ABSTRACT

Yoghurt remains a fermented milk of choice globally but its desirability is limited by quality attributes and syneresis. In this study, the effect of using exopolysaccharide (EPS) producing starter cultures and EPS on the quality attribute of yoghurt produced from cow milk was examined. Two starter cultures of EPS-producing LAB were used singly and in combination in three treatments portions (YEPS_La, Lactobacillus acidophilus yoghurt; YEPS_Ls, Leuconostoc suionicum; YEPS_La + YEPS_Ls, Lactobacillus acidophilus and Leuconostoc suionicum); Yxg, Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus to produced yoghurt while reference
yoghurt (RY) was obtained from the market and refrigerated stored at 4°C. Sensory, proximate composition, textural, rheological properties and whey separation were carried out after 1 and 28 days only, while physicochemical and microbiological were analyzed after 1, 7, 14 and 28 days. No significant difference (p < 0.05) between RY (8.60 ± 0.60), (7.21±0.10) and YEPS$_{La}$ + YEPS$_{La}$ (8.54 ± 0.71), (7.25 ± 0.21) in overall acceptability for day 1 and 28. Moisture (82.45 ± 0.12 - 81.31 ± 0.06%), fat (3.46 ± 0.01 - 3.42 ± 0.03%) and carbohydrate (13.05 ± 0.11 to 12.51 ± 0.10%) contents decreased while total solids (17.57 ± 0.12 - 17.97 ± 0.12%), ash (0.56 ± 0.02 - 0.57 ± 0.02%) and protein (3.74 ± 0.01 - 4.30%) contents increased respectively across the yoghurts. The result showed that the highest cohesiveness and syneresis was observed in YEPS$_{La} + YEPS_{La}$ (27.52 ± 0.63) and commercial yoghurt (29.10 ± 0.31), the lowest in Yxg (16.71 ± 0.21) and YEPS$_{La} + YEPS_{La}$ (21.50 ± 0.51). The highest viscosity was observed in YEPS$_{La} + YEPS_{La}$ across the rotation speeds. The pH and titratable acid ranged (4.28 – 4.50; 0.90 – 1.41) while the total bacteria colony count (5.5×10$^8$ – 11.0×10$^8$cfu/ml) during 28 days storage period. Overall, EPS produced by EPS-producing LAB both in-vitro and in-vivo improve texture, mouthfeel, viscosity and reduce syneresis in yoghurt. Combine cultures of Lactobacillus acidophilus and Leuconostoc suionicum and their EPSs competed favourably with conventional starter, and other stabilizing agents in cow milk yoghurt.

Keywords: Exopolysaccharide; palm sap; yoghurt; lactic acid bacteria; quality attribute.

1. INTRODUCTION

Yoghurt is a fermented milk and traditional biotechnology rich products’ that serve as a source of macronutrient; proteins, carbohydrates and fats, and micronutrients; vitamins, phosphorous and calcium [1,2]. As a fermented milk product, yoghurt is easily digestible, provides increased bioavailability of calcium in the intestine, and this uniqueness is attributed to lactic acid bacteria (LAB) activity during its production [2]. Yoghurt is obtained by milk fermentation, using a pure culture of LAB that produce coagulated milk product with lactic acid flavour and distinctive aroma [3]. Fermented dairy products have been traditionally fermented with LAB such as Lactobacillus, Lactococcus, Leuconostoc and Bifidobacterium species [4]. Conventionally, the common strains of Lactobacillus (Lactobacillus delbrueckii ssp. Bulgaricus) and Streptococcus (Streptococcus thermophilus) bacteria are added to raw milk or evaporated skimmed milk for probiotic yoghurt production.

The desirable qualities of yoghurt at the time of consumption are the milk evenness, appearance, mouthfeel and, to a lesser extent aroma and the presence of probiotics which should be viable and contain at least 6-7 Log cfu.g$^{-1}$of viable probiotic bacteria [5]. Syneresis remains the major technological defect of yoghurt. To this end, studies have reported the use of stabilizers, whey protein, gelatin, calcium and hydrocolloids to increase firmness and eliminate syneresis in yoghurt [6,7]. The rheological and textural properties of yoghurt are determined by the type of stabilizing agent and culture starter used [8]. Exopolysaccharide (EPS) has shown the capacity to increase viscosity, gel fracture, influence gel strength preventing syneresis, improved the organoleptic quality of fermented foods; in texture, taste perception, mouthfeel and stability [9,10]. Some strains of LAB can produce EPS including the conventional starters Lb. delbrueckii ssp. and S. thermophilus in different quantities based on their biosynthesis capacity [11]. EPSs from LAB share the status of generally regarded as safe (GRAS) with LAB being the source. It has been demonstrated that yoghurts made with EPS-producing cultures has a better texture than those made with non-EPS starters and additives.

To the best of our knowledge, no research work has been carried out to study the in Vitro effect of EPS from Lactobacillus acidophilus and Leuconostoc suionicum as a stabilizing agent in yoghurt. The aim of this research was to comparatively determine the physicochemical, rheological, microbiological and sensory properties of fermented cow milk by using EPS-producing starter cultures and EPS, separately and in composite using a conventional starter, xanthan gum and commercial yoghurt as control.

2. MATERIALS

2.1 Materials and Chemicals

Fresh cows’ milk was obtained from local pastoral farmers in Jimba Fulani settlement,
Kwara State (longitude 8.379966 and latitude 4.670199) and transported in an ice-packed to College Central Research Laboratory (CCRL) (Yabatech, Lagos, Nigeria). Commercial freeze-dried thermophilic yoghurt culture (Yogourmet®) and Xanthan gum were purchased from Bariga market, Bariga, Lagos State, Nigeria. A reference yoghurt sample was purchased from Shop Rite, Lagos. Gum-producing bacterial monocultures containing Lactobacillus acidophilus (accession number FJ556999.1) and Leuconostoc suionicum (CP015247) isolated from palm sap and their freeze-dried exopolysaccharides’ EPS as and EPSLs[11] were obtained from collections of the Molecular Biology laboratory unit, CCRL.

2.2 Methods

2.2.1 Preparation of starter culture

Yoghurts were prepared using a lyophilized strain mix of thermophilic lactic acid bacteria composed of Streptococcus thermophilus and Lactobacillus delbrueckii sub-species bulgaricus, which was activated at 42°C by dissolving 5 grams of starter in a 10 ml pasteurized milk for 5 min; the working culture was used for each 1 L working volume. Gum-producing bacterial monocultures; Lactobacillus acidophilus and Leuconostoc suionicum were revived by sub-culturing in 6% sucrose agar at 37°C for 24 h.

2.2.2 Yoghurt production and experimental design

Four litres of raw cow milk (gross composition: Lactose 4.63±0.03%; Fat 4.7±0.01%; Total solids 14.48±0.02%; Total protein 4.00±0.01%; pH 6.53±0.03 and Titratable acidity 0.18±0.00) was homogenized and pasteurized (85°C / 20 min). Next, the milk was divided into four equal volumes of 1 L each, stabilizer (Xanthan gum) was added to one volume (C: Yxg or control yoghurt) and EPS was added to the three volumes of the milk at a rate of 0.50% w/v at 70°C and the milk was then cooled to 42°C. Each of the volumes was inoculated with one of the previously revived cultures; Lactobacillus acidophilus, Leuconostoc suionicum, and with a mixed culture of Lactobacillus acidophilus and Leuconostoc suionicum, and Streptococcus thermophilus and Lactobacillus delbrueckii sub-species bulgaricus. The addition of bacterial starter cultures was 5 g/L (5% v/v) and bacteria inoculums were approximately 8.0 log10 CFU/mL. Each of the inoculated milk were poured into five 200 ml plastic containers, incubated at 42°C until the final pH reached pH ranging from 4.5 to 4.7 depending on the starter culture strain, and gently stirred with a sterile glass stem until homogeneity was obtained. After acidification, the samples of fermented milk were cooled to room temperature (28 ± 2°C) for 30 min and then stored at 4 ± 1°C for 28 days. In the experiment, we obtained four types of fermented milk (excluding the reference sample, RY) designated as follows: YEPSa – fermented by Lb acidophilus, YEPSLs – fermented by Leuconostoc suionicum, YEPSLas + YEPSLs – fermented by Lactobacillus acidophilus and Leuconostoc suionicum and Yxg – fermented by Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus(C: control). All samples were evaluated for sensory, proximate composition, textural, rheological properties and whey separation after 1 (24 h) and 28 days only, while physicochemical and microbiological were analyzed after 1, 7, 14 and 28 days of storage.

2.2.3 Analytical methods

2.2.3.1 Enumeration of microorganisms

The enumeration of LAB was done by counting colonies. The M17 Oxoid agar was used for enumeration of Streptococcus thermophilus under aerobic conditions (37°C / 72 h) [12]. Enumeration of Lactobacillus delbrueckii subspes bulgaricus and Fungi, following the method described by Adamu-Governor et al. [13]. Total colony count was expressed in colony-forming units per millilitre (CFU/mL).

2.2.3.2 Physicochemical analysis and proximate composition

The pH values and titratable acidities of the yoghurts were measured at a specific time interval daily and during the storage duration. 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 phenolphthalein indicator. Total ash was determined at 550°C in a muffle furnace (Union laboratories, England, UK) until a constant weight was obtained, and total solids (TS) were determined in a dry oven set 102 ± 2°C for 48 h until constant mass [14]. Crude protein and percentage carbohydrate contents were determined following the method described by Adamu-Governor et al. [13]. The solids-not-fat (SNF %) was done by subtracting the percent fat from percent total solids.
2.2.3.3 Apparent viscosity and whey separation analysis

Apparent viscosity of yoghurts was determined using a Brookfield viscometer (DV-E viscometer, Brookfield engineering Laboratory, Inc II commerce, Middleboro) [13]. The syneresis of yoghurts was determined based on spontaneous movement of whey out of the gel under the force of gravity as described by Alaa [9]. The quantity of whey expelled from the yoghurts was expressed as milliliters of drained whey, and the process was carried out in triplicate.

2.2.3.4 Sensory analysis

The sensory attribute analysis of yoghurts was carried out twice; the 1st and 28th days of storage at 4°C and as described by Yildiz and Ozcan [14] with modification, the attributes examined were; appearance, texture, odour, taste, colour and general quality. Twenty (20) assessors (ten male, ten female; ages 19-45 years) who had previous experience in the descriptive sensory evaluation of food products in the Central College Research Laboratory were trained in two sessions in the examination of yoghurt. The panelists scored the samples on a 9-point hedonic scale as described by Adamu-Governor et al. [13]

2.3 Statistical Analysis

All statistical analysis was performed using a one-ANOVA test to identify significant differences among the means (p < 0.05) with Duncan’s Multiple Range to ascertain where differences occur with the aid of SPSS (SPSS Inc. version 20. Chicago, IL. USA).

3. RESULTS AND DISCUSSION

3.1 Sensory Evaluation of Yoghurts

The results for sensory evaluation of yoghurt samples after 24 h and 28 days storage period are presented in Tables 1 and 2. For 24 h post-production of yoghurts, the result showed that reference yoghurt (RY) (8.55 ± 0.76; 8.65 ± 0.59) had the highest mean values and significantly different (p < 0.05) from other yoghurts in appearance and texture. Whereas, there was no significant difference (p < 0.05) among Yxg, YEPS\textsubscript{La}, YEPS\textsubscript{Ls}, and YEPS\textsubscript{La} + YEPS\textsubscript{Ls} in appearance. The lowest value for texture (6.95 ± 1.15) was observed in Yxg. This may be attributed to the high moisture content (82.45 ± 0.12) of Yxg, which correlated with its high syneresis value [15]. For aroma, sample RY (8.60 ± 0.60) had the highest mean score followed by YEPS\textsubscript{La} + YEPS\textsubscript{Ls} (7.86 ± 0.91), Yxg (7.32 ± 0.89) and YEPS\textsubscript{La} (7.25 ± 1.37) while YEPS\textsubscript{Ls} (6.90 ± 1.59) has the least score and the means differed significantly (p < 0.05). The high score of aroma recorded for RY in this study may be attributed to the acetaldehyde content [9].

The value for the overall acceptability of the samples showed that RY (8.60 ± 0.60) had the highest mean value followed by YEPS\textsubscript{La} + YEPS\textsubscript{Ls} (8.54 ± 0.71) and the lowest mean scores were observed in YEPS\textsubscript{La} (7.60 ± 0.88) and YEPS\textsubscript{Ls} (7.58 ± 0.84) and means of the two highest samples were significantly different from the other samples (p < 0.05). Expectedly, the acceptance of the yoghurt samples in terms of sensory properties decreased after the 28th day of storage which correlated with the panelists’ low scoring of the parameters evaluated as presented in Table 2. The decrease in the sensory properties of yoghurts has been attributed to the development of acidity during storage [16].

The highest mean score for appearance (7.00 ± 0.26) was observed in RY followed by YEPS\textsubscript{La} + YEPS\textsubscript{Ls} (6.60 ± 0.36), Yxg (6.24 ± 0.42) and YEPS\textsubscript{La} (6.05 ± 0.44) while YEPS\textsubscript{La} (5.95 ± 0.90) has the least mean among the yoghurts. Sample RY was significantly (p < 0.05) higher than the means of all other samples except YEPS\textsubscript{La} + YEPS\textsubscript{Ls}. Similarly, the highest mean scores for texture (7.45 ± 0.09) was observed in sample RY followed by YEPS\textsubscript{La} + YEPS\textsubscript{Ls} (6.13 ± 0.88), Yxg (6.01 ± 0.73), and YEPS\textsubscript{La} (6.00 ± 0.71) while YEPS\textsubscript{Ls} (5.75 ± 0.75) has the lowest mean value and the mean of sample RY differed significantly (p < 0.05) from the other yoghurt samples. The highest mean value for acceptability was observed in sample YEPS\textsubscript{La} + YEPS\textsubscript{Ls} (7.25 ± 0.21) that was not significantly different (p < 0.05) from RY (7.21 ± 0.10). This result agrees with Alaa [9] who reported that yoghurt made with EPS-producing starter cultures was the most accepted after 21 days storage period.

3.2 Proximate Composition of Yoghurts

The results of the proximate composition of yoghurt samples are shown in Tables 3 and 4. The mean values of proximate parameters varied significantly (p < 0.05) within and across the yoghurt samples after 24 h post-production as
presented in Table 3. The highest mean score for moisture content was observed in Yxg (82.45±0.12%) followed by YEPS_La + YEPS_Ls (81.98±0.40%) while the least mean score was observed in RY (81.31 ±0.06%) and these means differed significantly (p < 0.05). The moisture content of yoghurts observed in this study is in agreement with previously reported values of 82.04 to 82.99% [15], but lower than 86.7% [17]. The texture and mouthfeel of yoghurt are affected by the presence of higher moisture content [15,18].

For total solid content, the lowest mean value was observed in YEPS_La + YEPS_Ls (11.32±0.40%) which was marginally lower than the mean score observed in sample Yxg (11.36 ±0.06%), YEPS_La (11.49 ±0.02%) and YEPS_Ls (11.61 ±0.01%) and were all significantly (p < 0.05) lower than the mean in sample RY (18.11 ±0.12%). Except for commercial sample, the total solid content of yoghurts in this present study is lower than 15% [19] and 17.11% [20]. The difference in total solid of commercial yoghurt may be due to the addition of powdered milk during pasteurization of milk as a mechanism for controlling moisture and total solids ratio for desired yoghurt [21]. This same trend was observed in the mean scores for carbohydrate and non-solid-fat (NSF) contents. The lowest mean value for carbohydrate and NSF was found in sample Yxg (3.61±0.10% and 7.90 ±0.02%). The carbohydrate content of yoghurts in this study was found to be lower than previously reported values 5.2% [17], except RY in which case it is 13.05%. Also, the non-solid-fat content of yoghurts in this present study is lower than previously reported values of 8.50% [22] and 16.85 - 21.68% [23]. The lowest mean value for ash (0.36 ± 0.01), fat (2.12 ± 0.01%) and crude protein (2.58 ± 0.04%) was observed in commercial yoghurt (RY). No significant difference (p > 0.05) was observed in ash content among the yoghurt samples; Yxg (0.56 ± 0.01%), YEPS_La (0.56 ± 0.01%), YEPS_Ls (0.56 ± 0.02%) and YEPS_La + YEPS_Ls (0.56 ± 0.01%). The ash content of yoghurts in this study is in agreement with Matela et al. [23] who reported three ranges of ash content 0.28 - 0.31, 0.45 - 0.46 and 0.93 - 0.95 in commercial yoghurt samples. Higher ash content in yoghurts made with cow milk has been established [15,17]. The value of ash content in the samples is an index of mineral content needed for bone formation and body function [24]. A similar trend was observed in fat and crude protein content. The fat content of yoghurts in this present study is in agreement with previously reported values 1.49-3.50% [23] and lower than 3.59 % and 3.7% [15,17]. Also, the crude protein of yoghurts in this study is in agreement with reported values 1.29-3.52% from other studies [25] and lower than 4.50% [15].

Table 1. Sensory evaluation of Yoghurts for 24 h post-production

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appearance</th>
<th>Taste</th>
<th>Texture</th>
<th>Colour</th>
<th>Aroma</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>RY</td>
<td>8.55 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.40 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.65 ± 0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.45 ± 0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.60 ± 0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.60 ± 0.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yxg</td>
<td>7.50 ± 1.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.79 ± 1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.95 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50 ± 1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.32 ± 0.89&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.10 ± 1.10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>YEPS_La</td>
<td>7.60 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.05 ± 1.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.20 ± 1.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.70 ± 1.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.25 ± 1.37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.60 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>YEPS_Ls</td>
<td>7.79 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.30 ± 1.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21 ± 1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.00 ± 0.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.90 ± 1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.58 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>YEPS_La + Ls</td>
<td>7.95 ± 0.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.45 ± 1.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.33 ± 1.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.81 ± 0.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.86 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.54 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

YEPS_La (yoghurt+EPS+Lactobacillus acidophilus), YEPS_Ls (Yoghurt+ EPS +Leuconostoc suionicum), YEPS_La + YEPS_Ls (yoghurt + Lactobacillus acidophilus +Leuconostoc lactis + EPS), Yxg (yoghurt+ xanthan gum+Starter culture). Value are recorded in triplicate determination, means in the same column with different sets of superscript are statistically different (Duncan multiple range test)(p< 0.05)

Table 2. Sensory evaluation of yoghurts after 28 days storage period

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appearance</th>
<th>Taste</th>
<th>Texture</th>
<th>Colour</th>
<th>Aroma</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>RY</td>
<td>7.00 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.40 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.45 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.15 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.10 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.21 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Yxg</td>
<td>6.24 ± 0.42&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.79 ± 0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.01 ± 0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.35 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.15 ± 0.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.23 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>YEPS_La</td>
<td>6.05 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.25 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.40 ± 0.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.05 ± 0.87&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.25 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>YEPS_Ls</td>
<td>5.95 ± 0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.30 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.75 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.28 ± 0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.35 ± 1.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.15 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>YEPS_La + Ls</td>
<td>6.60 ± 0.36&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.75 ± 0.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.13 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.91 ± 0.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.26 ± 0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.25 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
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Table 3. Proximate composition of yoghurt produced from cow milk after 24 h storage period

<table>
<thead>
<tr>
<th>Sample</th>
<th>MC ± 0.06</th>
<th>TS ± 0.12</th>
<th>Ash ± 0.01</th>
<th>Fat ± 0.01</th>
<th>CP ± 0.01</th>
<th>CHO ± 0.01</th>
<th>NSF ± 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>81.31</td>
<td>17.57</td>
<td>0.36</td>
<td>2.12</td>
<td>13.05</td>
<td>15.45</td>
<td></td>
</tr>
<tr>
<td>Yxg</td>
<td>82.45</td>
<td>11.36</td>
<td>0.56</td>
<td>3.46</td>
<td>3.73</td>
<td>3.61</td>
<td></td>
</tr>
<tr>
<td>YEPS_{La}</td>
<td>82.20</td>
<td>11.49</td>
<td>0.56</td>
<td>3.39</td>
<td>3.72</td>
<td>3.82</td>
<td></td>
</tr>
<tr>
<td>YEPS_{Rs}</td>
<td>82.30</td>
<td>11.61</td>
<td>0.56</td>
<td>3.43</td>
<td>3.74</td>
<td>3.98</td>
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<tr>
<td>YEPS_{La} + YEPS_{Rs}</td>
<td>81.98</td>
<td>11.32</td>
<td>0.56</td>
<td>3.42</td>
<td>3.72</td>
<td>3.62</td>
<td></td>
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</tbody>
</table>

As expected, there were marginal decrease in the mean values for moisture, fat and carbohydrate content across the yoghurt samples after 28 days storage period [26]. Similarly, there was observable marginal increase in the mean value for total solid and crude protein as shown in Table 4. The level of moisture content of the yoghurt samples was in the range of (80.37 ± 0.15 - 81.31 ± 0.10%). The lowest score was recorded in YEPS_{La} + YEPS_{Rs} (80.37 ± 0.15%) and the highest score in RY (81.31 ± 0.10%). The reduced moisture content of yoghurt samples observed in this study could be attributed to increase of dry matter from microbial cell proliferation [27]. Also, decrease in moisture content has been attributed to increase in nutrients concentration [28]. The highest value for total solid was observed in RY (17.97 ± 0.10%) and differed significantly (p < 0.05) from other yoghurts. There was also slight increase in the values for ash content across the yoghurt samples as shown in Table 4. The marginal decrease recorded in the fat content of yoghurts may be attributed to the increased activities of the lipolytic enzymes of lactic acid bacteria which hydrolyzed fat component into fatty acid and glycerol during storage period. The increased crude protein content in the yoghurts observed in this study is consequent on microbial cell proliferation or anabolic polymer build – up [27]. Conversely, Thomas and Mills [29] reported decrease in protein content of yoghurts during storage. These authors attributed the decrease in protein content on the proteolytic activity of lactic acid bacteria hydrolyzing protein into peptides and amino acids. Carbohydrate content in yoghurts ranges from 3.29 – 12.51%, which was highest in RY (12.51 ± 0.11%) and lowest in Yxg (3.29 ± 0.07%) and differed significantly (p < 0.05) as presented in Table 4. The decrease in carbohydrate content may be due to the fermenting activities of microorganisms using them as carbon source through which it derived energy for all cellular activities [26,27]. For solid-not-fat, the lowest value was recorded in Yxg (7.75 ± 0.02%) and was significantly different (p < 0.05) from YEPS_{La} (8.19 ± 0.03%), YEPS_{Rs} (8.24 ± 0.01%) and YEPS_{La} + YEPS_{Rs} (8.21 ± 0.02%) and the highest in RY (15.87 ± 0.01%).

3.3 Instrumental Texture and Whey Analysis of Yoghurts

Textural properties of yoghurts varied significantly (p < 0.05) across the samples and during 24 h of storage period (Table 5). The highest mean value for firmness was observed in Yxg sample (30.30 ± 0.02), followed by RY (27.50 ± 0.10) and YEPS_{La} (25.57 ± 0.11) and they differed significantly (p < 0.05). There was no significant difference (p > 0.05) in firmness between YEPS_{La} (25.57 ± 0.11) and YEPS_{Rs} (25.50 ± 0.21), while the lowest value of firmness was observed in YEPS_{La} + YEPS_{Rs} (23.50 ± 0.00). This finding is similar to the findings of Hassan et al. [30] and Marshall and Rawson [31] who reported that yoghurt produced using EPS-producing starter had lower firmness to the control sample. The lowest cohesiveness was recorded in YEPS_{La} (22.11 ± 0.14) and the highest in YEPS_{La} + YEPS_{Rs} (33.61 ± 0.70). A significant difference (p < 0.05) was observed in the level of syneresis in the yoghurt samples which was highest in commercial yoghurt (RY) (38.50 ± 0.07) and lowest in YEPS_{La} + YEPS_{Rs} (29.50 ± 0.11). The difference in syneresis in yoghurts produced with EPS-producing cultures has been attributed to the differences in exopolysaccharides and their interaction with the protein network [32]. Generally, a significant decrease was observed in firmness, cohesiveness and syneresis in the yoghurts at the end of 28 days storage period (p < 0.05). This result is in agreement with a study conducted by Alaa [9], who reported that syneresis and firmness of yoghurt samples decreased during 21 days storage period. This may be due to an increase in acidity and casein hydration during the long period of storage that affects the firmness and syneresis of yoghurt.
The mean values of firmness of the yoghurt sample were in the range of (18.42 ± 0.21 - 24.40 ± 0.10). The lowest value was observed in YEPS$_{La}$ + YEPS$_{Ls}$ (18.42 ± 0.21) and the highest value in Yxg (24.40 ± 0.10). The higher value of firmness in yoghurts has been attributed to lower fat content and higher syneresis [34]. There was no significant difference (p < 0.05) in firmness between commercial yoghurt (20.59±0.28) and YEPS$_{Ls}$ (20.50 ± 0.71). For cohesiveness and syneresis, the highest value was observed in YEPS$_{La}$ + YEPS$_{Ls}$ (27.52 ± 0.63) and commercial yoghurt (RY) (29.10 ± 0.31), the lowest in Yxg (16.71 ± 0.21) and YEPS$_{La}$ + YEPS$_{Ls}$ (21.50 ± 0.51) respectively. Stronger gel composition in yoghurts has been attributed to higher cohesiveness [35], and cohesiveness affects the structural integrity of yoghurt [36]. Further, the higher syneresis value in commercial yoghurt in this study may be attributed to the rearrangements of casein particle in the gel network and the formation of a weak structure with an increased rate of spontaneous syneresis [37].

3.4 Apparent Viscosity Analysis of Yoghurts

The viscosities of yoghurt samples increased within the samples and a corresponding increased across the rotation speeds in this study (Fig. 1). It may be assumed that the addition of EPSs in vitro and the synthesis of EPS in vivo by gum-producing LAB increased the viscosity of yoghurts and enhanced the interactions within the protein network [13]. Increased in the apparent viscosity of yoghurts with EPS-producing starter cultures has been established [13]. These authors attributed the increased in apparent viscosity to the stretchability of EPS. The highest viscosity was observed in YEPS$_{La}$ + YEPS$_{Ls}$ sample and the lowest in RY for the 1st and 28th days, and across the rotation speeds. The difference in viscosities among yoghurts produced with EPS-producing strains has been attributed to the differences in molecular characteristics and the amount of EPS, and their ability to interact with protein [38]. Also, improved yoghurt texture and increased in the total EPS production by the combinations of two types of EPS-producing cultures has been reported [13].

3.5 Physicochemical Analysis of Yoghurts

The pH and titratable acidity of yoghurts are shown in Figs. 2 and 3. In this study, the pH of yoghurts showed a gradual decreased across the storage period. These results were in agreement with that obtained by Alaa [9]; Yildiz-Akgul [34] and Adamu-Governor et al. [13] who reported that the pH values of yoghurt samples gradually decreased during storage. The decreased in the pH values of yoghurts during storage has been attributed to the lactic acid produced by the lactic acid bacteria [14]. Also, the continuous enzymatic activity of yoghurts bacteria with low metabolic activity during storage has been reported [39]. The highest and lowest pH values were observed in YEPS$_{Ls}$ and reference yoghurt for day 1 and week 1. For week 2, 3 and 4, the lowest pH values were observed in YEPS$_{Ls}$ and the highest in YEPS$_{La}$. The differences in the pH of yoghurts have been attributed to storage condition and low acidity [15]. Furthermore, it may be assumed that these findings resulted from the different activities of the gum-producing LAB in the starter culture of the yoghurt. The viability of yoghurts’ bacteria in milk during fermentation and refrigerated storage is strain-dependent [40]. On the other hand, the titratable acidity of yoghurt samples increased progressively through the storage period.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MC</th>
<th>TS</th>
<th>Ash</th>
<th>Fat</th>
<th>CP</th>
<th>CHO</th>
<th>CHO</th>
<th>SNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>61.31 ± 0.10</td>
<td>17.97 ± 0.12</td>
<td>0.36 ± 0.01</td>
<td>2.10 ± 0.01</td>
<td>3.00 ± 0.04</td>
<td>12.51 ± 0.10</td>
<td>15.87 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Yxg</td>
<td>81.06 ± 0.09</td>
<td>11.17 ± 0.05</td>
<td>0.55 ± 0.01</td>
<td>3.42 ± 0.01</td>
<td>3.90 ± 0.01</td>
<td>3.29 ± 0.07</td>
<td>7.75 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>YEPS$_{La}$</td>
<td>80.89 ± 0.03</td>
<td>11.37 ± 0.02</td>
<td>0.56 ± 0.01</td>
<td>3.18 ± 0.01</td>
<td>4.05 ± 0.03</td>
<td>3.58 ± 0.03</td>
<td>8.19 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>YEPS$_{Ls}$</td>
<td>81.30 ± 0.01</td>
<td>11.55 ± 0.05</td>
<td>0.57 ± 0.02</td>
<td>3.31 ± 0.06</td>
<td>4.02 ± 0.01</td>
<td>3.65 ± 0.04</td>
<td>8.24 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>YEPS$<em>{La}$ + YEPS$</em>{Ls}$</td>
<td>80.37 ± 0.15</td>
<td>11.41 ± 0.30</td>
<td>0.57 ± 0.01</td>
<td>3.20 ± 0.03</td>
<td>4.30 ± 0.11</td>
<td>3.34 ± 0.12</td>
<td>8.21 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

YEPS$_{La}$ (yoghurt+EPS+Lactobacillus acidophilus), YEPS$_{Ls}$ (Yoghurt+ EPS +Leuconostoc suionicum), YEPS$_{La}$ + YEPS$_{Ls}$ (yoghurt +Lactobacillus acidophilus +Leuconostoc lactis + EPS), Yxg (yoghurt + xanthan gum+Starter culture). Value are recorded in triplicate determination, means in the same column with different superscripts are statistically different (Duncan multiple range test) (p< 0.05)
Table 5. Textural properties of yoghurts after 24 h and 28 days storage period

<table>
<thead>
<tr>
<th>Sample</th>
<th>Firmness (g)</th>
<th>Cohesiveness (g)</th>
<th>Syneresis (ml/100 g)</th>
<th>Firmness (g)</th>
<th>Cohesiveness (g)</th>
<th>Syneresis (ml/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference (RY)</td>
<td>27.50 ± 0.10&lt;sup&gt;a&lt;/sup&gt; 25.05 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.50 ± 0.07&lt;sup&gt;a&lt;/sup&gt; 20.59 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.05 ± 0.19&lt;sup&gt;b&lt;/sup&gt; 29.10 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yxg</td>
<td>30.30 ± 0.02&lt;sup&gt;d&lt;/sup&gt; 26.90 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.50 ± 0.10&lt;sup&gt;b&lt;/sup&gt; 24.40 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.71 ± 0.21&lt;sup&gt;a&lt;/sup&gt; 29.47 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YEPS&lt;sub&gt;La&lt;/sub&gt;</td>
<td>25.57 ± 0.11&lt;sup&gt;b&lt;/sup&gt; 28.25 ± 0.35&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31.60 ± 0.15&lt;sup&gt;b&lt;/sup&gt; 20.50 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.35 ± 0.15&lt;sup&gt;c&lt;/sup&gt; 24.50 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YEPS&lt;sub&gt;ls&lt;/sub&gt;</td>
<td>25.50 ± 0.21&lt;sup&gt;b&lt;/sup&gt; 22.11 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.50 ± 0.01&lt;sup&gt;b&lt;/sup&gt; 19.45 ± 0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>20.81 ± 0.25&lt;sup&gt;b&lt;/sup&gt; 25.50 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YEPS&lt;sub&gt;La + LS&lt;/sub&gt;</td>
<td>23.50 ± 0.00&lt;sup&gt;a&lt;/sup&gt; 33.61 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.50 ± 0.11&lt;sup&gt;a&lt;/sup&gt; 18.42 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.52 ± 0.63&lt;sup&gt;a&lt;/sup&gt; 21.50 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Fig. 1. Changes in viscosity of yoghurt samples

Increased in the amount of lactic acid and a decrease in the pH values of yoghurt during storage has been established [13]. Among the yoghurt samples, the highest titratable acidity was found in reference yoghurt (RY), and the lowest titratable acidity was observed in YEPS<sub>La</sub> at week 4. Higher titratable acidity in yoghurts has been attributed to the presence of high numbers and activities of conventional starter [14]. Earlier, Ashraf and Shah [41] reported that during storage, bacterial activity in yoghurts continues with active enzymes. Generally, the pH values of yoghurt samples recorded in this study are within the acceptable limit of good quality yoghurt.

3.6 Microbiological Analysis of Yoghurts

Total aerobic bacteria; lactic acid bacteria, enterobacterial groups and yeasts and molds count for yoghurt samples during the storage period at 4°C for 28 days are presented in Fig. 4. In this study, the total viable counts of lactic acid bacteria in reference yoghurt (RY) decreased with increasing storage periods. The reduction in the total viable bacteria counts in the commercial yoghurt may be attributed to the attainment of the highest count by viable yoghurt bacteria just after manufacturing of yoghurt and consequently decreased during the storage period [42]. In contrast, all the other yoghurt samples showed an increased in the total viable bacteria counts. Several studies have reported increase in the total yoghurts bacteria count with an increase in storage duration [43]. Further, increased in the total bacteria counts during storage period are dependent on bacterial species, the inhibitory effect of acidic conditions and high oxygen sensitivity [14]. Contrarily, Alaa [9] reported a decrease in the total viable counts of total...
aerobic count in yoghurt after 14 days of 21 days storage period. The highest mean value for total viable bacteria count was recorded in YEPS$_{La}$ + YEPS$_{Ls}$ sample and the lowest in reference yoghurt for week 1 to 4 except for day 1. The higher total viable bacteria count recorded by the mixed culture of gum-producing LAB (YEPS$_{La}$ + YEPS$_{Ls}$) in this study may be attributed to synergetic relationship between the bacteria culture and stimulation in the probiotic strain growth population in yoghurt [13]. Generally, the viable lactic acid bacteria recorded in this present study are in agreement with other authors [44; 45] and higher than the findings of Oladipo et al. [43] who reported microbial counts of $98 \times 10^6$ - $124 \times 10^6$ at week 4 of 6 weeks storage period. The presence of coliforms, yeasts and molds were not detected in all the yoghurt samples in this study. The absence of coliforms and molds in the yoghurts may be attributed to the inhibitory effect and antimicrobial property contain in the lactic acid produced by lactic acid bacteria [46], and good hygienic practice and conditions [9].

![Fig. 2. Changes in pH values of yoghurt samples](image1)

![Fig. 3. Changes in titratable acidity of yoghurt samples](image2)
Fig. 4. Changes in total viable counts of yoghurt sample

4. CONCLUSION

The use of EPS as a stabilizing agent and EPS-producing LAB starter cultures controlled the level of syneresis and increased viscosity of cow milk yoghurt. According to the results, the use of combine cultures of *Lactobacillus acidophilus* and *Leuconostoc suionicum* and their EPSs competed favourably with conventional starter, and other stabilizing agents in cow milk yoghurt in all parameters examined. The overall results showed that EPS improve texture, mouthfeel, viscosity and reduce syneresis in yoghurt. It was also evident that the use of EPS-producing starter cultures and EPS may well provide better textures for cow milk yoghurt than those imparted by additives.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and the producers of the products because I do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. This work was funded by the Tertiary Education Trust Fund (TETFUND) of the federal Ministry of Education, Nigeria

ETHICAL APPROVAL

The study did not involve any animal testing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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