter cultures and EPS as stabilizing agents increased viscosity and controlled the level of syneresis in yoghurt. A gradual increase in the total viable cell counts and a low post-acidification was obtained in yoghurts with EPS-producing starter cultures. Also, the use of combined cultures of *Lactobacillus plantarum* and *Leuconostoc lactis*, and their EPSs competed favourably with the commercial starter, and xanthan gum in whole evaporated cow skimmed milk-based yoghurt. Specifically, it was evident that the combination of *Lactobacillus plantarum* and *Leuconostoc lactis* cultures as starter, and their exopolysaccharides have shown potential for improving the quality attributes of yoghurt. Overall, the results indicated that EPS from LAB improve texture, mouth-feel, viscosity and reduce syneresis in yoghurt.

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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