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Invertase activities of lactic acid bacteria isolated from traditional fermented milk ("nono"), agadagidi and palm wine obtained from different locations in Ile-Ife, Osun-State, Nigeria

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Abstract

This study was aimed at the isolation and identification of Lactic Acid Bacteria (LAB) from fermented drinks and assaying for invertase enzyme produced by the bacteria. Fresh samples were collected from different sellers and locations in Ile-Ife and lactic acid bacteria were isolated from the samples using De Man and Rogosa Sharpe (MRS) agar and for the determination of microbial count. Standard morphological and biochemical tests were carried out for the characterization of the organisms. The 3, 5-Dinitrosalisylic Acid (DNSA) method was used to determine the enzyme concentration. A total of 36 Lactic acid bacteria were isolated. The mean microbial loads of all the isolates ranges from 2.0 x 10^3 cfu/ml to 6 x10^3 cfu/ml from "Nono", 1.0 × 10^3 cfu/ml to 1.3 × 10^4 cfu/ml from Agadagidi and 7.0×10^3 to 2.0 × 10^5 from Palm wine. They were identified as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus brevis, Pediococcus halophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus casei*. The assay for invertase enzyme revealed the presence of the enzyme in *Lactobacillus plantarum* (0.225mg/ml) from "Nono", *Lactobacillus plantarum* (0.75 mg/ml) and *Lactobacillus fermentum* (0.51 mg/ml) from Palm wine while *Lactobacillus plantarum* and *Lactobacillus brevis* showed 0.191 mg/ml and 0.248 mg/ml respectively from Agadagidi. This study showed the enzymatic activity of invertase in LAB that are present in the fermented drinks and are essential for numerous health benefits.

Keywords: Invertase activity; Fermented products; Lactic acid bacteria; Probiotics

1. Introduction

Lactic acid bacteria are heterogeneous group of microorganisms; they are gram positive, non-sporing, aero-tolerant, catalase-negative, non-respiring cocci or rods that produce lactic acid as the major end product of carbohydrate fermentation [1]. They are widely employed in the manufacture of fermented food products because of their safe metabolic activity, ability to improve the sensory and rheological properties of food materials, ability to decrease the pH of foods, thus inhibiting the growth of spoilage microorganisms [2, 3].

Lactic acid bacteria have been classified through various identification tests [4]. The general consensus is that there is a core group made up of four genera and they are; *Leuconostoc, Pediococcus, Streptococcus,* and *Lactobacillus.* Several new genera have been proposed by recent taxonomic revision and they include; *Oenococcus, Tetragenococcus, Lactococcus, Carnobacterium, Alloiococcus, Aerococcus, Enterococcus, Dolosigranulum, Enterococcus, Globicatella, Wiesella and Vagococcus* [1].

Invertase, also called beta- fructofuranosidase, is a glycoprotein with an optimum pH of 4.5 and stability at 50 °C. It is one of the simplest carbohydrases found in nature [5]. Invertase is widely distributed in the biosphere, especially in

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plants and microorganisms [6]. Invertase is a very useful enzyme used in the industrial processes such as food processing. Aside from beta-fructofuranosidase, invertase is also known as saccharase, glucorase and beta-h-fructosidase [7].

Palm wine is commonly called "emu" and "oguro" in western part of Nigeria. Palm wine may be distilled to produce a strong drink "ogogoro" (local gin) [8]. The sap of the palm trees is originally sweet and serves as a rich substrate for the growth of various types of microorganisms [9].

The sap undergoes spontaneous or wild fermentation which promotes the proliferation of yeasts and bacteria that bring about the conversion of the sweet substrate into several metabolites such as ethanol, lactic acid and acetic acid [10] Yeasts, lactic acid bacteria and acetic acid bacteria play the most important roles in the palm wine production [11].

'Agadagidi' is a product of alcoholic fermentation of rotten/decaying banana because of a good proportion of sugar it contains. Wine is one of the most recognizable high value added products from fruits. Banana (*Musa sapientum*) is an important staple food in Nigeria. It is a seasonal and highly perishable fruit.

Banana has a lot of nutritional benefits, thus demands in the market is high. They are highly recommended by doctors due to their impressive potassium content. It contains good amount of dietary fibre that helps normal bowel movement; thereby reducing constipation. It can also be used to manage anemia, heartburn, temperature control, ulcer, overweight etc. [12] 'Agadagidi' made from banana have opaque effervescent sweet-sour nature.

Fermented milk products can be produced traditionally by spontaneous fermentation or back slopping where a batch of previously fermented product is used to inoculate the new batch [3]. Spontaneous fermentation of milk is carried out by natural milk micro flora, and environmental contaminants [13]. Fermented milk products are also produced through the deliberate application of lactic acid bacteria, as starter culture, to initiate milk fermentation in the dairy industry [4].

Examples of traditionally fermented milk products around the world include; ""Nono"", "Wara", "Amasi" [14], "tempeh", "Dahi" [13], "dadih" [2], "Koumiss" and "Kurut" [15], "gariss" [16] and so on. This research was carried out to evaluate the invertase activities of Lactic acid bacteria from different fermented drinks.

2. Material and methods

2.1. Sample collection

Two samples of fresh Palmwine were collected from four different locations in Osun State. The locations are Mayfair, Ede-road, Lagere and AP area. Freshly prepared 'agadagidi' were collected from Sabo market and Mayfair market in Ile Ife while Nono samples were collected from different Fulani hawkers in Sabo, Ile-Ife, and Osun State. The samples were transported in sterile bottle to the laboratory where they were immediately analysed.

2.2. Microbiological Analysis

2.2.1. Media Used and Their Preparations

The MRS media was prepared following the manufacturers instruction. 65 g MRS was weighed using the weighing balance and dissolved in 1L of distilled water. 2% fungusol was added to the media to inhibit yeast growth. The resulting mixture was homogenized and then sterilized in an autoclave at 121^{o}C, 15Psi for 15 mins and poured into sterile petri-dishes [17].

2.3. Isolation of the Associated Bacteria

2.3.1. Serial dilution of sample

Samples were serially diluted, 1 ml of the samples was pipetted into 9 ml of sterile distilled water, shaken vigorously and then serially diluted up to 10^{-5}. 1 ml of dilution factor 10^{-3} was plated in duplicates using pour plate method on de Man Rogosa and Sharpe media. The plates were incubated for 24-48 hours at 37 °C under anaerobic conditions using the anaerobic jar [16]. After incubation, plates were examined for the growth of lactic acid bacteria. Colonies on each plate were counted and the microbial load in each of the plates was recorded. Representative colonies were randomly chosen. Isolates were sub-cultured and repeated streaking was done to obtain pure culture [18].

2.4. Inoculation and Enumeration

2.4.1. Characterization and Identification of Bacterial isolates

After 48 hours of incubation the plates were examined and colony forming units were enumerated. The colonies on the plates were examined and the morphology recorded. The pure culture of Lactic acid bacteria were preserved on MRS slant. The MRS media containing the lactic acid bacteria was stored at 4 °C and sub cultured every week to get a viable count of the organism [19].

Bacteria cultures were characterized and identified using various morphological and biochemical tests including: Gram Stain, Motility, Catalase, Coagulase, Indole, Citrate, Oxidase and Sugar Utilization Test. The motility test was done according to the technique described by [20] to distinguish motile bacteria from the non-motile one.

2.4.2. Assay for the Invertase Activity of Lactic Acid Bacteria

The invertase activities of the organisms were determined by using 3, 5-Di-nitro salicyclic acid (DNSA) reagent method of [21] as modified by [22] 1ml of the cultured supernatants was added to 1ml of the substrate containing 1.2% (w/v) substrate in 0.1M phosphate buffer at a pH of 6.0.

3. Results

Table 1 Total Lactic Acid Bacteria Count

Location	"Nono" (x104 cfu/ml)	Agadagidi (x104 cfu/ml)	Palm wine (x104 cfu/ml)
Location 1	2.5 ± 0.058	1.01± 1.447	4.0±0.091
Location 2	2.0 ± 0.041	1.11±3.456	2.0±0.129
Location 3	3.5 ±0.100	4.0±1.748	7.0±0.147
Location4	6.0 ± 0.082	3.5 ± 1.459	2.5±0.052

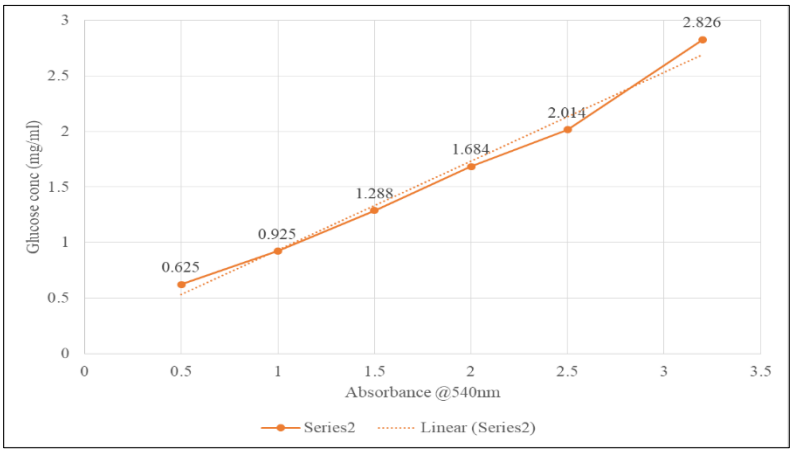

Figure 1 The readings of the assayed enzymes were then taken on the spectrophotometer and compared to the glucose standard curve by tracing out the values on the curve

Table 2 Biochemical Characteristics of Lab Isolates from "Nono"

Isolate codes	Cellular morphology	Gram's reaction	Catalase test	Oxidase test	Indole test	Methyl red test	Voges Proskauer	Citrate utilization	Gelatin liquefaction	Growth at 3 ℃	Growth at 20 ℃	Growth at 70 ℃	Growth in 4% NaCl	Growth at pH 3.4	Growth at 8.5	Casein hydrolysis	Starch hydrolysis	Glucose	Maltose	Mannitol	Lactose	Galactose	Sucrose	Raffinose	Arabinose	Xylose	Inositol	Raffinose	Trehalose	Probable identities
MSEI	C	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	+	-	+	+	*Pediococcus halophilus*
MSQ2	R	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+g	+	+	+	+	+	-	+	+	+	+	+	*Lactobacillus plantarum*
MSA1	R	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+g	+	+	+	+	+	-	+	+	+	+	+	*Lactobacillus plantarum*
MSA2	R	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+g	+	+	+	+	(+)	-	+	+	+	+	+	*Lactobacillus casei*
MSBI	C	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	*Pediococcus halophilus*
MSB2	R	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	*Lactobacillus bulgaricus*
MSR1	R	+	-	-	-	+	-	(+)	-	+	+	-	+	+	+	-	+	+g	+	+	+	+	+	+	+	+	(+)	+	+	*Lactobacillus Plantarum*
MSR2	R	+	-	-	-	+	-	(+)	-	+	+	-	+	+	+	-	+	+g	+	+	+	+	+	-	+	+	+	+	+	*Lactobacillus plantarum*
MSG1	R	+	-	-	-	+	-	(+)	-	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+	+	*Lactobacillus fermentum*
MNG2	C	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+g	+	-	+	+	+	-	+	+	-	+	+	*Pediococcus halophilus*
MNF1	R	+	-	-	-	+	-	+	-	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	+	+	*Lactobacillus fermentum*
MNJ2	R	+	-	-	-	+	-	+	-	+	+	-	+	+	+	-	+	+g	+	+	+	+	+	+	+	+	-	+	+	*Lactobacillus plantarum*

Table 3 Biochemical Characteristics of Lab Isolates from Palm Wine

Isolate code	Cell. Morph	Gram's rxn	catalase	Oxidase	Methyl red	VP	Indole	Citrate utiliz.	Gelatin liq.	4% NaCl	Starch hydr.	Sucrose	Lactose	Inositol	galactose	Casein hydr.	25 ℃	70 ℃	3.9 pH	9.4 pH	Glucose	Manitol	maltose	Raffinose	Arabinose	xylose	Rhamnose	Trehalose	Percent (%)	Probable Identity
AP1	R	+	-	+	+	_	_	+	_	_	+	+	+	_	+	_	+	_	+	+	+	+	+	+	+	+	+	83	*Lactobacillus fermentum*
AP2	R	+	_	_	_	_	_	_	_	_	+	+	_	_	+	_	+	_	+	+	+	_	+	+	+	+	+	88	*Lactobacillus fermentum*
LP1	R	+	_	_	+	_	_	+	_	_	+	+	+	_	+	_	+	_	+	+	+	_	+	+	+	+	+	88	*Lactobacillus casei*
LP2	R	+	_	_	_	_	_	_	_	+	+	+	+	_	+	_	+	_	+	+	+	_	+	+	+	+	+	96	*Lactobacillus fermentum*
LP3	R	+	_	+	+	_	_	_	_	_	+	+	+	_	+	_	+	_	+	+	+	_	+	+	+	+	+	88	*Lactobacillus plantarum*
LP4	R	+	_	_	+	_	_	_	_	_	+	+	+	_	+	_	+	_	+	+	+	_	+	+	+	+	+	92	*Lactobacillus plantarum*
MP1	R	+	_	+	+	_	+	+	_	_	+	+	+	+	+	_	+	_	+	+	+	+	+	+	+	+	+	92	*Lactobacillus plantarum*
MP2	R	+	_	_	_	_	_	_	_	_	+	+	+	_	+	_	+	_	+	+	+	_	+	+	+	+	+	92	*Lactobacillus plantarum*
MP3	R	+	_	_	_	_	_	_	_	_	+	+	+	_	+	_	+	_	+	+	+	+	+	+	+	+	+	88	*Lactobacillus plantarum*
MP4	R	+	_	_	+	_	_	_	_	_	+	+	+	_	+	_	+	_	+	+	+	+	+	+	+	+	+	88	*Lactobacillus fermentum*
MP5	R	+	_	+	+	_	_	+	_	+	+	+	+	_	+	_	+	_	+	+	+	+	+	+	+	+	+	83	*Lactobacillus casei*
MP6	R	+	+	_	_	_	_	_	_	+	+	+	+	_	+	_	+	_	+	+	+	+	+	+	+	+	+	96	*Lactobacillus casei*

Table 4 The Biochemical Characterization of Lactic Acid Bacteria Isolated From 'Agadagidi'

Isolate code	Cell. morph	Gram's rxn	Catalase	Oxidase	Methyl red	VP	Indole	Citrate utiliz.	Gelatin liq.	4% NaCl	Starch hydr.	Sucrose	Lactose	Inositol	Galactose	Casein hydr.	3 °C	25 °C	70 °C	3.9 pH	9.4 pH	Glucose	manitol	maltose	Raffinose	Arabinose	Xylose	Rhamnose	Trehalose	Percent (%)	Probable Identity
MC1	R	+	_	_	+	_	+	_	_	+	+	+	_	_	+	_	+	+	_	+	+	+	+	+	+	_	+	+	+	86	*Lactobacillus plantarum*
MC2	R	+	_	_	+	_	_	_	_	_	+	+	_	_	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	82	*Lactobacillus plantarum*
MS1	R	+	_	_	+	_	_	_	_	_	+	+	+	_	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	80	*Lactobacillus brevis*
MS2	R	+	_	_	+	_	_	+	_	_	+	+	_	_	+	_	+	+	_	+	+	+	+	+	+	_	+	+	+	86	*Lactobacillus plantarum*
MA1	R	+	_	_	+	_	+	_	_	+	+	+	+	+	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	91	*Lactobacillus plantarum*
MA2	R	+	_	_	+	_	+	_	_	_	+	+	+	_	_	_	+	+	_	+	+	+	+	+	+	+	+	+	+	87	*Lactobacillus plantarum*
MM1	R	+	_	_	+	_	_	+	_	_	+	+	+	_	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	86	*Lactobacillus brevis*
MM2	R	+	_	_	+	_	_	_	_	_	+	+	+	+	+	_	+	+	_	+	+	+	+	+	+	+	_	+	+	80	*Lactobacillus. plantarum*
MA1a	R	_	_	_	+	_	+	+	_	+	+	+	+	_	+	_	+	+	_	+	+	+	+	+	+	+	_	+	+	82	*Lactobacillus. fermentum*
MA2a	R	_	_	_	+	_	_	+	_	_	+	+	+	_	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	80	*Lactobacillus plantarum*
MC1a	R	_	_	_	+	_	_	+	_	_	+	+	_	_	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	82	*Lactobacillus fermentum*
MC2a	R	+	+	_	+	_	_	_	_	_	+	+	+	+	+	_	+	+	_	+	+	+	+	+	+	+	+	+	+	86	*Lactobacillus plantarum*

Table 5 Distributions of Lactic Acid Bacteria in All the Samples

LAB Isolates	Nono	Agadagidi	Palm wine	Total	%Occurrence
Pediococcus halophilus	3	-	-	3	8.33
Lactobacillus plantarum	5	8	5	18	50
Lactobacillus casei	1	-	3	4	11.11
Lactobacillus bulgaricus	1	2	-	3	8.33
Lactobacillus fermentum	2	2	4	8	22.22

Table 6 Results of Enzymatic Assay of Invertase Produced By Lactic Acid Bacteria Isolates

LAB Isolates	"Nono" (mg/ml)	Agadagidi (mg/ml)	Palm wine(mg/ml)
Lactobacillus fermentum	-	-	0.51±0.0088
Lactobacillus plantarum	0.225 ± 0.025	0.119±0.0006	0.75±0.0058
Lactobacillus brevis	-	0.248±0.0006	-

Table 7 Glucose Standard Curve

GLUCOSE CONCENTRATION (mg/ml)	ABSORBANCE @ 540nm
3.2	2.826
2.5	2.014
2.0	1.684
1.5	1.288
1.0	0.925
0.5	0.625

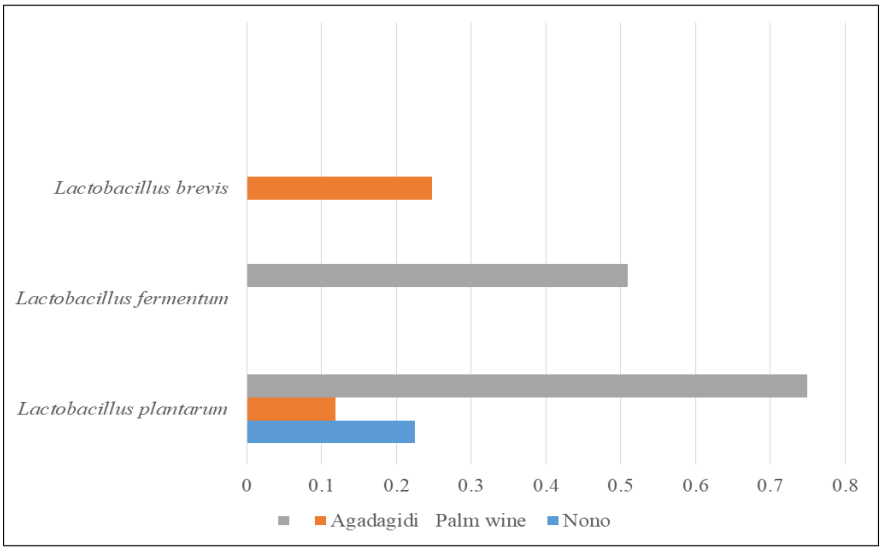

Figure 2 The chart showing the various enzymatic activities of invertase in Lactic Acid Bacteria isolated from the different sample

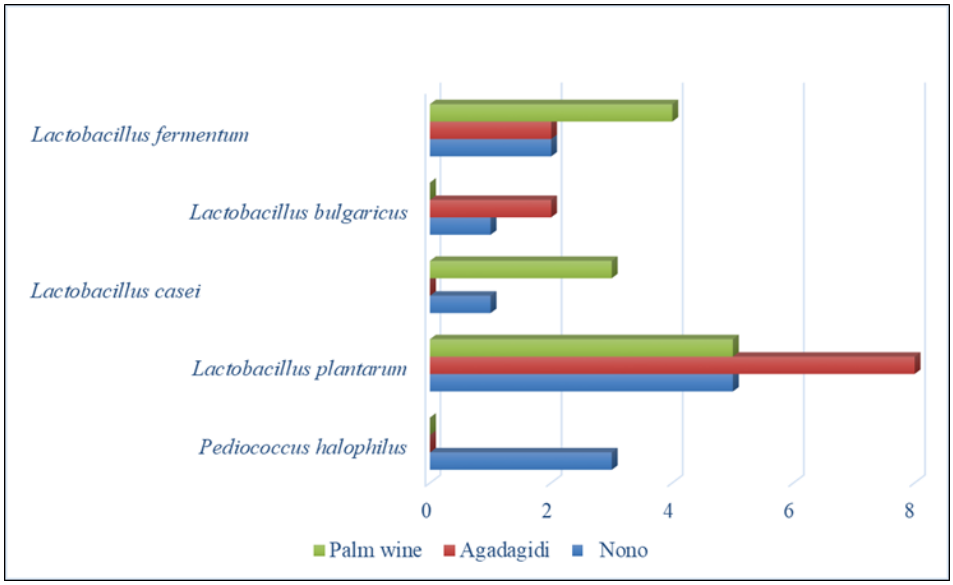

Figure 3 The distribution of Lactic Acid Bacteria isolated from the different samples

4. Discussion

The presence of Lactic acid bacteria in traditional fermented milk and wine products- where they are responsible for the fermentation of the milk and wine, and the improvement of the nutritional, sensory and rheological quality of the fermented milk and wine products- have been reported by many researchers [23].

Twelve isolates each were randomly isolated from the "Nono", Agadagidi and Palm wine samples respectively. Based on microscopic, cultural morphological, physiological and biochemical characterization, the isolated LAB were identified as *Pediococcus halophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus fermentum*, according to Bergey's Manual of Determinative Bacteriology, based on similarities in characteristics with standard strains [19].

It was observed that *Lactobacillus plantarum* had the highest occurrence in all the samples with 50% while *Pediococus halophilus* and *Lactobacillus bulgaricus* lowest occurence 8.33% each. According to [24], *Lactobacillus plantarum* was found to be the dominant species (41.6%) in fermented foods.

The mean lactic acid bacterial load of "Nono" samples ranges 2.0 x 10^3cfu/ml to 6.0 x 10^3cfu/ml. For Agadagidi samples, the count ranges 1.0× 10^3 cfu/ml to 1.7×10^4 cfu/ml and this is lower than earlier results from [25] which ranged from 1.6 x10^7 cfu/g to 7.1 x10^8 cfu/g. The microbial load for Palm wine samples ranges from 7× 10^3 cfu/ml to 2×10^5 cfu/ml and this differs from earlier results of [26] .This difference might be as a result of the inherent (intrinsic factors) and environmental characteristics of the food [27].

The International Commission for Microbiological Specification for Foods [28] states that ready-to-eat foods with plate count between 0 to 10^3 is acceptable, between 10^4 to ≤10^5 is tolerable while 10^6 and above is unacceptable. Hence, the 'agadagidi'; and Palm wine in Ile-Ife are of acceptable and tolerable microbiological quality [29].

The result of the enzyme assay showed *Lactobacillus plantarum* as the highest producer (0.75 mg/ml) with *Lactobacillus brevis* having a lower activity (0.191 mg/ml), although, there is just a slight difference in the production of invertase. Contrary to these reports, [30] reported higher results of invertase enzyme from other fermented foods and fungi but these organisms might not be safe.

It was observed that the LAB isolates- *Lactobacillus plantarum* was able to produce the enzyme invertase which breaks down sucrose into reducing sugars and this is in conformity with the results reported by [31].

Production of acid (lactic acid) as a form of its preservative properties is one of the common characteristic of lactic acid bacteria (LAB), this acid then exhibits antimicrobial activities on the food. This leads to the acidification of the

'agadagidi', 'Nono' and Palmwine and prevents it against spoilage microorganisms and proliferation of pathogens. LAB also produce antimicrobial metabolites called bacteriocins [32]

The acids and bacteriocins produced by Lactic Acid Bacteria have really great potential to be used in food preservation, which are considered as safe natural preservatives. Lactic acid bacteria influence the flavour, texture, shelf life, nutritive properties, health attributes and commercial values of industrially and traditionally fermented foods [33].

5. Conclusion

This research work has showed the invertase activity of the Lactic Acid Bacteria isolated from 'agadagidi'; Nono' and Palmwine sold within Ile Ife and its environs are safe for consumption as they are within the acceptable range in terms of microbial load of microorganisms.

Recommendation

Since there has been an established association between Lactic Acid Bacteria in fermented foods and numerous positive health effects such as stronger immunity, optimum digestivity and other beneficial nutrients, it is recommended that fermented food products are introduced into the daily diet for overall improved health. However, more work has to be done to ensure safer and more hygienic methods in fermenting food.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that there is no conflict of interest.

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