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Proximate, Phytochemical and Probiotic Properties of Some Fermented Foods

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ABSTRACT

Negative health problems raise from changes in food consumption patterns in society today leading to the emergence of diseases, which affect the digestive tract. This study was designed to determine the phytochemical, mineral, proximate and probiotic properties of some fermented foods. The fermented foods were produced by standard methods and the phytochemical, proximate and mineral compositions of the fermented foods were analysed using methods described by Association of Official Analytical Chemistry (AOAC). Sample (fermented foods) were cultured on de Man Rogosa and Sharpe (MRS) agar and the isolated Lactic Acid Bacteria (LAB) were screened for probiotic properties, including salt tolerance, protease activity, acid tolerance, adhesion and antimicrobial activity. The results revealed that the fermented foods contained different essential nutrients (carbohydrate, proteins), mineral (calcium, magnesium) and a variety of phytonutrients (flavonoids, saponins). The isolated LAB inhibits some pathogenic bacteria to varying degrees and had a survival rate to bile salt concentration ranged from 85 to 99% and 52% to 90%. The phylogenetic analysis and the 16S rRNA sequencing revealed that the LAB isolated belongs to the genera *Lactobacillus* and *Weissella* and were identified as *Lactobacillus plantarum* (two isolates) and *Weissella paramesenteroides.* The presence of phytochemicals (flavonoids, saponins, tannins, terpenoids, and phenolic acid) in the fermented foods makes it medicinal and can be used for the management of oxidative stress and the treatment of various diseases. These strains exhibited potential as probiotic producer and are promising candidates for application in pharmaceutical and food industry in the production of nutraceuticals and functional foods.

***Keywords**: Probiotics; phytochemical; proximate; antioxidant; antimicrobial*

1. INTRODUCTION

The enzymatic conversion of beverages or food through controlled microbial growth is refers to as fermented foods (Marco *et al.*, 2017). Some foods such as vegetables, cereals, meat, soybeans, fruits, fish and other legumes historically undergo fermentation In developing countries fermented foods are constituents of dietary and are treasured because value is being added by enhancing quality nutritionally and digestibility, guaranty safety of food, and are acceptable and accessible traditionally (Champagne *et al.*, 2018).

Changes in the consumption of food patterns in our society leads to emergence of negative health issues today leading to the new diseases, that affects the digestive tract. These cases arise because of changes in behavior, lifestyle and experience of the choice of foods we consumed. These problems can be overcome, when the population of beneficial bacteria dominant that of harmful bacteria (Sudan *et al.*, 2022). The consumption of adequate probiotics the number of beneficial bacteria will supersede that of harmful bacteria (Pathan, *et al.*, 2024). Probiotics are live microorganisms when administered improve/restore the gut microflora generally confer health benefit to the consumer (Maftei *et al.*, 2024). Probiotic functions by influencing the gut immune and intestinal epithelial cells details of these not yet

unveil (Sudan *et al.,* 2022). Probiotics can remedy the effects caused by immune disordered responses in the immune system of the host.

Bifidobacterium, Lactobacillus Bacillus, Leuconostoc, Lactococcus, Pediococcus, Streptococcus and *Enterococcus* strains are probiotics which are also known as lacticacidbacteria (LAB) (Cutting, 2011; García-Ruiz *et al.*, 2014; Le and Yang, 2018) are isolated from fermented products and human breast milk gut microbiota and feces (Fontana *et al.,* 2013; Syngai *et al.,* 2016; Tarrah *et al.,* 2019). Mycotoxins; fumonisins, aflatoxins and trichothecenes and can be remove from food products at preharvest, production and storage by LAB strains (Deepthi *et al.*, 2016; Poornachandra *et al.*, 2017). The functional characteristics of some fermented foods are formulated by strains isolated from traditional fermented foods that could retard the growth of pathogenic microorganisms and could prevent consumers from diseases (Campagnollo *et al.,* 2016; Batista *et al.,* 2017; Deepthi *et al.,* 2017). This study aimed to determine the phytochemical, proximate analysis and also to isolate LAB with probiotic characteristic from these fermented foods.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples (milk, maize, orange) were bought at Kure Central Market, Minna, Niger State transferred into a clean nylon bag and transported to Microbiology Laboratory, Federal University of Technology, Minna.

2.2 Fermented Foods Production

2.2.1 Wara (Cheese)

Sodom apple (*Calotropis procera)* stem were washed with distilled water and carefully weighed and a sterile knife was used to sliced stem. The sliced stem was mixed with distilled water; the mixture was sieved to collect the extract. Fifty milliliters (50 mL) of the extract was added to one litre (1 L) milk (raw) and then mixed and was sieved to remove sheaves and placed in a pot. The metallic pot was placed on a gas cooker at a regulated temperature of 68 ^{o}C for of 20 min for heating to take place. After the heating, clotting occur, the resulting mixture was cooled to renneting temperature of 31^{o}C. The curd was separated from the whey using a clean sieve cup (Ogunlade, 2019).

2.2.2 Orange Wines

Orange fruit were washed and manually peeled, a sterile knife was used to cut the orange into halves and their seeds were removed. Hand juice squeezer was used to press the cut oranges to extract the juice. Sterile hand Monilex blender was used to homogenize (blended) the juice and pulp was obtained. A clear juice is obtained from the homogenate manually using a sterile muslin cloth. The juice was allowed to ferment for 7 days, during fermentation period, sample were taken at 24 h interval for; isolation of LAB, proximate, phytochemicals and mineral analysis (Obasi *et al.*, 2017).

2.2.3 Ogi

The ogi was produced from maize adapting the method described by Eke- Ejiofor (2018) and Banwo *et al.* (2022). Five hundred gram (500g) of the grain were sucked in water (2 L) and allowed to ferment for 2 days. The fermented maize was separated from the sucked water by decanting. A blender was used to grand the maize resulting to slurry. The slurry was sieved using a fine muslin cloth. The by-products were discarded and the sediment starch (ogi) was allowed to ferment. The ogi was allowed to ferment for 7 days, during sample were taken at 24 h interval for; isolation of LAB, proximate, phytochemicals and mineral analysis.

2.3 Proximate Analysis

The method as described by Association of Official Analytical Chemistry (Sangta *et al.*, 2021) was used for general proximate analysis of the fermented food samples.

2.4 Phytochemical Analysis

The phytochemical was analyzed following procedures as explained by Sahira and Cathrine (2015).

2.5 Low pH Tolerance Test of LAB

Ten milliliter (10 ml) of MRS broth was used to inoculate 1 ml of 24 h culture (10^{6}cfu/ml) and was centrifuged at 5000 rpm for 10 minutes. Phosphate buffer (pH 7.2) was used to wash the pellet twice

and was suspended into adjusted MRS broth (5 ml) pH values of 3.0, 2.0 and 1.5 incubated for 24 h at 37°C. Afterwards, the culture (100 µl) and its 10^{10} diluent were plated on MRS (agar) and incubated for 24 h at 37°C. The control contained MRS broth and culture only without pH adjustment (Gheziel *et al.* 2019). Equation 1 was used to calculate the survival rate;

$$\text{Survival rate } (\%) = \frac{\text{Log CFN}_1}{\text{Log CFN}_0} \text{ X } 100 \quad (1)$$

N_1 = viable count N_0 = initial count

2.6 Bile Salts Tolerance

The overnight grown isolates 10^6cfu/ml was placed on the centrifuge for 10 min at five thousand revolution per minutes (5000 rpm). Phosphate buffer (pH 7.2) was used to wash the pellet twice and was suspended into 5 ml broth (MRS) containing bile salt concentration of 0.3% incubated for 24 h at 37ºC (Mulaw *et al.,* 2019). Afterwards, the culture (100 µl) and its 10^{10} diluent were plated on MRS (agar) and incubated for 24 h at 37°C. The control contained MRS broth and culture only without bile salt. Survival rate calculation as in equation 2 below;

$$\text{Survival rate } (\%) = \frac{\text{Log CFN}_1}{\text{Log CFN}_0} \text{ X } 100 \quad (2)$$

N_1 = viable count N_0 = initial count

2.7 Protease Activity

One percent (1%) fresh milk were incorporated into agar (MRS) plates and the isolates were inoculated on the plate and incubated for 48h at 37ºC. The absent of growth around the cultures shows positive to protease test (Gao *et al*., 2022). The two bacteria used as negative and positive control are *Klebsiella* spp. and *Pseudomonas* spp. respectively.

2.8 Adhesion Test

A plate (sterile stainless steel) was introduced into a testtube containing 450 µl of MRS broth inoculated with 24 h culture and incubated for 24 h at 37°C. The plate was removed and washed with 10 ml one percent (1%) peptone H_2O, and left for 5 min and was re-washed and vortexed for 3 min for detachment of bacterial cell to occur and was plated on MRS agar and incubated for 24 h at 37°C to determine the cell number by counting. The percentage adhered of cells were estimated by calculating the total initial cell numbers for each LAB.

2.9 Antimicrobial Activity against Bacteria Pathogens

One millilitre (1 ml) of 10^7 cfu/ml of 24 h grown culture was inoculated and incubated for 24 h at 37°C, afterward centrifuged (5000 rpm) for 10 min, the culture cell free supernatant was collected (crude extract). Brain heart infusion broth was used to innoculate the pathogenic bacteria (*E. coli, S. aureus, S. typhimurium, P. aeruginosa,)* and were incubation for 24 h at 37°C. 100 µl of the inoculum (pathogen) were evenly spread on the plates and allowed to air dry. A 5 mm diameter cork borer was used to bore a uniform wells, and filled with 100 µl crude (cell free supernatant) and incubated for 48 h at 37°C, and zone of inhibition (ZOI) were observed around the well. The zone was measured with venier caliper, 1 mm or more clear zone was considered positive (Benkova *et al.,* 2020).

2.10 DNA Sequencing

By using manufacturer's protocol, the amplified products amplicons were selected and purified A sequencing kit (big dye terminator cycle) was used to performed the sequencing and ethanol EDTA solution was used to purify and precipitate the unincorporated dye terminators. Buffer (HiDi formamide) was then used to resolve the pellets. Genetic Analyser (3130 x 1) was used to performed the sequencing. The 16s rDNA nucleotide sequences present in BLAST tool of Genbank at NCBI WHERE were used to compare the resulting pattern (Sharma *et al.,* 2020).

2.11 Data Analysis

Mean ± SEM (standard error of mean), one –way analysis of variance (ANOVA) SPSS software version 28, were used to analyzed the data obtained. The p values less and equal to 0.05 ($p < 0.05$) were significantly considered.

3. RESULTS

3.1 Mineral and Proximate Composition of the Fermented Foods

The results of proximate and mineral composition of the fermented foods show that the foods are nutritious as they contained different essential nutrients and mineral.

3.1.1 Wara Mineral and Proximate Composition

Wara mineral and proximate compositions were shown in Figure3.1 and Figure3.2. Magnesium, calcium and crude protein, carbohydrate were the dominant mineral and nutrients, with calcium and carbohydrate concentration of 672.84 mg/l and 4.17% respectively.

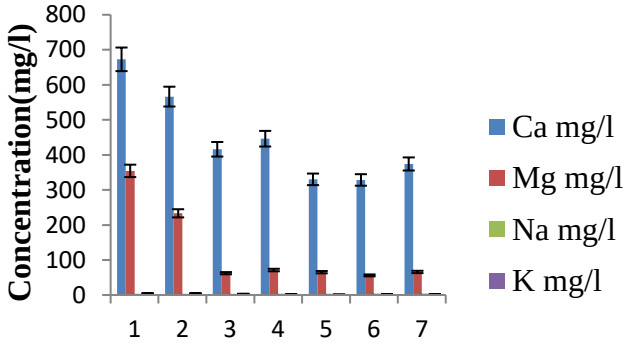

Figure 3.1: Wara Mineralcomposition at K=Potassium,Na=Sodium,Mg=Magnesium,Ca =Calcium

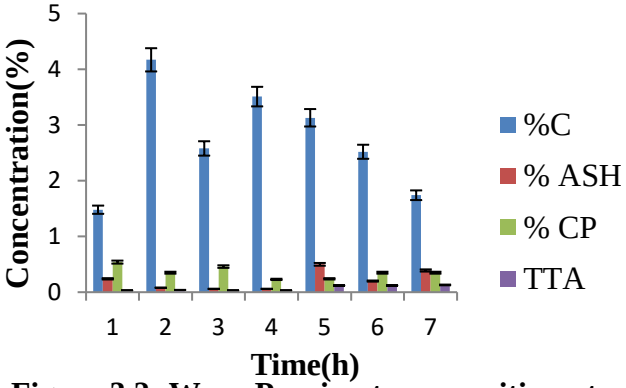

Figure 3.2: Wara Proximatecomposition at TTA=Titratableacidity, ASH=Ash, CP=Crudeprotein, C=Carbohydrate

3.1.2 Ogi Mineral and Proximate Composition

Ogi mineral and proximate composition is presented in Figure 3.3–3.4. Calcium is the dominant minerals at day 1 followed by Magnesium with concentration of 252.31 mg/l and 115.84 mg/l respectively and carbohydrate with the highest concentration of 2.89% at day 7.

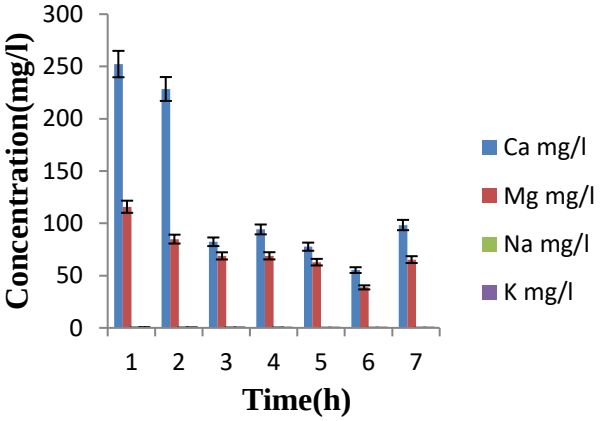

Figure 3.3: Ogi Mineralcomposition at K=Potassium,Na=Sodium,Mg=Magnesium, Ca=Calcium

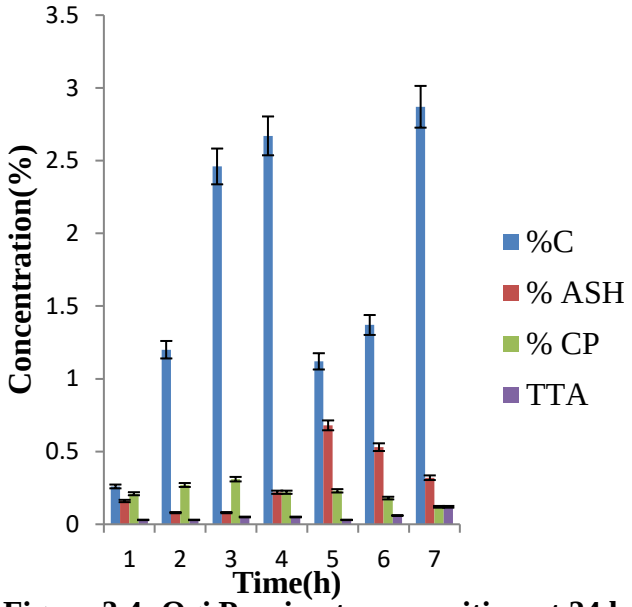

Figure 3.4: Ogi Proximatecomposition at 24 h

TTA=Titratableacidity, ASH=Ash, CP=Crudeprotein, C=Carbohydrate

3.1.3 Orangewine Mineral and Proximate Composition

Orange wine had the higher magnesium content of 470.19mg/l compare to others (ogi and wara) and carbohydrate of 5.79% as shown in Figure 3.5 – 3.6.

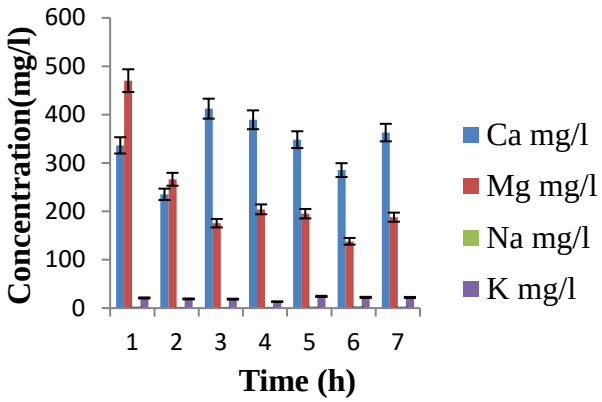

Figure 3.5: Orangewine

K=Potassium,Na=Sodium,Mg=Magnesium, Ca=Calcium

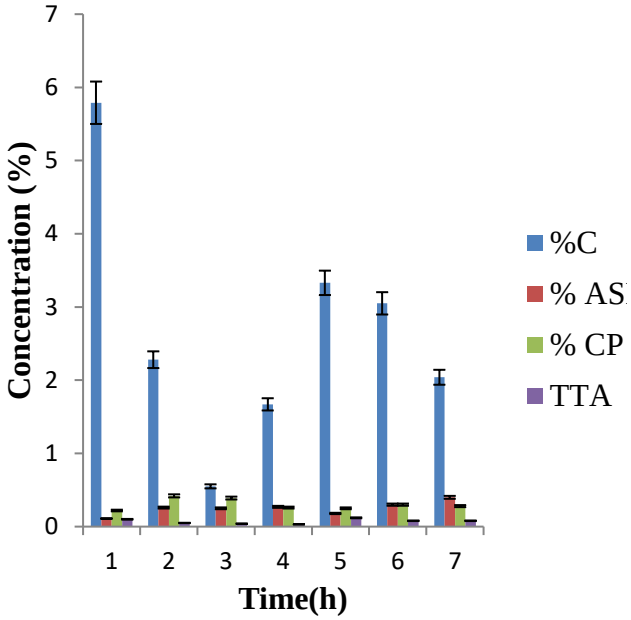

Figure 3.6: Orangewine Proximatecomposition at 24h

TTA=Titratableacidity, ASH=Ash, CP=Crude protein, C=Carbohydrate

3.2 Phytochemical Composition of the Fermented Foods

The fermented foods are well endowed with a variety of phytonutrients (Table 3.1). All the fermented foods contain flavonoids and saponins and some tannins, terpenoids, phenolic acid which have anti-inflammatory, cardiovascular, cholesterol lowering and cancer protections.

Table 3.1: Phytochemical composition of the fermented foods

Phytochemical	Fermented Foods		
	OR	OG	WA
Flavanoids	+	+	+
Phenolic acid	-	+	-
Terpenoids	+	+	-
Tannins	+	+	-
Anthraquinones	-	+	-
Cardiac steroidal	-	+	-
Saponins	+	+	+
Steroid	+	-	-
Cardiac glycosides	+	-	-

OG= Ogi, OR = Orange, WA = Wara, - = Absent, + = Present

3.3 Tolerance to Acidic Condition

Eleven LABS were isolated from the fermented foods and were tested for alkaline and acidic tolerance of 1.5-9 at 24h (Table 3.2). Four (4), nine (9) and all isolates survived pH 1.5, 2 and 3 respectively.

Table 3.2: pH Tolerance of Isolated LAB

Isolates Code	pH										
	1.5	2	3	4	5	6	6.5	7	8	8.5	9
OR5	-	+	++	+	+	+	+	+	+	+	+
OR6	-	+++	+++	+	+	+	+	+	+	+	+
WA15	-	++	+++	+	++	++	+	+	+	+	+
WA16	-	-	++	+	+	+	+	+	+	+	+
WA17	-	++	+++	+	+	++	+	+	+	+	+
WA18	++	++	+++	+	++	+	++	+	+	+	+
WA19	-	+++	+++	+	+	+	+	+	+	+	+
WA20	+++	+++	+++	+	+	++	++	+	+	+	+
WA21	+++	+++	+++	+	++	++	+	++	+	+	+
OG33	+	++	+++	+	+	++	++	+	+	+	+
OG34	-	-	+++	+	+	+	+	+	+	+	+

+++ = Maximum growth, ++ = Moderate growth, + = Minimum growth, - = No growth, OG = Ogi, WA = Wara, OR = Orange

3.4 Antimicrobial Activities of Bacterial Isolates

Antimicrobial activity against some pathogen (Table 3.3). The eleven isolates show some inhibitory potentials towards the pathogens. The average ranged at which the isolates inhibit the pathogens is 8.25 - 21.40 mm. Isolates OG33, WA21, WA20 had higher inhibition rate against *P. aeruginosa, Salmonella typhi, E. coli and Staphylococcus aureus* respectively.

Isolates Code	Diameter zone of inhibition(mm)			
	S.aureus	*E.coli*	*S.typhimurium*	*P.aeruginosa/*
OR 5	19.25 ± 0.03^{f}	20.85 ± 0.09^{h}	20.01 ± 0.01^{i}	20.06 ± 0.03^{fg}
OR 6	21.10 ± 0.06^{j}	16.00 ± 0.00^{b}	19.40 ± 0.06^{g}	21.00 ± 0.00^{i}
WA 15	10.05 ± 0.03^{a}	14.10 ± 0.06^{a}	16.75 ± 0.14^{c}	8.25 ± 0.14^{a}
WA 16	20.40 ± 0.12^{i}	17.35 ± 0.03^{d}	19.71 ± 0.02^{h}	21.00 ± 0.00^{i}
WA 17	20.20 ± 0.12^{h}	21.00 ± 0.00^{i}	19.15 ± 0.09^{f}	20.00 ± 0.00^{ef}
WA 18	19.15 ± 0.09^{f}	20.00 ± 0.00^{f}	20.85 ± 0.09^{j}	19.65 ± 0.03^{e}
WA 19	10.15 ± 0.09^{a}	16.05 ± 0.03^{c}	11.05 ± 0.03^{a}	18.50 ± 0.12^{d}
WA 20	14.00 ± 0.00^{c}	16.06 ± 0.03^{b}	19.05 ± 0.03^{f}	16.35 ± 0.20^{c}
WA 21	19.50 ± 0.06^{g}	17.00 ± 0.00^{c}	20.65 ± 0.03^{j}	20.05 ± 0.03^{fg}
OG 33	17.05 ± 0.03^{d}	20.08 ± 0.04^{f}	20.85 ± 0.09^{j}	20.40 ± 0.06^{gh}
OG 34	19.10 ± 0.06^{f}	17.00 ± 0.00^{c}	17.25 ± 0.14^{d}	18.65 ± 0.03^{d}
CH	22.00 ± 0.00^{ef}	23.00 ± 0.00^{g}	22.00 ± 0.00^{ef}	22.00 ± 0.00^{ij}

OR=Orange, WA=Wara, OG=Ogi, CH-Chloramphenicol. Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

3.5 Adhesion of LAB Isolates

The adhesion of the LAB stainless plate ranged from 32.83 to 37.70% (Table 3.4). This indicates that the isolates could withstand stress in the intestinal wall.

3.6 Bile Salts Tolerance

There are 80% growth rates of the four (4) high acidic tolerance LAB (OG33, W18, W20 and W21) at bile salts concentration of 0.1%, 0.3% and 0.5%. the isolates had maximum ranged 73%-99% (Table 3.4).

Table 3.4: Bile Salt Survival rate (%) and Adhesion rate (%) of LAB

Bile Salt Isolates	0.10 %	0.30 %	0.50 %	Adhesion (%)
WA18	73	73	52	33.51
WA20	99	94.5	44	35.84
WA21	99	91.5	90	37.33
OG33	85	85	80	32.83

WA= Wara**,** OG = Ogi, CA = Cabbage.

3.7 Identification of LAB by 16SrRNA Gene Sequencing

The three (3) isolates that possess probiotics properties were subjected to gene sequence with higher homology of 99.90%, 98.61% and 99.79 to *Lactobacillus plantarum*, *Weissella paramesenteroides* and *Lactobacillus plantarum* respectively.

Table 3.5: Sequence Identity of the LAB Isolates

Isolates code	Max Score	Total Score	Query Cover	E value	Identity (%)	Accession number
WA20	*Weissella paramesenteroides*	1764	100%	0	99.90%	OR541666
WA21	*Lactobacillus plantarum*	3562	99%	0	98.61%	OR541668
OG33	*Lactobacillus plantarum*	1746	99%	0	99.79%	OR541669

4. DISCUSSION

The fermented foods are rich in both minerals and proximate content which make them nutritious with higher medical values. The foods show high calcium and magnesium content of 672.8mg/l and 524.03mg/l respectively, Carbohydrate with highest concentration of 5.79 % and Na with the least content ranged from 0.21-0.31mg/l in all the fermented foods. The presence of titratable acidity and ash indicates that the food is rich in minerals. The fermented foods proved to be more nutritious as they contained higher proportions of the essential mineral and lower proportion of sodium (Na) in agreement with Kiczorowski *et al.* (2022). These mineral elements play very essential roles in enhancing metabolic processes in the body.

Fermented foods are well endowed with a variety of phytonutrients. The principal phytochemicals present in the fermented foods include flavonoids, saponins, phenolic acids and terpenoids. The fermented foods contain flavonoids, saponins and tannins which have anti-inflammatory and cardiovascular, cholesterol lowering and cancer protections similar to Haq *et al.* (2018) who reported that fermented food is source of bioactive phytochemical compounds like flavonoids, saponins and tannins which possess antioxidant potential. The high antioxidant potential of these fermented foods can be used for the management of oxidative stress and the treatment of various diseases.

pH is significant in the growth of probiotics, on this note the LAB isolated were subjected to different pH ranges of pH 1.5 - 9 to test their survivability potential. Four (4) isolates survived pH 1.5 after 24 h. The pH survivability potential is criteria for their function in the gastrointestinal tract of the consumers. This result is contrary to the previous study of Pundir *et al.* (2013) who reports that *Lactobacillus* isolate from curds, fruits, and vegetable could only survived pH 3.5-7.0. Reuben *et al.* (2020)

reported that *Lactobacillus* grows only at pH 2.5 - pH 8.5.

The selected LAB exhibited antagonism effect against *Salmonella typimurium, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* at varying degree. Isolates WA21, CA39, WA20 and OG33 displayed higher antagonistic effect against *P. aeruginosa, E. coli, Salmonella typhi* and *Staphylococcus aureus*, with the zone of inhibition of 21.1mm diameters. In consensus, Dejene *et al.* (2021) reported that LAB isolated from finfish and borde sample belongs to genus *Lactobacillus* were *Salmonella* spp., *E. coli* and *S. aureus* inhibitors with ranged of 15 - 17 mm. Gheziel *et al.* (2019) also isolated six *L. plantarum* strains from fecal samples that possesses high antagonistic activity against *E. coli* and *S. aureus.* The LAB possess inhibitory activity by antimicrobial substances production; acids (organic), bacteriocins, diacetyl, hydrogen peroxide and natural protective substances antiviral peptides and also by food competition with pathogenic bacteria (Souza, 2021).

The isolates possessed adherence property ranged from 32.83 to 37.70%., with these adhesions capacity the isolates would be able to colonized intestinal tract mucosa. In accordance with Mulaw *et al.* (2019) reported that LAB ranged from 32.75 to 36.30%. El-Jeni *et al.* (2020) also revealed adhesion to stainless steel plates to range from 32 - 35%.

All isolates survived bile salt concentration of 0.1 %, 0.3 % and 0.5 % with growth ranged from 73% to99%. Akalu *et al.* (2017) who isolated 30 LAB from fermented Kocho and Shamita and found that 17 isolates have high tolerance to 0.3% bile salt concentration. Not in agreement with Biswal *et al.* (2021); Haghshenas *et al.* (2023) that LAB could only possess 50% survival rate at 0.3% and no survival rate at 50% bile salts concentration.

The 16S rRNA sequencing identified the isolates under study as *Weissella paramesenteroides* and *Lactobacillus plantarum* (2 species), based on comparison of their nucleotides with those of the closest species on the Gene bank (NCBI), with similarity percentages greater than 98 % and E value of 0 for all isolates. Similarly, previous reports on genome sequencing and comparative genomics have revealed a high genomic diversity and flexibility of LAB, which is believed to contribute to its survival in diverse ecological niches (Leeuwendaal *et al*., 2022; Abdulhakim *et al*., 2023).

5. CONCLUSION

Fermented food as revealed is a good source of minerals (ca and magnesium) with reduced Na content, nutrient such as carbohydrae and protein are predominant and also contained bioactive phytochemical compounds.These foods could be included in our daily diet. The presence of phytochemicals such as flavonoids, saponins, phenolic acids and terpenoids makes the fermented food medicinal. The LAB isolated exhibited better characteristics to bile salt and acid tolerance, adherence rate, antibiotics susceptibility, and antimicrobial activity therefore are potential probiotic candidates for application in pharmaceutical, medicine and food industry in the management of oxidative stress, treatment of various diseases and the production of functional foods.

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