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Production of Probiotic Cabbage Juice by Lactic Acid Bacteria Isolated from Spoilt Cabbage

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Abstract

Probiotic cabbage juice was produced using Lactic acid bacteria (LAB) isolated from cabbage undergoing microbial spoilage. Seven (7) selected LAB isolate were identified on the bases of their colonial, biochemical, physiological characteristics and BD BBL Crystal panel identification system namely; *Pediococcus pentosaceus* (3), *Lactococcus plantarum* (1), *Streptococcus sanguinis* (1) and *Enterococcus faecium* (2). Activities of their probiotic were validated by screening for their growth at low pH, presence of bile and antibacterial property against pathogenic bacteria of clinical importance including *Salmonella typhi* , *Staphylococcus aureus* and *E. coli* using agar well diffusion method. Production of the probiotic cabbage juice was carried out using *P. pentosaceus* (C6) at 37oC for 72 hours. Production markers important for establishing critical control points such as *P. pentosaceus* (C6) population, pH, titratable acidity, reducing sugar, were measured during production. The probiotic cabbage juice was acceptable and liked in terms of its sensory attributes. The stability of the probiotic juice was determined under the storage conditions at 4°C for 4 weeks. *P. pentosaceus* (C6) showed the highest tolerance to pH 3.0 for 3 hours, while *P. pentosaceus* C4 and C6 showed better tolerance to 0.3% bile concentration for 4 hours. Furthermore, *P. pentosaceus* (C6) showed highest zone of inhibition against the three clinical bacteria isolates (15.00±1.41mm, 20.00±0.00mm and 18.00±0.00mm respectively). The pH reduced from 6.5±0.1 to 4.6±0.1, there was increase in titratable acidity from 0.13±0.0 to 0.61±0.1 and sugar concentration was reduced from 3.0 ±0.1(mg/ml) to 2.3±0.1 (mg/ml). The probiotic cabbage juice was acceptable and liked in terms of its sensory attributes. *P. pentosaceus* (C6) grew on the cabbage juice and reached a peak of 1.14±0.2 x109 CFU/ml after 72 hours. *P. pentosaceus* (C6) was viable in the samples during storage period (9.8×107 CFU/ml –3.1×107 CFU/ml). This study established that probiotic cabbage juice of good quality can be produced using *P. pentosaceus* having excellent nutritional content, antimicrobial, sensory and stability characteristics.

Introduction

Probiotics have been defined as living microorganisms which upon ingestion in adequate numbers exert positive health effects beyond inherent basic nutrition [1]. Probiotic strains have an ability to produce antimicrobial metabolites (organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins) [2]. Bacteriocins are ribosomal synthesized anti-microbial compounds that are produced by many different bacterial species including many members of the lactic acid bacteria (LAB). Majority of probiotic microorganisms belong to lactic acid bacteria genera Lactobacillus and Bifidobacterium. However, strains of *Pediococcus* have also been found as suitable candidates [3]. A number of probiotic strains have been introduced in the market in dietary and pharmaceutical forms. Research has shown that addition of probiotics to food provides several health benefits including reduction in the level of serum cholesterol, improved gastrointestinal function, enhanced immune system, and lower risk of colon cancer [4]. (Rafter, 2003). Fermented milk products have been conventionally considered as the most excellent carriers for probiotics; however, the use of milk-based products may be also limited by lactose-intolerance, allergies, dyslipidemia and vegetarianism. Hence, in recent time several raw materials have been extensively explored to determine if they are appropriate substrates to produce novel non--dairy functional foods, vegetables have been proposed as new products containing probiotic strains essentially [5]. Fruit and vegetable juices have been reported as a novel suitable carrier medium for probiotic because they do not contain any dairy allergens (e.g., lactose), and might help certain segments of the population having allergy to dairy products [6]. Most of the tropical fruits are important sources of antioxidants, vitamins, minerals, and dietary fibers, and they constitute as a very healthy part of a human diet. Technological advances have made it possible to alter some structural characteristics of fruit and Vegetables matrices by modifying food components in a controlled way [6]. (Luckow and Delahunty 2004). There is a genuine interest in the development of fruit juice based functional beverages with probiotics, because they have taste profiles that are appealing to all age groups, and are perceived as healthy and refreshing foods [7]. Furthermore, fruit juices are often supplemented with oxygen scavenging ingredients such as ascorbic acid, thus promoting anaerobic conditions that facilitate probiotication. Fruit juices contain high amounts of sugars that could encourage probiotic growth. Non-dairy substrates that have been used for lactic acid bacteria (LAB) fermentation include soy protein and cereals. In recent years, several researches have reported that fruits and vegetable juices could serve as basal medium for LAB fermentation [8]. Nowadays, according to FAO recommendations, an intake of five portions of fruit and vegetables per day is beneficial in terms of public health [9]. Cabbage it is a leafy vegetable crop that grows close to the ground. Cabbage is one of the most important vegetable crops under cultivation and is, in fact, one of the oldest vegetables grown [10]. It is grown in the tropical regions of the world and is a good source of vitamin A, B and C, minerals and carotene [11]. In Nigeria the most commonly grown varieties of cabbage are the Green, Red and Chinese cabbage respectively in their order of demand. The favorable climatic condition contributes immensely to the commercial and subsistence production of these three (3) varieties [12]. Cabbage contains bioactive molecules such as glucosinolates and polyphenolic compound such as flavonoids [13]. Glucosinolates are of interest because their enzymatic degradation releases physiologically active compound which contributes to the bioactivity of cabbage and to the characteristics flavor and taste in vegetable food products. The biosynthesis of these compound is modified due to the environmental growth condition. Glucosinolates are modified by autolysis and food processing methods such as cooking and fermenting and cutting or chewing: the components are degraded and decomposed and variety of break down products (B-DP) are formed. The hydrolysis products of gluconsinolates include isothiocynates, sulforaphane, goitrin and nitriles which have been shown to have pronounced biological effects in animals' models in human and in *in vitro* studies [14]. The compounds may induce detoxification enzyme in mammalian cells thereby acting as cancer – preventing agent [15]. Also, cabbage proven to have health promoting benefit to human body [16]. In many parts of the world, cabbage is used mainly in salads, as a fresh food item, but is also cooked with other foods, and is suitable for processing into products such as shredded cabbage that is subjected to bacterial fermentation controlled with salt resulting in the production of acid cabbage also known as Sauerkraut [17]. Sauerkraut is a very good example of fermented cabbage, during the fermentation, acid produced acts as preservative in addition to development of desired flavor [18]. Sauerkraut is very popular in America and European countries. It is often eaten as an adjuvant with other foods to make them more appetizing, enhance the flavor and digestibility of other foods. It has been reported that the isothiocyanates produced during Sauerkraut fermentation prevent the growth of cancer cells [19].

Fermentation of cabbage juice plays an important role in preservation, production of wholesome nutritious foods in a wide variety of flavors, aromas and textures which enriches the human diet.

The existing literature lacks comprehensive exploration into the utilization of lactic acid bacteria isolated from spoilt cabbage for the production of probiotic cabbage juice. While there is a growing interest in probiotics and functional foods, there is limited research specifically addressing the potential benefits and feasibility of utilizing lactic acid bacteria derived from spoilt cabbage in probiotic beverage production.

Production of probiotic cabbage juice by lactic acid bacteria isolated from spoilt cabbage contributes significantly to the existing knowledge by filling the identified research gap. It provides novel insights into the use of unconventional sources of lactic acid bacteria, specifically those obtained from spoilt cabbage, for the development of a probiotic-rich beverage. The research explores the fermentation process, microbial dynamics, and the nutritional profile of the resulting cabbage juice. Furthermore, the study may offer valuable information on the potential health benefits associated with the consumption of probiotic cabbage juice, thus expanding the scope of functional foods in the field of food science and nutrition.

Therefore, the need to produce probiotic cabbage juice which serves many benefits such as food security and better social well-being of the people living in the country cannot be overemphasized

The aim of this research is to produce probiotic cabbage juice using Lactic acid bacteria isolated from spoilt cabbage.

Material and Methods

Sample Collection

Spoilt cabbage (*Brassica olearaceae* var. capitata) was randomly collected from different vegetable sellers in Samaru Market, Zaria Kaduna State and transported immediately to the laboratory Department of Microbiology, Ahmadu Bello University Zaria, for further analysis. Isolation and Identification of Lactic acid Bacteria

Preparation of Media

Man Rogosa Sharpe (MRS) (Oxoid LTD, Basingstoke, England) using pour plate technique agar was prepared according to manufacturer instructions and sterilized by autoclaving at 121°C for 15 min.

Isolation of Lactic Acid Bacteria

Twenty-five (25g) of spoilt cabbage was inoculated in to 225 ml of peptone water and incubated at 37°c for 18-24 hours. One ml (1ml) from the culture was serially diluted in 9ml of sterile normal saline contained in a sterile test tube. The culture was diluted to a factor of 5 (10^{-5}). One ml (1ml) from the diluent (10^{-5}) was inoculated into the prepared MRS agar using pour plate technique. The preparations were incubated at 37°C for 48hrs. Distinct colonies were subculture into the prepared agar plates using streak method [20].

Morphological identification of the Bacteria Isolates

Gram staining

A single colony from each isolate was smeared in a drop of distilled water on a glass slide and heat fixed by passing it through a flame on the Bunsen lamp. The heat fixed isolates were flooded with two drops of crystal violet and allow to stay for one (1) minute. Furthermore, the glass slide was tilted and rinsed gently with distilled water and air dried. The isolates were flooded with

sufficient (1%) lugols iodine to rinse off excess water and were allowed to stay for 30 sec. The isolates were decolorized with 95% ethanol until color ceases to run out of the smear. The slides were tilted and rinsed with water. Finally, the smear was counter stained with safranin, allowed to stay for 30 second and rinsed with water and allowed to air dried. All isolates were viewed under microscope at 100x objective using oil immersion. The color, shape and size of the cells were observed.

Spore Staining

A single colony from each isolate of 24 hrs old culture was smeared in a drop of distilled water on a glass slide. Slides were covered with Malachite green and slides were heat to steaming by passing it over flame for 5 minutes. Slides were allowed to stand for 5 minutes, and rinsed carefully under slowly running tap water. Slides were counter stained with safranine for 2 minutes, after which they are rinsed carefully and stand to dry. All slides were viewed under microscope at 100x objective using oil immersion.

Biochemical and Physiological Identification of the Bacteria Isolates

Conventional identification of the isolates was performed using the following biochemical and physiological characteristics: [21].

Catalase Test

Freshly prepared colony of 24hrs old culture was used, 3% hydrogen peroxide solution was dropped on a clean glass slide using a sterile loop the bacteria colony was emulsify directly on the clean glass slide containing the hydrogen peroxide. Catalase positive reaction is characterized by the formation of oxygen bubbles that indicate the bacteria that produce the catalase enzyme. The isolates, which did not give gas bubbles, were choosen for further biochemical characterization

Arginine Hydrolysis Test

Arginine MRS medium and Nessler's reagent were used in order to see ammonia production from arginine. MRS broth containing 0.3% L-arginine was transferred into tubes as 5 ml and inoculated with 1% overnight cultures. Tubes were incubated at 37 °C for 24 h. After incubation, 100µl of cultures were transferred onto a white background. The same amount of Nessler's reagent was pipetted on the cultures. The change in the color was observed. Bright orange color indicated a positive reaction while yellow indicated the negative reaction. A control, which did not contain arginine

Gas ($CO_{2)}$ Production of the Bacteria isolates from Glucose

In order to determine the homo-fermentative and hetero-fermentative characteristics of the isolates, CO_2 production from glucose was observed. Sterile MRS broths and inverted Durham tubes were prepared and inoculated with the colonies of gram-positive isolates then the test tubes were incubated at 37 °C for 24hrs. Gas production in Durham tubes were observed after 2hrs of incubation.

Growth of the Bacteria Isolates at Different Temperatures

Five (5ml) of prepared MRS broth containing bromocresol purple indicator, was transferred into tubes. Then colonies of Gram--positive isolates were inoculated into the tubes and incubated for 24-48 hours at 10°C, 15°C, 25°C and 45°C. During these incubation time cells growth at each temperature were observed from change of color from purple to yellow.

Growth of the Bacteria Isolates at Different NaCl Concentrations

Isolates were tested for their tolerance against different NaCl concentrations of 2.0%, 4.0%, 6.5%, 8.0% and 10.0% NaCl (w/v). Test mediums containing bromecresol purple indicator were prepared and 5 ml was transferred into each test tube. Tubes were inoculated and then incubated at 37 °C for 24hours days, growth was observed by change of color from purple to yellow.

Growth of the Bacteria Isolates at Different pH

Growth in media with pH 3.5, 4.5, 5.5, 6.5, 7.5 and 8.0. MRS broth was prepared and was adjusted to various pH and sterilized. The broth was inoculated and all the set were incubated at 37°C for 24 hours. Growth was observed by presences of turbidity.

Sugar Fermentation Ability of the Bacteria Isolates

A series of test tubes containing sterile MRS broth with 1% each of membrane-filter-sterilized (0.45μm) single fermentable sugar was inoculated with the test organisms. The sugar fermentation tubes were incubated at 37°C for 24 hours. At the end of the incubation period, all tubes were examined for acid following the addition of methyl red indicator and then compared with the control for interpretation [22]. A total of fifteen (15) sugar were used for the fermentation tests, these include; Glucose, Fructose, Maltose, Mannitol, Mannose, Galactose, Sucrose, Lactose, Sorbitol, Arabinose, Rhamnose, Raffinose, Trehalose Ribose and Xylose.

Confirmation of Bacteria Isolates Using BD BBL Crystal Identification System

Confirmation of the LAB isolate was done using BD BBL Crystal Identification System (Becton, Dickinson and Co U.S.A). The BD BBL Crystal ID kits is comprising of a BD BBL Crystal ID panel lid, BD BBL Crystal bases and BD BBL Crystal inoculum fluid tubes. The lid contains 29 dehydrated substrate and a florescence control on tips of plastic prongs. The base has 30 reaction wells. Test inoculum is prepared with the inoculum fluid and is used to fill all 30 wells in the base. When the lids were aligned with the base and snapped in place the test inoculum rehydrates the dried substrate and initiates test reactions. Following incubation period wells were observed for color changes or presence of fluorescence that result from metabolic activities of the microorganisms. The resulting patterns of the 29 reactions are converted into ten (10) digit profile number that is used as bases for identification. Biochemical and enzymatic reaction patterns for the Twentynine BD BBL Crystal ID substrate for a wide variety of microorganisms are stored in the BD BBL Crystal ID data base. Identification is derived from a comparative analysis of the reaction patterns of the test isolates to those held in the database.

Principle of the Procedure

The bacterial isolates were identified to species level using BD BBL Crystal panel identification kits. Purified bacteria isolates were cultivated on MRS agar plates, the isolates were transferred into a tube of BD BBL inoculum fluid and the turbidity of the suspension was determined by comparing to 0.5 McFarland. The inoculum fluid was use to fill the well of the BD BBL base and BD BBL lid align the targeted area of the base. Panels are incubated facing down at 37°C for 24h. The test used in the system are based on microbial utilization and degradation of specific substrates detected by various indicator system. Enzymatic hydrolysis of fluorogenic substrates containing coumarin derivatives of 4-methylumbelliferon (4MU) or 7-amino –methylcoumarin (7-AMC) results in increased fluorescence that is easily detected visually with a UV-light source. Chromogenic substrates Biochemical Identification upon hydrolysis produce color changes that can be detected visually. In addition, there are test that detect the ability of an organism to hydrolyze, degrade, reduce or utilize a substrate in the BD BBL Crystal ID systems

Probiotic Properties of The Isolates

Major selection criteria (resistance at low pH, tolerance against bile salt and the antimicrobial activity) were chosen for the determination of probiotic properties of isolates.

Tolerance to Low pH

Resistance at pH 3 is often used *in vitro* assays to determine the resistance to stomach pH

The staying time of foods in the small intestine is believed to be 3 hours, this time limit was taken into account for this purpose. Ac-

tive cultures (incubated for 16-18 hours) were used. Cells were harvested by centrifugation for 10min at 5000 rpm and 4°C. Pellets were washed once in phosphate-saline buffer (PBS at pH7.2). Then cell pellets were suspended in PBS (pH 3) and incubated at 37 °C. Growth was monitored by absorbance at OD620nm [23].

Tolerance to Bile Concentration

The mean intestinal bile concentration is believed to be 0.3% (w/v) and the staying time of food in small intestine is suggested to be 4 hours, the experiment was applied at this concentration of bile for 4 hrs. The medium MRS containing 0.3% bile was inoculated with active cultures and incubated for 16-18 hrs. During the incubation for 4 hours growth was monitored by absorbance at OD620. [23].

Evaluation of Antibacterial Activity

Antibacterial activities of the isolates were determined by the agar diffusion method. The tested bacteria were incubated in nutrient broth at 37° C for 24 hours. One percent (1%) of the bacteria to be tested for sensitivity (indicator bacteria) were inoculated into 20 ml of nutrient agar and poured into the Petri dishes. To detect antibacterial activity of the isolates, MRS containing only 0.2% glucose was used. Ten (10) ml of broth was inoculated with each isolate and were incubated at 37 °C for 48 hours. After incubation, a cell-free solution was obtained by centrifuging (6000 rpm for 15 min). Supernatants were neutralized by 1N NaOH to pH6.5, supernatant of each isolate was checked for antibacterial activity against potentially pathogenic bacteria inoculated in mullar-hiton agar. Then 100µl of cell free supernatants was filled in 8-mm diameter sealed wells cut in the mullar-hiton agar. Once solidified, the dishes were stored for two hours in a refrigerator.

The inoculated plates were incubated for 24 hours at 37°C, and the diameter of the inhibition zones was measured by calipers in millimeters [24].

Production of Probiotic Cabbage Juice

Cabbage juice was obtained using a juicing machine and sterilized by autoclaving for 10 minutes at 121°C. The fermentation experiment was conducted in 250 ml Erlenmeyer flaks containing 100ml cabbage juice. All samples were inoculated with a 24-h culture (1.5×10^8 CFU/mL) and incubated at 37° C for 72h.The probiotic cabbage juice were taken at intervals of 0, 24, 48, and 72 h during the fermentation process for analyses [25].

The pH of the cabbage juice was measured using a pH meter. For acidity measurement,3 drops of 1% phenolphthalein was added to 10ml of samples and titrated by addition of 0.1 N NaOH. The acidity (%) calculated was taken as lactic acid, using this formula

$$Acidity\,(\%)\;LacticAcid = \frac{TitrexChemicalfactor}{10}x100$$

The reducing sugars was measured by adding 0.3 ml of the diluted sample to a tube, applying 0.9 ml of dinitrosalicylic acid (DNS) reagent, thoroughly mixing, heating in boiling water for 5 min, and cooling on ice (Miller,1954). The absorbance was measured at 546 nm using spectrophotometer. The reducing sugar content was determined using a glucose standard curve.

Viable cells (CFU/ml) were determined by the standard plate count method using medium [26]

Proximate Composition of the Fermented Probiotic Cabbage Juice

The proximate composition of the fermented probiotic juice that were determined includes Moisture content, Ash content, Crude fiber, Crude protein and Carbohydrate content as described by Association of Official Analytical Chemists [27].

Sensory Evaluation of the Fermented Probiotic Cabbage Juice

Samples of the probiotic Cabbage juice was served to 10 panelists. The panelists were asked to rate the sample based on taste, aroma, color and appearance. The ratings were prepared on a 5- point hedonic scale ranging from 1- Strongly like to 5 – Strongly dislike [28].

Storage Stability of the Fermented Probiotic Cabbage Juice

After 72 h of fermentation at 37 ^{0}C, the fermented samples were stored at 4^{0}C for 4 weeks. Samples were taken at weekly intervals, and the stability of probiotic cultures in fermented probiotic cabbage juice was determined and expressed as colony forming units (CFU/ml) [25].

Data Analysis

Results obtained were presented as tables, charts and graphs where necessary. All experiment were carried out in duplicate also each sample was analyzed in duplicate. The results are expressed as mean ± S.D (standard deviation) The Graph pad prisms statistical computer package was used to analyze the experimental data.

Result

The macroscopic and microscopic characteristics of the isolates is shown in Table 1. On de Man Rogosa Sharpe (MRS) agar plates, the bacteria colonies isolated were observed to be greyish white, whitish to creamy in color, with smooth surface appearance and entire edge. The bacteria colonies isolated were observed to be Gram positive non-spore forming cocci.

The result presented in Table 2 also shows the biochemical and physiological characteristics of the bacterial isolates. All the bacterial isolates were catalase negative and did not produce gas in the presences of glucose, but they all produced ammonia from arginine except isolate C2. Isolate C1, C4, C6, C7 and C8 grew at 10°C, 25°C ,35°C ,40°C and 45°C. Isolate C2 grew at 40°C but unable to grow at 45 °C while isolate C5 only grew at 25°C and 40°C. Isolate C1, C4, C6, C7 and C8 tolerated 2% NaCl, 4% NaCl, 6.5% NaCl and 8% NaCl concentrations, isolate C2 tolerated 2% NaCl and 4%NaCl concentration, while isolate C5 tolerated only 2% NaCl. The isolates where unable to grow in pH 3.5, but isolate C1, C4, C6, C7 and C8 grew in pH 4.5 and 8.5 while isolate C2 and C5 grew on pH 4.5 and 7.5. The result of sugar fermentation test carried out on all the bacterial isolates and the result shows that all bacteria isolates utilized glucose, fructose, maltose, sucrose and mannose. Isolate C1, C4 and C6 showed negative result for mannitol and sorbitol. Isolate C2 were negative for galactose, The result of sugar fermentation test carried out on all the bacterial isolates and the result shows that all bacteria isolates utilized glucose, fructose, maltose, sucrose and mannose. Isolate C1, C4 and C6 showed negative result for mannitol and sorbitol. Isolate C2 were negative for galactose, Arabinose, raffinose, ribose and xylose. Also, isolate C7 and C8 showed negative results for rhamnose as shown in Table 3. The results of sugar fermentation and biochemical characteristics were compared with [29] Bergey's Manual (2nd edition Vol. 3) and the isolates were presumptively identified as Lactic acid Bacteria.

Table 1: Cultural and Morphological Characteristics of the Bacteria Isolates

Isolates	Growth on MRS agar	Cell morphology	Gram reaction	Spore formation	Presumptive identity
C1	Smooth greyish Colonies	Cocci/round	+	-	*Pediococcus spp*
C2	Whitish colonies, circular, smooth with an entire margin	Cocci/round	+	-	*Latocuccus spp*

C4	Smooth greyish Colonies	Cocci/round	+	-	*Pediococcus spp*
C5	Smooth whitish colonies with entire edge	Cocci/chains	+	-	*Streptococcus spp*
C6	Smooth greyish Colonies	Cocci/round	+	-	*Pediococcus spp*
C7	Smooth creamy colonies with entire edge	Cocci/round	+	-	*Enterococcus spp*
C8	Smooth creamy colonies with entire edge	Cocci/round	+	-	*Enterococcus spp*

Key: MRS;de Man Rogosa Sharpe,+, Positive ,- Negative

Table 2: Biochemical and Physiological Characterization of Bacteria Isolates

				Biochemical reactions			
				Growth at different temperature	Growth at different Nacl Concentration	Growth at different pH	Inference
Isolates	Catalase	Ammonia from Arginine	CO_2 from Glu	10oC 15oC 25oC 35oC 40oC 45oC	2% 4% 6.5% 8.0%	3.5 4.5 5.5 6.5 7.5 8.5	
C1		+		+ + + + + +	+ + + +	- + + + + +	*Pediococcus spp*
C2		-		+ + + + + -	+ + + +	- + + + + -	*Latococcus spp*
C4		+		+ + + + + +	+ + + +	- + + + + +	*Pediococcus spp*
C5		+		- - + + + -	+ - - -	- - + + + -	*Streptococcus spp*
C6		+		+ + + + + +	+ + + +	- + + + + +	*Pediococcus spp*
C7		+		+ + + + + +	+ + + +	- + + + + +	*Enterococcus spp*
C8		+		+ + + + + +	+ + + +	- + + + + +	*Enterococcus spp*

Keys: +; Postive, -; Negative. GLU; Glucose

Table 3: Sugar Fermentation Ability of the Bacteria Isolates

Isolates	Glu	Lac	Fru	Mal	Mant	Sugar Man	Suc	Gal	Sor	Ara	Rha	Raf	Tre	Xyl	Rib	Inference
C1	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	*Pediococcus spp*
C2	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	*Latococcus spp*

C4	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	*Pediococcus spp*
C5	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	*Streptococcus spp*
C6	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	*Pediococcus spp*
C7	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Enterococcus spp*
C8	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Enterococcus spp*

Key: +; Positive, _; Negative. (GLU); Glucose, (Lac); Lactose, (FRU); Fructose, (MAL); Maltose, (MANT); Manitol, (MAN); Mannose, (SUC); Sucrose, (GAL); Galactose, (SOR); Sorbitol, (ARA); Arabinose, (RHA); Rhamnose, (RAF); Raffinose, (TRE); Trehalose, (XYL); Xylose, (RIB); Ribose.Confirmation of the Bacteria Isolates using BD BBL Crystal Panel Identification System

The Lactic acid bacteria isolates were confirmed using BD BBL Crystal panel identification kits. The results obtained are shown in Table 4.

Table 5 shows the identity of Lactic acid bacteria isolated from the spoilt Cabbage samples.

Screening for their Probiotic Properties

The confirmed and identified Lactic acid bacteria were further screened for their probiotic properties. Figure 1 showed the tolerance pattern of the Lactic acid bacteria isolates at pH 3.0 for 3 h. Isolate C6 (*Pediococcus pentosaceus)* showed highest tolerance at 0 hour with an optical density of 0.43 and at 3 hrs it also had an optical density of 1.10 while isolate C8 (Enterococcus *faecium)* has the lowest value at 0hr with an optical density of 0.18 and at 3hrs the isolate had an optical density of 0.19. The isolates were tested for tolerance to 0.3% bile concentration for 4 h as shown in Figure 2. Among all the Lactic acid bacteria isolate, C4 (*Pediococcus pentosaceus)* and C6 (*Pediococcus pentosaceus*) were the most tolerant isolates at 0.3% bile concentration for 4h. At 0-hour isolate C4 and C6 has the highest value with optical density 0.52 and 0.44 and at 4 hours the isolates had the highest value with the optical density 1.21 and 1.23 respectively.

Table 4: Confirmation of Bacteria Isolates Using BD BBL Crystal Panel Identification System

	Isolates Substrates																													
	FCT	FHO	FGC	FGS	FGN	FGA	FPH	FTR	FAR	FVA	FPY	FIS	LAC	PLN	PCE	PAM	BGL	PHO	TRE	SUC	ARA	MNT	GLR	MAB	MTT	FRU	ONPG	ARG	ESC	URE
C1	-	-	+	+	-	+	+	+/-	-	+/-	+/-	-	+	-	+/-	+/-	+/-	-	+	+	+	+	+	-	+	+	+/-	+	+	-
C2	-	-	+	-	-	+	+	+	+	+	-	+	+	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	+/-	-
C4	-	-	+	+/-	-	+	+	+	-	-	+	+/-	+	+/-	+/-	+/-	+/-	-	+	+	+	+	+/-	+	+	+	+/-	+	+	-
C5	-	-	-	+/-	-	-	+	+	+	+/-	-	+	+	-	-	-	-	+/-	-	-	-	+/-	-	-	-	+	-	+	-	-
C6	-	+/-	+/-	-	-	+/-	+	+	-	+	-	+	+	-	-	-	-	-	+/-	-	-	+/-	-	-	-	+	-	+	+	-
C7	-	-	+	+/-	-	+	+	+	+	-	+	-	+	-	+	+/-	+	-	+	+	+	+	+	+	+	+	+/-	+	+	-
C8	-	-	+	+	-	+	+	+	+	-	+	-	+	-	+/-	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-

Key: +; Positive, - Negative; +/- Variable. (FCT); Fluorescent negative control, (FHO);4MU –Phosphate,(FGC);4MU-β-D-glucoside, (FGS);4-MU-α-D-glucoside, (FGN);4MU-β-D-glucuronide, (FGA);4MU-N-acetyl-β-D-glucosaminide, (FPH);L-phenylalanine, (FTR); L-tryptophan, (FAR); L-arginine, (FVA);L-valene, (FPY); L-pyroglutamine acid, (FIS); L-isoleucine, (LAC); Lactose, (PLN); Proline&Leucine-p-nitroanilide, (PCE);P-nitrophenyl-β-D-cellobioside, (PAM);P-nitrophenyl-α-D-maltoside, (BGL); P-nitrophenyl-β-D-glucoside , (PHO); P-nitrophenyl-phosphate, (TRE);Trehalose, (SUC);Sucrose, (ARA);Arabinose, (MNT); Mannitol,(GLR); Glycerol (MAB);Methyl-α-&-β-glucoside, (MTT);Maltotriose, (FRU);Fructose,(ONPG);O-nitrophenyl-β-D-galactoside, (ARG); Arginine, (ESC);Esculine, (URE); Urea

Table 5: Identity of Lactic acid Bacteria Isolated from Spoilt Cabbage Samples

Sample codes	Identity of bacteria isolated Specificity (%)	
C1	*Pediococcus pentosaceus*	98.8
C2	*Lactococcus plantarum*	87
C4	*Pediococcus pentocaseus*	96
C5	*Streptococcus sanguinis*	56
C6	*Pediococcus pentocaseus*	97.6
C7	*Enterococcus faecium*	98
C8	*Enterococcus faecium*	98

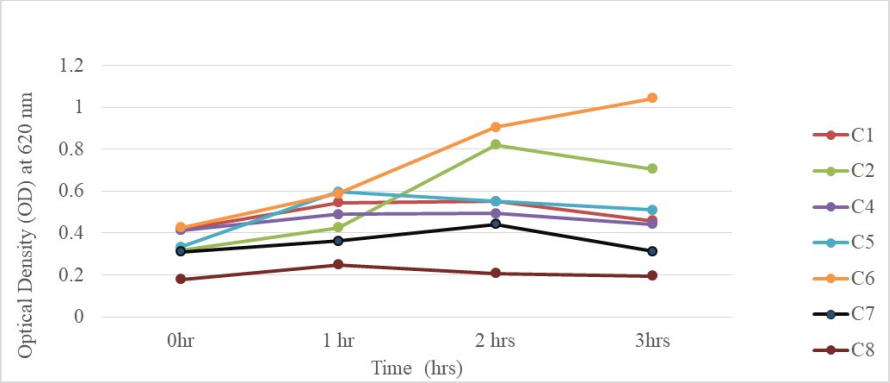

Figure 1: Screening for Probiotic Property of Lactic acid Bacteria Isolates Using Growth at pH 3.0 for 3 hours.

Key: C1 = *Pediococcus pentosaceus* C2 = *Lactococcus plantarum* C4 = Pediococcus pentocaseus C5 = *Streptococcus sanguinis* C6 = *Pediococcus pentocaseus* C7 = *Enterococcus faecium* C8 = *Enterococcus faecium*

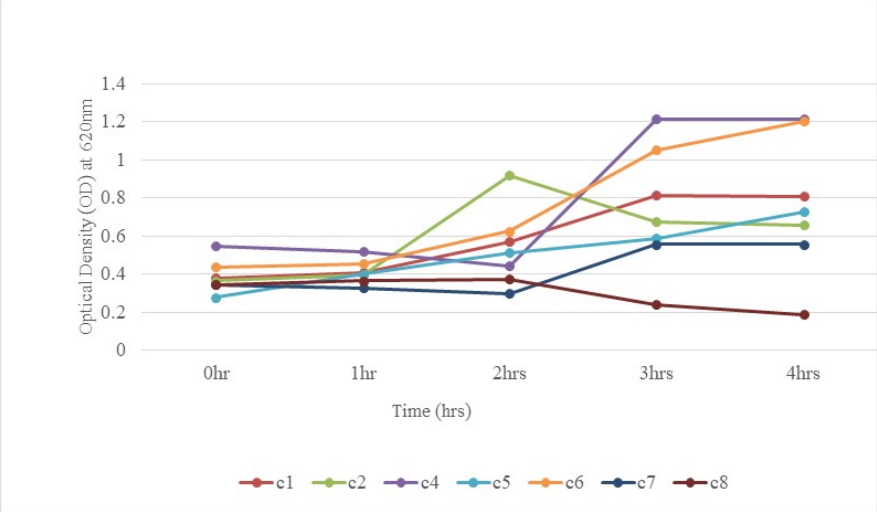

Figure 2: Screening for Probiotic Property of Lactic acid Bacteria Isolates Using Growth at 0.3% Bile Concentration for 4 hours

Key: C1 = *Pediococcus pentosaceus* C2 = *Lactococcus plantarum* C4 = Pediococcus pentocaseus

C5 = *Streptococcus sanguinis* C6 = Pediococcus pentocaseus C7 = *Enterococcus faecium* C8 = Enterococcus faecium

The Lactic acid bacteria isolates were tested for their antagonistic properties against potentially pathogens of clinical origin. For this purpose, strains were tested against pathogenic organism (*Salmonella thyphimurium*, *Staphylococcus aureus* and Escherichia

coli). Isolate C6 (*Pediococcus pentosaceus*) has highest zone of inhibition against *Salmonella thyphimurium*, *Staphylococcus aureus* and *E. coli*. (15.00±1.41, 20.00±0.00 and 18.00±0.00 respectively) as shown in Table 6

Table 6: Antagonistic Properties of the Lactic acid Bacteria against Pathogenic Organisms

	Isolates/ Zone of inhibition (mm)						
Test organisms	C1	C2	C4	C5	C6	C7	C8
Salmonella typhi	13.00±1.41a	13.00±1.41a	12.00±2.83a	11.00±1.41a	15.00±1.41a	14.00±1.41a	14.00±1.41a
Staphylococcous aureus	14.00±1.41a	18.00±1.41b	17.00±1.41b	11.00±1.41a	20.00±0.00b	13.00±1.41a	16.00±0.00a
E.coli	15.00±0.00a	17.00±1.41b	18.00±1.41b	15.50±2.12b	18.00±0.00ab	16.00±0.00a	17.00±1.41a

Values are mean of duplicate (± Standard Deviation). The experimental values within columns that do not have a common superscript are significantly different ($p<0.05$)

Key: C1 = *Pediococcus pentosaceus* C2 = *Lactococcus plantarum* C4 = Pediococcus pentocaseus C5 = *Streptococcus sanguinis* C6 = Pediococcus pentocaseus C7 = *Enterococcus faecium* C8 = *Enterococcus faecium*

Out of all the Lactic Acid Bacteria that were isolated, isolate C6 (*Pedicoccous pentosaceus*) was found to be the best based on the criteria used for the screening process and it was used in the production of probiotic cabbage juice.

Production of Probiotic Cabbage Juice by *Pediococcous pentosaceus*

The pH of the juice reduced from 6.5 to 4.6 at 72 hours of fermentation. The total titratable acid during the fermentation process increased from 0.13 g/dm^3 to 0.61 g/dm^3, after 72 hours. There was reduction in the reducing sugar from 3.0 mg/ml to 2.3mg/ml. *Pediococcous pentosaceus* produced a viable cells 1.14 ± 0.2 x 10^9 there was difference in the parameters tested in the fermented probiotic juice when compared to the unfermented juice after 72 hours of fermentation. The results obtained were presented in Table 7.

Proximate Composition of the Fermented Probiotic Cabbage Juice

The proximate analysis of the fermented probiotic cabbage juice and unfermented cabbage juice after 72 h of fermentation shows that the fermented probiotic juice has ash content of 0.15% ,crude fiber 0.37% fat content 1.44% ,crude protein 1.06% and carbohydrate content 2.29% while the unfermented juice contains higher ash content of 0.16%, fat content of 2.28%, carbohydrate content of 3.86 % and lower protein content of 0.81 when compared to the fermented probiotic juice as shown in Table 8.

Sensory Evaluation of the Fermented Probiotic Cabbage Juice

The fermented probiotic juice was accepted and liked in terms of sensory attributes after 72 hours of fermentation. The aroma of the fermented probiotic juice was acceptable and 70% liked, the taste was 67 % moderately liked, the colour and appearance was 60 % liked while the taste of the unfermented juice was 40% moderately liked, 30 % strongly disliked and 3% liked. The result obtained was presented in Figure 3.

Table 7: Production of Probiotic Cabbage Juice by Pediococcus pentocaseus

Fermented Cabbage juice					Unfermented Cabbage juice			
Time (hrs)	pH	TTA (Lactic acid) g/dm3	RS (mg/ml)	Viable cells(cfu/ml)	pH	TTA (Lactic acid) g/dm3	RS (mg/ml)	Viable cells(CFU/ml)
0	6.5±0.1a	0.13±0.0a	3.0±0.1a	3.6±0.1x108a	6.4±0.1a	0.12±0.1b	3.0±0.1c	-
24	5.5±0.1b	0.37±0.1ab	2.6±0.1ab	2.08±0.3x109b	6.2±0.1a	0.11±0.1b	2.9±0.1c	-
48	4.9±0.1c	0.46±0.1ac	2.4±0.1b	1.40±0.1x109c	6.3±0.1a	0.12±0.1b	2.8±0.1c	-
72	4.6±0.1c	0.61±0.1bc	2.3±0.1b	1.14±0.2x109c	6.2±0.1a	0.10±0.1b	2.8±0.1c	-

Values are mean of duplicate (± Standard Deviation). The experimental values within columns that do not have a common superscript are significantly different ($p<0.05$).

Key: TTA; Total Titratable Acidity, RS; Reducing Sugar.

Table 8: Proximate Composition of the Fermented Probiotic Cabbage Juice and Unfermented Cabbage Juice

Parameters	Fermented Cabbage juice Amount (%)	Unfermented Cabbage juice Amount (%)
Moisture content	94.69	92.63
Crude protein	1.06	0.81
Crude fiber	0.37	0.26
Fat content	1.44	2.28
Ash content	0.15	0.16
Carbohydrate content	2.29	3.86

Table 9: Storage Stability of Fermented Probiotic Cabbage Juice Produced by Pediococcus *pentosaceus* at 4°C for Four Weeks

Time (weeks)	Viable count (CFU/ml)
1	9.8x107
2	7.4x107
3	5.2x107
4	3.1x107

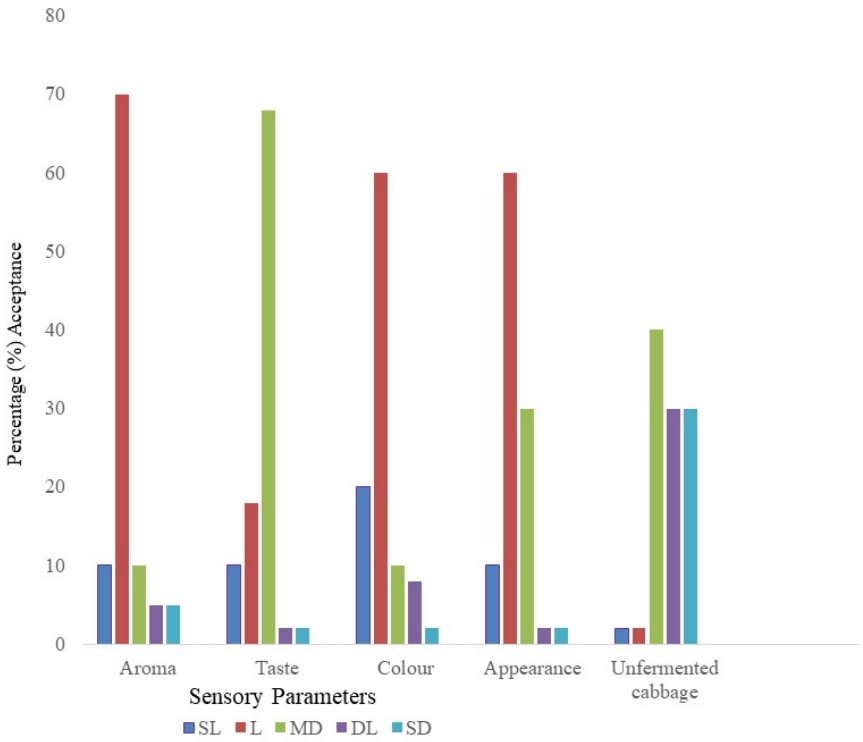

Figure 3: Sensory Evaluation of the Fermented Cabbage Juice Produce by Pediococcus pentocaseus.

Key: SL; Strongly liked, L; Liked, MD; Moderately, DL; Disliked, SD; Strongly disliked.

Discussion

Spoilt cabbage samples collected from Samaru Market, Zaria were used for the isolation of LAB. The isolates were identified and confirmed as *Pediococcus pentosaceus*, *Lactococcus planetarum*, *Streptococcus sanguinis* and *Enterococcus faecium* based on cultural, morphological, biochemical and BD BBL Crystal panel identification kit. The research carried out by Kopupula and Bhukya [30] similarly confirmed the isolation of *Lactobacillus acidophilus*, *L. brevis, Pediococcus spp. and Leconostoc spp* from spoilt cabbage, fruit and kitchen waste.

The ease of isolation of these LAB from spoilt cabbage could be due to the richness in the nutritional composition of cabbage. The use of lactic acid bacteria isolated from spoilt cabbage represents a novel and unconventional source of probiotics. This finding contributes to the ongoing efforts to diversify probiotic strains and broaden the range of sources for these beneficial microorganisms.

The major selection criteria used to determine probiotic properties of LAB are resistance to low pH (3.0) for 3 h, tolerance against bile salt (0.3%) and the antimicrobial activity [31]. In this study, *Pediococcus pentosaceous* show the highest tolerance at pH 3.0. for 3h. This may be as a result of their high adhesion ability to intestinal epithelium and high colonization ability for human intestine cells [32]. This is in agreement with the work of Yuksekdag and Aslim [33], who studied the viability of *Pediococcous* strains at pH 1- 3, and reported growth.

Tolerance of the Lactic acid bacteria was examined at 0.3% bile salt concentration for 4 hours, *Pediococcus pentosaceous* (C4) and (C6) were the most stable isolates withstanding 0.3% bile salt concentration. This may be as a result of bile salt that causes the increase in permeability of bacterial cell membranes, as the membranes are composed of lipids and fatty acids [34]. This is in agreement with the work of Reethu et al. [35] who worked on the *in vitro* study of potential probiotic *Pediococcus pentosaceus* isolated from idli batter and biomass production using whey. Also, this finding is similar to the work of Noohi et al. [36] that reported

0.3-0.4% bile salt following evaluation of potential probiotic characteristics and antibacterial effects of strains of *Pediococcus* species.

The LAB isolates were tested for their antagonistic properties against potentially pathogenic bacteria of clinical origin (*Salmonella typhi* , *Escherichia coli* and *Staphylococcus aureus*). All the probiotic (LAB) isolates have antimicrobial activities on the potentially pathogenic bacteria, from the results *Pediococcus pentosaceus* isolate C6 has the highest antimicrobial activity on the potentially pathogenic bacteria, while isolate C5 *Streptococcus sanguinis* has the lowest antimicrobial activity. Isolate C6 *Pediococcus pentosaceus* inhibited the growth of *Staphylococcus aureus* (20.00±0.00 mm ZOI) followed by E coli (18.00±0.00 mm ZOI) and *Salmonella typhi* (15.00±1.4 mm ZOI) respectively. This may be due to production of metabolites (bacteriocin, organic acids such as lactic acid, acetic acid) that have activity against pathogenic bacteria. This is in agreement with the work of Rasha et al. [31] who worked on the characterization of LAB isolated from Dairy products. Production of bacteriocins is another characteristic property of Lactic acid Bacteria, this antimicrobial compound inhibits the growth of microorganism causing food spoilage and used as natural preservatives in foods and also provides protection against harmful pathogens in intestinal tract [37]. These potential health benefits associated with the consumption of probiotic cabbage juice have been linked to improvements in gut health, immune function, and overall well-being. Highlighting these benefits in the context of the study's findings reinforces the positive impact of incorporating such functional foods into the diet.

There was statistical difference in the fermented cabbage juice ($P < 0.05$) and no statistical difference in the unfermented juice ($P>0.05$) with respect to the tested parameters. This may be due to the activity of *Pediococcus pentosaceus* in the fermentation medium which brought about the differences in the tested parameters when compared with the unfermented medium, the fermented probiotic cabbage juices showed a decrease in pH, increase in acidity, and reduction in the cell viability and concentration of sugars at different time intervals (0, 24, 48 and 72 h). The pH decreases in the fermented cabbage juices after 24 h may be due to carbohydrate fermentation that led to increased acidity due to formation of organic acids. However, no significant change was noticed at 72 h, in pH, reducing sugar and viable count but there was significant increase in titratable acid. This may be as a result of more fermentation taking place with decrease in pH over time. This finding is in agreement with the work of Muneeb et al.,[38] who studied the growth of potentially probiotic LAB in carrot and beetroot juice and reported decrease in pH increase in titratable acid, decrease in reducing sugar and viable cells after 72h of fermentation following the activities of LAB in the probiotic juice. It has been proposed that cell viability is a factor which depends on the substrate, the oxygen content, status of nutrients and the final acidity of the matrix used [39].

The cabbage juices supported the growth of *Pediococcus pentosaceus* and showed viable count (1.14 x109 CFU/ml) at 72 h of fermentation. Cell viability was observed in the cabbage juice during fermentation. It was observed that cabbage juices, without any nutrient supplementation, served as good culture media and matrix for the growth of *Pediococcus pentosaceus*. This agreed with Yoon et al. [40] and Vijaya Kumar et al. [41] who reported that fruit juices and vegetables as a good medium for cultivating probiotics. Insights into the fermentation process and microbial dynamics during the production of probiotic cabbage juice contribute to our understanding of the mechanisms behind the formation of functional compounds. This knowledge is valuable for optimizing fermentation processes and ensuring consistent quality in functional food production.

The crude protein of the fermented probiotic cabbage juice was higher than the unfermented cabbage juice, this could be attributed to increase in microbial mass during fermentation of the cabbage juice which my result to extensive hydrolysis of the protein molecules to amino acid and other simple peptides. This is in agreement with the findings of Ojokoh and Orekoya [42] who worked on the Effect of fermentation on the proximate composition of the Epicarp of watermelon. High protein content of the fermented probiotic juice has a good implication in respect to protein deficiency which in return might compliment protein from other plant food. [43]. The percentage of crude fat in the fermented probiotic cabbage juice is lower than the unfermented juice this could be as a result of breakdown of fatty acid and glycerol by *Pediococcus pentosaceous* which in return might increase the taste odour and aroma of the fermented probiotic cabbage juice.

In agreement with the work conducted by Ojokoh and Orekoya [42] low fat content recorded after fermentation of the epicarp of watermelon resulted in the breakdown of fatty acid and glycerol by fermenting microorganism. Owing to the low percentage of fat in the fermented probiotic cabbage juice as compared to the unfermented cabbage juice it may therefore be recommended for loss or maintenance of weight, supply of nutrients and lowering of blood pressure.

The decrease in carbohydrate content of the fermented probiotic cabbage juice could be attributed to the conversion or break down of the carbohydrate to simple sugars which are used by the fermenting microorganisms for growth and metabolisms. This observation is related to the research of Rafiq et al. [44] who stated that reduction in carbohydrate content of fermented probiotic carrot juice is caused by utilization of sugar by fermenting organisms during their metabolisms.

The reduction in ash content in the fermented probiotic cabbage juice may be due to utilization of minerals for metabolic activities by the fermenting microorganism. This is in agreement with the work of Igbabul et al. [45] who stated that reduction in the proximate composition of ash content in fermented mahogany bean is as result of utilization of minerals by fermenting microorganisms.

This study assesses the nutritional composition of the probiotic cabbage juice, discussing the nutritional benefits, it has potential health advantages associated with consuming this particular functional food.

The fermented cabbage juice had good sensory scores when compared to unfermented juices. The acidity, mouth feel, aroma, taste, color and overall appearance were changed in fermented juices. The fermented Probiotic cabbage juice had better overall organoleptic quality and appearance than the unfermented cabbage juices. This may be due to the production of catabolic product and aroma compounds and this could contribute to the sensory attribute of the Probiotic cabbage juice. This is in support of the work of Giraffa et al., [46] who work on the Applications of probiotic bacteria to the Pickle and other vegetable products.

Storage stability of the fermented cabbage juice produced showed that probiotic *Pediococcus pentosaceus* was viable during 4 weeks of storage at 4o C. The viability ranged from 3.1x 107 -9.8 x 107 CFU/ml and the highest viability was recorded at 1st week of storage (9.8 x 107CFU/ml). High cell viability recorded in this work may be as a result of modification in cytoplasmic membrane thereby regulating membrane fluidity at cold temperature and oxygen metabolized. This support the findings of Yoon et al. [41] , that viability of probiotic organisms is dependent on the level of oxygen in products, oxygen permeation of the package, fermentation time, and storage temperature. The viable cell counts of the *Pediococcus pentosaceus* in the cabbage juice was higher than 106 CFU/ml after 4 weeks of cold storage at 4°C. However, it is important to have a significant number of viable LAB in the product for maximum health benefits [47]

Conclusion

Conventionally the isolates were identified cultural, morphological, bicochemical,Physiological and confirmed by BD BBL panel identification kits as *Pediococcus pentosaceus Lactococcus plantarum, Streptococcous sanguinis and Enterococcus faecuim.*

The confirmed and identified Lactic acid bacteria were further screened for their probiotic crystal

properties. *Pediococcus pentosaceus* was able to tolerate pH 3.0 for 3h, also 0.3% bile concentration for 4h and has antimicrobial properties against potentially pathogenic bacteria of clinic isolates. *Staphylococcus aureus* (20.00±0.00 mm ZOI) followed by E coli (18.00±0.00 mm ZOI) and *Salmonella typhi* (15.00±1.4 mm ZOI) Probiotic cabbage juice was produced by *Pediococcus pentosaceus* after 72 hrs of fermentation with pH (4.6), total titratable acid (0.6 g/dm3), reducing sugar (2.3mg/ml), viable cells (1.14x109 CFU/ml) after 72hrs of fermentation. Also, the proximate composition of the fermented cabbage juice contains appreciably good sources of nutrients as revealed by the results of the proximate analysis when compared to the unfermented cabbage juice. For fermented cabbage juice; (94.69% moisture content, 1.06 crude protein, 0.37 crude fiber 1.44% fat, 0.15% ash content,

2.29% carbohydrate content). *Pediococcus pentosaceus* was stable in the cabbage juice during 4 weeks of cold storage at 40C. and was still viable (3.1x107 CFU/ml) during storage.

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