EVALUATING THE VIABILITY OF *Lactobacillus species* IN WATERMELON JUICE

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.
IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY

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CERTIFICATION

I hereby certify that this work was carried out by Efosa Peace LAWRENCE with matriculation number LSC1705574 at the Department of Microbiology, Faculty of Life Sciences, University of Benin City, under my supervision

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_____ _____

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DEDICATION

I hereby dedicate this project to God Almighty my creator, my father, my source of inspiration, wisdom, knowledge and understanding.

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ABSTRACT

Watermelon fruit is a suitable medium for the production of probiotic juice due to its nutritional content, high amounts of sugar and the lack of competing starter. However, its utilisation as a probiotic drink is under-utilized. This research was carried out to evaluate the viability of three *Lactobacillus* species in watermelon juice. Watermelon juice was freshly prepared, sterilized and inoculated under aseptic condition. Incubation was carried out at 4 ^{o}C and 28±2^{o}C and uninoculated juice served as controls at both temperatures. Samples of juice were obtained every 24 hr for 7 days and tested for parameters which include pH, titratable acidity (%), average titre value (cm^3) and viable cell counts (cfu/ml). Sensory analysis of the probioticated juice was also carried out using the hedonic scale. The pH of the probioticated juice was measured using a pH meter, titration methods were used to determine titratable acidity and average titre value while the pour plate method was used to determine viable cell count. A significant reduction in pH was observed in samples stored at 28±2 ^{o}C with LB1 having the highest reduction in pH (5.20±0.06 – 3.60±0.06) and LB3 having a slight reduction (5.20±0.12 – 3.70±0.12). There was rapid increase in average titre value at 28±2^{o}C with LB1 having the highest increase (5.00 - 15.16) and LB3 having the lowest average titre value (5.00 – 14.72 cm^3). There was only a slight increase of titre values in samples stored at 4^{o}C. This led to a corresponding increase in titratable acidity for both *Lactobacillus* species; LB1 (0.32 – 0.99%) and LB3 (0.32 – 0.94%) at 28±2 ^{o}C. However, at 4 ^{o}C , a slight increase was observed for the three *Lactobacillus* species. At 28±2 ^{o}C, there was an observable increase in viable cell counts with LB1 having the highest mean count (0.03 x 10^8 – 168 x 10^8) and LB2 having the lowest mean count (0.03 x 10^8 – 123 x 10^8 cfu/ml). Samples stored at 4 ^{o}C had little or insignificant increase during production. Samples stored at 4 ^{o}C had a better acceptability in terms of taste, colour and appearance compared to those stored at 28±2 ^{o}C with LB1 having the poorest acceptability. There are several challenges to overcome in the probiotication of watermelon juice which include the survival of probiotics, storage temperature, and their effects on sensory attributes. Longer shelf-life study should be investigated in the future to evaluate the acceptability of this juice.

CHAPTER ONE

INTRODUCTION

Foods have many roles such as satisfying hunger, providing necessary nutrients, improving health, promoting a state of physical and mental well-being as well as preventing or reducing nutrition-related diseases. Moreover, consumers' awareness towards the association between food and health has flare up interest in "healthy foods" in recent years (Shah and Prajapati 2013). In addition to the traditional nutritional effects, "functional foods" exert beneficial health effects on body. Well-recognized examples of functional foods are those containing bioactive compounds like dietary fibers, oligosaccharides, vitamins, minerals and active "friendly" bacteria, called probiotics that promote the equilibrium of intestinal microflora (Jankovic *et al*., 2010; Shah and Prajapati 2013).

At present, there is growing consumer interest on the benefits of foods with functional properties, as these foods have improved nutritional value, promoting better health and disease risk reduction. At this time, the food industry is promoting functional juices rich in vitamins, minerals, and antioxidants to help maintain good health. In recent years, the great interest for viable microorganisms that promote or support a beneficial balance of autochthonous microbial population of gastrointestinal tract. Probiotics may be consumed in different forms, comprising foods, mainly in fermented state and pharmaceutical products, mainly as capsules or in microencapsulated forms (Menshutina *et al*., 2010). A particular feature of probiotic cultures is that they regulate the balance of the gut bacterial population, presumably by competition for epithelium contact sites and nutrients and also by modulation of pH value. They may also induce synthesis of vitamins such as riboflavin. In addition, probiotic cultures are also suggested to stimulate the immune system (Koc *et al*., 2010).

According to Tripathi and Giri (2014), intrinsic food parameters like titratable acidity, pH, molecular oxygen, water activity, presence of salt, sugar, artificial flavoring and coloring agents, and chemical or microbial preservatives like hydrogen peroxide and bacteriocins; processing parameters like extent of heat treatment, incubation temperature, cooling rate, volume, and packaging mat are the main factors that could limit probiotic viability and survival in juices.

The usage of milk-based products may be restricted by lactose intolerance, allergies, dyslipidemia, and vegetarianism. Fermented milk products have traditionally been thought of as the best carriers for probiotics. As a result, a number of raw materials have recently undergone intensive research to see if they might serve as suitable substrates for the production of novel non-dairy functional foods. Fruit and vegetable juices have essentially been described as a novel acceptable carrier medium for probiotics; beverages based on fruits, cereals, vegetables, and soybeans have been presented as new products containing probiotic strains (Tripathi and Giri, 2014). Fruit and vegetable juices are kept and stored through fermentation, which also enhances the goods' nutritional and sensory qualities. The suitability of fruit and vegetable juices as a medium for creating new probiotic beverages from fruits like barberry and black cherry, noni, guava, pineapple, pear, grape, pomegranate, and carrot, as well as vegetables like beet, sweet potato, and potato has been the subject of numerous studies (Khezri *et al*., 2016). (Dipjyoti *et al*., 2015). However, compared to dairy products, the survival of probiotics in fruit and vegetable-based matrices is more difficult. The acidic environments in these medium require protection for the probiotic microorganisms. The most commonly employed probiotics includes different strains from *Lactobacillus acidophilus*, *Lb. helveticus, Lb. casei, Lb. paracasei, Lb. johnsonii, Lb. plantarum, Lb. gasseri, Lb. reuteri, Lb. delbrueckii* subsp. *bulgaricus, Lb. crispatus, Lb. fermentum, Lb. rhamnosus, B. bifidum, B. longum, B. adolescentis, B. infantis, B. breve, B. lactis, B. laterosporus,* and other species like

Escherichia coli Nissle, *Streptococcus thermophilus, Weissella* spp., *Propionibacterium* spp., *Pediococcus* spp., *Enterococcus faecium, Leuconostoc* spp. and *Saccharomyces cerevisiae* var. *boulardii* (Nagpal *et al*., 2012).

1.1 Aim and Objectives

The aim of this study was to evaluate the viability of *Lactobacillus* species in watermelon juice.

The specific were to;

i. fortify and ferment watermelon juice with *Lactobacillus* species previously isolated from yoghurt.
ii. determine the pH, average titre, and titratable acidity and viable cell count of the probioticated watermelon juice.
iii. evaluate the sensory characteristics of the probioticated watermelon juice.

CHAPTER TWO

LITERATURE REVIEW

Fruits are concentrated source of natural components. These natural components are plant derived materials performing a key role in maintaining human health, especially in disease

prevention, growth and development. In the recent era, phyto-nutrients, particularly from fruits and vegetables, are becoming popular due to consumer awareness regarding their health enhancing potential (Naz *et al*., 2013). Plants and plant-based compounds are the basis of many of the modern pharmaceuticals used today for the treatment of various dreadful diseases.

Watermelon (*Citrullus lanatus*) is one of the world's most important vegetables, grown both for its fruit and the vegetative parts which are highly nutritious. It belongs to the cucurbit family commonly known as Cucurbitaceae; it is an important horticultural crop, often known for its sweet and juicy fruit, grown commercially in areas with long frost-free warm climates all over the world (Prohens and Nuez, 2008). Watermelon global consumption is greater than that of any other cucurbit (Paris, 2016). China is the leading producer of watermelon in the world; it provides one third of the total watermelons in the world, followed by Iran, Turkey, Brazil, Egypt, United States, Uzekistan, Algeria, Russia and Vietnam completes the top ten producers in the world (FAOSTAT, 2022).

2.1 Physical Characteristics of Watermelon

It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and yellow flowers. It is grown for its edible fruit, which is a special kind of berry botanically called a pepo. The watermelon fruit has deep green smooth thick exterior rind with grey or light green vertical stripes. Inside the fruit is red in colour with small black seeds embedded in the middle third of the flesh (Dia *et al*., 2016). Watermelons range in shape from round to oblong. Rind colours can be light to dark green, with or without stripes. Flesh colours can be dark red, red or yellow. India grows approximately 25 commercial varieties, a few of which have delightfully interesting names: New Hampshire Midget, Madhuri 64, Black Magic, Sugar

Baby, Asahi Yamato, Arka Jyoti, Arka Manik, Improved Shipper, Durgapura Meetha and Durgapura Kesar to name a few. Watermelon varieties fall into three broad classes based on how the seed was developed: open-pollinated, F1-hybrid and triploid or seedless (Kumar *et al*., 2013).

2.2 Nutritional Value of Fresh Watermelon

Watermelon is one of the commonly consumed fruits in many countries. Watermelon contains more than 91% water and up to 7% of carbohydrates. It is a rich source of lycopene and citrulline. Watermelon rind contains more amounts of citrulline than the flesh. Additionally,
watermelon has a number of essential micronutrients and vitamins (Reetu and Maharishi, 2017).

2.3 Health Benefits of Watermelon

2.3.1 Heart health

Watermelon contains high levels of lycopene that is very effective in protecting cells from damage and lowering the risk of heart disease. Watermelon extracts help to reduce hypertension and lower blood pressure in obese adults. Watermelon fruit is also a good source of potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure. Thus, it prevents against stroke and coronary heart diseases (Dia *et al*., 2016).

2.3.2 Anti-inflammatory and antioxidant support

Anti-inflammatory foods can help with overall immunity and general health. The lycopene in watermelon makes it an anti-inflammatory fruit. Lycopene is an inhibitor for various

inflammatory processes and also works as an antioxidant to neutralize free radicals (Edwards *et al*., 2003). It also contains a good amount of vitamin-B6 (pyridoxine), vitamin–C and manganese. Consumption of food rich in vitamin–C helps the body develop resistance against infectious agents and scavenge harmful oxygen-free radicals. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase. Watermelon is an excellent source of Vitamin A, which is a powerful natural antioxidant. It is one of the essential vitamins for vision and immunity.

2.3.3 Hydration and digestion

Watermelons are the perfect example of a food that can help you stay hydrated. Watermelons are nature gift to beat summer thirst due to rich in electrolytes and water content. The watermelon contains fibre, which encourages a healthy digestive tract and helps keep you regular (Reetu and Maharishi, 2017).

2.3.4 Skin and hair benefits

Vitamin A helps keep skin and hair moisturized and it also encourages healthy growth of new collagen and elastin cells. Vitamin C is also beneficial in this regard, as it promotes healthy collagen growth (Reetu and Maharishi, 2017).

2.3.5 Cancer prevention

Like other fruits and vegetables, watermelons may be helpful in reducing the risk of cancer through their antioxidant properties. According to the National Cancer Institute, lycopene helps in reducing prostate cancer cell proliferation. Consumption of natural fruits rich in vitamin-A is known to protect from lung and oral cavity cancers (Reetu and Maharishi, 2017).

Most people throw away the watermelon rind and seeds. Rind not only contains plenty of health promoting and blood-building chlorophyll, but the rind actually contains important amino acid citrulline than the flesh. Citrulline is a non-protein amino acid and was first identified from watermelon. Citrulline is used in the nitric oxide system in humans and has antioxidant and vasodilatation roles (Kumar *et al*., 2013). According to Reetu and Maharishi (2017), citrulline improves circulation by reducing muscle soreness and heart rate. Many people prefer seedless watermelon varieties, but black watermelon seeds are quite healthy and edible. They contain iron, zinc, protein, and fibre.

2.4 Watermelon Juice

Watermelon juice is commonly consumed in Mexico and can be found in many American bars as a mixer for alcoholic beverages. Due to its low acidity and growing conditions, watermelon is regarded as a potentially hazardous food (Oladele, 2015). In the fruit juice industry, juice is typically pasteurized by high temperature short time (HTST) pasteurization. This process uses plate heat exchangers to heat the sample quickly at least 78°C. Although this method is effective at inactivating microorganisms and enzymes, it can cause detrimental effects on the quality of the juice. Heat treatment may cause color change, separation of particles, and a change in flavor and/or smell (Isibor and Ugwumba, 2014). If heat treatment is not performed rapidly or at a reasonably low temperature, the juice will begin to separate due to the destruction of pectin (Kainga, 2013). Compared with thermal pasteurization, non-thermal processing offers the advantages of low process temperatures which results in a better retention of flavors and nutrients. Color and appearance are the first characteristic a consumer sees while shopping in a grocery store. Color may have an influence on a consumer's purchase intent. Fruit juices are generally packaged in clear plastic bottles with

large labels so consumers can see the juice's color. Thermal pasteurization can cause pigments to degrade which may the color and appearance of fruit juice (Ajewole, 2015).

2.5 Preservation of Watermelon Juice

Watermelon pulp contains from 1.1 to 4.7 g of L-citrulline kg^{-1} of fresh weight (Tarazona-Dıaz *et al*., 2011). These variations could be due to preharvest and postharvest factors, which can affect the final concentration in juices. Therefore, watermelon juice should undergo an industrial process to guarantee its quality and safety during the shelf life of the product. One effective way of limiting microbial growth is to increase the juice's acidity by adding an acidifier in addition to other treatments such as high hydrostatic pressure pasteurization or conventional thermal treatments. Thermal treatments are still used by most of the fruit juice processing industries due to their simplicity and efficiency. Heat treatments are conventionally used to inactivate enzymes, ensure safety, and extend the shelf life of juices, but undesirable changes such as loss of bioactive compounds and sensory quality reduction are often induced (Tarazona-Díaz, *et al*., 2017). Reducing peroxidase (POD) activity in watermelon juice is important for avoiding color deterioration, off-flavor formation, and loss of nutrients (Aguayo *et al*., 2017). On the other hand, decreasing the pectin methyl esterase (PME) and polygalacturonase (PG) activities could limit the degradation of pectins, decreasing the losses in viscosity and cloud stability (Liu *et al*., 2012). Previously, Tarazona-D´ıaz and Aguayo (2013) described that centrifugation and pasteurization (87.7ºC for 20 sec) of an acidified watermelon juice significantly reduced the red color, bioactive compounds (lycopene, antioxidant capacity, and total polyphenols), and sensory quality of the juice.

2.6 Probiotics in fruit and vegetable juices

As defined by FAO/WHO (2002), probiotics are live microorganisms (mainly bacteria and few yeast strains) that confer a beneficial health effect on the host if administered in appropriate amounts. 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. Beverages based on fruits, cereals, vegetables and soybeans have been proposed as new products containing probiotic strains; essentially, fruit and vegetable juices have been reported as a novel suitable carrier medium for probiotics (Tripathi and Giri, 2014).

Naturally, fruits and vegetables are rich in carbohydrates, dietary fibers, vitamins minerals, polyphenols and phytochemicals; referred as healthy foods (Sutton, 2007). Numerous researchers reported on the beneficial health effects of juices; for example, aqueous extracts of kiwifruit and avocado had very less cytotoxicity plus high anti-inflammatory activity in a Crohn's gene- specific assay (Pakbin, 2014). Similarly non-aqueous extracts of kiwifruit, avocado and blueberry had high anti-inflammatory activity, with slightly higher cytotoxicity than the aqueous extracts. Fenech *et al.* (2015) demonstrated the positive effect of the intake of nine micronutrients that can be easily found in fruits viz. calcium, retinol, vitamin E, folate, nicotin acid, riboflavin, pantothenic acid, β-carotene and biotin on genome damage and repair. Therefore, juice fortification with probiotics and/or prebiotics is a challenge and a frontier goal, as juices could combine nutritional effects in addition provide specific health benefit through added probiotic strain. Furthermore, fruit juices have shown negative effects on some pathogenic microorganisms, conversely improves the growth of beneficial bacteria. The

berries, such as blueberry, blackberry and raspberry, possess antimicrobial effects towards many foodborne pathogens (Ranadheera *et al*., 2014).

While looking for different food matrices, many researchers have investigated the suitability of various fruit and vegetable juices, such as tomato, mango, orange, apple, grape, peach, pomegranate, watermelon, carrot, beet root and cabbage juices as raw material for the production of probiotic juices or related beverages. The most commonly employed probiotics includes different strains from *Lactobacillus acidophilus*, *Lb. helveticus, Lb. casei, Lb. paracasei, Lb. johnsonii, Lb. plantarum, Lb. gasseri, Lb. reuteri, Lb. delbrueckii* subsp. *bulgaricus, Lb. crispatus, Lb. fermentum, Lb. rhamnosus, B. bifidum, B. longum, B. adolescentis, B. infantis, B. breve, B. lactis, B. laterosporus,* and other species like *Escherichia coli* Nissle, *Streptococcus thermophilus, Weissella* spp., *Propionibacterium* spp., *Pediococcus* spp., *Enterococcus faecium, Leuconostoc* spp. and *Saccharomyces cerevisiae* var. *boulardii* (Nagpal *et al*., 2012).

2.7 Major factors affecting probiotic survival in juices

The health benefit of probiotics mainly relies upon their concentration in foods plus on their ability to endure the unfavorable conditions of the gastrointestinal tract. Maintaining the viability (at least 10^6-10^7 cells/ml) and activity of probiotics in food products at the end of shelf-life are two important criteria to be fulfilled in fruit juices, too. The low pH of fruit juices is a shortcoming in favoring the total viable counts and activities of probiotics (Vasudha and Mishra, 2013). However, probiotic viability is strain-dependent, i.e. some strains of *Lb. plantarum, Lb. acidophilus* and *Lb. casei* can grow in fruit matrices due to their tolerance to acidic environments (Peres *et al*., 2012).

Several factors could limit probiotic viability and survival in juices. As suggested by Tripathi and Giri (2014), the major influencing parameters can be categorized as,

(1) intrinsic food parameters, such as titratable acidity, pH, molecular oxygen, water activity, presence of salt, sugar, artificial flavoring and coloring agents, and chemical or microbial preservatives like hydrogen peroxide and bacteriocins;
(2) processing parameters- extent of heat treatment, incubation temperature, cooling rate, volume, packaging materials and storage techniques;
(3) microbiological factors which mainly include kind of probiotic strains, compatibility of different strains, inoculums proportion and rate.

Among all these, pH is one of the chief significant factor affecting the probiotic viability. Fruit juices naturally have a low pH and high level of organic acids, which increases the concentration of undissociated form. It is presumed that combined action of acidic environment and the intrinsic antimicrobial activity of accumulated organic acids affect probiotic bacteria. Among various probiotics, lactobacilli are generally found to resist and survive in fruit juices with pH ranging from 4.3 to 3.7, while bifidobacteria are less acid tolerant; even about pH 4.6 is unfavorable for their survival (Tripathi and Giri, 2014). On the other hand, this trend differs with the kind of probiotic strain. For instance, strains of *lactobacillus* and *Bifidobacterium* revealed wide differences regarding acid resistance into orange, pineapple and cranberry juice, the strains screened survived for longer in orange and pineapple juice than cranberry (Sheehan *et al*., 2007).

Lactobacillus casei, Lb. rhamnosus, Lb. paracasei display a great robustness surviving at levels above 7.0 log cfu/ml and 6.0 log cfu/ml in orange and pineapple juice, respectively for at least 3 months. However, after thermal pasteurization at 76 ^{o}C for 30 sec and 90 ^{o}C for 1 min, an additional 5 min high-pressure treatment (400 MPa) observed that these strains were not able to withstand the treatments required to achieve >6.0 log cfu/ml in juice (Sheehan *et al*., 2007).

Nualkaekul and Charalampopoulos (2011) investigated the factors that affected *B. longum* survival in model solutions and in fruit juices (orange, pineapple, grapefruit, blackcurrant, strawberry and pomegranate). The orange, pineapple, grapefruit and blackcurrant juices reduced (less than 0.8 log CFU/ mL) viability of bifidobacteria, with the highest cell count found in orange and pineapple juice while storage at 4°C after six week. Further, the decrease in grapefruit was only 0.5 log CFU/mL, despite of the low pH (3.21) and the high concentration of citric acid (15.3 g/L) suggesting some controversial effects of pH. The probiotic was below the detection limit after one week in pomegranate and four weeks in strawberry juice. These results are suggestive of the synergistic as well as antagonistic action of some parameters on the survival of bacteria. Fruits are naturally rich in phenolic compounds, which are strongly found to affect the viability of probiotic bacteria. Some food components like proteins and dietary fiber could protect cells from acidic stress at low pH. According to several researchers, the incorporation of LAB into fruit juices with low pH may boost the resistance of bacteria to subsequent stressful acidic conditions, such as those observed in gastrointestinal tract (Ranadheera *et al.*, 2014).

A major challenge during fortification of probiotics in fruit juices or beverages is the product acceptance by consumers (Ellendersen *et al.*, 2012). The kind of microorganism and juices type, storage conditions, and addition of other compounds may influence on the sensory traits of finished product. The addition of pleasant aroma and volatile ingredients may be able to "mask" the presence of probiotics. Fermented juices with sugar had more acceptable taste and flavor than the sugar free juice; further, when sucrose was added at the beginning of fermentation, flavors seemed to be reduced and the taste was more acceptable (Sivudu *et al*., 2014). Luckow *et al.* (2006) mentioned that the addition of tropical fruit juices, mainly pineapple, but also mango or passion fruit (10% v/v), might optimistically contribute to the

aroma and flavor of the final product and might avoid the identification of probiotic off-flavors by consumers.

2.8 Fortification with prebiotics

The most attractive and straightforward way to improve probiotic stability in fruit juice could be the fortification with some prebiotics such as dietary fiber, cellulose or with some ingredients able to exert a protective effect within the fruit juice. In connection to this, Rakin *et al.* (2007) enriched beetroot juice and carrot juice with brewer's yeast autolysate before fermentation with *Lb. acidophilus*. It was noticed that autolysate enhanced the growth of *Lb. acidophilus* during the fermentation, decreased fermentation time, enriched the juices with minerals, vitamins, amino-acids, and antioxidants as well as positively influenced probiotics survival. Another group of researchers fortified juices with glucans and demonstrated that in apple juice, oat flour with 20% of β-glucan could protect *Lb. rhamnosus* during refrigerated storage (Sohail *et al*., 2012).

2.8 Storage under refrigeration, use of antioxidants and microencapsulation

The level of oxygen within the package foods during storage should be as low as possible in order to avoid oxidative damage to the probiotics, however the extent of sensitivity is strongly strain variable. Oxygen induces an oxidative damage by the creation of reactive oxygen species (ROS) like H_2O_2 or superoxide ion. Commonly, it is noticed that bifidobacteria are more sensitive than LAB (Nag and Das, 2013; Tamminen *et al*., 2013).

Several authors suggested the modification of product atmosphere by raising the content of carbon (IV) oxide (CO_2) in the headspace (Corbo *et al*., 2014). Additionally, antioxidant compounds could help to limit the harmful effects of oxygen. In this connection, a group of researchers evaluated the effects of different amounts of (+)-catechin, green tea

epigallocatechin gallate, and green tea extracts on the growth and survival of *B. longum* ATCC 15708, *B. longum* subsp. *infantis* ATCC 15697 and *Lb. helveticus* R0052, having different oxygen sensitivities (Gaudreau *et al*., 2013). They found that the growth of *Lb. helveticus* was strongly enhanced. Moreover, fortification of vitamin-E improved the stability of *Lb. casei* CRL 431 in the food matrix during 20 week storage period at 25°C.

The laboratory are highly sensitive to fluctuation in storage temperature. The viability of probiotic strains in fruit juices is also found to influence refrigeration, this could promise a longer survival, whereas a thermal abuse could demonstrate a harmful effect. Different authors proposed numerous strategies to resolve such issues. Microencapsulation technologies have also been successfully applied using various matrices to protect the probiotic bacterial cells from the damage caused by the external environmental factors. For instance, a novel microencapsulation method reduced the acidification and improved the viability of probiotic strains *Lb. rhamnosus* and *Lb. acidophilus* at 25°C for at least 9 days in orange juice (Sohail *et al*., 2012). In a recent investigation, *Lb. acidophilus* immobilized in Ca-alginate carried out normal banana puree fermentation and resulted in a novel probiotic fruit product. In tomato juice, *Lb. acidophilus* immobilized in Ca-alginate showed a higher survival rate than free cells during cold storage at 4°C. Further, the overall acceptance of immobilized cell fermentation was higher than free cells as noticed by the sensory evaluation during storage (King *et al*., 2007). Chaikham (2015) investigated the effect of alginate encapsulation with Thai herbal extracts including cashew flower, pennywort and yanang on the viability of probiotic *L. casei* 01b, *Lb. acidophilus* LA5 and *B. lactis* Bb-12 bacteria suspended in mulberry, maoberry, longan and melon juices. It was noticed that the survival rate of *L. casei* 01 cells entrapped with 0.05% (w/v) cashew flower extract were notably higher than those encapsulated with pennywort and yanang extracts, after 30 days storage.

On the other hand, Gaanappriya *et al*. (2013) evaluated the viability of encapsulated *Lb. plantarum* in sapodilla, grapes, orange and watermelon juices which successfully maintained the viable probiotic count at 7 log CFU/mL or more for one month. Ding and Shah (2008) emphasized that microencapsulated probiotic bacteria were more stable in compare to free probiotic cells in fruit juices. In principle, the encapsulated probiotics (*Lb. rhamnosus, Lb.acidophilus, Lb. paracasei, Lb. plantarum, Lb. salivarius, B. longum, B. lactis* type Bi-04 and Bi- 07) were protected from the acidic environment of the orange juice, did not allow a strong viability loss and showed a residual cell count of 5 log CFU/ mL even after 6 weeks. Some studies reported that microencapsulation might provide a more favorable anaerobic environment for susceptible probiotic strains, as well as a physical barrier from the harsh acidic conditions of the fruit juice (Ding and Shah, 2008).

Elvina and Wee (2018) investigated non-dairy probiotic drink was produced through the fermentation of watermelon juice using *Lactobacillus plantarum* ATCC 8014. Watermelon juice was supplemented with or without inulin or fructooligosaccharides (FOS) and fermented for 6 hr at 37°C. The effect of two weeks of refrigerated storage at 4°C on the physicochemical characteristics and viability of *L. plantarum* were measured. The viability of *L. plantarum* with or without inulin or FOS supplementation was maintained at about 11 log CFU/mL until week 2 of refrigerated storage. The pH of fermented watermelon juice continued to decrease, whereas lactic acid concentration continued to increase until week 1 of storage. The supplementation of inulin or FOS increased the total soluble solids of the juice. There was notable change of color in all fermented watermelon juices measured using colorimeter. The concentration of glucose, fructose and citric acid measured using High Performance Liquid Chromatography (HPLC) varied over the two weeks of storage.

Naga *et al.* (2014) evaluated the suitability of watermelon and tomato juice as a raw material for production of probiotic mixed juice by growing on *Lactobacillus fermentum* and

Lactobacillus casei. Experiments were conducted in 250 mL flasks, each containing 100 mL of mixed juice in equal proportions, sterilized for 15 min at 120ºC, inoculated separately with 24 h old broth cultures (~10^6cfu/mL), incubated at both 30°C and 37°C and analyzed for pH, acidity, sugar content and viable cell counts for a period of 72 hr. They grew better at 30°C for the first 24 h with an increase in cell number (>1.8 log cfu/mL). However, the viable cell counts from both the temperatures were not much different (0.7 log cfu/mL) after 72 hr. Both strains produced a similar amount of titratable acidity expressed as lactic acid. But the titratable acidity produced was about two times higher at 30°C than that produced at 37°C (0.7% vs. 0.4% lactic acid). After four weeks of cold storage at 4ºC*, L. fermentum* grown at lower temperature (30°C) and *L. casei* grown at higher temperature (37°C) survived better. The addition of sucrose at the beginning of fermentation increased the amount of titratable acidity by at least two times (>1.8% lactic acid) and causing the decrease in cell viability while it was stored at 4ºC for four weeks.

2.9 Hedonic rating test: Sensory analysis of foods/ beverages

Sensory quality of food products is of great importance to both the producer or processor and the consumer. Good quality products attract the consumer by satisfying his aesthetic and gustatory senses. Therefore, it is always the endeavor of the processor to produce the best quality product or produce a product having certain qualities accepted by the consumer in a product already available in the market (Lawless and Heymann, 2010).

There are different sensory test methods to suit specific purposes. One of the frequent requirements of the processor while developing a product is to find out the relative acceptability of his product compared to three or four market sample or to develop a product

close to the best in the market. One of the simplest sensory test methods to generate such information is the Hedonic rating test (Avsar *et al*., 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection and Preparation

Watermelons were purchased from a local vegetable market in Uselu, Benin City and kept at 4ºC prior to use. The watermelons were thoroughly washed with distilled water to remove dirt, dust and then rinsed with water. The watermelons were cut into quarters and the flesh was scooped out and cut into small cubes. The cubes were placed on a sterile laboratory scale and weighed 5 kg, 500 ml of distilled water juice processor and filtered through a muslin cloth with a sieve (0.8 to 1.1 mm pore size) to separate watermelon juice and cake containing seed a total volume of 4 L of watermelon juice was produced.

3.2 Sterilization of materials

The watermelon fruits were washed with pure ethanol to remove micro flora on the surface of the fruit prior to juice extraction. All glassware and knives were sterilized at 160°C for 1 hr in an oven, all other equipment was sanitized with hypochlorite prior to usage.

3.3 Preparation of innoculum

McFarland standard was mixed on a vortex mixture prior to examination and the McFarland standard was aliquoted into a tube that was the same size and diameter as the tube used to prepare the test suspension. The test suspension was prepared by obtaining a fresh pure culture of the test organisms (*Lactobacillus* species) and inoculating a suitable broth in the presence of good lighting, the turbidity of the test suspension was visually compared with that of the McFarland standard by comparing the clarity of the lines of the Wickerham card. Organisms were inoculated until turbidity matches that of the standard (McFarland, 1907).

3.4 Media Preparation

Lactobacillus species that were previously isolated from yoghurt samples which were maintained in MRS broth at 4 ºC before usage. This served as the starter culture for the inoculation of the watermelon juice.

3.5 Pasteurization of watermelon juice

The extracted juice was then filtered and placed in sterile screw-top glass bottles. The filtered watermelon juice was pasteurized in a covered water bath with high temperature short time (72 ºC for 15 sec).

3.6 Probiotication of watermelon juice

Erlenmeyer flasks (1000 ml) each containing 500 ml of pasteurized watermelon juice was inoculated with 1 ml culture of lactobacilli (>10^5) CFU/ ml and was further incubated at 37º C for 24 hr. Enumeration of the cells was done using pour plate method from the stock solution. Plates were incubated at 37º C in a 5% CO_2 atmosphere for 24 hr (Yoon *et al*., 2006).

3.7 Physicochemical and microbiological analyses

3.7.1 pH Determination

The pH of probioticated mixed watermelon juice was measured with a Benchtop pH meter (WTW series, Inolab, Germany).

3.7.2 Titratable acidity

The titratable acidity was determined as described by Friedrich (2001) using colorimetric acidity titration as follows: Equal parts of deionized distilled water (ddH_2O) were added to the solid samples and macerated in a blenders at 100rpm for 2min. before centrifuging for

5min, at 2500rpm at room temperature. To 10ml of the supernatant sample solution in a clean Erlenmeyer flask were added 5 drops of 1% phenolphthalein indicator solution and a magnetic stir bar before stirring on a magnetic stir plate. Then 0.1N NaOH was carefully titrated against the sample solution to the end point of pH 8.2 until a faint but definite pink colour, which was stable for 5 to 10 sec was obtained. The titratable acidity was calculated using the equation:

% acid =

$$\frac{[\text{mls NaOH used}]\text{x } [0.1 \text{ N NaOH}]\text{x } [\text{milliequivalent factor}]\text{x}[100]}{grams\ of\ sample}$$

Where V is volume (ml) of NaOH solution used for

titration

N = Normality of NaOH solution, meq.

wt is milliequivalent weights of acid: lactic acid (0.090)

Vs= sample volume

The analysis was performed in triplicate to find the mean

Titratable acidity of each sample.

Total acidity, expressed as percent lactic acid and was determined by titrating the mixed juice samples with 0.1 N NaOH to pH 8.2, using phenolphthalein as indicator.

3.7.3 Determination of Viable Cell Counts

Viable cell counts were obtained by serial dilution with saline until 10^{-8} dilution was reached. Aliquots of 0.1 ml of dilution were plated in triplicates on Petri-dishes containing MRS agar.

The Petri-dishes were incubated for 24 hr at 37˚C. Plates containing 20 - 350 colonies were measured and recorded as colony forming units (CFU/mL). The appearances on agar as irregular light brown colonies ranging in diameter from 0.9 to 1.5 and characterizes such as small circular, rough, dull white and convex colonies (Okoro *et al*., 2017).

3.8 Sensory Analysis

The organoleptic properties of the probioticated juice was evaluated by a panel of 10 persons, the taste, colour, odour, and appearance or texture was evaluated by the panel. Samples were scored based on a 9-point hedonic scale with corresponding descriptive terms ranging from 9(like extremely) to 1(dislike extremely).

3.9 Statistical analysis

All experiments were conducted in triplicate and the results are expressed as mean. Data from Hedonic scale was analyzed using descriptive statistics namely percentage and mean value.

CHAPTER FOUR

4.1 RESULTS

The probiotication of watermelon juice with *Lactobacillus* species was conducted in 7 day period. The inoculated watermelon juices were stored at 28±2^{o}C and 4^{o}C, with uninoculated juice serving as controls in both temperatures. Physicochemical and microbiological analyses such average titration value, pH, titratable acidity and the viable cell count were determined

at 24 hr interval. Hedonic scale chart was designed and distributed to respondents in order to determine the colour, smell and taste of the probioticated juice.

Table 1 shows the average titre values for LB1 at 28±2°C temperature ranged from 5.00 - 15.16cm^3 from day 0 to 7 compared to control which ranged from 4.90 - 5.00 cm^3. At 4°C, the average titre values ranged from 5.00 - 10.28 cm^3 which was higher than the control group. At 28±2°C, the average titre values for LB2 ranged from 5.00 - 15.19 cm^3 and at 4°C, values ranged from 5.00 to 10.12 cm^3 which was higher than the control (4.90 - 5.00 cm^3). For LB3 samples, the average titre value ranged from 5.00 – 14.72 cm^3 at 28±2°C, while values for samples stored at 4°C samples ranged from 5.00 – 9.85 cm^3 which was higher than the control (5.00 – 4.90 cm^3).

Table 2 shows the results for pH changes from day 0 to day 7. At 28±2°C, the pH values of LB1 samples ranged from 5.20±0.06 – 3.60±0.06 and at 4°C, pH values ranged from 5.20±0.12 – 4.20±0.17 which was higher than the 28±2°C samples and the control noticed a slight reduction of pH (5.20±0.06 – 5.00±0.11). For LB2 samples stored at 28±2°C, pH values ranged from 5.20±0.06 – 3.60±0.12 and at 4°C, pH ranged from 5.20±0.06 – 4.30±0.12. For LB3 samples stored at 28±2°C, pH values ranged from 5.20±0.12 – 3.70±0.12 and at 4°C, pH values ranged from 5.20±0.12 – 4.40±0.12 and the control had a pH values ranging from 5.20±0.06 – 5.00±0.12.

Table 3 shows results for the percentage of titratable acidity (%) from day 0 to day 7. At 28±2°C, the percentage of titratable acidity ranged from 0.45% - 1.36% while at 4°C, values ranged from 0.45% – 0.93% and control; 0.45% – 0.44% for LB1 samples. LB2 samples stored at 28±2°C had the highest titratable acidity (0.45% - 1.37%) and at 4°C, values ranged from 0.45% - 0.91% while control samples had reduction of titratable acidity (0.45% - 0.44%. For LB3 samples stored at 28±2°C,a high percentage of titratable acidity was observed

(0.45% - 1.32%) and at 4°C, values ranged from 0.45% - 0.89% while control samples had reduction in titratable acidity (0.45% - 0.44%).

Table 4 shows the viable cell counts of probioticated watermelon juice for day 0 to day 7. For LB1 samples stored at 4°C, viable cell counts ranged from 0.03 x 10^8 – 18.80 x 10^8 cfu/ml which was less than those stored at 28±2°C (0.03 x 10^8 - 168 x 10^8cfu/ml) and in control samples, no viable cell count was observed. The viable cell count increased in the LB2 samples stored at 4°C (0.03 x 10^8 – 14.30 x 10^8) more than the samples stored at 28±2°C (0.03 x 10^8 - 123 x 10^8) and no viable cell count was observed in control samples. LB3 samples stored at 4°C (0.03 x 10^8 – 12.50x 10^8) had more viable cell count than the samples stored at 28±2°C(0 .03 x 10^8 - 163 x 10^8).

Table 5 shows the sensory evaluation of the fermented watermelon juices. The colour for samples LB1, LB2 and LB3 remained consistent throughout the evaluation. For LB1 samples stored at 4°C, the smell and taste ranged from 9 – 6 which was higher than those stored at 28±2°C (8 - 2). For LB2, the smell and taste for the samples stored at 4°C ranged from 8 – 6 but significantly higher than those stored at 28 ±2°C (8 - 2). At 28 ±2°C, the LB3 samples ranged from 8 – 3 but there was considerable increase at 4°C (9 – 6). The control at 4°C maintained its sensory properties from start to end of the evaluation while the control at 28 ±2°C ranged from 8 – 6.

Table 1: Average titre values for probioticated watermelon juice

		AVERAGE TITRE VALUE (cm^3)							
Organisms	**Temperature**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
LB 1	Control	5.00	5.00	5.00	4.90	4.90	4.90	4.90	4.90
	28±2°C	5.00	11.20	13.20	13.31	13.85	14.65	15.10	15.16
	4 °C	5.00	5.40	5.41	8.26	9.20	9.65	10.25	10.28
LB 2	Control	5.00	5.00	5.00	4.90	4.90	4.90	4.90	4.90
	28±2°C	5.00	12.90	13.50	13.50	13.50	14.50	14.55	15.19
	4 °C	5.00	5.36	5.39	8.19	8.25	9.42	9.79	10.12
LB 3	Control	5.00	5.00	5.00	4.90	4.90	4.90	4.90	4.90
	28±2°C	5.00	12.55	12.46	13.42	13.55	14.10	14.61	14.72
	4 °C	5.00	5.38	5.45	8.15	8.30	9.22	9.53	9.85

Table 2: pH changes for probioticated watermelon juice

Organisms	Temperature	pH values							
		Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
LB 1	Control	5.20±0.06	5.20±0.06	5.20±0.06	5.00±0.11	5.00±0.11	5.00±0.11	5.00±0.11	5.00±0.11
	28±2°C	5.20±0.06	5.20±0.06	4.00±0.11	4.00±0.06	3.90±0.06	3.75±0.03	3.60±0.06	3.60±0.06
	4 °C	5.20±0.12	5.10±0.06	5.00±0.06	4.80±0.12	4.60±0.06	4.40±0.12	4.20±0.12	4.20±0.17
LB 2	Control	5.20±0.06	5.20±0.06	5.20±0.06	5.10±0.01	5.00±0.12	5.00±0.06	5.00±0.12	5.00±0.06
	28±2°C	5.20±0.06	4.10±0.12	4.00±0.06	4.00±0.17	3.90±0.12	3.80±0.12	3.70±0.12	3.60±0.12
	4 °C	5.20±0.06	5.10±0.06	5.00±0.02	4.90±0.02	4.80±0.12	4.60±0.12	4.40±0.17	4.30±0.12
LB 3	Control	5.20±0.06	5.20±0.06	5.10±0.17	5.00±0.12	5.00±0.12	5.00±0.12	5.00±0.12	5.00±0.12
	28±2°C	5.20±0.12	4.10±0.12	4.10±0.12	4.00±0.06	4.00±0.06	3.80±0.6	3.70±0.12	3.70±0.12
	4 °C	5.20±0.12	5.10±0.12	5.00±0.12	4.90±0.46	4.80±0.12	4.70±0.12	4.50±0.12	4.40±0.12

Mean±Standard deviation

Table 3: Titratable acidity percentage (%) values for probioticated watermelon juice

Organisms	Temperature	Percentage of titratable acid (%)							
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
LB 1	Control	0.45	0.45	0.45	0.44	0.44	0.44	0.44	0.44
	28±2°C	0.45	1.00	1.19	1.20	1.25	1.32	1.36	1.36
	4 °C	0.45	0.49	0.49	0.74	0.83	0.89	0.92	0.93
LB 2	Control	0.45	0.45	0.45	0.44	0.44	0.44	0.44	0.44
	28±2°C	0.45	1.16	1.22	1.22	1.22	1.31	1.31	1.37
	4 °C	0.45	0.48	0.49	0.74	0.74	0.85	0.88	0.91
LB 3	Control	0.45	0.45	0.44	0.44	0.44	0.44	0.44	0.44
	28±2°C	0.45	1.13	1.12	1.21	1.22	1.27	1.31	1.32
	4 °C	0.45	0.48	0.49	0.73	0.75	0.83	0.86	0.89

Table 4: Viable cell count (cfu/mL) for probioticated watermelon juice

Organisms	Temperature	Viable cell count (10^8cfu/ml)							
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
LB 1	Control	0	0	0	0	0	0	0	0
	28±2°C	0.03	9.40	16.50	21.50	140.00	197.00	173.00	168.00
	4 °C	0.03	0.68	1.21	1.52	1.94	2.31	11.50	18.80
LB 2	Control	0	0	0	0	0	0	0	0
	28±2°C	0.03	9.00	17.00	19.10	121.00	141.00	130.00	123.00
	4 °C	0.03	0.82	1.37	1.51	1.69	2.03	10.50	14.30
LB 3	Control	0	0	0	0	0	0	0	0
	28±2°C	0.03	8.70	16.3	17.7	111.00	138.00	171.00	163.00
	4 °C	0.03	0.93	1.45	1.50	1.81	1.97	9.80	12.50

Table 5: Sensory evaluation of watermelon juices

		Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7		
		Control	4 ºC	28±2 ºC	**Control**	4 ºC	28±2 ºC	Control	4 ºC	28±2 ºC	Control	4 ºC	28±2 ºC	Control	4 ºC	28±2 ºC	Control	4 ºC	28±2 ºC	Control	4 ºC	28±2 ºC	Control	4 ºC	28±2 ºC
LB1	Colour	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	Smell	7	7	7	6	7	6	6	7	5	6	6	5	5	6	4	5	6	4	5	6	3	5	6	2
	Taste	8	9	8	7	8	6	7	8	6	7	8	6	6	7	4	6	7	4	6	7	3	6	7	3
LB	Colour	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
2	Smell	7	7	7	7	6	6	7	6	5	6	6	5	5	6	4	5	6	4	5	6	3	5	6	2
	Taste	8	8	8	6	7	7	6	7	6	6	7	6	6	6	5	6	6	4	6	6	3	6	6	3
LB	Colour	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
3	Smell	7	7	7	6	7	6	6	7	5	6	6	5	5	6	4	5	6	4	5	6	3	5	6	2
	Tast	8	9	8	7	8	6	7	8	6	7	8	6	6	7	4	6	7	4	6	7	3	6	7	3

Key: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = Like slightly, 5 = Neither like or dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much 1 = Dislike extremely

CHAPTER FIVE

5.1 Discussion

The viability of *Lactobacillus* species in watermelon juice was evaluated in this study. The following physiochemical and microbiological analysis were carried out, pH, average titration, titratable acidity and viable cell count. *Lactobacillus* species used for inoculation of watermelon juice were previously isolated from yoghurt. The watermelon juices were stored at 28±2 ^{o}C and 4 ^{o}C and monitored with a control. Hedonic scale chart was designed and distributed to respondents in order to determine the colour, smell and taste. The probiotication of *Lactobacillus* species on watermelon juice was conducted for 7 days.

An increase in average titration values was observed. The average titration values from LB1, LB2 and LB3 at 28±2 ^{o}C was evidently higher than samples stored at 4 ^{o}C. This indicates active fermentation of fruit juice by *Lactobacillus* species which is in accordance to Elvina and Wee (2018) who investigated non-dairy probiotic drinks produced through the fermentation of watermelon juice using *Lactobacillus planatarum* ATCC 8014.

Also, watermelon experimental results for pH changes, the samples stored at 28±2 ^{o}C, a reduction of pH (5.20±0.06 - 3.60±0.06). This finding is similar to that of Tripathi and Giri (2014) during their study of probiotic functional foods. The pH of fermented watermelon juice continued to decrease. Among all major factors that could limit probiotic viability and survival in juices, pH is one of the chief significant factors affecting the probiotic viability. Fruit juices naturally have a low pH and high level of organic acids, which increases the concentration of undissociated form. It is presumed that the combined action of acidic environment and the intrinsic antimicrobial activity of accumulated organic acids affect probiotic bacteria. Among various probiotics, lactobacilli are generally found to resist and survive in fruit juices with pH ranging from 4.3 to 3.7 (Tripathi and Giri, 2014).

The viable cell count (cfu/mL for 0-7 days) indicated that difference in temperature(4 ºC and 28±2 ºC) plays an important role in the multiplication of the lactobacilli. Viable counts for all samples stored at 4 ºC were significantly lower than those stored at 28±2 ºC which indicated that the viable cell count of *Lactobacilli* do not thrive better in cooler temperature conditions. These findings are in line with Elvina and Wee (2018) who also stated that the effect of two weeks of refrigerated storage at 4°C on the physicochemical characteristics and viability of *L. plantarum* were measured. Naga *et al.* (2014) evaluated the suitability of watermelon and tomato juice as a raw material for production of probiotic mixed juice, they found out that *Lactobacillus fermentum* and *Lactobacillus casei* grew better at 30°C for the first 24 hr with an increase in cell number (>1.8 log cfu/mL). However, the viable cell counts from both the temperatures were not much different (0.7 log cfu/mL) after 72 hr.

The results of sensory analysis revealed that there was no colour change in the fermented watermelon juice. For the other sensory properties such as taste, odour and smell it was observed that the day 0-2, the feedback from the respondents were liked extremely and fairly positive for day 3-7 for the samples stored at 4ºC but for samples stored at 28±2ºC it was discovered that the day 0-2 were positive (liked,) day 3-5 were moderate and towards the end of the evaluation, the respondents gave negative (disliked) feedback. The control (28±2ºC) followed the same pattern as the sample stored at 4ºC but with little enthusiasm from the respondents, but the control stored at 4ºC remained the same throughout the evaluation with much enthusiasm from the respondents when they were sampling it. Changes in sensory properties were due to changes in pH, average titre values, titratable acidity and viable cell count. As fermentation progressed, sensory properties began to decline especially at 28±2ºC, hence the need for appropriate storage medium.

5.2 Conclusion

Probiotic watermelon juice can be successfully produced using *Lactobacillus* species. The viability of the probiotic was maintained even during 7 days of storage at 4^{o}C. The changes in pH, taste and smell of the probiotic watermelon juice were demonstrated during the 0 – 7 days of storage at both 4^{o}C and 28±2^{o}C. Longer shelf-life study of 4 – 6 weeks and sensory evaluation test to evaluate the acceptability of this drink should be investigated in the future.

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APPENDIX I

Nutritive value per 100 g of watermelon

Components	**Nutrient Value**	**Percentage of Recommended Daily Allowance**
Energy	30 Kcal	1.5%
Carbohydrates	7.6 g	6 %
Protein	0.6 g	1%
Total Fat	0.15 g	0.5%
Dietary Fiber	0.4 g	1%
Vitamins		Niacin
0.178 mg	1%	-
Pantothenic Acid	0.221 mg	4.5%
Vitamin A	569 mg	19%
Vitamin C	8.1 mg	13.5%
Electrolytes		Potassium
112 mg	2.5%	
Iron	0.24 mg	3 %
Manganese	0.038 mg	1.5 %
Zinc	0.10 mg	1%
Phyto-nutrients		-
Carotene-alpha	303 µg	-
Lycopene	4532 µg	-

Source: Reetu, I. and Maharishi, T. (2017)

APPENDIX 11

DEPARTMENT OF MICROBIOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENIN CITY

HEDONIC SCALE RATING FOR WATERMELON JUICE

Please, taste the sample juice and tick (∨) to indicate each of the characteristics using the number in the box to express your opinion on the juices. You can taste the juice sample more than once.

Instruction.

The number in the box explain the following:

9 = like extremely, 8 = like very much, 7 = like moderately, 6 = Like slightly, 5 = Neither like or dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much 1 = Dislike extremely

	9	8	7	6	5	4	3	2	1
COLOUR									
SMELL									
TASTE									

APPENDIX III

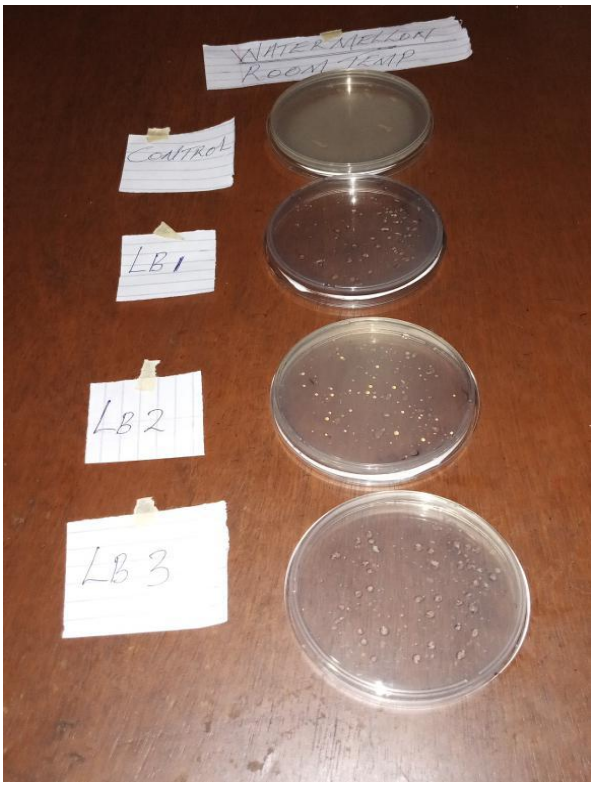

Plate 1: *Lactobacillus species* at 28±2 °C

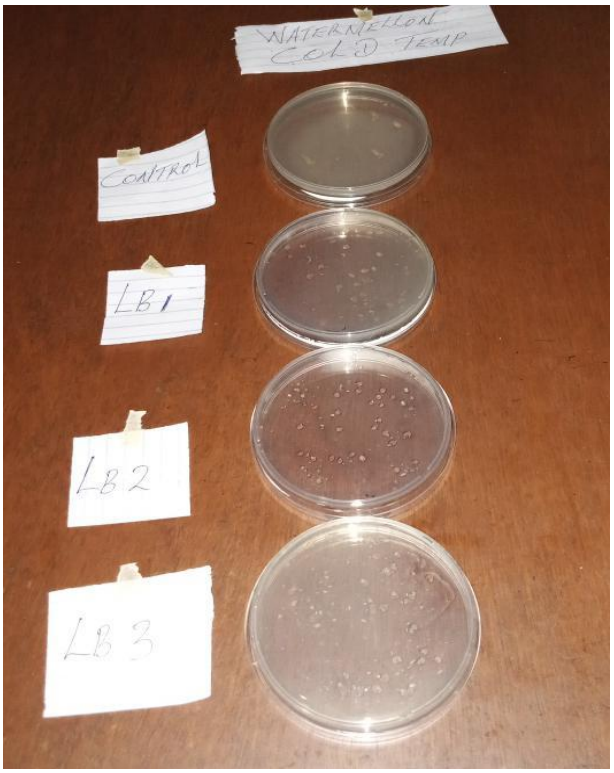

Plate 2: *Lactobacillus species* at 4 °C

Plate 3: Samples of the probioticated watermelon juice using *Lactobacillus species*

Plate 3: The researcher identifying colour change during titration