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# Antioxidant Activities of Exopolysaccharides Produced by Lactic Acid Bacteria Isolated from Commercial Yoghurt Samples

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## Abstract

An antioxidant is a substance that inhibits the oxidation of other molecules caused by free radicals. The inbuilt antioxidant systems possessed by living organisms are generally not enough to prevent them from oxidative damages and the uses of synthetic antioxidants also have some harmful effects. This study was aimed at evaluating the antioxidant activities of exopolysaccharides produced by lactic acid bacteria isolated from yoghurt. Lactic acid bacteria (LAB) were isolated from six different brands of commercially available yoghurt using deMan Rogosa Sharpe (MRS) agar. The LAB isolates were identified based on morphological and biochemical analyses and were screened for exopolysaccharide (EPS) production. The LAB isolates screened positive were used for EPS production in a liquid medium and the EPS produced were purified and quantified using standard methods. Antioxidant activities of the EPS were evaluated by determining the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferric ion reducing power, and total phenolic contents. Data obtained were analysed using Analysis of Variance. Total lactic acid bacterial count obtained from the yoghurt samples ranged from 0 - $3.9 \times 10^4$ CFU/mL with sample A (Fan Yoghurt) having the highest LAB count ($3.9 \times 10^4$ CFU/mL). The isolated LAB and their incidence rate were *Lactobacillus plantarum* (25.49%), *L. delbrueckii* (19.61%), *L. fermentum* (15.69%), *L. acidophilus* (13.73%), *Leuconostoc mesenteroides* (11.76%), *Lactococcus lactis* (7.84%), and *Lactobacillus casei* (5.88%). Fifty-one out of the 64 LAB isolates were screened positive for EPS production and only six were able to produce substantial quantity of EPS ranging from 127.4 - 208.5 mg/L. The exopolysaccharides produced by *L. fermentum* had the highest DPPH radical scavenging activity (62.90%) while that of *L. plantarum* had the lowest (23.10%) at a concentration of 1000 μg/mL. Also, the EPS produced by *L. fermentum* recorded the highest ferric ion reducing power (12.89 mg AAE/mL) at 1000 μg/mL while that of *L. plantarum* had the lowest (5.62 mg AAE/mL). At 1000

μg/mL, the total phenolic contents of the EPS samples ranged from 1.41 - 1.58 mg GAE/mL, and the EPS produced by *L. fermentum* had the highest (1.58 mg GAE/mL) while those produced by *L. paracasei* had the lowest (1.41 mg GAE/mL). This study revealed that the exopolysaccharides produced by the LAB isolates showed high antioxidant activities with respect to their DPPH free radical scavenging activity, ferric ion reducing power and total phenolic contents.

## Keywords

Antioxidant Activities, Exopolysaccharides, Lactic Acid Bacteria, Commercial Yoghurt

## 1. Introduction

Yogurt is among the most common dairy products consumed around the world [1]. It is mainly obtained from fermentation of fresh milk or reconstituted milk with lactic acid bacteria (LAB) [2]. Yogurt is considered a probiotic because it contains live microorganisms (usually LAB) that provide significant nutritional and therapeutic values. It stabilizes gut microflora, produces antimicrobial compounds, reduces serum cholesterol and stimulates the immune system [3].

Lactic acid bacteria (LAB) are widespread in nature and are found primarily in the environments where there are high level of carbohydrates, peptides, amino acids and vitamins. They are Generally Recognized as Safe (GRAS) bacteria that have been used in the food industry for centuries [4]. They are widely used as starter cultures during fermentation of milk. They occur naturally as indigenous microflora in fermented dairy products such as yoghurt [5]. Lactic acid bacteria are able to produce exopolysaccharides in the surrounding medium as a slime or on the surface of bacterial cells to form a capsule [6].

Exopolysaccharides (EPS) are biosynthetic polymers secreted by microorganisms that consist of mainly carbohydrates [7]. EPS from lactic acid bacteria contributes a gelatinous texture and good taste to fermented dairy products. Microbial EPS have been reported to have good rheological properties as well as biological activities such as anti-cancer, anti-inflammatory, immune-stimulatory, and antioxidant activities [8]. The presence of EPS or EPS-producing starter cultures in fermented products influences several important sensory properties, including mouth thickness, shininess, clean cut, ropiness and creaminess [9].

Oxidative stress (damage) refers to elevated intracellular levels of free radicals that cause damage to lipids, proteins, and DNA [10]. Free radicals are unstable molecules that the body produces as a reaction to environmental and other pressures. Oxidative stress results when there is an imbalance in the ratio of oxidant/antioxidant in favour of oxidant factors. Oxidative damage plays a significant pathological role in many human diseases and aging process [11].

Antioxidants are substances that can prevent or delay damage to cells caused by free radicals [12]. Most living organisms possess enzymatic defenses (supe-

roxide dismutase, glutathione peroxidase, glutathione reductase), non-enzymatic antioxidant defenses (glutathione, thioredoxin, Vitamin C, Vitamin E), and repair systems to protect them against oxidative stress [13]. These natural antioxidant systems are generally not enough to prevent living organisms from oxidative damage [12].

The human body is under constant attack from oxidative damage. Oxygen in the body splits into single atoms (free radicals) with unpaired electrons. These atoms scavenge the body to seek out other electrons to become a pair. This results in DNA hydroxylation, protein denaturation, lipid peroxidation, and apoptosis, ultimately compromising cells' viability. The uses of natural antioxidants from food sources are highly recommended rather than synthetic antioxidants which have been restricted because of their toxic and carcinogenic effects [14].

Yoghurt is one of the most important dairy products with promising antioxidant activities. This is due to its ability to synthesize antioxidant molecules as well as probiotic bacteria (LAB) which have been reported to exhibit antioxidant activities [3]. There has been an increasing interest in the exploitation of EPS produced by lactic acid bacteria for their biological activities including antioxidant activities. The aim of this study is to evaluate the antioxidant activities of exopolysaccharides produced by lactic acid bacteria isolated from commercial yoghurt samples.

## 2. Materials and Methods

### 2.1. Sample Collection

Six brands of commercially available yoghurt were purchased from local markets in Abeokuta. The brand names of the samples were Destiny Yoghurt (Sample A), L & Z Yoghurt (Sample B), Viju Yoghurt (Sample C), Cedaa Yoghurt (Sample D), Elite King Yoghurt (Sample E), and Hollandia Yoghurt (Sample F) while Fan Yoghurt served as the Control sample because the brand is the mostly consumed brand. The samples were defrose at room temperature (28˚C) and taken immediately to the laboratory for analyses.

### 2.2. Isolation of Lactic Acid Bacteria

This was done using the method described by [15]. Ten millilitre of each sample was added to 90 mL sterile distilled water and then serially diluted to 5 dilution factor ($10^{-5}$). Using pour plate method, 0.1 mL inoculum was inoculated on sterile deMan Rogosa Sharpe (MRS) agar and then incubated anaerobically at 37˚C for 48 hours. After the incubation period, the visible colonies were counted and representative colonies were sub-cultured on sterile MRS agar to obtain pure cultures. This was done in triplicate.

### 2.3. Preservation of Lactic Acid Bacteria Isolates

Pure cultures of LAB isolates were maintained on sterile MRS agar slants and were stored at 4˚C inside a refrigerator for subsequent use.

## 3. Characterization of the LAB Isolates

The LAB isolates were identified based on morphological and biochemical characterizations.

### 3.1. Morphological Characterization

Gram Staining, Spore Staining, Capsule Staining and Motility Test were determined on all samples using the standard procedures of Fawole and Oso [16].

### 3.2. Biochemical Characterization

Biochemical tests carried out includes: Catalase Test using standard methods of Fawole and Oso [16], Coagulase Test, Oxidase test, Hydrogen sulphide ($H_2S$) production test, and Methyl Red Test Cheesbrough [17], also Voges-Proskauer (VP) Test, and Fermentation of sugars are determined using the procedures of Cheesbrough [17] Growth at Different Temperatures were monitored following the method described by Fawole and Oso [16].

### 3.3. Screening of the LAB Isolates for EPS Production

The LAB isolates were screened for EPS production as described by Adebayo-Tayo and Onilude [18]. The LAB isolates were cultured on a compounded EPS screening medium consisting of MRS agar (70 $g{\cdot}L^{-1}$), sucrose (20 $g{\cdot}L^{-1}$), bromocresol purple (0.12 $g{\cdot}L^{-1}$), and sodium azide (0.2 $g{\cdot}L^{-1}$). This medium was sterilized at 121˚C for 15 minutes in an autoclave and was dispensed into sterile Petri dishes after cooling. The plates were inoculated and then incubated anaerobically at 37˚C for 24 - 48 hours. The un-inoculated plates served as control. The presence of a yellow colouration after incubation indicated an EPS producing potential of the LAB isolate.

### 3.4. Production of EPS in Liquid Medium

This was done using the method of Adebayo-Tayo and Onilude [18]. The isolates were sub-cultured into sterile MRS broth and incubated overnight at 30˚C for 16 - 18 hours. Then 10 ml inoculum each was transferred into 200 mL conical flask containing 90 mL of modified exopolysaccharide selection medium (mESM). Fermentation was allowed to proceed by placing the flask in a 25˚C shaking water bath at 35 rpm for 48 hours. The mESM contained 5% skim milk, 0.35% (w/v) yeast extract, 0.35% (w/v) peptone, and 5% (w/v) glucose.

### 3.5. Purification and Quantification of the EPS Produced

The EPS was isolated and purified using the method described by Garcia-Garibay and Marshall [19]. The fermentation medium was treated with 17% (w/v) of 80% trichloroacetic acid solution and centrifuged at 16,000× g at 4˚C for 30 min. The clarified supernatant was concentrated 5 times by evaporation using a rotary evaporator. The EPS was then precipitated by adding 3 volumes of cold absolute

ethanol, and stored overnight at 4˚C. The recovered precipitates were redissolved with distilled water and dialyzed against the same solution for 24 h at 4˚C. The exopolysaccharides produced were quantified and expressed in mg/L. The purified EPS obtained were then freeze-dried and stored at 4˚C.

### 3.6. Determination of Antioxidant Activities of the EPS

The EPS was assayed for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferrous chelating activity, inhibition of lipid peroxidation, and reducing power.

#### 3.6.1. Determination of DDPH Free Radical Scavenging Activity

DPPH free radical scavenging activity was determined using the method of Son and Lewis [20]. Two millilitre DPPH in ethanol (500 mM) was added to 2 mL of the EPS, the mixture was shaken vigorously and allowed to stand in the dark at 28˚C for 30 min. The absorbance was measured at 517 nm. Ethanol was used as blank, while DPPH solution in ethanol serves as the control. The free radical scavenging activity of the samples was expressed as:

$$\%\ \text{DPPH Activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

#### 3.6.2. Determination of Ferric Ion Reducing Power

Ferric ion reducing power was determined according to the method of Vijayalakshmi and Ruckmani [21]. The EPS samples in different concentrations ranging from 200 - 500 µg/mL were added to 2.5 mL of 0.2 M sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide solution. The mixture was vortexed and then incubated at 50˚C for 20 min using a vortex shaker. After incubation, 2.5 mL of 10%, w/v trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Then 2.5 mL of the supernatant was mixed with 2.5 mL deionised water and 0.5 mL of 0.1% ferric chloride. The absorbance of the solution was measured at 700 nm using a UV spectrophotometer. Ascorbic acid was used as positive reference standard. The ferric ion reducing power were expressed as milligrams of Ascorbic Acid Equivalent (AAE) per mL of EPS sample.

#### 3.6.3. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu's method as described by Nabavi *et al.* [22]. The EPS samples (0.5 mL of different concentrations) were mixed with 1 mL of Folin Ciocalteu's phenol reagent (5 ml, 1:10 diluted with distilled water). After 5 min 10 mL of a 7% aqueous $Na_2CO_3$ (4 ml, 1 M) was added to the mixture followed by the addition of 13 mL deionised water and mixed thoroughly. The mixture was allowed to stand in the dark at 23˚C for 30 min after which the absorbance was measured at 750 nm using a UV spectrophotometer. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The total

phenol values were expressed as milligrams of Gallic Acid Equivalent (GAE) per mL of EPS sample.

### 3.7. Data Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20.

## 4. Results and Discussion

### 4.1. Isolation and Lactic Acid Bacterial Count

The total lactic acid bacterial count obtained from the yoghurt samples ranged from 0 - $3.9 \times 10^4$ CFU/mL. Sample A (Fan yoghurt) had the highest LAB count ($3.9 \times 10^4$ CFU/mL) followed by Sample C (L & Z yoghurt) ($2.6 \times 10^4$ CFU/mL) while zero LAB count was obtained from Samples D, E, F and G (Viju yoghurt, Cedaa Yoghurt, Elite King yoghurt and Hollandia Yoghurt respectively) (Table 1). Sixty-four LAB isolates were obtained from all the yoghurt samples. Twenty-one were obtained from Fan yoghurt, 13 and 30 isolates were obtained from Destiny and L & Z yoghurt respectively.

Yoghurt as a fermented dairy product is known to contain probiotics, predominantly lactic acid bacteria. In this study, lactic acid bacteria were isolated in different numbers from the yoghurt samples with *Lactobacillus plantarum* having the highest incidence rate. This result is in agreement with Ishola and Adebayo-Tayo [15] who reported that *L. plantarum* was predominant among the lactic acid bacteria isolated from yoghurt and other fermented dairy products such as *Nono*, *Fura* and *Wara*. On the other hand, Vantsawa *et al.* [23] reported that *Lactobacillus delbrueckii* was predominant among the lactic acid bacteria isolated from Nigerian fermented cow milk (*Nono*).

### 4.2. Screening of the LAB Isolates for EPS Production

Out the 64 LAB isolates screened for EPS production, 51 isolates were positive *i.e.* produced yellow colouration. Seventeen isolates were positive from sample A (A1 - A17), 8 from sample B (B1 - B8) and 26 from sample C (C1 - C26) (Table 2).

**Table 1.** Total lactic acid bacterial count of yoghurt samples.

| Sample Code | Brand Name | LAB count ($\times 10^4$ CFU/mL) |
|---|---|---|
| A | Fan Yoghurt | 3.4 ± 0.4 |
| B | Destiny Yoghurt | 1.9 ± 0.2 |
| C | L & Z Yoghurt | 2.6 ± 0.2 |
| D | Viju Yoghurt | - |
| E | Cedaa Yoghurt | - |
| F | Elite King Yoghurt | - |
| G | Hollandia Yoghurt | - |

Values are mean of triplicate readings ± Standard error.

**Table 2.** Screening of LAB isolates for EPS production.

| S/N | Sample | Isolate code | Result (Colour change) |
|---|---|---|---|
| 1 | | A1 | + |
| 2 | | A2 | + |
| 3 | | A3 | + |
| 4 | | A4 | + |
| 5 | | A5 | + |
| 6 | | A6 | + |
| 7 | | A7 | + |
| 8 | | A8 | + |
| 9 | | A9 | + |
| 10 | | A10 | + |
| 11 | A (Fan Yoghurt) | A11 | + |
| 12 | | A12 | + |
| 13 | | A13 | + |
| 14 | | A14 | + |
| 15 | | A15 | + |
| 16 | | A16 | + |
| 17 | | A17 | + |
| 18 | | A18 | − |
| 19 | | A19 | − |
| 20 | | A20 | − |
| 21 | | A21 | − |
| 22 | | B1 | + |
| 23 | | B2 | + |
| 24 | | B3 | + |
| 25 | | B4 | + |
| 26 | | B5 | + |
| 27 | | B6 | + |
| 28 | B (Destiny Yoghurt) | B7 | + |
| 29 | | B8 | + |
| 30 | | B9 | − |
| 31 | | B10 | − |
| 32 | | B11 | − |
| 33 | | B12 | − |
| 34 | | B13 | − |

**Continued**

| | | | |
|---|---|---|---|
| 35 | C (L & Z Yoghurt) | C1 | + |
| 36 | | C2 | + |
| 37 | | C3 | + |
| 38 | | C4 | + |
| 39 | | C5 | + |
| 40 | | C6 | + |
| 41 | | C7 | + |
| 42 | | C8 | + |
| 43 | | C9 | + |
| 44 | | C10 | + |
| 45 | | C11 | + |
| 46 | | C12 | + |
| 47 | | C13 | + |
| 48 | | C14 | + |
| 49 | | C15 | + |
| 50 | | C16 | + |
| 51 | | C17 | + |
| 52 | | C18 | + |
| 53 | | C19 | + |
| 54 | | C20 | + |
| 55 | | C21 | + |
| 56 | | C22 | + |
| 57 | | C23 | + |
| 58 | | C24 | + |
| 59 | | C25 | + |
| 60 | | C26 | + |
| 61 | | C27 | − |
| 62 | | C28 | − |
| 63 | | C29 | − |
| 64 | | C30 | − |

Key: +: Presence of yellow colouration; −: Absence of yellow colouration.

They were further screened for their ability to produce EPS in liquid medium. Majority of the LAB (79.69%) isolated in this study possess an EPS-producing potential. Similar report has been made by Adebayo-Tayo and Onilude [18] who reported that only 2 out of 191 LAB isolated from fermented dairy and non-dairy products could not produce exopolysaccharides. Contrarily, Sanni *et al.* [24] and Savadogo *et al.* [25] reported that only 16% and 26% of the isolated LAB could produce EPS respectively.

### 4.3. Characterization of the EPS-Producing LAB Isolates

Table 3 shows the morphological and biochemical characterization of the 51 EPS-producing LAB isolates. The isolates were Gram positive, non-spore forming and non-motile rods or cocci. They showed moderate or scanty growth on MRS agar. The isolates were catalase, oxidase and coagulase negative; hydrogen sulphide and Voges Proskauer positive. Some strains were able to grow at 4% NaCl, 15˚C and 45˚C while all the strains grew at 37˚C. The isolates were all fermentative rather than being oxidative in nature and able to ferment different sugars.

The isolated LAB and their incidence rate were *Lactobacillus plantarum* (25.49%), *L. delbrueckii* (19.61%), *L. fermentum* (15.69%), *L. acidophilus* (13.73%), *Leuconostoc mesenteroides* (11.76%) *Lactococcus lactis* (7.84%) and *Lactobacillus casei* (5.88%).

The selected isolates were identified as *Lactobacillus delbrueckii, L. plantarum*, *L. paracasei* and *L. fermentum*.

### 4.4. Production and Quantification of EPS in Liquid Medium

Out of the 51 LAB isolates used for EPS production in a liquid medium, only 6 produced significant amount of EPS. The quantity of the EPS produced ranged from 127.4 - 208.5 mg/L. A17 (*Lactobacillus plantarum*) produced the highest quantity while B3 (*L. fermentum*) produced the least amount of EPS (Table 4). Figure 1 shows the purified EPS produced by the LAB isolates in a liquid medium.

The quantity of exopolysaccharides produced varied as only six LAB isolates could produce significant amount of EPS in liquid medium. This study showed that *L. plantarum and L. fermentum* produced the highest quantity. This result is in agreement with [15] in which the quantity of EPS produced ranged from 120 - 1390 mg/L and *L. fermentum* was the highest EPS producer among the isolated lactic acid bacteria. In another study, Sanusi (2018) reported a smaller quantity of EPS production (10.1 - 24.8 mg/L) by lactic acid bacteria isolated from yoghurt in which *L. brevis* produced the highest quantity. However, *L. plantarum* isolated from *Fufu* (a fermented non-dairy product) produced a higher quantity (199.1 mg/L) of EPS.


Figure 1. Purified EPS produced by the LAB isolates in liquid medium. Key: A1: *Lactobacillus delbrueckii*; A17: *L. plantarum*; B3: *L. delbrueckii*; B7:*L. acidophilus*; C19: *L. fermentum*; C25:*Leuconostoc mesenteroides*.

**Table 3.** Morphological and biochemical characterization of the LAB isolates.

| Isolate code | Gram reaction | Shape | Spore formation | Capsule formation | Motility | Catalase | Oxidase | Coagulase | $H_2S$ production | Voges Proskauer | Methyl red | Growth at 4% NaCl | Homo/Hetero fermentation | Growth at different temperature | | | | Sugar fermentation | | | | | | | | | | | | | Probable name |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | 10˚C | 15˚C | 37˚C | 45˚C | Mannose | Glucose | Sucrose | Lactose | Maltose | Fructose | Rhamnose | Galactose | Raffinose | Arabinose | Xylose | Mannitol | Sorbitol | |
| A1 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| A2 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| A3 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| A4 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| A5 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillus fermentum* |
| A6 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| A7 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | + | − | + | + | − | + | + | + | + | − | + | + | + | − | *Leuconostoc mesenteroides* |
| A8 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | + | − | + | + | − | + | + | + | + | − | + | + | + | − | *Leuconostoc mesenteroides* |
| A9 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| A10 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| A11 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillus fermentum* |
| A12 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| A13 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| A14 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillus fermentum* |
| A15 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| A16 | + | C | − | − | − | − | − | − | + | + | + | − | Hm | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactococcus lactis* |
| A17 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| B1 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillus fermentum* |
| B2 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| B3 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| B4 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillusfermentum* |
| B5 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| B6 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| B7 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| B8 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | + | − | + | + | − | + | + | + | + | − | + | + | + | − | *Leuconostoc mesenteroides* |
| C1 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | + | − | + | + | − | + | + | + | + | − | + | + | + | − | *Leuconostoc mesenteroides* |
| C2 | + | C | − | − | − | − | − | − | + | + | + | − | Hm | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactococcus lactis* |
| C3 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| C4 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| C5 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| C6 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |

**Continued**

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C7 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| C8 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillusf ermentum* |
| C9 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| C10 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillus fermentum* |
| C11 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | *Lactobacillus plantarum* |
| C12 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | − | − | | *Lactobacillus casei* |
| C13 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | − | − | | *Lactobacillus casei* |
| C14 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | − | + | | *Lactobacillus plantarum* |
| C15 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| C16 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| C17 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | + | − | + | + | − | + | + | + | + | − | + | + | + | − | *Leuconostoc mesenteroides* |
| C18 | + | C | − | − | − | − | − | − | + | + | + | − | Hm | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | | *Lactococcus lactis* |
| C19 | + | R | − | − | − | − | − | − | + | + | − | − | Hm | − | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | *Lactobacillus fermentum* |
| C20 | + | R | − | − | − | − | − | − | + | + | + | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | − | + | | *Lactobacillus plantarum* |
| C21 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |
| C22 | + | R | − | − | − | − | − | − | − | + | − | − | He | − | − | + | + | + | + | + | + | + | + | − | + | + | + | − | − | − | *Lactobacillus acidophilus* |
| C23 | + | C | − | − | − | − | − | − | + | + | + | − | Hm | − | − | + | − | + | + | + | + | + | + | + | + | + | + | + | − | + | | *Lactococcus lactis* |
| C24 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | − | + | + | + | + | + | + | + | + | + | + | − | − | | *Lactobacillus casei* |
| C25 | + | R | − | − | − | − | − | − | + | + | − | − | He | − | − | + | + | − | + | + | − | + | + | + | + | − | + | + | + | − | *Leuconostoc mesenteroides* |
| C26 | + | R | − | − | − | − | − | − | + | + | − | + | He | − | − | + | + | + | + | + | + | + | + | − | + | + | − | − | − | − | *Lactobacillus delbrueckii* |

**Table 4.** Quantity of EPS produced by the LAB isolates.

| Isolate code | Name | Quantity of EPS (mg/L) |
|---|---|---|
| A1 | *Lactobacillus delbrueckii* | 191.4 |
| A17 | *Lactobacillus plantarum* | 208.5 |
| B3 | *Lactobacillus delbrueckii* | 127.4 |
| B7 | *Lactobacillus acidophilus* | 177.8 |
| C19 | *Lactobacillus fermentum* | 198.0 |
| C25 | *Leuconostoc mesenteroides* | 154.2 |

Key: A1-*Lactobacillus delbrueckii*; A17: *L. plantarum*; B3: *L. delbrueckii*; B7: *L. acidophilus*; C19: *L. fermentum*; C25: *Leuconostoc mesenteroides*.

### 4.5. DPPH Free Radical Scavenging Activity of the EPS

There was an increase the percentage DPPH scavenging activities with increases in the concentration of the EPS (Figure 2). All the EPS samples as well as the standard (ascorbic acid) recorded their highest DPPH scavenging activities at a concentration of 1000 µg/mL. The EPS produced by C19 (*Lactobacillus fermentum*) had the highest percentage DPPH scavenging (62.90%) while that of A17 (*L. plantarum*) had the least percentage DPPH scavenging (23.10%) at 1000 µg/mL.

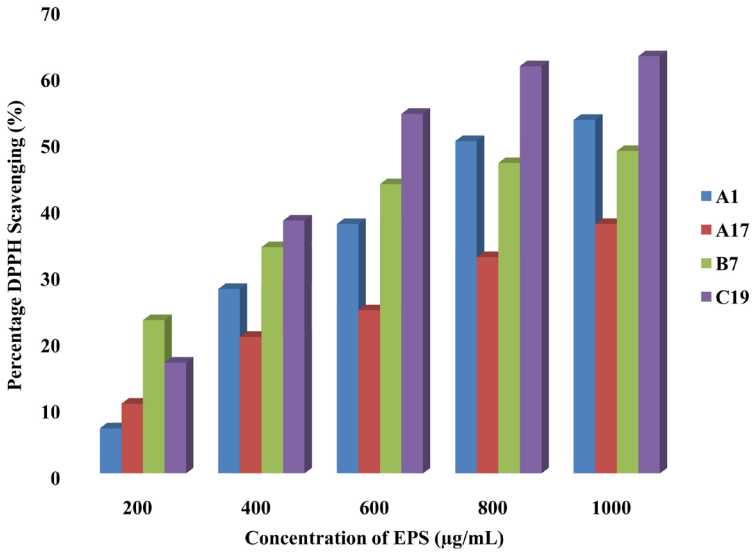


**Figure 2.** Percentage DPPH scavenging of the isolated EPS.

The percentage DPPH scavenging of the ascorbic acid (96.90%) was higher than those of the EPS samples.

The results showed that all the EPS samples exhibited a concentration-dependent DPPH radical scavenging activities. The percentage DPPH scavenging were highest at 1000 µg/mL. This result is in agreement with Zhang *et al.* [26] in which it was reported that EPS produced by *Lactobacillus plantarum* showed a DPPH scavenging activity of 52.23% at 1000 µg/mL. Similarly, Ghalem [27] documented a DPPH radical scavenging of 24.25% from EPS produced by yoghurt starter culture. In contrast, Xu *et al.* [28] reported that the DPPH radical scavenging activity of the EPS isolated from *Bifidobacterium animalis* could increase with the increasing EPS concentration and reach to a similar activity with ascorbic acid.

### 4.6. Ferric Ion Reducing Power of the EPS

Figure 3 shows the ferric ion reducing power of the isolated EPS. All the EPS samples showed an increasing ferric ion reducing power with an increase in the concentration and the highest ferric ion reducing power was recorded at 1000 µg/mL. C19 (*Lactobacillus fermentum*) had the highest ferric ion reducing power of 12.89 mg AAE/mL while A17 (*L. plantarum*) had the lowest (5.62 mg AAE/mL).

Ferric ion reducing power is another important assay in estimating antioxidant activity. The antioxidant activity is established on the capability of the antioxidant fractions in the EPS solutions to reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction that includes single electron transfer [29]. Qiao *et al.* [30] reported a direct correlation between antioxidant activity and the ferric ion reducing power. In this study, the EPS produced by *Lactobacillus fermentum* recorded the highest ferric ion reducing power (12.89 mg AAE/mL) at 1000 µg/mL. This value is higher than those obtained by Adebayo-Tayo *et al.* [31] in which the ferric ion reducing power obtained from EPS produced by wild type and mutant *Weisella confuse* strains ranged from 0.21 - 1.85 mg AAE/mL.

### 4.7. Total Phenolic Content of the EPS

Figure 4 shows the total phenolic content of the isolated EPS. All the EPS samples showed an increasing total phenolic content as the concentration of the EPS sample increased. The highest total phenolic content was recorded at 1000 µg/mL while the lowest was recorded at 200 µg/mL. At 1000 µg/mL, C19 (*Lactobacillus fermentum*) had the highest total phenolic content of 1.58 mg GAE/mL while B7 (*L. acidophilus*) had the lowest (1.41 mg GAE/mL). Several studies have revealed that total phenolic contents of compounds are associated with their antioxidant activities. This was due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers, and also may have a metallic chelating potential [32]. The total phenolic contents of the EPS samples ranged from 1.23 - 1.58 mg GAE/mL. A higher total phenolic contents ranging from 14.23 - 16.34 mg GAE/mL was reported from stirred yoghurt fortified with pomegranate peel extracts [1].

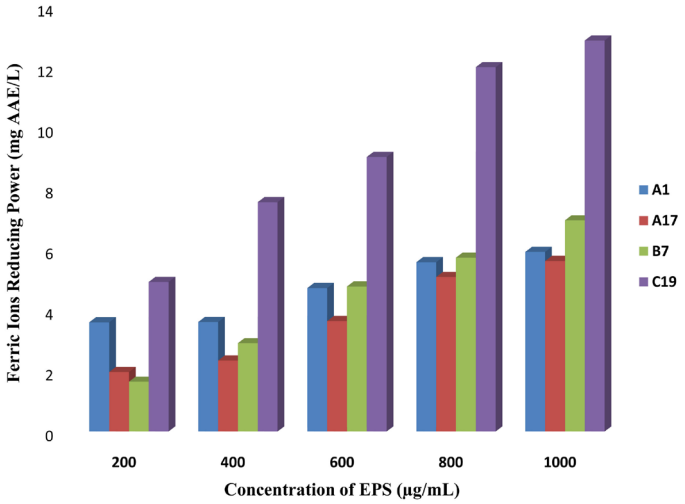


Figure 3. Ferric ion reducing power of the EPS.

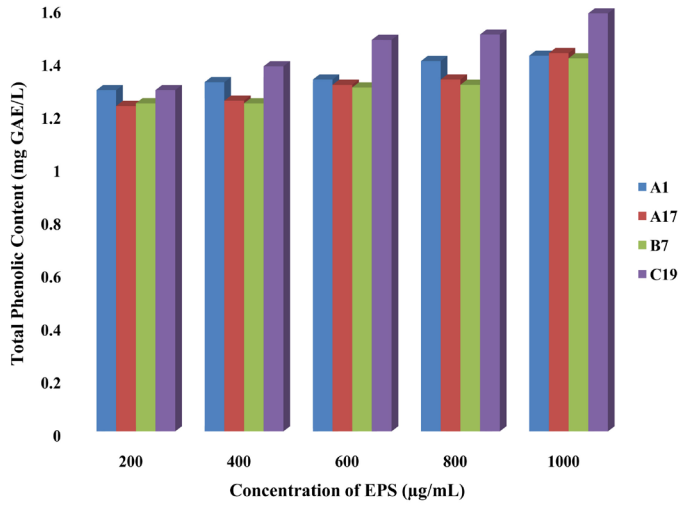


Figure 4. Total phenolic content of the EPS samples.

## 5. Conclusion

This study revealed that some commercial yoghurts have lactic acid bacteria while some did not have. This implies that those yoghurt samples with zero LAB did not use lactic acid bacteria as starter culture during yoghurt production which definitely affect the quality of the final product. Therefore, the yoghurt samples that have enough lactic acid bacteria can be regarded as genuine yoghurts. The predominant lactic acid bacteria isolated from the commercial yoghurt samples were *Lactobacillus plantarum*, *L. delbrueckii*, *L. fermentum* and *L. paracasei*. These lactic acid bacteria were able to produce substantial quantity of exopolysaccharides in liquid medium. This study also showed that the exopolysaccharides produced by the LAB isolates has high antioxidant activities with respect to their DPPH radical scavenging activity, ferric ion reducing power and total phenolic contents.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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