

Potential Application of Exopolysaccharides from *Lactobacillus delbrueckii* FASHADFF1 (LDYG2) and *Weissella confusa* FASHADFF1 (WCFF1) in Sourdough Bread Production

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Abstract

Baking of sourdough is a common practice and has the advantage of improving the nutritional value, sensory qualities and increasing the shelf life of the bread. This study therefore focus on the antimicrobial and antioxidant capacity of exopolysaccharides form Lactic Acid Bacteria (LAB) and its application in sourdough production. The Lactobacillus delbrueckii LDYG2 and Weissella confusa WCFF1 were collected from the culture collection Centre and the LABs were maintained in De Man, Rogosa and Sharpe (MRS) broth. Modified Exopolysaccharide Selection Medium (mESM) was used to produce the EPS while the total sugar concentration was determined using phenol-sulfuric acid method. The antibacterial, antioxidant, proximate, physical, organoleptic properties and the shelf life of the SDB produced were also evaluated. The quantity of EPS produced by LDYG2 and WCFF1 ranged from 4743.75 - 5090.03 g/L. Eight different sugars were present in both EPSLD and EPSWC with high antibacterial activity (24 mm and 23 mm) against B. cereus and S. aureus respectively. EPSLD and EPSWC had antioxidant capacity increased in a dose dependent (0.5 - 10 mg/mL) manner. EPSWCSDB had the highest proximate content except for moisture content. There was a significantly different ($P \le 0.05$) in the shelf life extension of the sourdough bread. WCEPSSDB was generally accepted in terms of colour, aroma, taste, texture and palatability. EPS produced by L. delbrueckii (EPSLD) and W. confusa (EPSWC) has antimicrobial and antioxidant capacity and can be used in production of nutraceutical sourdough bread with an improved shelf life and high consumer acceptability.

Keywords

Lactic Acid Bacteria, Exopolysaccharide, Antimicrobial, Antioxidant, Sourdough Bread, Shelf Life

1. Introduction

Recently, there has been great concern about the capacity of Lactic Acid Bacteria (LAB) to produce extracellular polysaccharides so as to enhance the nourishment of sourdough bread (SDB). EPS from microbial origin are high molecular weight polymer released by most microorganisms [1]. The EPS produced referred to all forms of bacterial polysaccharide that are loosely adhere to the microbial cell wall (slimy exopolysaccharide) or tightly adhered to microbial cell wall (capsular exopolysaccharide) [2]. Lactobacillus delbrueckii and Weissalla confusa are heterofermentative LAB strains that produce lactic acid and acetic acid in the preparation of the bread thereby giving a sour taste [3]. EPS from LAB are widely utilized in medicine, dairy product industrial fermentation and established starter culture [4] [5]. LAB is generally regarded as safe, thus leading to their wide industrial application such as food biothickeners [6] [7]. Certain LAB EPS exhibited beneficial health effects in human such as prebiotic effects, immunomodulating, antitumoral and cholesterol-lowering ability [8] [9]. Interestingly, EPS also interacts with human immune system by providing healing effects in bowel [10].

Sourdough can be fermented with LAB or the EPS produced by LAB. Wheat bread such as whole grain or high fibre flour is generally considered to be a good source of energy and vital nutrients for the human body. Sourdough bread is an intermediate product of bread preparation and it contains microorganisms that are metabolically active. Sourdough is a mixture of flour, and water with lactic acid bacteria or EPS which produces acid or sharp taste. Sourdough bread made from cereal or wheat doughs has been successfully used to improve the quality of gluten-free bread [11]. Traditional bread made from this sourdough bread is known to have a soft, elastic texture, palatable taste and long shelf life. It has also proven to improve the texture and palatability of cereal products [12]. Improvement in the quality, taste and flavor of wheat bread is a result of acidification process which affects the application of sourdough and slow staling [13]. Most intrinsic properties of SDB rely on the metabolic activities of its resident LAB. The vital activities during SDB fermentation include lactic fermentation, acetic fermentation, proteolysis, synthesis of volatile compounds, anti-mould activity and antiropiness. Mostly, the effects of EPS in SD have been observed by decrease in pH value which is caused by the release of organic acid. Exopolysaccharides obtained from Lactobacillus delbrueckii and Weissalla confusa also

have notable economic and therapeutic potential for the development of nutrient-rich food products with long-lasting health benefits which include antimicrobial and antioxidant agents. The usefulness of the EPS in the industry due to their physicochemical properties has gained immense commercial value. EPS play important role in human health owing to its antitumor, antioxidant, anti-ulcer and antimicrobial activities [14]. One of the most serious public health challenges in this present world is antimicrobial resistance. Most microbial infections no longer respond to common antimicrobial drugs thereby creating lots of serious public health challenges [15]. Free radicals and other reactive oxygen species play a crucial role in oxidative damage to the cell which could to cell injury and death. This has been associated with pathogenesis of various chronic diseases such as carcinomas, coronary heart disease, and many other hearth problems related to advancing age. Superoxide, hydroxyl radical, and hydrogen peroxide are among the highly reactive species that have been identified and are known to be the major cause of ill effects in humans, including cancer, atherosclerosis, rheumatoid arthritis, and neurodegenerative diseases. These ill effects are caused by the effects and level of damage caused by reactive oxygen species on biological molecules such as DNA, lipids, and proteins [16]. There is increased concern regarding the safety and toxicity of synthetic antioxidants which challenged made researchers to find natural antioxidants that do not adversely affect human health [17]. The addition of EPS from LAB in the baking of SDB, as compared with the more normal wheat bread involving only baker's yeast has been shown to create positive effects on wheat bread standard by reducing loaf volume delay in crumb firmness and staling, reduced anti-nutritional factors, high resistant starch formation in bread, and reduce antifungal activities, hence preventing spoilage [18] [19]. The study was therefore aimed at investigating the antimicrobial and antioxidant ability of Exopolysaccharide (EPS) produced by Lactobacillus delbrueckii LDYG2 (LDEPS) and Weissella confusa WCFF1 (WCEPS) and its potential in sourdough bread (SDB) production.

2. Material and Methods

2.1. Collection of LAB Isolates

Stored molecularly characterized cultured *Weissella confusa* FASHADFF1 and *Lactobacillus delbrueckii* FASHADYG2 from previous studies isolated from fermented *fufu* and yoghurt were collected from Industrial Unit, the University of Ibadan, Nigeria [20]. The LAB was subcultured in De Man, Rogosa and Sharpe (MRS) broth [21] (Table 1).

Table 1. List of LAF	sion numbers.	
	Identification	NCBI Conobank

Inclator and an	Identification	NCBI Genebank	Fermented	
Isolates codes	by BLAST	accession no.	Source	
WCFF1	Weissella confuse	MH790313	fufu	
LDYG2	Lactobacillus delbrueckii	MH790315	Yoghurt	
20].				

2.2. Physico-Chemical Analysis of the LAB

2.2.1. Quantitative Estimation of Lactic Acid

Lactic acid Production by the LAB strains was carried [22]. Ten milliliter of the supernatant from the MRS broth culture of WCFF1 and LDYG2 was titrated against freshly prepared 250 Mm NaOH with the addition of phenolphthalein indicator (1 mL). The percentage lactic acid (%, v/v) titratable acidity was calculated:

Titratable acidity =
$$\frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times ME}{\text{Volume of Sample used}} \times 100$$

1 N NaOH is equivalent to 9.008 mg of lactic acid for each mL.

Where Volume (mL), *N* = normality and *ME* = Equivalence Factor.

2.2.2. Quantitative Determination of Hydrogen Peroxide of the LAB

The H_2O_2 produced by the LAB was determined by adding 20 mL of freshly prepared Sulphuric acid (H_2SO_4) into 25 mL of supernatant from the MRS broth culture of WCFF1 and LDYG2. Followed by titration with 0.1 N potassium permanganate (KMnO₄). Changes in the sample colour were regarded as the end point. The H_2O_2 production was calculated as follows:

Hydrogen peroxide =
$$\frac{V_{\text{KMnO}_4} \times N_{\text{KMnO}_4} \times ME}{V_{\text{H}_2\text{SO}_4} \times V_{\text{sample}}} \times 100$$

Each milliliter of 0.1 N KMnO₄ is equivalent to 1.701 mg of H_2SO_4 . Where Volume (mL), N = normality and ME = Equivalence Factor.

2.2.3. pH Determination of the LAB

A well sanitized pH probes of Kent pH meter (Kent Industrial Measurement Ltd. Survey) model 7020 equipped with a glass electrode was used to determine the pH change of each LAB. The pH reading was done in duplicate.

2.3. Production, Extraction and Quantification of Exopolysaccharides by the LAB

Weissella confusa FASHADFF1 and Lactobacillus delbrueckii FASHADYG2 were prepared [23]. 0.5 mL of the stock frozen culture was transferred to 10 mL of MRS broth and incubated for 48 hrs at 37°C. The resulting culture was transferred (2% v/v) into Exopolysaccharide Selection Medium (mESM) (5% skimmed milk (Oxoid), 0.35% yeast extract (oxoid), 0.35% peptone (Difco) and 5% glucose (BDH)), incubated at 37°C for 148 hrs and then centrifuged at 7000 xg for 30 mins at 4°C. The EPS was precipitated at 4°C by the addition of 2 volume of chilled ethanol (100%). The resulting precipitate was collected after centrifugation (7000 xg for 30 mins at 4°C). The precipitate was dissolved in distilled water and dialyzed at 4°C for 48 hours [24]. Total neutral carbohydrate was determined using phenol-sulphuric acid. Briefly, 0.2 mL of the EPS sample as diluted in 2.0 mL distilled water. 0.2 mL of 6% phenol and 5.0 mL of 95% (v/v) sulphuric acid was added quickly and stirred after 10 min of standing. The absorbance was obtained at 490 nm using UV-spectrophotometer using distilled water as blank and glucose as standard.

2.4. Analysis of the Monosaccharide Composition of the EPSWC and EPSLD

The determination of the monosaccharide composition of EPSWC and EPSLD was done using HPLC equipped with Refractive Index Detector (Knauer, Germany) [25]. The purified EPSWC and EPSLD samples (1 mg) were diffused in water, rehydrated and hydrolyzed with 2 M trifluroacetic acid at 120°C (120 min/mL). The hydrolysate was derivatized with 1-phenyl-3-methyl-5-pyrazolone, analyzed by High Performance Liquid Chromatography (HPLC) (Agilent Technologies, ilmington, USA) and a Shim-pak VPODS column (4.6 × 150 mm) with detection by absorbance monitoring at 245 min. The mobile phase contains Sodium phosphate (82%, 50 mM, pH 7.0) and 18% acetonitrile (v/v), and the sample was eluted at 1.0 mL/min flow rate using a detector. The area curves was measured using an integrator and the sugars were compared with a known retention time of the EPS samples.

2.5. Antimicrobial Potential of the EPSWC and EPSLD

The antimicrobial activity of the EPSWC and EPSLD was investigated by agar diffusion assay [26]. Overnight incubation cultures of the indicator microorganisms (*B. cereus* ATCC 6348, *S. typhi* ATCC 33,458, *E. coli* ATCC 25,922, *K. pneumonia* ATCC 13,883, *S. dysentriae* ATCC 6736 and *S. aureus* ATCC 6033) were diluted to 10^6 cfu/mL and spread on 10 mL Mueller-Hinton Agar (Lab M Ltd., UK) in a petri dish. 7 mm wells were cut on the dried cultured agar plate using a sterile cork-borer and each well was imbued with EPSWLD and EPSMLD (10 µL) and incubated for 24 h at 37°C. The zones of inhibition (ZOI) around the wells were recorded.

2.6. Antioxidant Potential of EPSWC and EPSLD

The DPPH scavenging activities of the EPS were determined [27]. A freshly prepared solution of 3 mL of ethanol (Sigma Chemical Co.) and 0.1 mM DPPH (5 mL) was mixed with EPS concentration (50, 100, 250, 500 and 1000 mg/mL). The solution was kept in the dark at 37° for 20 min and absorbance was taken at 517 nm using Analytik Jena US LLC Spectophtometer. Ascorbic acid was used as positive control. A change in colour indicates DPPH radical scavenging activity. The percentage of scavenged DPPH radical was derived using the equations below:

Scavenging Activity (%) =
$$\left[1 - \left(A_{\text{sample}} - A_{\text{blank}}\right) / A_{\text{control}}\right]$$

where A_{sample} is the absorbance of the sample, A_{blank} is the absorbance of the blank and A_{control} is the absorbance of the control.

Determination the Ferric reducing power of the EPS with slight modification

was done [27] [28]. The mixture of 1 mL of distilled water, 2.5 mL phosphate buffer (2 mM, pH 6.6) and 2.5 mL of 1% of Potassium ferricyanide (2.5 mL) was mixed with different concentration of EPS (200 - 1000 mg/mL). The mixture was kept at 50°C for 20 min. 10% trichloroacetic acid (2.5 mL) was added to the mixture and spin at 3000 rpm for 10 min. 2.5 mL distilled water and 1% Ferric chloride (0.1 mL) was added. After 10 min of incubation, the absorbance was read at 700 nm using ascorbic acid was used as standard.

Total Antioxidant Activity of the EPS samples was determined with slight modification [29]. 1.235 g of Ammonium molybdate (4 mM), 7.45 mL Sulfuric acid (0.6 M), 0.9942 g of Sodium Sulphate (28 mM) were mixed in 250 mL distilled water. The mixture was used as the total antioxidant capacity reagent. 0.1 mL of 50, 100, 250, 500 and 1000 mg/mL EPS was dissolved in 1 mL of total antioxidant capacity reagent. Absorbance was taken at 695 nm after 15 min with ascorbic acid been used as standard.

Hydrogen Peroxide scavenging capacity was investigated with slight modification [30]. Solution of Hydrogen peroxide (10 mM) was prepared in 0.1 M Phosphate buffer (pH 7.4). 1 mL of different concentration of 50, 100, 250 and 1000 mg/mL EPS was added to 2 mL of the Hydrogen peroxide solution. The mixture was allowed to incubate for 10 min and the absorbance of the reaction mixture was taken at 230 nm at 30°C against blank solution using UV-spectrophotometer. Ascorbic acid was used as standard.

Scavenging activity (%) = $(A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}} \times 100$

2.7. Production of the Sourdough Bread Using EPSWC and EPSLD

Sourdough bread was produced in line with [31] [32]. Briefly, 100 g of the wheat flour was mixed using four different compositions:

A: 100 g flour, 50 mL sourdough starter and a pinch of salt (control).

B: 100 g flour, 50 mL sourdough starter, 50 mL LDEPS and a pinch of salt.

C: 100 g flour, 50 mL sourdough starter, 50 mL LDEPS and WCEPS and a pinch of salt.

D: 100 g flour, 50 mL sourdough starter, 50 mL WCEPS and a pinch of salt.

All ingredients used were mixed thoroughly and kneaded for 10 min at high speed in an electric mixer (Binatone electric mixer, Model: HM-350S, United Kingdom). The mixture stood for 30 - 40 min with 50 mL sterile distilled water to give moist dough. The dough was kneaded until a small piece of dough can be stretch between four fingers without breaking. The prepared dough was cut into 600 g dough, placed in a baking pan and allowed to proof (38°C, RH = 85%) for 45 min after been covered lightly with foil paper. It was baked in an electric deck oven (India) at 180°C for 40 minutes. The bread was sliced, packed in plastic bags, and stored at 25°C for further analysis after cooling for 3 hrs [32] [33]. The sourdough bread were used for determination of the height, weight, crack, crust, colour, texture, shelf-life, proximate and sensory analysis.

2.7.1. Proximate Analysis

The moisture, protein, fat, ash and crude fibre content of the bread was analysed [22].

2.7.2. Height of the Prepared Sourdough Bread

The height of the bread samples were measured using tape rule in Centimeter (cm). The loaves were measured in triplicates and the average values were reported [34].

2.7.3. Weight of the Prepared Sourdough Bread

The weight of the sourdough bread was determined using a weighing balance (Phoenix Weighing Scale, Model: SMART-5Q, New Delhi).

2.7.4. Determination of Shelf-Life of Bread Samples

The sourdough bread were enveloped in a clean plastic bags and kept at room temperature ($28^{\circ}C \pm 2^{\circ}C$) on a sterile shelf to study the shelf life (in days) until growth of mold become visible [34].

2.7.5. Sensory Evaluation

The panel of judges (Nine membered) comprising of students and laboratory assistants familiar with sourdough bread carried out the sensory evaluation on the sourdough bread prepared using EPSWC and EPSLD in the Department of Microbiology, University of Ibadan, Ibadan, Nigeria. All panelists were requested to score the samples based on the appearance, taste, aroma, crust, clolour, crack and overall acceptability of the product. The rating was shown on a 9-point Hedonic scale ranging from 9-Extremely like to 1-Dislike extremely.

2.8. Analysis of Data

Data were statistically analyzed using one way ANOVA procedure of SPAA (version 11.0, Chicago, IL). Data were considered statistically significant when P \leq 0.05. Values are expressed as the mean ± SD of the three replicates of each experiment.

3. Results and Discussion

The pH development, lactic acid and hydrogen peroxide produced by the two LAB strains during fermentation is shown in **Table 2**. LDYG2 had the lowest pH (3.9) with greatest amount of lactic acid (2520 g/L) and Hydrogen peroxide (8.1 \times 10³). A predominant role of LAB has been noticed in dairy products, since

Table 2. The pH development, lactic acid and hydrogen peroxide produced *L. delbrueckii*(LDYG2) and *W. confusa* (WCFF1).

S/N	Isolate code	рН	Lactic acid (g/L)	Hydrogen peroxide (g/L) 10 ⁻³
1	LDYG2	3.9 ± 0.28^{a}	$2520\pm0.41^{\rm a}$	8.1 ^a
2	WCFF1	4.5 ± 0.13^{b}	$2110\pm0.11^{\text{b}}$	7.6 ^b

LAB portrays high quality of safety, storage security and good sensory potentials [35]. They are significantly important in improving health issues due to the tremendous health beneficial microflora in the intestinal tract [36]. Most LAB also have the ability to synthesize functional exopolysaccharides [37]. The quantity of lactic acid produced by the LAB is reasonablethereby leading to the low pH [38]. The sufficient acidity produced by the LAB help to improve the flavor and aroma of fermented food. Due to the lofty quantity of lactic acid and low pH produced by LAB, it was concluded that fermented food increases shelf life stability [39].

The EPS yields by EPSWC and EPSLD were investigated. The EPS yield by *W. confusa* and *L. delbrueckii* ranged from 4743.75 - 5090.03 g/L. Statistical analysis showed significant difference between the EPS produced by the LAB. *L. delbrueckii* (LDEPS) however, had the higher yield of EPS (5090.03 g/L). It was reported that *L. delbrueckii* produced the highest amount of EPS (5580.72 mg/L) [40]. Lactic acid bacteria isolated from sourdough generally belongs to the genius *Lactobacillus*, *Leuconostoc*, *Pediococcus* or *Weissella*. *Lactobacillus* and *Weissella* strains were often observed in sourdough [19].

The HPLC analysis of the monosaccharide composition of the EPSLD and EPSWC samples is shown in **Figure 1(a)** and **Figure 1(b)** while the various sugars and content in the EPS produced by EPSWC and EPSLD is shown in **Table 3**. Eight sugar moieties (mannose, glucose, galactose, mannose, rhamnose, arabinose, fructose and xylose) were present. There was a significant difference ($P \le 0.05$) in the sugar content of the EPS produced by *Lactobacillus delbrueckii* and *Weissella confusa*. The sugar concentration varied from 2.17 - 40.96 mg/100g for EPSLD and 2.32 - 43.87 mg/100g for EPSWC. The highest sugar content was glucose (40.96^a) followed by galactose (21.44^b) for LDEPS while Galactose (43.87^a) had higher in EPSWC. However, ribose, arabinose and mannose had the least sugar concentration for both LDEPS and WCEPS. The eight sugars observed in the EPSWC and EPSLD was similar to the work [41] who recorded that galactose and glucose are dominant. It was also revealed that glucose and

S/N	Monosaccharide composition	Sugar concentration of EPSLD (mg/100g)	Sugar concentration of EPSWC (mg/100g)
1	Ribose	$2.17^{\rm h}$	$1.98^{ m h}$
2	Xylose	4.58 ^e	4.25 ^d
3	Arabinose	4.14^{f}	2.19 ^g
4	Rhamnose	4.05 ^g	3.70 ^e
5	Fructose	10.59°	9.83°
6	Glucose	40.96 ^a	23.48 ^b
7	Mannose	9.8 1 ^d	2.32^{f}
8	Galactose	21.44 ^b	43.87 ^a



Figure 1. (a): HPLC Chromatogram of EPSWC, (b): HPLC Chromatogram of EPSLD.

galactose (neutral sugars) occurred frequently in bacteria exopolysaccharide [42]. The present of eight different sugars in EPSLD and EPSWC is an indication that the EPS was heteropolymeric in nature which is an indication that it is heteropolysaccharide. Glucose (14.386 mg/100g) and glucose (12.478 mg/100g) as the highest sugar concentration [43].

Figure 2 shows the antibacterial activity of purified EPSLD and EPSWC. Significant difference ($P \le 0.05$) was recorded in the susceptibility of the pathogenic microorganisms. EPSLD and EPSWC revealed activity on some pathogenic microorganisms ranging from 4.0 mm to 24.0 mm and 0.4 mm to 23.0 mm. EPSLD was highly resistance to *B. cereus* (24 mm) and *S. aureus* (23 mm) while *S. typhi, E. coli, K. pneumonia* and *S. dysentriae* were resistant to the EPS. Ability of the LAB EPS to act as antimicrobial agents could be as due to the organic acids, fatty acids, hydrogen peroxide, short fatty acids, bacteriocin and diacetyl produced by LAB [44] [45] reported that exopolysaccharides isolated from *L. plantarum* R315 inhibited *Staphylococcus aureus, Listeria monocytogens, Bacillus cereus* and *Shigella sonnei.* Few studies had shown that EPS from LAB had potent inhibitory ability on diverse test pathogenic microorganisms' in-vitro. Antimicrobial mechanisms such as impairing cell division, disrupting the cell wall and cytoplasmic membrane and decomposing DNA could be possibly be responsible for their antimicrobial activity [46] [47].

The antioxidant activities of the EPS produced by *Lactobacillus delbreuckii* and *Weissella confusa* were evaluated using four different radical scavengers (DPPH, Ferric reducing power capacity, total antioxidant activity and OH⁻) as shown in **Table 4**. There was a significant difference ($P \le 0.05$) between the DPPH, Ferric reducing power capacity (FRAP), total antioxidant activity and H₂O₂. Increase in EPS concentration leads to higher antioxidant activity. The DPPH activity for EPSLD and EPSWC ranged from 27.5% - 69.3% and 37.7% - 64.6% respectively at concentration on 0.5 - 10 mg/mL. The highest antioxidant activity of 69.3% was found for EPSLD at a concentration of 10 mg/mL while for EPSWC, the highest antioxidant activity was observed at 64.6% at 10 mg/mL. However, EPSLD produced the highest antioxidant activity. The ferric reducing power scavenging activity of EPSLD and EPSWC ranged from 1.23% to 1.89% and 1.26% to 1.85% respectively at concentration ranging from 0.5 - 10 mg/mL. EPSLD however, had the highest reducing power scavenging potential. The total antioxidant capacity of EPSLD and EPSWC ranged from 1.46% - 1.88% and



Figure 2. Antimicrobial potential of EPSLD and EPSWC.

Conc. (mg/L)	DPPH (%)		FRAP (%)		Total Antioxidant capacity (%)		H ₂ O ₂ (%)	
	LDEPS	WCEPS	LDEPS	WCEPS	LDEPS	WCEPS	LDEPS	WCEPS
50	27.6 ^e	37.7 ^e	1.23 ^e	1.26 ^e	1.46 ^e	1.21 ^e	45.3 ^e	40.9 ^e
100	38.7 ^d	46.2 ^d	1.68 ^d	1.28 ^d	1.63 ^d	1.47 ^d	63.8 ^d	67.6 ^d
250	49.4 ^c	55.6°	1.75 ^c	1.69 ^c	1.73 ^c	1.64 ^c	75.6 ^c	76.9 ^c
500	58.2 ^b	56.3 ^b	1.80 ^b	1.70 ^b	1.77 ^b	1.75 ^b	80.9 ^b	77.2 ^b
1000	69.3ª	64.6 ^a	1.89 ^a	1.85 ^a	1.88 ^a	1.80 ^a	90.5 ^a	86.7 ^a
Ascorbic acid (mg/L)	19.9 ^f	30.8 ^f	0.59 ^f	0.66 ^f	0.62 ^f	0.25 ^f	0.79 ^f	21.8 ⁷

Table 4. The antioxidant activities of the LDEPS and WCEPS.

Mean of values with the same superscript on the same column are not significantly different (P \leq 0.05).

1.21% - 1.80% at concentration of 0.5 - 10 mg/mL. EPSLD had the highest scavenging potential followed by WCEPS. H₂O₂ scavenging activity for EPSLD and EPSWC ranged from 45.3% - 90.5% and 40.9% - 86.7% with EPS concentration ranging from 0.5 to 10 mg/mL. The highest antioxidant potential was observe in EPSLD (90.5%) at the concentration of 10 mg/mL. It was generally observed that EPSLD and EPSWC had the higher scavenging activity than ascorbic acid. Hydroxyl radicals has the ability to react with practically all biological molecules (lipids, carbohydrates, proteins) thereby making it a powerful oxidant [48]. It was also reported that there is a reduction in absorbance rate when DPPH and proton-donating substances combines to an antioxidant thereby scavenging the free radicals. The ferric ion is known to form a stable product because of its electron donor and reducing power ability for neutralizing free radicals [49]. It was also recorded that L. plantarum with K₃Fe(CN)₆ have reductive capacity. This study contradict the report of [50] who recorded that *L. plantarum* had the lowest ferric iron reducing power (5.62 mg AAE/mL) while L. fermentum had the highest. The sensitivity of free ferrous iron to oxygen results in superoxide and ferric iron thereby producing hydrogen peroxide [51]. Total antioxidant capacity depends majorly glycosidic bonds configuration and structural characteristics due to the presence of some antioxidant constituents such as proteins, peptides and microelements in the crude EPS resulting in potent antioxidant effectiveness [52]. A vigorous scavenging ability of EPS from L. plantarum YW32 (5 mg/mL) towards hydroxyl and superoxide radicals was reported [53].

Determination of the proximate analysis and sensory evaluation of EPSLD and EPSWC

The proximate analysis of sourdough bread produced using EPSLD and EPSWC is shown in **Figure 3**. There was significant difference in the crude protein, crude fat, ash, carbohydrate and moisture content of the sourdough bread



■ Crude Protein ■ Crude Fat ■ Ash ■ Carbohydrate ■ Moisture content

Figure 3. The proximate analysis of sourdough bread produced using LDEPS and WCEPS. Key: A—sourdough bread without EPS (control), B—sourdough bread from EPSUC, C—sourdough bread from EPSLD and EPSWC. D—sourdough bread from EPSLD.

produced by EPSLD and EPSWC. The crude protein and crude fat ranged from 10.07% - 10.49% and 1.01% - 1.64% while ash content and carbohydrate content ranged from 1.78% - 1.88% and 8.42% - 59.90%. Generally, sourdough bread EPSWC had the highest crude protein, crude fat, ash and carbohydrate content followed by sourdough bread produced from EPSLD and EPSWC while the least was produced by the control sourdough bread. The moisture content ranged from 27.52 - 29.68 with sourdough bread EPSWC having the least moisture content while the highest moisture content was observe in the control sourdough bread.

The physical properties of sourdough bread are shown in **Table 5**. The height, weight and shelf-life of the bread ranged from 1.53 cm - 2.07 cm, 145.7 g - 211.3 g and 4 - 7 respectively. Sample B sourdough bread (EPSLD) had the highest physical properties followed by sample D sourdough bread while sample A (control) had the least. The ability of the EPSWCSDB to produce high protein, fat, ash, carbohydrate and low moisture content was because *Weissella* strains are known to be effective gluco-oligosaccharide producer as well as the right starter for sorghum and wheat sourdoughs [54]. From this research work, it was obvious that fermentation had positive influence on SDB due to its quality mineral composition. Apart from improvement, leavening of the dough properties and addition of flavor to bread, the application of EPS to SDB also include texture improvement, delayed consistency and staling, stave off spoilage and improve nutritional quality of wheat. It was concluded that the benefits were attributed to the LAB and yeast present naturally in sourdough.

The sensory evaluation of the sourdough bread produced by LDEPS and WCEPS is shown in **Figure 4**. Scores for colour and aroma is between 4.89 - 6.70 and 4.56 - 6.44. The highest score was recorded in sample A (EPSWC sourdough



Figure 4. Organoleptic properties of sourdough bread produced with EPS from selected LAB strains. Key: A—sourdough bread without EPS (control), B—sourdough bread from EPSWC, C—sourdough bread from EPSLD and EPSWC, D—sourdough bread from EPSWC.

Daramatara (04)	Sourdough Samples					
rafameters (%)	A (Control)	В	С	D		
Height (cm)	1.53 ^d	2.07 ^a	1.84 ^c	2.05 ^b		
Weight (g)	145.7 ^d	211.3ª	198.6 ^c	209.4 ^b		
Crack	Medium	Medium	Medium	Medium		
Crust Colour	Dark brown	Dark brown	Dark brown	Dark brown		
Texture	Hard	Hard	Hard	Hard		
Shelf life	4 ^c	7 ^a	6 ^b	7 ^a		

Table 5. Physical properties of sourdough bread samples.

Key: A—sourdough bread without EPS (control), B—sourdough bread from EPSWC, C—sourdough bread from EPSLD and EPSWC, D—sourdough bread from EPSLD.

bread) while the least was exhibited sample A (control) and sample D (EPSLD) sourdough bread. Score for taste and texture ranged from 2.66 - 5.40 and 3.67 - 6.30. The highest was recorded in sample B (EPSWC sourdough bread), the least was recorded in sample A (control) an Sample C (EPSWC and EPSLD sourdough bread). The score for palatability ranged from 3.00 - 6.70. The highest was recorded in sample B (EPSWC) and Sample D (EPSLD) sourdough bread while the leas was recorded in sample C (control) sourdough bread. The EPS from *Weissella confusa* increased the textural characteristics and quality of the bread [55]. The crack, crust colour and texture of the SDB samples are medium, dark brown and hard. The result obtained could be due to the activation acidity-induced proteolytic enzymes present in flour, which solubilizes gluten thereby reducing the hardness [56]. Water is been released from the gluten network through proteolysis which allows the activity of amylase and other enzymes pre-

sented in the dough to be increased. Furthermore, the more elastic gluten structure in the SDB was produced mainly by heterofermentative LAB. It was revealed that crumb hardness of the SDB depends on the concentration of sourdough and strains used as the sourdough starter [57]. A similar observation using *Lactobacillus brevis* and *L. plantarum* for SDB was also recorded [58]. The EPS from LAB improve the taste, flavor of fermented product via proteolysis, and lipolysis which result in the formation of acetate ethanol, diacetyl and acetyldehyde [59]. The EPS is also known to enhance the weight, texture and shelf-life of SDB [60]. The acetic acid and lactic acid produced by the LAB strains may be responsible for creating desirable taste and odor in SDB. Dough acidification is a very important factor for initiating proteolysis, which has a major role in developing different flavor and odor in sourdough [61].

4. Conclusion

The result from this research work revealed that Weissella confusa FASHADFF1 and Lactobacillus delbrueckii FASHADYG2 isolated from fermented foods are capable of producing EPS. Glucose and galactose were the dominant sugars present in the EPS which indicates that it was heteropolysaccharide. EPSLD and EPSWC have good antimicrobial due to their ability to inhibit all the test pathogens. It was generally observed that EPSWC and EPSLD had the highest scavenging activity than the ascorbic acid. The application of EPS to sourdough bread produced benefits including leavening, improvement of the dough properties, flavor, texture and the nutritional value. Weissella confusa improved the textural characteristics and quality of the bread. The crack, crust, colour and texture of the SDB samples are medium, dark brown and hard. However, sourdough bread produced from EPSWC had higher proximate and physical properties thereby making the sourdough bread generally accepted. This finding highlights the favorable role of EPS in SDB. It revealed that the EPS from Weissella confusa FASHADFF1 and Lactobacillus delbrueckii FASHADYG2 improved the quality and shelf-life of sourdough bread. Further research is required to investigate and compare the impact of mineral composition of the sourdough bread produced with EPS and without EPS.

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Conflicts of Interest

Authors declared no potential conflict of interest exists.

Authors' Contribution

Adebayo-Tayo, B.C. and Fashogbon R.O.—research concept and design; Fashogbon R.O. and Ogunleye G.E.—collection and/or assembly of data. Fashogbon R.O. and Akintunde M.O.—data analysis and interpretation; Fashogbon R.O.—writing the article; Adebayo-Tayo, B.C. and Fashogbon R.O.—critical revision of the article; Adebayo-Tayo, B.C. and Sanusi, J.—final approval of the article.

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