ASSESSMENT OF THE NUTRITIONAL QUALITY OF LACTIC ACID BACTERIA FERMENTATION OF TIGER NUT (C*YPERUS ESCULENTUS*) MILK FOR YOGHURT PRODUCTION

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A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREEOF MASTER OF SCIENCE (M.Sc.) IN MICROBIOLOGY OF THE OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA

2017

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POSTGRADUATE THESIS

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YEAR: 2017

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This is to certify that this research study was carried out by Omolola Bosede AKINYEMIas part of the requirements for the award of Master of Science (M.Sc.) degree in Microbiology of the Obafemi Awolowo University, Ile-Ife

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Dr. (Mrs) A. O. Oluduro

(Head of Department) **Date**

DEDICATION

My work is dedicated to my dependable and caring father Lawrence Oluyemi Akinyemi. I love and owe you alot. Thanks Dad.

ACKNOWLEDGEMENTS

I sincerely appreciate the Almighty God for His Grace and Mercies on my life throughout the period of this programme. He is the author of my life. I am grateful Lord.

My heartfelt gratitude goes to my supervisor, Dr. (Mrs.) S.M.Adeyemo, for the sistership and mentorship role played throughout the period of execution of this research work.

My sincere gratitude goes to the Head, Department of Microbiology, Dr. A.O. Oluduro for her astute and meticulous running of the department which has made the completion of this program easy.

I thank all lecturers in the Department of Microbiology; Prof. G.O. Babalola, Prof. D.A. Akinpelu, Prof. B.O. Omafuvbe, Dr. A.O. Shittu, Dr. N. Torimiro, Dr. K.O. Awojobi, Dr. J.O. Omololu-Aso, Dr.Mrs. M.O. Japhet, Mrs. C.D. Fashina, Mr. O.A. Adeshina, Mr. O. Adedeji, Mr. O.O. Olumide, Mr. T. Fadare, Mr. T. Abioye, Mr. O. Aregbesola and Mrs. O. Bamigbade for their help and support during the period of this study. Special gratitude to you Mrs. O.T. Awotipe and other technical and non-teaching staff of the Department for being there throughout the whole program, as well as all other technical staff members.

In addition, I am deeply indebted to my colleagues Miss Jumoke Omoolorun, Miss. Ayoola Adegbesan, Miss. Comfort Shotunde, and all Postgraduate students in the Department. Finally, to my special friends(Gbenga Ayodele, Hadassah Forum, DCA, Patience). I am very grateful.

Finally, I am most grateful to my wonderful parents. I sincerely acknowledge you, Mr. and Mrs. Akinyemi and my wonderful siblings Ifeoluwa, Damilola, Feyisola and Oluwanifemi for their support, prayers, love and affection throughout the period of this research.

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ABSTRACT

This study investigated the technological and bio-preservative properties of lactic acid bacteria (LAB) as starter culture in the fermentation of tiger nut milk for highly nutritive yoghurt production. It also evaluated the shelf life, sensory attributes and nutritional qualities of tiger nut yoghurt produced with LAB strains. These were with a view to obtaining a relatively less expensive yoghurt rich in dietary nutrients.

Tiger nuts and *Sorghum bicolor*samples were purchased at Sabo market, Ile-Ife, Osun State, Nigeria.*S. bicolor* grains were steeped in water for *og*i production for 5 days to isolate LAB and from yoghurt samples.The LAB were obtained and purified by successive subculturing on MRS agar and subjected to biochemical test. Gram positive and catalase negative isolates wereconfirmed as LAB. Tiger nuts were sorted, washed, milled and sieved to obtain the milk. The LAB strains with desirable properties were selected as potential starter for this study. The tiger nut milk extract was divided into 3 portions for fermentation. One was fermented with *Lactobacillus plantarum* while the second was fermented with *Lactobacillus fermentum and* the third fermented spontaneously. Physicochemical properties such as pH, titratable acidity, proximate and anti-nutritional factors were monitored in the raw tiger nut, tiger nut yoghurt using standard procedures. Sensory and organoleptic evaluation of the starter produced yoghurt were assessed by a panel of 50 consumers of yoghurt and the nutritional properties was compared with commercially sold yoghurt by proximate analysis.

A total of 47 LAB were isolated from the samples and identified as *L. plantarum,L. acidophilus, L.fermentum, L. casei, L. pentosus, L. cellobiosus, L. lactis,*and *S. thermophilus.* The technological properties showed *L. plantarum*from sorghum and *L. fermentum*from yoghurt as the best starter and they showed high exopolysaccharide production and positive bacteriocin production against test isolates (*S. aureus* ATCC 43300 and *E.coli* NCIB 86). Diacetyl, lactic acid and hydrogen peroxide production ranged from 0.42 - 0.53 g/L, 11.90 -18.1 g/L and 0.34 - 0.70 g/L respectively. There was a reduction in the anti-nutritional factors in the raw tiger and fermented yoghurt (phytate 65 - 05 mg/100g, protease inhibitor 0.3 - 0.00 mg/100g; tanins 35 - 05 mg/100g). The microbial load of LAB ranged from $3x10^6$- $3x10^8$ cfu/mL during fermentation while the enzymatic activities of the starters varies. The nutritional analysis of the raw and fermented tiger nut yoghurt showed an increase in the protein content 6.6 - 15.2 mg/g; ash content 3.7 - 8.3 mg/g; riboflavin 0.09 - 3.01 mg/g, niacin 3.5 - 6.5 mg/g and thiamine 0.19 - 4.19 mg/g. The organoleptic assessment showed the starter mediated yoghurt had prolonged shelf life when compared with the spontaneous fermented yoghurt.

This study concluded that *Lactobacillus plantarum* and *Lactobacillus fermentum* could be used asstarter culture to improve the nutritional composition and extend the shelf life of tiger nut yoghurt.

CHAPTER ONE

1.0 INTRODUCTION

Fermentation is a method that has been used for thousands of years to provide longer shelf life for perishable foods and to increase the flavour and odour of final food products. Fermented milks have long been used as the main vehicles for probiotic strains. They have been used for incorporation of probiotic microorganisms and may offer a number of advantages compared with naturally processed milks (Gomes *et al*., 2011; Minervini *et al*., 2012). In addition to improving gut health, probiotics may play a beneficial role in several medical conditions, including lactose intolerance, cancer, allergies, hepatic disease, *Helicobacter pylori* infections, urinary tract infections, hyperlipidemia and assimilation of cholesterol (Ejtahed *et al*., 2011).

Probiotic microorganisms that are known to be beneficial to human health can be ingested through fermented dairy products, enrichment of various foods with these bacteria and consumption of pharmaceutical products that are obtained by using viable cells (lyophilized preparations and tablets). Fermented dairy products are crucial to the human diet. Today, due to the increasing demand for safe and functional foods, consumption of new and enriched foods has shown growth to higher rates (Yerlikaya, 2014).

Different bacteria can tolerate different temperatures, which provide vast scope for a range of fermentations (Lee *et al*., 2002). While most bacteria have a temperature optimum of between 20 to 30 ºC, there are some (the thermophiles) which prefer higher temperatures (50 to 55 ºC) and those with colder temperature optima (15 to 20 ºC). Most lactic acid bacteria work best at temperatures of 18 to 22 ºC. The Leuconostoc species which initiate fermentation have an optimum of 18 to 22 ºC. Temperatures above 22 ºC, favour the Lactobacillus species (Mike and Sue, 2011).

Lactic acid bacteria tolerate high salt concentrations (Mike and Sue, 2011). The salt tolerance gives them an edge over other less tolerant species and allows the lactic acid fermenters to begin metabolism, which produces acid and further inhibits the growth of non-desirable organisms. Leuconostoc is noted for its high salt tolerance and for this reason, initiates the majority of lactic acid fermentations (Gomes *et al*., 2011).

In general, bacteria require a fairly high water activity (0.9 or higher) to survive. There are a few species which can tolerate water activities lower than this, but usually the yeasts and fungi will predominate on foods with a lower water activity (Mike and Sue, 2011).The optimum pH for most bacteria is near the neutral point (pH 7.0). Certain bacteria are acid tolerant and will survive at reduced pH levels. Notable acid-tolerant bacteria include the Lactobacillus and Streptococcus species, which play a role in the fermentation of dairy and vegetable products (Gomes *et al*., 2011).

Some of the fermentative bacteria are anaerobes; while others are aerobes require oxygen for their metabolic activities. Some, lactobacilli in particular, are microaerophilic. That is they grow in the presence of reduced amounts of atmospheric oxygen. In aerobic fermentations, the amount of oxygen present is one of the limiting factors. It determines the type and amount of biological product obtained the amount of substrate consumed and the energy released from the reaction (Lee *et al*., 2002; Mike and Sue, 2011).

All bacteria require a source of nutrients for metabolism (Adeyemo and Onilude 2014). The fermentative bacteria require carbohydrates either simple sugars such as glucose and fructose or complex carbohydrates such as starch or cellulose. The energy requirements of microorganisms are very high restricting the amount of substrate available and in turn affect their growth (Mike and Sue, 2011).Yoghurt is the most consumed healthy and nutritious food around the world Bhardwaj *et al.* (2008). Therefore, it offers an appropriate potential to convey nutritious ingredients to human diet. Research shows that most people in developing or underdeveloped countries suffer from micronutrient deficiency and enriched food products can dramatically reduce the nutritional diseases (Galvev *et al*., 2015).

The nutritional value of any material depends on its components. Because of the presence of precious compounds in milk, yoghurt is of great importance (Walstra *et al*., 2010). Traditionally, yoghurt is obtained from fermentation of cow milk and other dairy products. However, in developing countries such as Nigeria, dairy products are scarce and relatively expensive for low income earners. In addition, strict vegetarians and lactose intolerant people are limited to consuming animal based yogurt. It is of utmost importance therefore, to consider other non-dairy sources as alternative substrates for yogurt production. An inexpensive, readily available milk substitute extracted from locally available plant food such as tiger nut could play important role in alleviating protein malnutrition in the developing world.

Lactic acid bacteria have been used in fermented foods due to their beneficial influence on nutritional, organoleptic, shelf-life characteristics and also used in food preservation where LAB's can acidify the food resulting in inhibition of spoilage and pathogenic bacteria (Nishant *et al*., 2011). Some LAB display crucial antimicrobial properties with respect to food preservation, safety and also has the potential to combat gastrointestinal pathogenic bacteria such as *Escherichia coli* and *Salmonella* sp (Kumar *et al*., 2012).