A comparison of Probiotic and Standard yogurt based on branding (premium and basic brands), consumer preference, sensory evaluation, microbiological and nutritional analysis.

By

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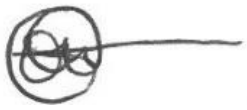
A thesis submitted in partial fulfilment for the requirements for the degree of MSc (by Research) at the University of Central Lancashire.

March 2016

STUDENT DECLARATION

I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution.

I declare that no material contained in the thesis has been used in any other submission for an academic award and is solely my own work.

Signature of Candidate: 

Type of Award: MSc (by Research).

School: The School of Sports and Wellbeing.

ABSTRACT

Yogurt is a type of dairy product and it is made by the addition of starter cultures like *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Specific cultures like *Lactobacillus acidophilus* and *Bifidobacterium* (probiotic cultures) are also sometimes added to it to make the yogurt probiotic.

The aim of this research is to compare probiotic and standard yogurts on the basis of branding (premium and basic brands), microbiological analysis, nutritional analysis, consumer preference, and sensory evaluation. This research is a novel one and very important in the field of dairy science because although some research has been done in comparing probiotic and standard yogurts e.g. (Hussain, Attiq-ur-Rahman, et al. 2009), there is no research that compares them on so many levels.

Four yogurt products were purchased from different supermarkets in Preston, UK. Microbiological analysis (such as total viable counts and gram stain), nutritional analysis (such as macro and micro nutrient compositions), consumer preference analysis (by questionnaire), and sensory analysis (by blind tasting) were conducted using the yogurts purchased. SPSS and Microsoft excel were used to analyze the results.

The non-probiotic yogurts had a higher total viable count than the probiotic ones and *Lactobacillus* and *Streptococcus* spp. were confirmed in all the samples. The research also showed that the nutritional composition of the products varied irrespective of the cultures present (probiotic or standard) and brand (premium or basic) of the yogurts. The results also showed that most of the participants had little or no idea about the meaning of probiotics and that the major reasons that influenced their yogurt choice was cost, availability and taste. The results of the sensory evaluation showed that the non-probiotic yogurts were rated higher than the probiotic ones in terms of taste, texture, appearance and overall quality.

More awareness should be done to ensure that consumers know the importance of probiotic products and probiotic yogurts should be sold at reasonable prices to promote sales. Further research need to be done on a wider scale and with different yogurt products both commercially and laboratory manufactured.

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ACKNOWLEDGEMENT

I would like to acknowledge first and foremost God who has been the brain behind this research. I would also like to acknowledge my Director of studies Professor Carol Wallace, my other supervisors Professor Nicola Lowe and Dr Brigit Ramsingh. You have all been a blessing to me and have made my entire research journey fun rather than stressful.

I would also like to thank my colleagues Dr Marisol Warthon-Medina and Suruchi Pradhan for your immense support and guidance during my research. I would like to thank Dr Stephanie Dillon, Steve, Tamar Gracia-sorribes, and all the other technicians and supervisors at the nutritional laboratory for all their help with my nutritional analysis. I would like to thank Dr Steve Beeton and Vikesh Chhabria for all the help and support during my microbiological analysis.

I would also like to thank all the participants for my sensory analysis for taking time out to help with my research. I would like to thank the catering staff at Foster building for helping me with the setup of the venue for the tasting and for help with the distribution of the posters and storage of the yogurt samples.

I would also like to thank the Dean of this prestigious university for giving me the opportunity to study this program and for making all the necessary facilities ready for my use.

Finally, I would like to thank my parents for giving me the opportunity to study here, for their support and prayers and for taking care of my every need. I love you dearly and will try never to disappoint you.

Thank you all so much and God bless you.

LIST OF ABBREVIATIONS

PCA - Plate Count Agar

MRS - De Man, Rogosa and Sharpe

TVC – Total Viable Count

LAB - Lactic Acid Bacteria

± - With or without

N/A - Not Available

CLA - Conjugated Linoleic Acid

FDA - Food and Drug Administration

WHO - World Health Organization

FAO - Food and Agriculture Organization

UCLAN - University of Central Lancashire

BAHSS - Business, Arts, Humanities and Social Science

SPSS - Statistical Package for the Social Science

MC - Moisture Content

TS – Total Solids

FC- Fat Content

Na - Sodium

Ca – Calcium

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1. YOGURT

Yogurt is a type of dairy product. Dairy products refer to all products produced from milk. The word *yogurt* comes from Turkey and refers to a tart, thick milk (culturesforhealth.com, 2014). Yogurts are made by the addition of certain bacteria cultures called starter cultures. Yogurt represents a very significant dairy product around the world. It is a semisolid fermented product made from a heat treated standardized milk mix by the activity of a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Chandan, White et al. 2006). These bacteria ferment lactose present in the milk and produce lactic acid.

Figure 1.1. Various types of yogurt

Source: Google images (https://www.google.co.uk/search?q=yogurt&newwindow=1&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiloJKko9jKAhXDuw8KHarJAIcQ_AUIBygB) 2014

Based on the UK market, yogurts come in different types such as;

Based on bacteria cultures: Probiotic yogurt and Natural or Standard yogurt

Based on fat: Non-fat, Low-fat, Whole yogurt

Based on texture: Frozen, Semi-solid, and Liquid yogurt, Greek style, Stirred curd, etc.

Based on flavors: Caramel, Vanilla, Strawberry, etc.

Based on Inclusions: Nut, Fruit pieces, Muesli, etc.

There has been a significant rise in the popularity of yogurt in recent years (Illupapalyam, Smith et al. 2014). In North America, the purchase of probiotic yogurts grew from 11% in 2006 to 19% in 2008 while in Europe between 2002 and 2007, yogurt consumption equally grew by 13% in Western Europe and 18% in Eastern Europe (Granato, Branco et al. 2010). Yogurt is derived from the Turkish word "Jugurt" describing any fermented food with acidic taste (Hussain, Attiq-ur-Rahman, et al. 2009). According to a proposal by the European commission for a council regulation, it establishes that 'yogurt' is a product obtained by the fermentation of milk with cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii sp. bulgaricus* (Guarner, Perdigon et al. 2005). Yogurt is a fermented milk product with a slight sour taste, smooth viscosity and refreshing flavour (Hekmat, Reid 2006). It is a conventional food that is best known for its therapeutic, nutritional and sensory properties such as relief from constipation (Illupapalyam, Smith et al. 2014). It is a coagulated milk product resulting from lactic acid fermentation in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Hussain, Attiq-ur-Rahman, et al. 2009; Adolfsson, Meydani et al. 2004; Mani-López, Palou et al. 2014). Lactic acid bacteria (LAB) are non-sporing, gram-positive, catalase negative organisms that are devoid of cytochromes and of non-aerobic habit but are aero tolerant, fastidious, acid tolerant, and strictly fermentative; lactic acid is the major end product of sugar fermentation (Holzapfel, Haberer et al. 2001). The microbiological cultures added to yogurts give it its texture and aroma (Hussain, Attiq-ur-Rahman, et al. 2009) as described below.

Yogurt is one of the most consumed dairy products in the world. It is a good source of dietary calcium and this is why it was well accepted when it was introduced in the American diet during the 1940's (Mani-López, Palou et al. 2014). It is produced in different forms and has different names depending on the part of the world (Hussain, Attiq-ur-Rahman, et al. 2009). The fermentation of milk converts some lactose to lactic acid which acts as a preservative in the yogurt giving it a slight sour taste, and increases the use of calcium and other minerals by the host and improves the gut microflora and this in turn improves digestion (Hekmat, Reid 2006).
Yogurts are unique in that they can contain both starter and probiotic cultures and as such different microbial combinations can be used (Mani-López, Palou et al. 2014). The major difference between standard and probiotic yogurt is the addition of probiotic organisms like *Bifidobacterium bifidum*. Probiotic yogurts are also believed to confer (in addition to nutritional

benefits) certain health benefits like gut health, decreased risk of diarrhoea, and improved bowel movement (Adolfsson, Meydani et al. 2004).

1.1.1. MANUFACTURE OF YOGURT

Yogurt is traditionally manufactured from milk by adding starter cultures. It can be made with milk (low fat, non-fat, or whole), fruits, sweeteners and flavourings (Mani-López, Palou et al. 2014).

Yogurt starter cultures are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* mixed in ratio 1:1. The principle behind yogurt production is fermentation of lactose (milk sugar) to lactic acid by these organisms (Saxelin, Grenov et al. 2011; Dave, Shah et al. 2000). The coccus (*Streptococcus*) grows faster than the rod (*Lactobacillus*) and is primarily responsible for acid production while the rod adds flavour and aroma. The associative growth of the 2 organisms results in lactic acid production at a rate greater than that produced by either when growing alone, and more acetaldehyde, the chief volatile flavour component of yogurt is produced by *L. bulgaricus* when growing in association with *S. thermophilus*. Figure 2 shows a typical representation of the yogurt production process.

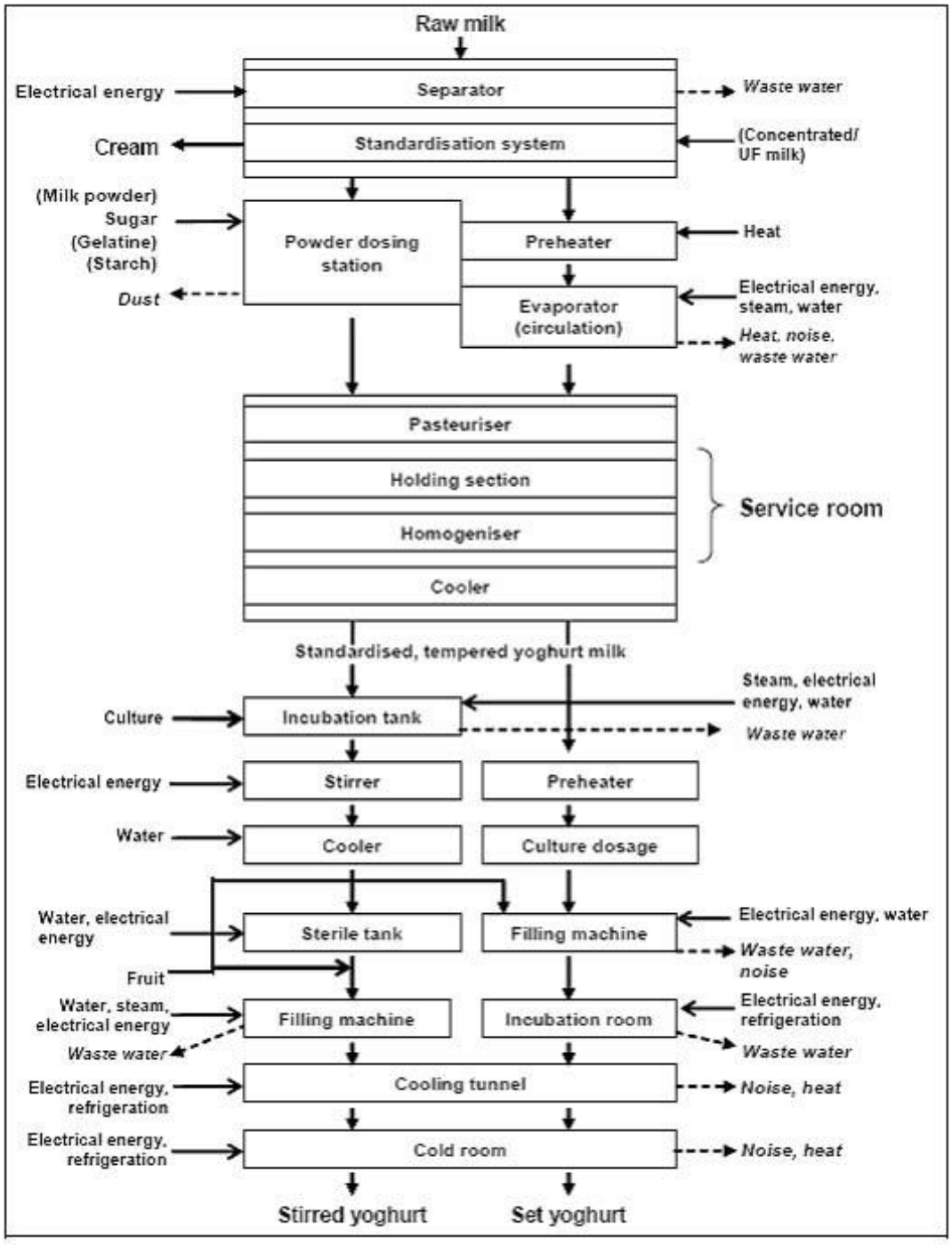


Figure 1.2. Process Flow Diagram of Yogurt

Source: University of Guelph (http://wiki.zero-emissions.at/index.php?title=Yogurt_production) 2013.

1.1.2. NUTRITIONAL COMPOSITION OF YOGURT

The nutritional composition of the milk from which the yogurt is derived is the basis of the nutritional composition of the yogurt, and the nutritional composition of milk is affected by several factors which include feed, stage of lactation, genetic and individual mammalian differences, and environmental factors such as the season of the year (Adolfsson, Meydani et al. 2004). Some variables that affect the processing of milk include temperature, exposure to light, duration of heat exposure, and storage conditions (Adolfsson, Meydani et al. 2004). These variables also affect the nutritional value of the final product (Adolfsson, Meydani et al. 2004). Changes in milk constituents that occur during lactic acid fermentation, strains and species of bacteria used in the fermentation process, the type and source of the milk solids that may be added before the fermentation process, and the duration and temperature of the fermentation process could also affect the final nutritional and physiological composition and value of the finished yogurt product (Adolfsson, Meydani et al. 2004).

Dairy products are generally known to be a good source of proteins, calcium, potassium, phosphorus, magnesium, zinc, and some B vitamins such as riboflavin, niacin, vitamin B6 and B12 (Adolfsson, Meydani et al. 2004).

Lactose

A good source of the disaccharide, lactose, in the human diet is dairy products and foods containing dairy ingredients (Adolfsson, Meydani et al. 2004). The lactose is hydrolyzed by the intestinal brush border beta galactosidase (lactase) into glucose and galactose and the monosaccharides are then absorbed and used as energy sources (Adolfsson, Meydani et al. 2004). The lactose content of yogurt mix is generally approximately 6% before fermentation (Adolfsson, Meydani et al. 2004). The hydrolysis of 20% - 30% of the disaccharide lactose to its absorbable monosaccharide components glucose and galactose is an example of a bacteria induced change that occurs during fermentation (Adolfsson, Meydani et al. 2004). In addition, a portion of the glucose is converted to lactic acid and depending on other ingredients added, this hydrolysis results in lower concentration of lactose in yogurt than milk and this particularly explains why yogurt is better tolerated by persons with lactose maldigestion than milk (Adolfsson, Meydani et al. 2004).

Protein

The addition of non-fat dry milk during processing and concentration of yogurt leads to an increase in the protein content of the final product which makes the protein content of commercial yogurt generally higher than that of milk (Adolfsson, Meydani et al. 2004). It is argued that proteins from yogurt are generally more digestible than proteins from milk and this is due to the conditional predigesting of milk proteins in yogurt. This argument is supported by the evidence of a higher free amino acid content especially proline and glycine in yogurt than milk (Adolfsson, Meydani et al. 2004). Coagulation of casein during fermentation is as a result of heat treatment and acid production and these contribute to the greater protein digestibility of yogurt than milk (Adolfsson, Meydani et al. 2004). Whey proteins and caseins in yogurt are rich sources of essential amino acid (Adolfsson, Meydani et al. 2004). Proteins in yogurt are of excellent biological quality as milk proteins due to the nutritional value of milk proteins being well preserved during fermentation processes (Adolfsson, Meydani et al. 2004).

Lipids

Milk fats experience some biological changes during fermentation and lipase activity results in the release of minor amounts of free fatty acids (Adolfsson, Meydani et al. 2004). Lipid hydrolysis has little effect on the attributes of non- fat or low- fat varieties of yogurt and more on whole- fat yogurts (Adolfsson, Meydani et al. 2004). Yogurts are shown to have a higher concentration of conjugated linoleic acid (CLA) a naturally occurring trans fatty acid obtained from omega 6 essential fatty acid than the milk from which it was processed (Adolfsson, Meydani et al. 2004; Han, Lee et al. 2012). CLA is present in several foods including meat, vegetables and dairy products and its content in dairy products is highly dependent on the geographical region, seasonal variations, initial content of raw milk, type of starter cultures, temperature, production process and ripening process (Serafeimidou, Zlatanos et al. 2012). CLA has been reported to have anti-carcinogenic, antiadipogenic, antidiabetogenic, antiatherosclerotic and immunostimulatory properties (Adolfsson, Meydani et al. 2004; Han, Lee et al. 2012).

pH

Yogurt is an acidic food substance. As a result of its acidic pH, it ionizes calcium and this facilitates intestinal calcium uptake (Adolfsson, Meydani et al. 2004). Yogurt according to the

FDA specifications should have a pH of 4.6 or lower (Olugbuyiro, Oseh 2011). The low pH may also reduce the inhibitory effect of dietary phytic acid on calcium bioavailability (Adolfsson, Meydani et al. 2004). The low pH of yogurt also reduces the risk of pathogens present in the product (Adolfsson, Meydani et al. 2004).

Other nutritional elements

Other compositional elements of yogurt include B vitamins and minerals. There is a much greater risk of losing vitamins than minerals during the processing of yogurt due to the greater sensitivity of vitamins to the environmental factors than minerals (Adolfsson, Meydani et al. 2004). Some of the processing factors known to affect vitamin content in dairies in general include pasteurization and heat treatment, agitation, ultrafiltration, and oxidative conditions (Adolfsson, Meydani et al. 2004). Bacteria cultures during fermentation are also known to influence vitamin content in yogurt production (Adolfsson, Meydani et al. 2004). Some LAB strains are known to synthesis B vitamins and careful use of these cultures can correct the loss of vitamin B-12 (Adolfsson, Meydani et al. 2004). Yogurt is a good source of minerals like calcium and phosphorous. Dairy products such as cheese, yogurt, milk, ice cream, etc. provide majority of the bioavailable calcium in the typical western diet (Adolfsson, Meydani et al. 2004). Calcium and magnesium are present in yogurt mostly in their ionic forms as a result of the lower pH of yogurt (Adolfsson, Meydani et al. 2004). Calcium plays a role in bone formation and mineralization (Adolfsson, Meydani et al. 2004). Due to the lower lactose content of yogurt to milk, the bioavailability of these minerals may slightly be negatively affected (Adolfsson, Meydani et al. 2004).

Yogurts are said to contain live cultures and these cultures could be starter or probiotic. However, although yogurts contain these live cultures at the time of manufacture, some yogurts are heat treated and this causes some of the live bacteria present to die. This therefore makes it difficult to tell which yogurt has actual live cultures in them. There is no European standard dedicated to yogurt specification and Europe has adopted the codex standards. An example of a standard on live yogurt is; For the finished yogurt product to meet the National Yogurt Association (for USA) Criteria for ‘live and active culture yogurt’, it must contain live lactic acid

bacteria in amounts > or = 10^8 organisms/g at the time of manufacture and the cultures must remain active at the end of the stated shelf life (Adolfsson, Meydani et al. 2004). The FDA administration 2008 standard for identity of yogurt drinks specifies that >8.25% milk solid non-fat and fat levels to satisfy non-fat yogurts (<0.5%), low-fat yogurt (2%), or yogurt (>3.25%) before the addition of other ingredients (Allgeyer, Miller et al. 2010). However the document does not specify the bacteria levels accepted in yogurt and this serves as a limitation in the current industry.

1.2. NON-PROBIOTIC YOGURT

Non-probiotic yogurts are also referred to as 'Standard', 'Natural' or 'Traditional' yogurts (Hekmat, Reid 2006; Hussain, Attiq-ur-Rahman, et al. 2009). They are defined as a fermented milk product to which no probiotic live cultures have been added.

Starter cultures are usually used to control and initiate fermentation processes (Mani-López, Palou et al. 2014). The type of starter culture, time, and temperature of fermentation are some of the variables that determine the flavor, texture, and final pH of the product (Mani-López, Palou et al. 2014). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the starter cultures used in yogurt production (Ranathunga, Rathnayaka 2013). The major benefit of standard yogurt in addition to its nutritional composition is that the starter cultures give the yogurt its unique flavours and aroma (Han, Lee et al. 2012).

1.3. PROBIOTIC YOGURT

1.3.1. HISTORY OF THE DEFINITION OF PROBIOTICS

The term "Probiotic" is derived from the Greek language and it means 'for life' (Myers 2007; Gismondo, Drago et al. 1999). It was first used in 1965 by Lilly and Stillwell to describe 'substances secreted by one organism which stimulates the growth of another' and this made it to be contrasted with antibiotics (Schrezenmeir, Michael de Vrese 2001; Lilly, Stillwell 1965). In

1971, Sperti used probiotics in relation to tissue extracts that stimulates microbial growth (Schrezenmeir, Michael de Vrese 2001; Ezema, 2013). Parker defined probiotics as 'organisms and substances that contribute to the intestinal microbial balance' and he was the first person to define probiotics in the sense by which it is used today (Schrezenmeir, Michael de Vrese 2001; Gibson, Fuller 2000). Fuller in 1989 defined probiotics as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (Schrezenmeir, Michael de Vrese 2001; Gibson, Fuller 2000; Fuller, 1989; Saarela, Mogensen et al. 2000). This definition is similar to the modern definition but the use of the words 'animals' and 'feeds' suggest that probiotics are only meant for animals benefits.

In 1992, Havenaar et al defined probiotics as 'a variable mono or mixed culture of microorganisms which applied to animal or man beneficially affects the host by improving the properties of the indigenous microflora (Schrezenmeir, Michael de Vrese 2001; Holzapfel, Haberer et al. 1998). This definition was better than Fuller's definition as it made more room for probiotics in human diet. In 1996, Salminen defined probiotics as 'a live microbial culture or cultural dairy product which beneficially influences the health and nutrition of the host' (Schrezenmeir, Michael de Vrese 2001; Sun, Yang et al. 2009). This definition is not acceptable because it implies that only dairy products are considered probiotic but other non-dairy products including meat and vegetables also contain probiotics. Schaafsma in 1996 defined oral probiotics as 'living microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition' (Schrezenmeir, Michael de Vrese 2001; Ajmal, Ahmed 2009). However, the most acceptable definition of probiotics according to Schrezenmeir et al was given by Havenaar and Huis et al. veld and they defined probiotics as 'a preparation of or a product containing viable, defined microorganisms in sufficient numbers which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health benefits in this host' (Schrezenmeir, Michael de Vrese 2001; Ezema, 2013). This definition encompasses a lot of variables including

- The organisms need to be in sufficient amount
- It could be any product and not just dairy products
- 'Colonization' implies that there may be no need to periodically reintroduce the bacteria into the host by any means

- The use of the phrase ‘alteration of the microflora’ instead of ‘improvement of the properties of the microflora’ due to the fact that the properties of the indigenous microflora were not defined and the evidence of benefit is only shown by health effects.

Probiotics according to FAO/WHO (2002) are defined as live beneficial microorganisms which when administered in the right amount confer a health benefit on the host (Mani-López, Palou et al. 2014; Illupapalyam, Smith et al. 2014; Pishva, Hassanna et al. 2014; Granato, Branco et al. 2010; Gueimonde, Salminen et al. 2006; Quigley, 2010; Reid 2008). They can also be defined as living microorganisms which on ingestion in significant amounts exert health benefits beyond inherent basic nutrition (Adolfsson, Meydani et al. 2004). The ministry of health in Italy define probiotics as microorganisms which once ingested in the right amount, have beneficial effect on the organism (Aureli, Capurso et al. 2011). They are also defined according to Kuo et al. as a sufficient number of living microbial species that may have a positive effect to alter the microflora of the host and improve health conditions (Kuo, Wang et al. 2013).

For a definition of probiotic to be accurate it must contain the following;

- The organisms must be live
- Organisms must be administered in the right amount
- Organisms must be beneficial not harmful
- A host (man or animal) and
- Confer health benefits

The development of probiotic yogurts was to exert beneficial effects on the health status of the consumer (Schillinger, 1999). They contain selected lactic acid bacteria (LAB) (e.g. *Lactobacillus acidophilus)* isolated from the human intestine (Schillinger, 1999). In Germany, probiotic yogurts are known as ‘bio-yogurt’ (Schillinger, 1999). Probiotics must be non-pathogenic and be resistant to gastric acid digestion and to bile salts to be able to reach the intestine intact (Pishva, Hassanna et al. 2014). Some of these strains are obtained from intestinal microbiota of healthy humans while others are non-human strains used in the fermentation of dairy products (Pishva, Hassanna et al. 2014). It is believed by many that the ideal probiotic should remain viable in the level of the intestine and should adhere to the intestinal epithelium to confer significant health benefits (Pishva, Hassanna et al. 2014; Del Piano, Morelli 2006).

1.3.2. BENEFITS OF PROBIOTIC YOGURT

Probiotic therapy is based on the notion that there exist a normal 'healthy microflora', but this has not been defined except as microflora without the existence of a pathogenic bacteria or growth (Adolfsson, Meydani et al. 2004). Probiotic bacteria are added to the human diet to replenish the organisms lost through defecation and to slow down or reverse the process of beneficial organisms lost as a result of ageing (Hekmat, Reid 2006). Although numerous studies have suggested the beneficial therapeutic effects of lactic acid bacteria on gut health, results have however been inconsistent and this may be as a result of differences in the strains of lactic acid bacteria used, routes of administration, and the investigational procedures used in these studies (Adolfsson, Meydani et al. 2004). The major property of probiotic bacteria is the ability to survive passage through a gastrointestinal tract and persist for a sufficient time in the gut to provide health benefits (Hekmat, Reid 2006).

To prevent the gastrointestinal side effects associated with oral antibiotic therapy, bacterial probiotics are regularly administered to humans (Courvalin, 2006). This could be in form of food or drugs. This however has its disadvantages which could include;

- The possibility of resistance transfer from probiotic to human bacterial pathogen either directly or indirectly via the commensal flora (Courvalin, 2006).
- The probiotics themselves can acquire resistant genes from human commensals (Courvalin, 2006).
- In immunologically compromised individuals, there could be a rare case of infection due to probiotics and this could result in the availability of a limited number of effective antibiotics to treat the patient (Courvalin, 2006).

Some functional, nutritional, health and therapeutic benefits of probiotic bacteria include: Table 1.1.

Table 1.1: Benefits of probiotic bacteria

BENEFITS	**SOURCES**
Lower frequency and duration of diarrhoea associated with antibiotics, *Clostridium difficile*, rotavirus infection, chemotherapy, and to a reduced extent traveler's diarrhea.	Schrezenmeir, Michael de Vrese 2001; Hekmat, Reid 2006; Holzapfel, Schillinger 2002.
Helps to improve diarrhoea in infants.	Adolfsson, Meydani et al. 2004; Hekmat, Reid 2006; Holzapfel, Schillinger 2002; Nomoto 2005
Stimulation of humoral and cellular immunity.	Schrezenmeir, Michael de Vrese 2001.
Decrease in unfavorable metabolites e.g. pro-carcinogenic and ammonium enzymes in the colon.	Schrezenmeir, Michael de Vrese 2001.
Modification and stimulation of the immune system.	Hekmat, Reid 2006; Holzapfel, Schillinger 2002.
Reduction of *Helicobacter pylovii* infection.	Schrezenmeir, Michael de Vrese 2001; Kuo, Wang et al. 2013; Sullivan, Nord 2002.
Reduction of allergic symptoms and reaction in the gastrointestinal tract.	Schrezenmeir, Michael de Vrese 2001; Adolfsson, Meydani et al. 2004; Kaur, Chopra et al. 2002; Prescott, Bjorksten 2007.
Relief from constipation.	Schrezenmeir, Michael de Vrese 2001; Holzapfel, Schillinger 2002.
Relief from irritable bowel syndrome and inflammatory bowel disease.	Schrezenmeir, Michael de Vrese 2001; Adolfsson, Meydani et al. 2004; Moayyedi, Ford et al. 2008; Gueimonde, Salminen et al. 2006; Kurniawan, Simadibrata 2011.
Restoration of healthy vaginal microbiota.	Hekmat, Reid 2006.
Beneficial effects on mineral metabolism especially bone stability and density.	Schrezenmeir, Michael de Vrese 2014.
Prevention of cancer.	Schrezenmeir, Michael de Vrese 2001; Adolfsson, Meydani et al. 2004; Shah, Lankaputhra 1997.
Improvement in lactose utilization in lactose malabsorbers.	Shah, Lankaputhra 1997; Adolfsson, Meydani et al. 2004; Hekmat, Reid 2006.
Management of normal gut flora.	Shah, Lankaputhra 1997.
Reduction of cholesterol and triacylglycerol plasma concentration.	Schrezenmeir, Michael de Vrese 2001; Shah, Lankaputhra 1997; Hekmat, Reid 2006; Scheinbach 1998.
Treatment of Minimal Hepatic Encephalopathy	Bajaj, Saeian et al. 2008.
Production of vitamins and important digestive enzymes.	Holzapfel, Schillinger 2002.

Mechanisms by which probiotics exert their therapeutic effects include:

1. Modulation of barrier function (Pishva, Hassanna et al. 2014).
2. Mucosal trophic action (Pishva, Hassanna et al. 2014; Holzapfel, Schillinger 2002).
3. Inhibition of pathogenic bacteria (Pishva, Hassanna et al. 2014).
4. Blockade of epithelial attachment and invasion by pathogenic bacteria (Pishva, Hassanna et al. 2014).
5. Modulation of intestinal cytokine production (Pishva, Hassanna et al. 2014).
6. Anti-inflammatory properties (Pishva, Hassanna et al. 2014).
7. Enhancement of digestion and absorption of food (Pishva, Hassanna et al. 2014).

As a result of these potential benefits, probiotics are very attractive to a range of consumer groups (men, women, and children), however very little data and research has been done on consumer preference and the reason behind this for probiotic yogurt.

Theoretically, genetically modified probiotic organisms may be responsible for four types of side effects: systematic infections, risk of deleterious metabolic activities, risk of adjuvant side effects and of immunomodulation, risk of gene transfer (Salminen, Wright et al. 1998). However, genetically modified probiotic organisms are currently not available for food use and this makes their theoretical adverse effect not considered (Salminen, Wright et al. 1998).

1.3.3. PROBIOTIC BACTERIA USED IN FOOD APPLICATIONS

Probiotics are used in a number of different food applications including meat, dairy and non-dairy applications. The most commonly used probiotics are lactic acid bacteria and non-pathogenic yeast (Quigley, 2011). A significant portion of probiotic cultures used in food industries are the genus *Bifidobacterium* and *Lactobacillus* because they are non-pathogenic (Liu, Chen et al. 2013). Some examples of organisms that have been demonstrated to have beneficial health effects on humans and animals include *Lactobacillus, Bifidobacterium, Escherichia coli* Nissle 1917, *Clostridium butyricium, Streptococcus salivarius thermophilus*, and a non-pathogenic yeast *Saccharomyces boulardii* (Pishva, Hassanna et al. 2014). Lactobacillus and Bifidobacterium are the most common types of probiotics used (Allgeyer,

Miller et al. 2010; Pishva, Hassanna et al. 2014). Some other strains include *Lactobacillus reuteri RC-14*, *Lactobacillus rhamnosus GR-1* (Hekmat, Reid 2006). Strains of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were introduced over 20 years ago because of the advantage of consuming active LAB that is adapted to the intestine and to produce mildly acidified yogurts (Schillinger, 1999).

Other genera that are used as probiotics include *Streptococcus*, *Bacillus*, and *Enterococcus* but there are concerns surrounding the safety of such probiotics because these genera contain so many pathogenic species especially *Enterococcus* (Pishva, Hassanna et al. 2014).

Dairy products are the main commercial probiotic foods and this is due to the buffering capacity of milk which ensures the survival of probiotics during fermentation and storage (Mani-López, Palou et al. 2014). Yogurts and fermented milks are the most widespread dairy probiotic products (Mani-López, Palou et al. 2014). Studies have shown that most probiotic foods have low amounts of probiotic levels and the organisms are unable to survive during the storage periods in yogurt (Han, Lee et al. 2012). Factors that affect viability of probiotic bacteria in yogurt include acidity, pH, hydrogen peroxide, oxygen content, organic acid concentration, milk composition, temperature and time of holding during manufacture, transport and storage of yogurt (Han, Lee et al. 2012).

According to Mani-Lopez et al. microbial food cultures have two main roles:

1. Food processing and
2. Product development

However, with probiotic cultures, a further role of providing beneficial, health and therapeutic effects is anticipated (Mani-López, Palou et al. 2014).

1.4. DIFFERENCES BETWEEN PROBIOTIC AND NON-PROBIOTIC YOGURT

The major difference between probiotic and standard yogurt is the introduction of probiotic organisms. Probiotic yogurts also confer in addition to nutritional benefits certain health benefits

including gut health, decreased risk of diarrhoea, and improvement of bowel movement. Other significant differences include: Table 1.2.

Table 1.2: Differences between probiotic and non-probiotic yogurt

Probiotic	**Non-probiotic**
It contains probiotic cultures like *Lactobacillus acidophilus* and *Bifidobacterium*	It contains starter cultures like *Lactobacillus bulgaricus* and *Streptococcus thermophilus*
It could contain both probiotic and starter cultures	It contains only starter cultures
It has in addition to nutritional properties, therapeutic and health benefits	It has nutritional properties
It helps in the reduction of acidity in yogurt	It gives the yogurt its unique aroma and texture

1.5. BRANDING

Branding is a technique used by the food industry to create a recognizable image to attract consumers and boost product sales (Keller, Kailema et al. 2012). A brand is not just a given name, it must be in line with the rest of the setup such as logo, tagline, poster, website, positioning and many others (Abidin, Effendi et al. 2014). A strong brand must also be able to associate with any opportunities for its own benefit (Abidin, Effendi et al. 2014). An example is Islamic branding. This is the type of branding that fulfils all aspects of the brands for the Muslim consumers (Mohd, Wan et al. 2014). It is defined according to three constructs: country of origin, target audience and whether it is Halal (Mohd, Wan et al. 2014). Branding must be able to give the total experience and keep the promises made by the brand owner (Abidin, Effendi et al. 2014).

Branding is a marketing strategy and markets have four principles i.e. prices, products, places, and promotion (Ailawadi, Keller 2004; Auttarapang, 2011). Branding encompasses reputation, cost, quality, consumer satisfaction, advertisements, etc. (Editorial, 2004). Reputation can be defined as the expression of corporate conduct aimed to differentiate the company from

competitors in the perception of competitive rivalry (Czinkota, Kaufmann et al 2014). To achieve this, a marketing strategy employed by companies is corporate branding (Czinkota, Kaufmann et al 2014). Reputation is also defined as a set of aggregate perceptions and evaluations developed by stakeholders and induced by the process of corporate branding that favor the company against its competitors (Czinkota, Kaufmann et al 2014). Corporate branding is a situation where a company uses a particular name for all its products e.g. McDonald's and Muller (Seo, Jang 2013). Branding therefore encompasses behavioral branding, corporate branding which is the corporate identity and product brand which refers to visual identity of the product (Czinkota, Kaufmann et al 2014).

Brand personality is the human characteristics or traits that can be attributed to a brand and it includes sincerity, excitements and competence (Ailawadi, Keller 2004). Brand architecture involves defining both brand boundaries and brand relationships and its roles includes; to clarify all products and service offerings and improve brand awareness with consumers (Ailawadi, Keller 2004). It also involves the motivation of consumer purchase by enhancing brand image of product and services (Ailawadi, Keller 2004).

Types of brands

Based on manufacturing: Retail brands and Manufacturer or national brands

Based on cost: Basic and premium brands.

Based on manufacturing: According to the American marketing association definition of a brand, a retail brand identifies the goods and services of a retailer and differentiates them from those of competitors (Ailawadi, Keller 2004). Retail owners have to compete with national brands for sales (Editorial, 2004). In doing so they improve customer services but this does not guarantee exceptional sales (Editorial, 2004). To promote sales, some retail brands give out discounts (i.e. sales promotion discounts) and this is also a form of food marketing (Hamlin, Lindsay 2012). Manufacturers respond to retail brands by decreasing cost, cutting prices, increasing promotions, introducing discount brands, etc. (Ailawadi, Keller 2004).

Based on cost: Yogurts have different prices attached to them depending on the packaging, content, and manufacturer decision. Basic brands refers to the non- expensive type of yogurt while premium brands refers to the expensive or luxury types of yogurts. These types usually sell at twice or triple the normal price of the basic brands.

Marketing

Marketing influences consumer food purchasing and behavior (Colby, Johnson et al. 2010). Nutrition marketing is defined as any marketing (TV, radio, or food labels) of food or beverages using health or nutrition information beyond minimum requirements i.e. a health claim (Colby, Johnson et al. 2010). Marketing strategies are based on the target groups i.e. children, ethnic group (e.g. Islamic group), teenagers, adults, and elderly (Jamal, Peattie et al. 2012). One of the ways of marketing a product is by advertisements. Food advertisement is a form of marketing strategy (Forman, Halford et al. 2009). Advertisements can be done on radio, television, posters, newspaper, etc. Television provides one of the first and most intimate experiences of commercial food promotion (Boyland, Halford 2013). This is because television remains one of the most powerful sources of communication (Boyland, Halford 2013). Television advertising is thought to be effective in building strong brands (Boyland, Halford 2013). In advertising a product, the use of celebrities, attractive packaging and location influences the product brand and consumer perception of the product.

Packaging

Packaging is a communication tool in branding (Abidin, Effendi et al. 2014). Packaging is essential in the food industry both for protecting the product from contamination and portraying the brand image (Mahalik, Nambiar 2010). The packaging of a product influences the consumer decision (Koenig-Lewis, Palmer et al. 2014). Packaging influences the purchasing behavior of the consumer through communication, functionality and environment where the communication aspects refers to graphic design, information and brand promotion (Arslanagic, Pestek 2013). Packaging depends on the type of products been packaged (Wikstrom, Williams et al. 2014; Mahalik, Nambiar 2010). Packaging involves the packaging material, the labelling, colour of the packaging, and visual effects of the packaging (Velasco, Salgado-Montejo et al. 2014).

Packaging is a powerful marketing tool that should effectively capture the attention of the consumer and effectively communicate with them (Velasco, Salgado-Montejo et al. 2014). Packaging include the type of material used e.g. plastic (flexible and light weight), plastics (transparency, softness, and heat seal ability), glass and metal (high value products, stronger and corrosion resistant) (Mahalik, Nambiar 2010). As the world is becoming more conscious of the environment, environmentally degradable and recyclable packaging should be used (Koenig-Lewis, Palmer et al. 2014). Package design must function as the aesthetic means of communicating to people from different backgrounds, interests and experience therefore an awareness of anthropology, sociology, psychology, ethnography and linguistics can benefit the design process and appropriate the design choices (Abidin, Effendi et al. 2014). Target audience, geography, product preference, gender, and age target must be considered before designing a packaging design (Abidin, Effendi et al. 2014). The use of promotional characters e.g. cartoon characters have a huge influence on children choice and food preference (Hebden, King et al. 2011). Inadequate packaging of food could lead to food wastage and to food safety/spoilage issues (Williams, Wikstrom et al. 2012; Babalis, Ntintakis et al. 2013). Effective packaging can reduce food loss directly or indirectly (Williams, Wikstrom et al. 2012; Abrams, Evans et al. 2015).

Although a range of factors are important in branding, there is limited information on the differences between basic and premium brands of yogurt in terms of product characteristics and nutritional performance.

1.6. CONSUMER ACCEPTABILITY AND SENSORY EVALUATION

Yogurt is one of the most commonly consumed dairy product in the world and its sensory attributes have a large effect on consumer acceptability (Allgeyer, Miller et al. 2010; Ranathunga, Rathnayaka 2013: Cruz, Cadena et al. 2012). The most important factors in fermented dairy products containing probiotics are to ensure probiotic viability and determination of sensory and physical property changes that may occur especially if a mixture of LAB is used as a starter culture or probiotic (Mani-López, Palou et al. 2014). The most prominent factors that influence the quality and acceptance of yogurt is flavor and texture and they are affected by several factors such as incubation temperature, starter culture, processing

conditions (heat treatment and homogenization), and compositional properties of the milk base (Soukoulls, Panaglotldls 2007)

While probiotic bacteria confer certain health benefits to the consumer, they could influence the product by developing different flavours and textures (Mani-López, Palou et al. 2014). Most probiotic cultures do not tend to modify sensory attributes of the products to which they are added. However, some certain starter cultures (*L.delbrueckii* subsp *bulgaricus*) have been evaluated by consumers as being too acidic so probiotic cultures are known to develop preferred flavors e.g. ABT cultures (Mani-López, Palou et al. 2014). ABT cultures are a mixture of Lactobacillus acidophilus, Bifidobacterium and Streptococcus thermophiles and it is usually packaged in a freeze-dried condition (Flávia, Buriti et al. 2007).

Consumer acceptability and product quality are important to increase product sales of different types of yogurt (Hussain, Attiq-ur-Rahman, et al. 2009; Cruz, Cadena et al. 2012). Consumer acceptance of probiotic or non-probiotic yogurt depends on its product quality (Ranathunga, Rathnayaka 2013). The quality of yogurt is difficult to standardize due to its various forms, varieties, manufacturing methods, ingredients, and consumer preferences (Hussain, Attiq-ur-Rahman, et al. 2009). Physical characteristics such as viscosity, smoothness, and firmness and chemical properties such as pH and organoleptic characteristics should be at standard levels i.e. optimum levels for consumer preference (Ranathunga, Rathnayaka 2013).

Yogurts generally contain live cultures but the addition of probiotic cultures like *Lactobacillus acidophilus* or *Bifidobacterium* spp. is what differentiates standard yogurts from probiotic yogurts. Based on its nutritional and microbiological and properties, yogurts are very beneficial to the health as they have lots of therapeutic benefits including relief from constipation, relief from irritable bowel syndrome and improvement of the gut microflora. However, in spite of the benefits of yogurts it has to be produced and packaged properly to ensure purchase by consumers hence branding of the yogurt products should be properly done to ensure it is attractive to consumers.

Based on the above, the aim of the research is to compare probiotic and standard yogurts on the basis of brands (basic and premium brands), microbiological analysis, nutritional analysis,

sensory evaluation and consumer preference. The secondary aim of the research is to determine if there is a significant difference in the properties of probiotic and non-probiotic yogurt and premium and basic brands of these yogurts. The following are the major objectives of the research:

1. To determine the cultures (probiotic and natural) used in the production of premium and basic brands of natural and probiotic yogurts.
2. To compare the level of nutrients in natural and probiotic yogurts and to determine if there is a difference in the nutritional properties of natural yogurts with the addition of probiotic cultures.
3. To determine consumer preference (by questionnaires and sensory evaluation) between premium and basic brands of probiotic and natural yogurts and to gain an understanding of the reasons underlying consumer choices.

Based on the above aims and objectives, this research hopes to prove the following hypotheses:

- That the commonly available yogurts in retail stores contain the stated organisms and nutritional composition (written on the label) in the product and in the appropriate amount.
- That the fat content of a yogurt product affect the choices and preference of consumers.
- That the addition of probiotic cultures influences the nutritional composition of the products.
- That there is a significant difference between probiotic and natural yogurt or basic and premium yogurts in terms of texture, taste, appearance, and overall quality and this difference is easily noticeable.
- That there is a significant difference (microbiological, nutritional and sensory quality) between premium and basic brands of yogurts and also probiotic and non-probiotic yogurts.
- That consumers understand the meaning of probiotic and its significance and that this influences their yogurt choices.

CHAPTER TWO

MATERIALS AND METHODS

For this research, both qualitative and quantitative analysis were carried out. The microbiological, nutritional and majority of the consumer based analysis are quantitative while a small part of the consumer based analysis is qualitative.

The research design methods used were used after careful consideration of the research questions and were chosen as the best methods to answer the research questions and hypotheses. Some of the hypotheses are answered by specific methods as detailed below whilst others, e.g. 'That there is a significant difference (microbiological, nutritional and sensory quality) between premium and basic brands of yogurts and also probiotic and non-probiotic yogurts', are answered by several methods collectively.

Four samples of premium and basic brands of yogurts (probiotic and standard) were bought from different supermarkets in Preston, UK. All samples were given numbers rather than using brand names. The characteristics of the samples are shown in Table 2.1 below.

Table 2.1: Characteristics of the yogurt samples used for analysis.

Yogurt samples	**Characteristics**
Sample A	Basic/non-expensive (cost 45 pence), non-probiotic, low fat, non-flavoured, manufacturer brand.
Sample B	Basic/non-expensive (cost 55 pence), probiotic, low fat, non-flavored, retail brand.
Sample C	Premium/expensive (cost £1.16p), non-probiotic, whole yogurt, non-flavoured, retail brand.
Sample D	Premium/expensive (cost £1.47p), probiotic, whole yogurt, non-flavoured, manufacturer brand.

2.1. CHEMICALS, REAGENTS, MATERIALS, EQUIPMENT'S AND INSTUMENTS

Below is a table (Table 2.2) showing the type of reagents, chemicals, materials and equipment's used in the various analysis.

Table 2.2: Materials, reagents, chemicals, and equipment used in the various analysis.

Analysis	Equipment and Instruments	Chemicals, reagents and materials
Microbiological analysis	Autoclave, colony counter, incubator, fridge, microscopic slides, microscope, inoculating loop and needle, measuring cylinder, weighing balance, Bunsen burner, McCartney bottles, beaker, conical flasks, laminar cabinet, pipette, spatula, forceps, and Durham tubes	MRS agar and broth, M17 agar and broth, motility test medium, PCA agar, oxidase reagent, nutrient agar, peptone water, starch, grams safranin, grams iodine, crystal violet, phenol red, hydrogen peroxide, ethanol, distilled water, and yogurt samples.
Nutritional analysis	Hot air oven, beaker, pipette, weighing balance, spatula, pH meter, measuring cylinder, crucibles, Sortec apparatus with thimbles and aluminum container, digestion tubes, Kjeltec apparatus, bomb calorimeter, oxygen cylinder, and volumetric flask.	Hydrochloric acid, sodium hydroxide, petroleum ether, boric acid indicator, concentrated sulphuric acid, Kjeldahl selenium catalyst tablets, benzoic acid, distilled water, deionized water, and yogurt samples.
Consumer preference and Sensory evaluation	Disposable cups and spoons.	Yogurts samples.

2.2. SAMPLE COLLECTION

All samples were purchased at different supermarkets in Preston. Four samples each were used for microbiological analysis, nutritional analysis and sensory evaluation. The samples were bought and immediately stored in the fridge at -4^0C until use. All samples purchased were non-flavoured.

2.3. CONSUMER PREFERENCE AND SENSORY EVALUATION

Ethics approval was given by the Uclan ethics committee (BAHSS Ethics Committee) to conduct this research. The population size was a convenient population size as the participants were approached without any preference to gender, ethnicity or type. The venue used was the Scholar Bar located in Foster building. Posters and personal invitation were the methods used to recruit participants for this research. The questionnaire was made after careful research of similar works (such as Ranathunga, Rathnayaka 2013: Tarakçi, Küçüköner 2003: Olugbuyiro, Oseh 2011) and modifications made to the methods used in these research to suit the nature of this particular research and population group. Appendix F and G show the questionnaire of the consumer preference and sensory evaluation respectively.

This method used for the consumer and sensory analysis was chosen to prove the hypothesis which states 'that there is a significant difference between probiotic and natural yogurt in terms of texture, taste, appearance, and overall quality and this difference is easily noticeable', and 'that consumer's understand the meaning of probiotic and its significance and that this influences their yogurt choices'.

Consumer preference: 102 participants (staff and students) filled out a questionnaire evaluating several things including their yogurt preference, frequency of yogurt consumption, and understanding of the term probiotics.

Sensory evaluation: Sensory evaluation of four samples was conducted by 102 participants (staff and students). Each participant was given 4 samples at each serving with the sample size large enough so that participants could re-taste the product if they so desired. The samples were

placed in uniform disposable containers that did not affect the properties of the products. The participants were asked to evaluate the samples based on taste, texture, appearance, and overall quality. They were also asked to try to identify which of the products were probiotic, non-probiotic, premium/expensive brands, and basic/non-expensive brands. The participants were placed at individual stations and no verbal communication as allowed between them during the evaluation.

2.4. NUTRITIONAL ANALYSIS

The following methods hope to prove the hypothesis which states 'that the fat content of a yogurt product affect the choices and preference of consumers', and 'that the commonly available yogurts in retail stores contain the stated organisms and nutritional composition (written on the label) in the product and in the appropriate amount'. It also hopes to prove the hypothesis which states 'that the addition of probiotic cultures influences the nutritional composition of the products'.

pH: The principle of this test is to check for the level of acidity or alkalinity of the sample. The original sample was used. About 5g of the sample was placed into beakers and using a pH meter, the pH was determined (Hussain, Attiq-ur-Rahman, et al. 2009). The test was done in triplicate.

Moisture content (MC): The principle of this test is to determine what can be evaporated off from the sample. The test was done in triplicate. 2.000g of the sample was dried to a constant weight at 105^0C for 16 hrs. It was then reweighed and the loss of weight was represented as a percentage of the original weight of the sample (Hussain, Attiq-ur-Rahman, et al. 2009).

For the remainder of the nutritional analysis, all four samples used were initially dried at 105^0C (Figure 3) for approximately 16 hours and then blended using a fine blender to ensure a homogenous consistency. The dried samples were then analyzed using the following tests with the exception of pH and moisture content.

Figure 2.1. Drying of yogurt for analysis.

Step 1: Yogurt after drying at 1050C for 16 hours.

Step 2: Powdered yogurt after blending.

Total solids (TS): Total solids was obtained from moisture analysis. The weight of the residue obtained from moisture content was expressed as percentage total solids using the formula below (Olugbuyiro, Oseh 2011):

$$TS = \frac{\text{(Weight of dish +Dry yoghurt) - (Weight of dish)}}{\text{Weight of the sample}} \times 100$$

Fat content using Soxtec apparatus (FC): The principle of this test is to determine the part of the food that can be extracted with petroleum ether as fats are soluble in non-polar solvent whereas other parts of the food are not. The reference material used was Skim milk powder. The test was done in six replicates. 2.000g of the sample was weighed into thimble covered with cotton wool and metal collar and placed onto the metallic holder and racked upwards. 40ml of petroleum ether was placed in a container which was then placed onto the heated shelf below the thimbles. The glass condensers was then clamped to the containers and the taps opened to allow condensed solvent drain back into the containers. The thimbles were then lowered into the boiling solvent and extraction was done for 30 mins. The thimbles were then racked upwards and extraction continued for 15 mins after which the taps were closed to prevent condensed solvent draining back into the containers. The air pumps were then turned on to ensure evaporation and the heating continued for 45mins to remove the solvent. After cooling and drying, the container plus fat was weighed and the fat represented as a percentage of the weight of the food (Hussain, Attiq-ur-Rahman, et al. 2009).

Protein determination using Kjeltec apparatus: The principle of this test is to determine the level of nitrogen present in the sample as the level of nitrogen is proportional to the level of protein in the sample. Skim milk powder was the reference material standard used. The sample is digested

in sulphuric acid and catalyst and the organic compounds are oxidized to water and carbon dioxide and the nitrogen converted into ammonium. The sample is made alkaline and the ammonia is distilled off and titrated with standard acid and the volume of acid used is proportional to the amount of nitrogen present in the sample. 0.5g of the sample was weighed into a digestion tube, 2 catalyst tablets and 15ml of conc. H_2SO_4 was added. It was then digested till it was clear, allowed to cool and then placed in the kjeltec apparatus to determine the crude protein (Hussain, Attiq-ur-Rahman, et al. 2009). The test was done in triplicates. The protein content was calculated with the formula

$$\% \text{ Protein} = 0.875 \times \frac{V}{W}$$

V = volume (ml) of 0.1 HCL

W = weight of sample

Energy determination using Bomb calorimeter: The principle of this is to determine the energy content of the sample which is calculated from the rise in temperature and the determined thermal capacity of the apparatus. Benzoic acid was used as an internal standard to calibrate the instrument and determine the Thermal Capacity (TC). 1.000g of the sample was weighed into a crucible and a 10cm fuse wire was fixed tightly between the calorimeters electrodes in such a way that it touches the top of the food. Oxygen was then filled into the bomb and it was placed in the calorimeter and the wires were attached to the terminals. The calorimeter was started and the temperature was monitored for 10mins until it was stable. The test was done in triplicates. The energy content was calculated with the formula

$$\text{Energy content (j/g)} = \frac{\text{TC} \times \text{Temp. Rise}}{W}$$

TC = thermal capacity in Joules per ^0C

Temperature rise = final temperature – initial temperature

W = weight of sample

Determination of mineral content (Na and Ca) using ICP-OES: The reference standard material used was Skimmed milk powder. 1.000g of yogurt sample was weighed and digested overnight at room temperature by adding 5ml of nitric acid in a closed 50ml graduated tube. After overnight incubation, the samples were incubated in an oven at 60^0C-70^0C for an hour after which the samples were cooled to room temperature and the final volume was made up to 25ml by adding deionized water. This solution was analyzed to total Ca and Na using ICP-OES.

2.5. MICROBIOLOGICAL ANALYSIS

Microbiological analysis was conducted using aseptic techniques. These methods were used to prove the hypothesis which states 'that the commonly available yogurts in retail stores contain the stated organisms and nutritional composition (written on the label) in the product and in the appropriate amount'.

Inoculation

Two methods of inoculation were used

Spread plate Method: This involves the aseptic transfer of 1ml of inoculum onto agar and using a sterile spreading glass to spread the inoculum evenly.

Streaking Method: This involves the use of a loop to streak a distinct colony on agar. The loop is flamed after each row of streaks.

Media Sterilization

Several types of liquid and solid media were used and sterilized by autoclaving at 121^0C for 15 minutes.

Liquid Media: The liquid media include MRS broth, Modified MRS broth (Contains MRS and 0.5g/L of L-Cysteine hydrochloride), M17 broth, and Peptone water.

Solid Media: They include Nutrient agar, MRS agar, Modified MRS agar (Contains MRS and 0.5g/L of L-Cysteine hydrochloride), Plate count agar, M17 agar, and Motility test medium.

Identification of Bacteria in the Samples

Several serial dilutions were made initially to get the perfect dilution factor for the products. The dilutions made ranged from 10^4 to 10^6. After several dilutions were carried out on the products, 10^6 dilution was chosen to be the best and was used to analyze the samples.

Total Viable Count: This is to check the total number of viable microorganisms in a product. The appropriate amount of PCA was prepared and autoclaved at 121^0C for 15mins. It was then poured into sterile Petri-dishes and allowed to gel. The samples were diluted (10^6) and then inoculated into the agar. The method of culturing used was spread plate. The plates were then inverted and incubated at 37^0C for 18-24hrs. This test was done in triplicate. The results were taken and recorded.

Culturing on MRS Agar and Broth: This agar is used for the enumeration of *Lactobacillus spp.* (Ashraf, Shah 2011).The appropriate amount of MRS agar was prepared and autoclaved at 121^0C for 15 mins (Davidson, Duncan et al. 2000; Lamoureux, Roy et al. 2002). It was then poured into sterile petri dishes and allowed to gel. The samples were then diluted (10^6) and then inoculated into the agar. The method of culturing used was spread plate. The plates were inverted and incubated at 37^0C for 48 hours. Each of the colonies was then sub-cultured 3 times onto fresh MRS agar by streaking to obtain pure colonies. *Lactobacillus* appeared as large clear colonies. The pure colonies were then sub-cultured into MRS broth. The presence of *Lactobacillus* makes the broth turbid. The positive tubes were then sub-cultured into MRS agar and the pure *Lactobacillus* strain was then sub-cultured into MRS agar slant and maintained at -4^0C.

Culturing on M17 Agar and Broth: This agar is used for the enumeration of *Streptococcus thermophiles* (Ashraf, Shah 2011; Gueimonde, Delgado et al. 2004).The appropriate amount of M17 agar was prepared and autoclaved at 121^0C for 15 mins (Davidson, Duncan et al. 2000; Lamoureux, Roy et al. 2002). It was then poured into sterile petri dishes and allowed to gel. The samples were then diluted (10^6) and then inoculated into the agar. The method of culturing used was spread plate. The plates were inverted and incubated at 37^0C for 48 hours. Each of the colonies was then sub-cultured 3 times onto fresh M17 agar by streaking to obtain pure colonies. *Streptococcus* appeared as white colonies. The pure colonies were then sub-cultured into M17 broth. The presence of *Streptococcus* makes the broth turbid. The positive tubes were then sub-

cultured into M17 agar and the pure *Streptococcus* strain was then sub-cultured into M17 agar slant and maintained at -4^{0}C.

Culturing on MRS Agar and Broth with the addition of L-Cysteine hydrochloride (Modified MRS): The appropriate amount of MRS agar was prepared and 0.5g/L of L-Cysteine hydrochloride added to it to inhibit the growth of lactobacillus (http://www.researchgate.net/post/Can_I_get_Bifidobacteria_to_grow_in_MRS). It was autoclaved at 121^{0}C for 15 mins. It was then poured into sterile petri dishes and allowed to gel. The samples were then diluted (10^6) and then inoculated into the agar. The method of culturing used was spread plate. The plates were inverted and incubated at 37^{0}C for 48 hours. Each colonies were then sub-cultured 3 times onto fresh modified MRS agar by streaking to obtain pure colonies. The pure colonies were then sub-cultured into modified MRS broth. The presence of *Bifidobacterium* makes the broth turbid. The positive tubes were then sub-cultured into modified MRS agar and the pure *Bifidobacterium* strain was then sub-cultured into modified MRS agar slant and maintained at -4^{0}C.

Of the samples that showed positive growths on the various plates, 2 colonies were chosen and then sub cultured three (3) times to obtain pure colonies. These were then used as the primary cultures. The pure colonies were then inoculated into the respective broth and incubated. The primary cultures were then used to conduct biochemical tests to confirm the organisms present.

Biochemical tests

Various biochemical tests are used to identify Lactic acid bacteria. The following are the test carried out;

Catalase test: The principle behind this test is to check if the organism produces the enzyme catalase as a catalyst to breakdown hydrogen peroxide into oxygen and water. Hydrogen peroxide was added to the organism. Production of gas shows a positive result. *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are catalase negative (Tannock, 1999; Pyar, Peh 2014; Nielson, 2010).

Motility test: The principle behind this test is to know if the organism is motile or not i.e. presence of flagella.The appropriate amount of Motility test medium agar was prepared in bijou bottles, autoclaved at 121^0C for 15mins and then allowed to gel in a standing position. The organism was then stabbed in the agar and incubated at 30^0C-37^0C for 18-24hrs. Growth outside the stab mark shows a positive result. *Bifidobacterium* and *Streptococcus* are non-motile (Charteris, Kelly et al. 1997) while *Lactobacillus* could be motile or non-motile depending on the specie (Tannock, 1999; Pyar, Peh 2014; Nielson, 2010).

Oxidase test: The principle behind this test is to check for the ability of the organism to produce oxidase. Oxidase reagent was poured on a Whatman no. 2 filter paper. A loopful of the organism was smeared on a portion of the filter paper. A color change of deep blue-purple shows a positive result. *Lactobacillus, Bifidobacterium* and *Streptococcus* are oxidase negative (Tannock, 1999; Pyar, Peh 2014; Nielson, 2010; Fluka, 2015).

Starch hydrolysis test: The principle behind this test is to check for the ability of the organism to hydrolyze starch to produce amylase. The appropriate amount of nutrient agar was prepared and 4g of starch was added to the agar. It was then autoclaved at 121^0C for 15mins. The agar was poured in plates and allowed to gel. The organisms were streaked on the agar and then incubated at 37^0C for 18-24 hrs. The plate was then flooded with Grams iodine. If starch is hydrolyzed and starch is no longer present, the medium will have a clear zone next to the growth.

Sugar fermentation test: The principle behind this test is to check the ability of the organism to utilize the sugar. The appropriate amount of peptone water was prepared in bijou bottles containing Durham tubes and the sugar was added. It was then autoclaved at 121^0C for 15mins. The organisms were inoculated into the bottles and then incubated at 37^0C for 18-24 hrs. About 5 drops of phenol red was added as an indicator. Yellow coloration and gas production shows a positive result. The sugars used were glucose, lactose, sucrose, and fructose. *Lactobacillus* is positive to all the sugars used (Bhardwaj, Puniya et al. 2012).

Gram stain

Gram staining was done on all the organisms to check for their morphological characteristics. The organism was smeared on a microscopic slide, air dried and then heat fixed. 5 drops of crystal violet was added and the slide was allowed to stand for a minute. It was then washed

briefly and then 5 drops of grams iodine was added and rinsed off after 30 seconds. 75% ethanol was then used to decolourize and the slide was then washed off immediately. 5 drops of safranine was added and washed off after a minute. The slides was then observed under a microscope. *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are gram positive (Tannock, 1999; Pyar, Peh 2014; Nielson, 2010). *Lactobacilli* are gram positive, non-spore-forming rods ranging from coccobacilli to long, slender bacilli while *Bifidobacterium* are gram-positive pleomorphic rods, ranging from uniform to branched, bifurcated Y and V forms, spatulate or club shaped (Tannock, 1999). *Streptococcus* are gram positive, facultative anaerobes and chain cocci (Hale, 2013).

2.6. STATISTICAL APPROACH AND ANALYSIS

SPSS 22.0 (Statistical Package for the Social Science) and Microsoft Excel were the statistical programs used for this research. The graphs were generated using Microsoft Excel while other statistical analysis were performed using SPSS. The Kolmogorov-Smirnov test was used to assess the normality of the data distribution. Data generated from the biochemical analysis of the nutrient composition of the yogurts were shown to be normally distributed, therefore parametric tests were used to identify statistically significant differences between the sample means. One way analysis of variance (ANOVA) was the parametric test of choice because of the comparison between multiple categories. A post hoc test (Bonferroni Alpha) was used to identify which of the group mean values were significantly different from one another. The cut off for significance was set *a priori* at a value of $P<0.05$. Chi square test (non-parametric) was used to identify associations between the consumer preference and sensory evaluation categories.

CHAPTER THREE

RESULTS

3.1. CONSUMER PREFERENCE AND SENSORY EVALUATION

3.1.1. PARTICIPANTS CHARACTERISTICS

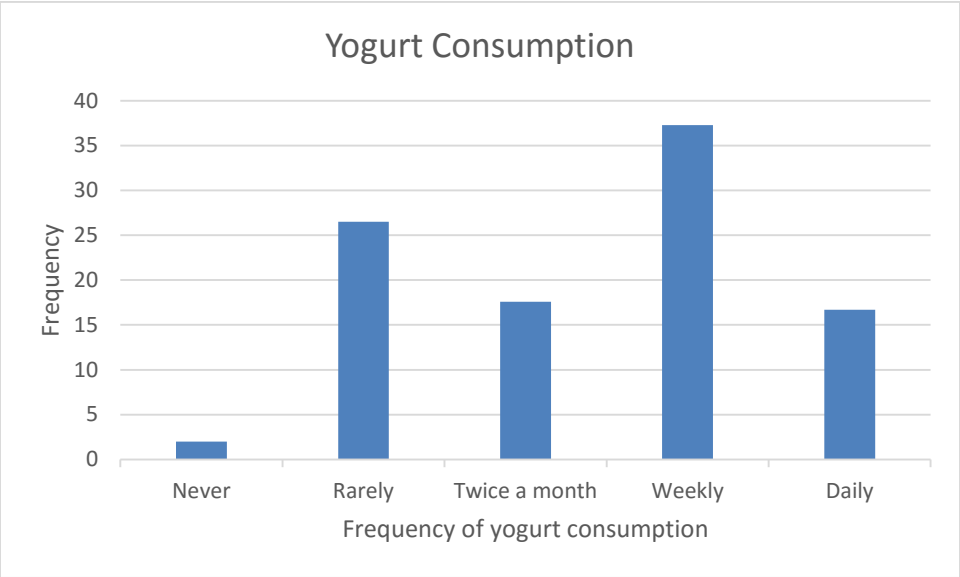
Table 3.1. Shows the participants characteristics of gender and age.

Participants characteristics	Number (Percent)
Gender	
Male	55 (53.9%)
Female	47 (46.1%)
Age range	
18-25	50 (49.0%)
26-35	23 (22.5%)
>36	29 (28.4%)

3.1.2. CONSUMER PREFERENCE AND KNOWLEDGE

Figures 3.1-3.7 shows the stated consumer preferences.

Figure 3.1



From the results in figure 3.1 above, 2% of the participants stated that they never consumed yogurts. 17.6% consumed yogurts at least twice a month, 37.3% consumed yogurts weekly and 16.7% consumed yogurts daily.

Figure 3.2.

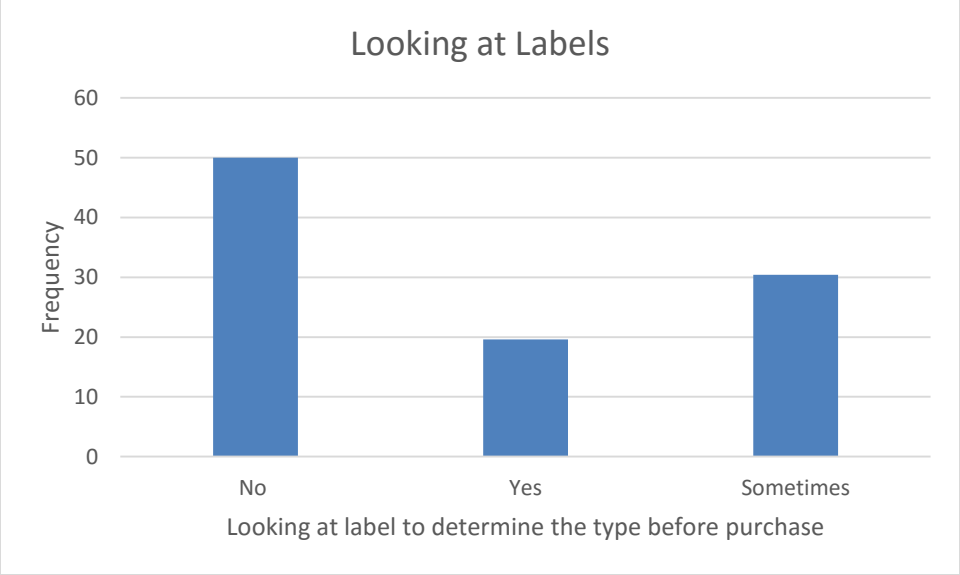
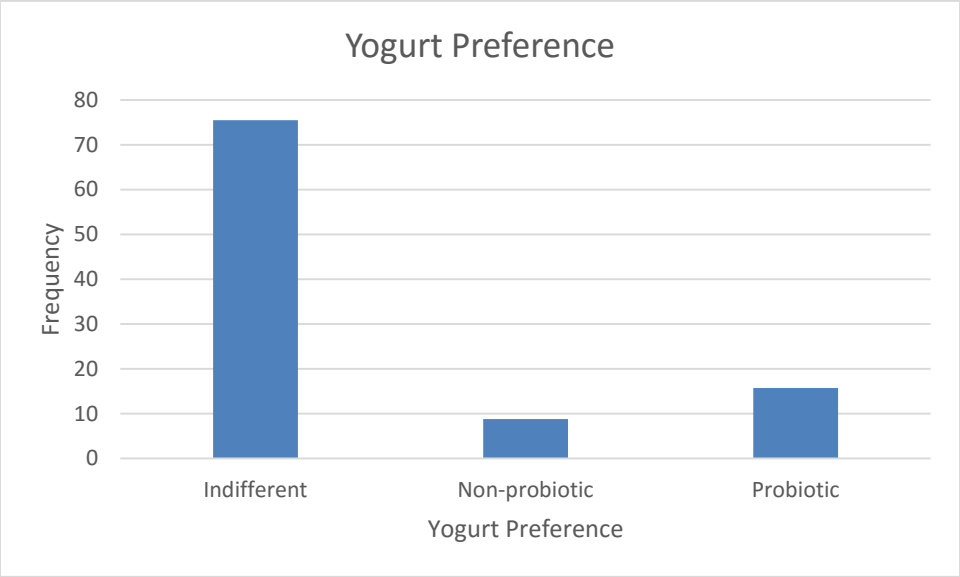


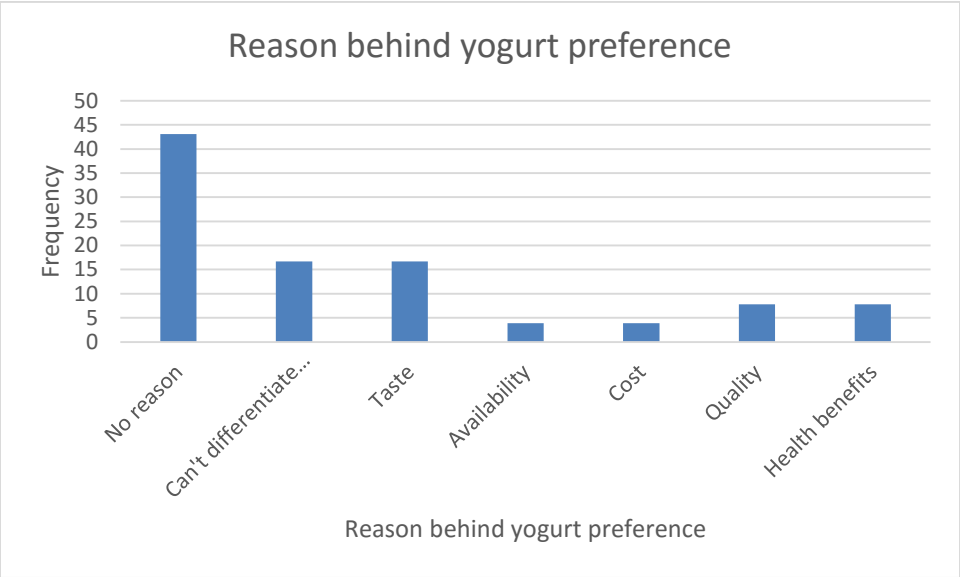
Figure 3.2 shows that 50% of the participants did not look at the label of a yogurt product before purchasing it. 30.4% sometimes looked at the label before purchase and 19.6% definitely looked at the label before purchasing a yogurt product.

Figure 3.3.



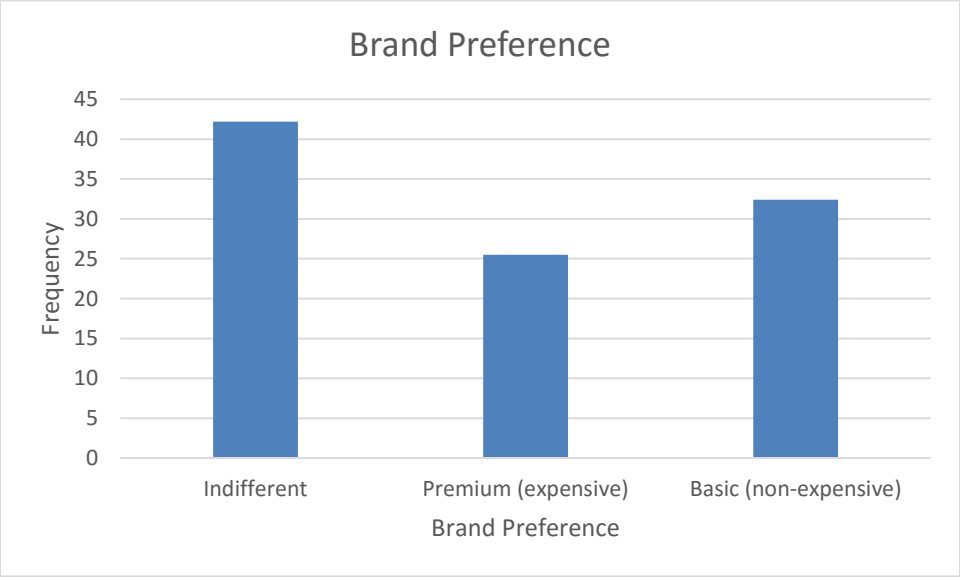
From the above result in figure 3.3, 75.5% of the participants were indifferent about their yogurt preference. 15.7% preferred probiotics, while 7.8% preferred non-probiotics.

Figure 3.4.



The above figure 3.4 shows that 43.1% of the participants had no reason for their yogurt preference. A few participants (16.7%) said their preference was based on taste whilst a similar number of participants (16.7%) couldn't differentiate between probiotic and non-probiotic yogurts. Only a small number (7.8%) said their choices was based on perceived health benefits. Other reasons given includes availability (3.9%), cost (3.9%) and quality (7.8%).

Figure 3.5.



The above results (figure 3.5) show that majority of the participants (42.2%) were indifferent about their brand preference and another significant group (32.4%) preferred the non-expensive brands. Only 25.5% of the participants preferred expensive brands.

Figure 3.6.

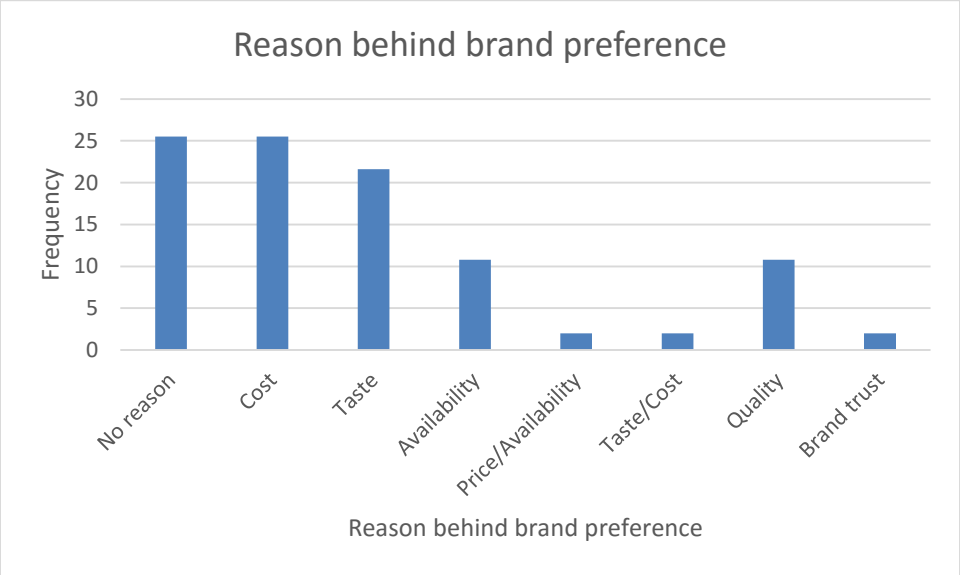
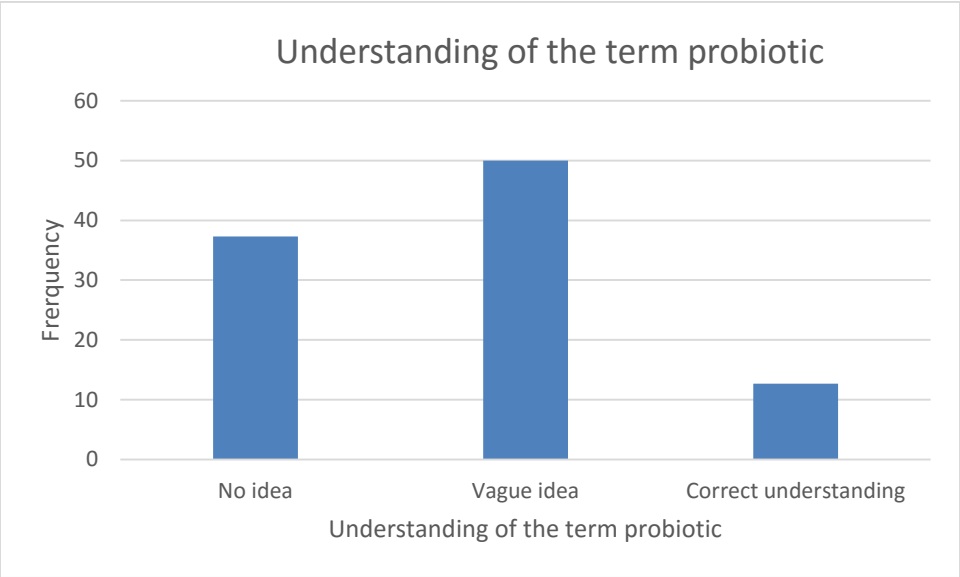


Figure 3.6 shows that 25% of the participants had no reason for their brand preference. 25.5% gave cost as their reason and 21.6% gave taste as their reason for choosing a particular brand. Other reasons given includes availability (10.8%), price/availability (2%), taste/cost (2%), quality (10.8%) and brand trust (2%).

Figure 3.7.



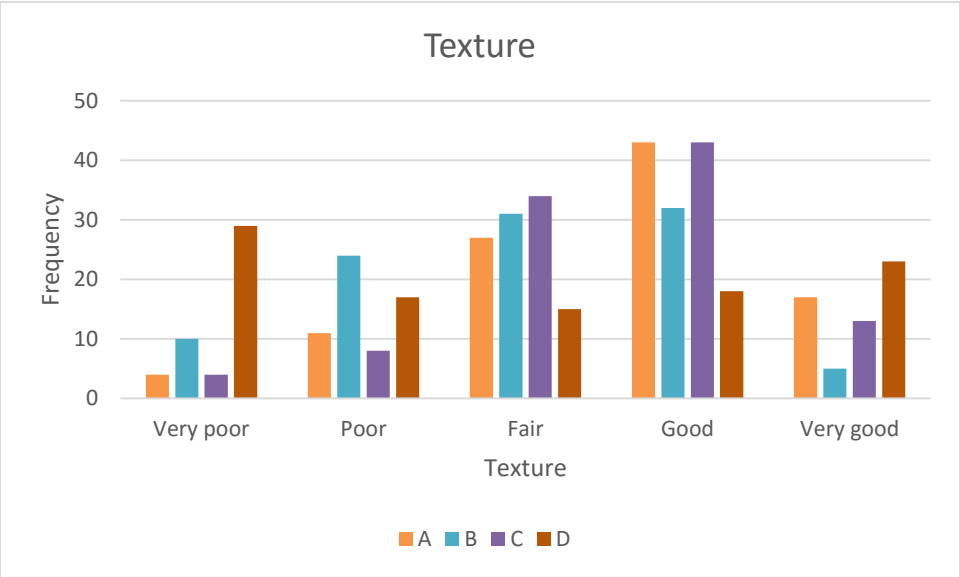
According to figure 3.7, 37.3% of the participants had no understanding of the term probiotics. 50% had a vague understanding of the term while only 12.7% had a correct understanding of the term.

From the results above (Figures 3.1-3.7), it is observed that many of the participants consumed yogurt weekly and that many did not look at the label before purchase of the said yogurts. It is also seen that many of the participants were indifferent about their yogurt and brand preference, however a lot preferred non-expensive brands and cost and taste were the primary reason for this preference. Also most of the participants had little or no idea about the meaning of probiotics.

3.1.3. SENSORY EVALUATION

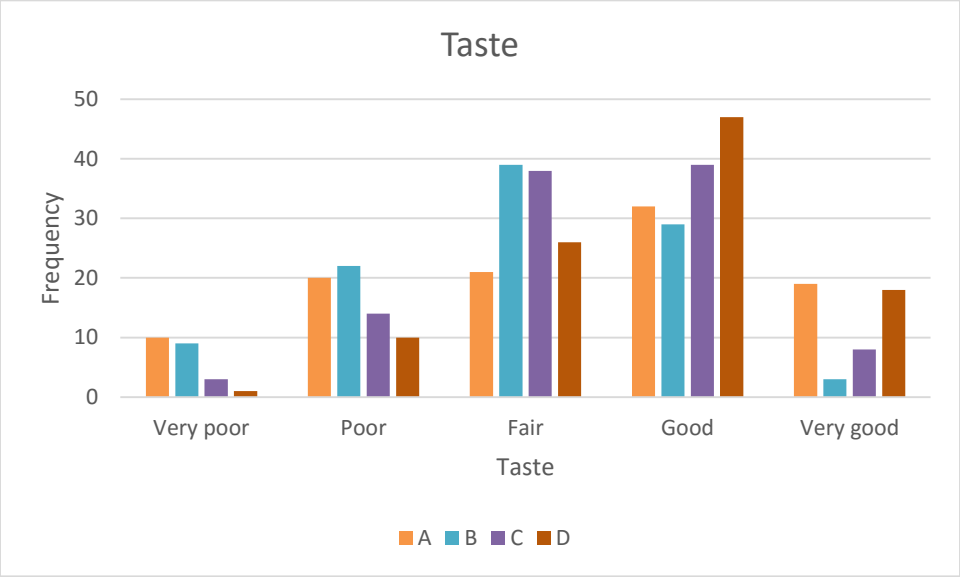
Figures 3.8-3.11 shows the frequency of texture, taste, appearance and overall quality of the yogurt samples.

Figure 3.8



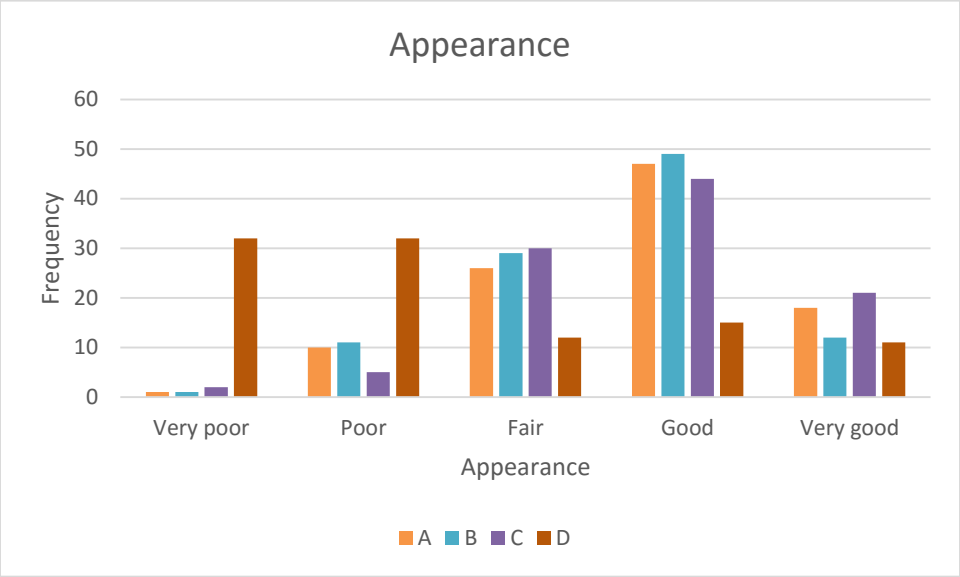
Summarizing the result above in figure 3.8 into poor and good, participants rated sample A as 14.7% poor or very poor and 58.8% good or very good. Sample B was rated 33.3% poor or very poor and 36.3% good or very good. Sample C was rated 11.8% poor or very poor and 54.9% good or very good, while sample D was rated 45.1% poor or very poor and 40.2% good or very good.

Figure 3.9



Summarizing the result in figure 3.9 above into poor and good, participants rated the taste of sample A as 29.4% poor or very poor and 50% good or very good. Sample B was rated 30.4% poor or very poor and 31.4% good or very good. Sample C was rated 16.7% poor or very poor and 46.1% good or very good, while sample D was rated 10.8% poor or very poor and 63.7% good or very good.

Figure 3.10



Summarizing the result in figure 3.10 above into poor and good, participants rated the appearance of sample A as 10.8% poor or very poor and 63.7% good or very good. Sample B was rated 11.8% poor or very poor and 59.8% good or very good. Sample C was rated 6.9% poor or very poor and 63.7% good or very good, while sample D was rated 62.8% poor or very poor and 25.5% good or very good.

Figure 3.11

Summarizing the result in figure 3.11 above into poor and good, participants rated the overall; quality of sample A as 16.7% poor or very poor and 52.1% good or very good. Sample B was rated 25.5% poor or very poor and 41.2% good or very good. Sample C was rated 11.8% poor or very poor and 51.1% good or very good, while sample D was rated 48% poor or very poor and 31.4% good or very good.

Figure 3.12-3.15 shows the results of participants on guessing which of the samples were probiotic, non-probiotic, expensive and non-expensive.

Figure 3.12.

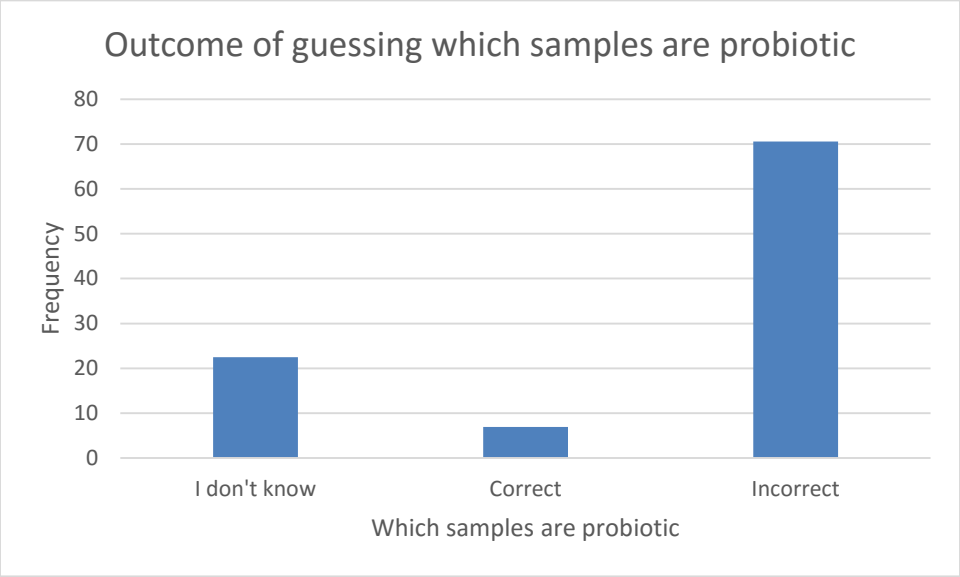


Figure 3.13.

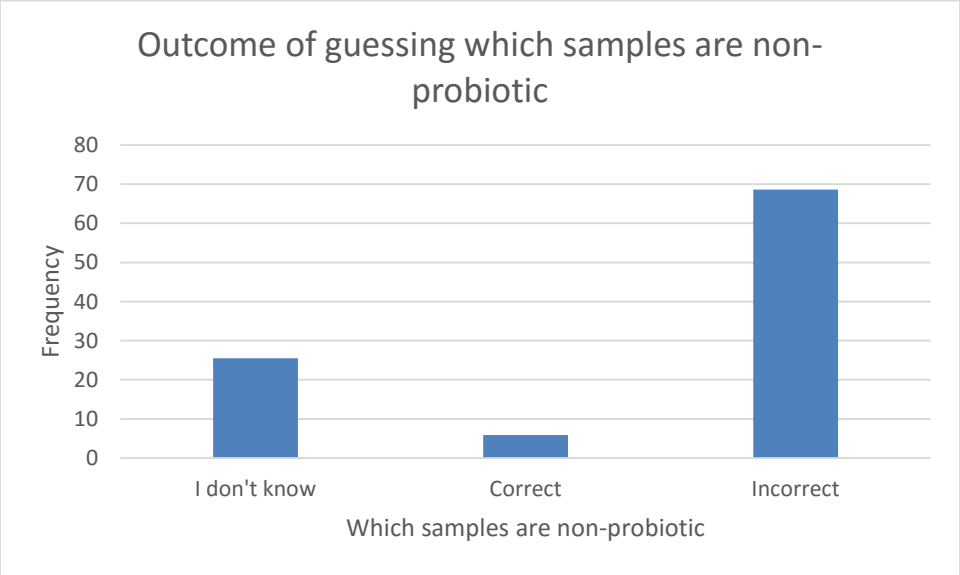


Figure 3.14

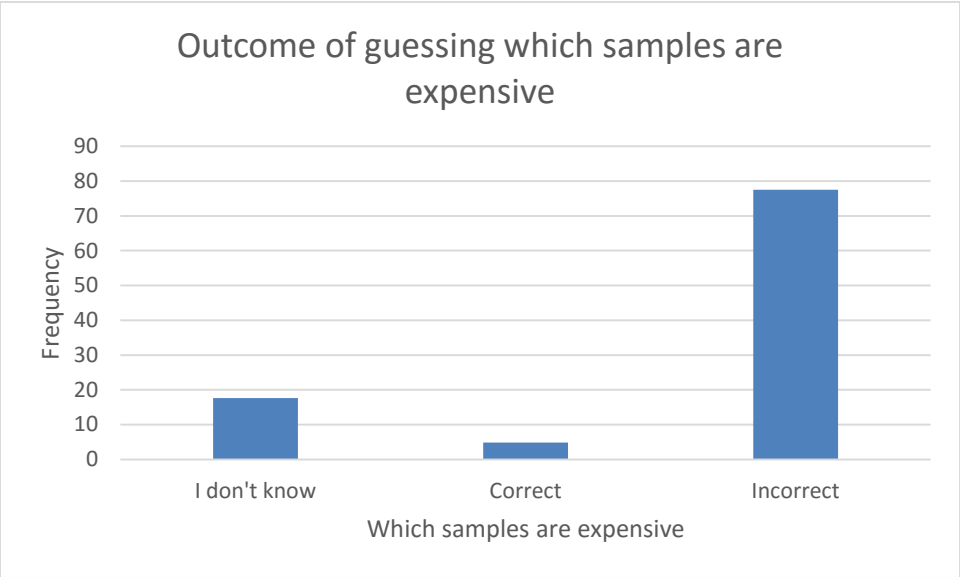
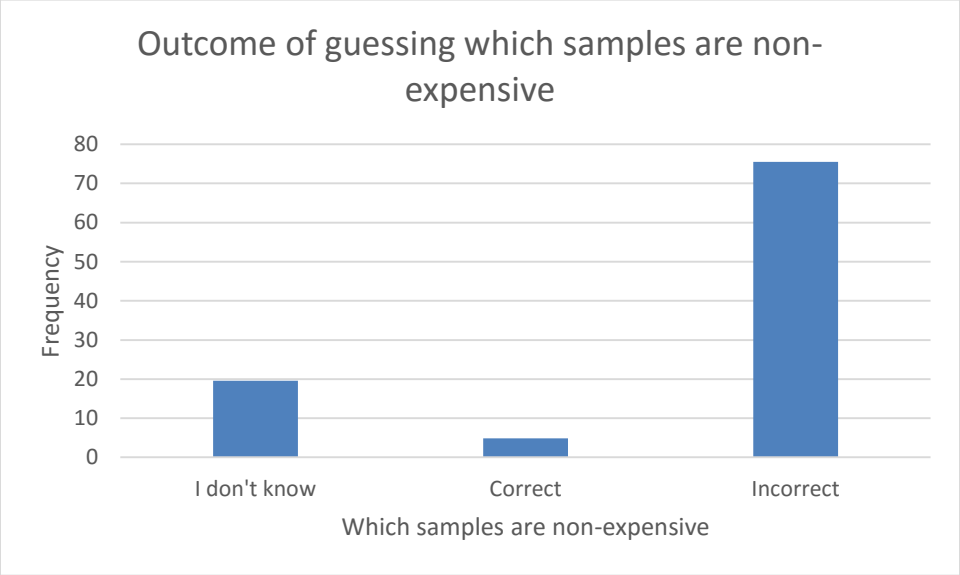


Figure 3.15.



The result (Figures 3.12-3.15) showed that most participants could not tell the difference between probiotic and non-probiotic yogurts neither could they differentiate between premium/expensive and basic/non-expensive brands.

Analysis to determine if there is any association between the consumer preference and the sensory evaluation was done using Chi square test but the result showed that there were no significant association between them. Table 3.2 shows the results.

Table 3.2. Chi square test for the association between yogurt or brand preference to the texture, taste, appearance and overall quality scores of the samples.

	Chi square significance (Yogurt preference)	**Chi square significance (Brand preference)**
Texture A	0.809	0.096
Texture B	0.024	0.438
Texture C	0.310	0.815
Texture D	0.301	0.085
Taste A	0.043	0.433
Taste B	0.108	0.067
Taste C	0.285	0.732
Taste D	0.118	0.391
Appearance A	0.952	0.778
Appearance B	0.364	0.996
Appearance C	0.952	0.979
Appearance D	0.651	0.801
Overall Quality A	0.442	0.787
Overall Quality B	0.059	0.463
Overall Quality C	0.239	0.207
Overall Quality D	0.358	0.962

Note: P value is $P<0.05$

The result showed that there was no significant association between preferred brand or yogurt type and the rating scores from the blind sensory evaluation analysis.

3.2. NUTRITIONAL ANALYSIS

Table 3.3-3.6 show the results of the nutritional composition of the yogurt in its wet form according to the labelling on the pack of the product, the nutritional composition of the products in its wet form after analysis in the laboratory, the nutritional composition of the reference material (skim milk), and the nutritional composition of the products after drying respectively.

The analysis were done in triplicates with the exception of the fat content as this was carried out in six replicates. The results shown below are the mean value.

Table 3.3. Nutrient composition per 100g of the yogurt (wet) according to the labelling on the pack.

Label	Product	Energy(KJ)	Protein(g)	Fat(g)	Calcium(mg)	Sodium(g)
Sample A	Basic non-probiotic	259KJ	4.7g	1.8g	N/A	0.05g
Sample B	Basic probiotic	258KJ	4.9g	1.5g	149mg	N/A
Sample C	Premium non-probiotic	520KJ	4.2g	9.5g	N/A	N/A
Sample D	Premium probiotic	285KJ	3.7g	3.7g	119mg	N/A

N/A- Not available

Table 3.4. Nutrient composition per 100g of the yogurt (wet) after analysis in the laboratory.

Product	Product	Energy	Protein	Fat	Calcium	Sodium	% MC	% TS	pH
Sample A	Basic non-probiotic	310KJ	4.6g	0.98g	1.97g	0.68g	86.5	13.6	4.40
Sample B	Basic probiotic	258.7KJ	4.5g	0.54g	2.12g	0.67g	87.5	12.5	4.31
Sample C	Premium non-probiotic	512.1KJ	3.3g	5.08g	1.03g	0.33g	81.4	18.6	4.19
Sample D	Premium probiotic	309.9KJ	3.8g	3.32g	1.51g	0.59g	87.2	12.8	4.30

MC- Moisture content, TS- Total solids

From table 3.4 above, there are differences between the results on the label and the results obtained in the lab. For energy of sample A, on the label it is 258KJ while after analysis in the lab, the result was 310KJ. For the rest of the nutritional compositions of the samples, there is a slight difference between the label values and the lab values.

Table 3.5. Nutrient composition of the Reference material (Skim milk) used (Mean ± Standard deviation)

Analysis	Certified value (g/100g)	Lab results
Crude protein	38.2	32.97±0.84
Fat	0.96	0.075±0.06
Sodium (Na)	4.19	3.48±0.07
Calcium (Ca)	13.9	12.59±0.02

According to table 3.5 above, there are differences in the results obtained in the lab compared with that which had been certified by the manufacturing company. The reason for this could be as a result of difference in methodology. However, most of the results obtained are very close to the certified value which shows that the authenticity of the methodology used. The fat content obtained in

the laboratory is significantly lower than the certified value and this therefore raises doubt about the authenticity of the method used for fat content.

Table 3.6. Nutrient composition of the dry products (Mean ± Standard deviation).

Product	Product	Energy	Protein	Fat	Calcium	Sodium	%MC	%TS	pH
Sample A	Basic non-probiotic	19991.04^{ab} ±713.6	32.2^{ab} ±0.1	6.8^{ab} ±0.3	13.7^{a} ±0.4	4.7^{ab} ±0.2	86.5^{abd} ±1.6	13.6^{abd} ±0.2	4.4^{abcd}±0.01
Sample B	Basic probiotic	20254.04^{ab} ±56.95	30.7^{ab} ±1.5	3.7^{ab} ±1.03	14.5^{b} ±0.4	4.6^{ab} ±0.1	87.5^{abd} ±0.1	12.5^{abd} ±0.1	4.3^{abcd} ±0.1
Sample C	Premium non-probiotic	27980.8^{c} ±205.3	18.9^{c} ±0.1	37.5^{c} ±3.3	7.6^{c} ±0.1	2.4^{c} ±0.1	81.4^{c} ±1.0	18.6^{c} ±1.01	4.3^{abcd} ±0.1
Sample D	Premium probiotic	24002.4^{d} ±56.95	26.5^{d} ±0.2	22.9^{d} ±4.02	10.3^{d} ±0.2	4.1^{d} ±0.3	86.6^{abd} ±1.2	12.8^{abd} ±0.03	4.3^{abcd} ±0.006

Note- Means with different subscripts within the same column are significantly different (P<0.05) using ANOVA and Post Hoc test.

From table 3.6 above, sample C and D has a higher fat content than sample A and B. For the energy content, sample A and B differ from sample C and D. The same is the case for protein and sodium contents. For the Moisture and Total solids composition, sample A, B and D are similar. Only sample C differ in composition. All the samples have similar pH compositions and they all differ in calcium compositions.

3.3. MICROBIOLOGICAL ANALYSIS

Total viable count was done using PCA (Plate count agar) and a serial dilution of 10^{-6}. Table 3.7 shows the Total viable count of the yogurt.

Table 3.7. Total viable count on PCA (10^{-6})

Product	**Product**	**1**	**2**	**3**	**Mean (cfu)**
Sample A	Basic non-probiotic	148	112	120	1.27×10^{-8}
Sample B	Basic probiotic	45	34	10	2.67×10^{-7}
Sample C	Premium non-probiotic	28	7	0	1.17×10^{-7}
Sample D	Premium probiotic	1	0	2	1.33×10^{-6}

CFU- Colony forming units

The table 3.7 above results shows the total amount of live organisms present in the products. The results show that the basic brands have more organisms present in them than the expensive brands.

Table 3.8 shows the growth of the samples on MRS, M17, and modified MRS agar.

Table 3.8. Growth of yogurt on MRS, M17, and modified MRS agar (10^{-6})

Product	**Product**	**MRS**	**M17**	**Modified MRS**
Sample A	Basic non-probiotic	+	+	+
Sample B	Basic probiotic	-	+	-
Sample C	Premium non-probiotic	-	+	-
Sample D	Premium probiotic	+	+	+

+ = Positive, - = Negative

From the above results in table 3.8, M17 showed positive growth on all the samples. MRS and modified MRS only showed positive growth on samples A and D. This is unexpected as all the samples are expected to have a positive growth on MRS agar as this medium is specific to *Lactobacillus* which is expected to be present in all the samples.

Sample A and D showed growth on both MRS and modified MRS agar plates. However samples B and C showed no growth. All samples showed a positive growth on M17 agar.

3.3.1. BIOCHEMICAL AND GRAM STAINING TESTS

The tables showing the results of the biochemical and gram staining tests can be found in appendix H. From the biochemical and gram staining results, the organisms identified were *Streptococcus* and *Lactobacillus* species. However, it was not confirmed if *Bifidobacterium* was present in the samples.

CHAPTER FOUR

DISCUSSION

This research aimed at comparing probiotic and standard yogurts based on branding, nutritional analysis, microbiological analysis, consumer preference and sensory analysis. All the analysis stated above were done and the results obtained were compared with previous research done but due to limited research done in this field, this research importance is further emphasized. The results obtained are further discussed below

4.1. CONSUMER PREFERENCE

From the results of Figure 3.1 above, a large number (26.5%) of participants rarely consumed yogurts and another significant group (37.3%) consumed yogurts weekly. A lot of participants (50%) according to Figure 3.2 also did not look at labels before purchasing yogurt products and this shows that most consumers buy what they like or what they are attracted to rather than for the sake of the nutritional value. This was further confirmed by the participant's yogurt and brand preferences. 75.5% of the participants were indifferent about their yogurt and brand preference (Figure 3.3 and 3.5), 15.7% preferred probiotics while 7.8% preferred non-probiotic yogurts. Several reasons were given for the above choices (Figure 3.4 and 3.6) while some of the participants had no reason for their yogurt and brand preference. Inability to differentiate between probiotic and non-probiotic yogurts was a significant reason (16.7%) for the participant's yogurt preferences and this was further established in the sensory evaluation as most of the participants could not tell the difference between probiotic and non-probiotic yogurts as well as expensive and non-expensive yogurts (Figure 3.12-3.15). Another major reason for brand and yogurt preference was cost (29.4%), availability (14.7%) and taste (38.3%) and this is in-line with Van Loo et al findings (Van Loo, Diem et al. 2013) which showed that cost, taste, and availability are important to consumers as these are the main factors that influence their decision for purchase of a particular yogurt. Cost being part of the major reasons for brand and yogurt preference could be because most of the participants were students who depended on their parents for their source of income and as such pricing was essential. Another reason could be because most non-probiotic yogurts cost less than the probiotic ones.

Other reasons given for the participant's yogurt and brand preference included quality, brand trust and health benefits. Only 7.8% of the participants choose health benefits as their reason for their yogurt preference and this suggests that most of the participants do not value the importance of the potential health benefits that probiotic yogurts provide. This also suggests that many of the participants may not understand the health benefits of probiotic yogurts and this fact was also established in the results of the 'Understanding of the term probiotic' question as majority of the participants had little or no idea of the meaning of probiotics (Figure 3.7). If the participants understood the health benefits of probiotic yogurts, it is likely that more might express a preference for it. As stated above, Figure 3.7 showed that majority of the participants (87.3%) had little or no understanding of the term 'probiotic' and only a few (12.7%) had a correct understanding of the term. As a matter of fact, when the participants were asked verbally about their understanding of probiotics, most indicated they had never heard the term before and as such had no idea what it meant. This suggests that more awareness need to be carried out to inform consumers especially the young adults about the importance of probiotics to the body and also what a great source of probiotic yogurts are as more people know about it, the more likely people are to purchase probiotic yogurts hence further information and education programs for consumers would be beneficial in this area.

The results of the consumer preference partially nullifies the hypothesis which says that consumers understand the meaning of probiotics and its significance and that this influences their yogurt choices. This is so because from the results, only a few understood the meaning of probiotics and the major factors that influenced consumer choice of yogurts were cost, availability and taste.

4.2. SENSORY EVALUATION

From the results, sample D (expensive probiotic) had the lowest rating in terms of overall quality, texture, taste and appearance. The remaining samples A, B, and C were mostly rated as good in terms of overall quality, texture, taste, and appearance. However the non-probiotic (A and C) samples were rated higher than the probiotic (B and D) samples in terms of texture, taste, appearance, and overall quality. This is in contrast to the findings of Hussain et al. (Hussain, Attiq-ur-Rahman, et al. 2009) which showed that probiotic overall acceptability was higher than non-probiotic overall acceptability that the reverse is the case in this research. This could be as a

result of the difference in the particular brands of samples or ingredients used in the manufacture of the samples.

From the results (Figure 3.11), the score of the overall quality of the non-expensive, non-probiotic sample (A) rated good was 53.1% while that of the expensive non-probiotic sample (C) was 51.1%. The score of the overall quality of the non-expensive, probiotic sample (B) rated good was 41.2% while that of the expensive probiotic sample (D) was 31.4%. This shows that for the non-probiotic product, the overall acceptability of the expensive brand is lower than the non-expensive one. For the probiotic product, the overall acceptability of the expensive brand is also lower than the non-expensive brand. This shows that the price of a product does not necessarily determine a higher quality of the product when compared to cheaper ones. These results are in line with the findings of Mendez et al. who determined that there were no differences in objective quality between manufacturer and store brands with regards to technologically less complex categories like food and drinks (Mendez, Oubina et al. 2008). However in terms of overall quality, sample D (expensive probiotic) was rated the poorest by 48% and sample A (cheap non-probiotic) was rated the best by 53.1%. This shows that the participants preferred the non-probiotic non-expensive yogurt sample over the expensive probiotic sample.

In terms of Taste (Figure 3.9), sample D (expensive probiotic) was the most preferred as it had the highest rating of 63.7% while sample B (non-expensive probiotic) was rated the least preferred by 30.4%. This suggests that the expensive probiotic had a better taste than the non-expensive one. The expensive non-probiotic sample (C) was rated 46.1 % good and the non-expensive non-probiotic sample (A) was rated 50% good by the participants. The above result suggest that the taste of a yogurt sample vary irrespective of the probiotic content or the cost. The taste invariably is a product of the manufacturing process and ingredients used.

For Appearance (Figure 3.10), sample A and C (non-expensive non-probiotic and expensive non-probiotic yogurts respectively) had the highest positive ratings of 63.7% good. Sample B (non-expensive probiotic) was the next best with 59.8% good. Sample D (expensive probiotic) however had the worst ratings with 62.8% poor. This results suggests that the participants thought that there were differences in the appearance of the different yogurts. The appearance of the yogurts is a factor of the manufacturing process as the cultures present in yogurts affect the

taste, aroma, and nutrient compositions of the yogurts rather than the appearance (Ranathunga, Rathnayaka 2012).

For the Texture (Figure 3.8), sample A (non-expensive non-probiotic) had the highest positive ratings of 58% good and sample C (expensive non-probiotic yogurts) had the second best rating with 54.9% good. Sample B (non-expensive probiotic) was rated 36.3% good and 33.3% poor by the participants. Sample D (expensive probiotic) was also rated 40.2% good and 45.1% poor by the participants. This suggests that for samples B and D (probiotics), the view on texture was varied depending on the participant's preference as some liked the texture while others did not. However the non-probiotic yogurts were preferred to the probiotic ones by a larger number of participants. According to the results, the non-probiotic yogurts had a better thickness than the probiotic yogurts. This result differs from the result by Olugbuyiro et al. which says that probiotic yogurts have greater viscosity/texture than non-probiotic ones (Olugbuyiro, Oseh 2011).

The result also showed that most participants could not tell the difference between probiotic and non-probiotic yogurts neither could they differentiate between premium/expensive and basic/non-expensive brands. These findings show that although there are differences between the probiotic and non-probiotic yogurts, these differences could not help the participants determine which were probiotic or non-probiotic. This means that although the addition of probiotics to yogurt provide certain health benefits (Koop-Hoolihan 2001; O'Brien, Crittenden et al. 1999; Klaenhammer, Kullen 1999), these organisms seem to have little effect on the taste of the yogurts hence the inability of consumers to differentiate between probiotic and non-probiotic. Some research has shown that probiotic yogurt can have greater viscosity/thickness/texture than non-probiotic ones (Olugbuyiro, Oseh 2011), but this research shows that most consumers did not prefer the texture of the probiotic yogurts compared to the non-probiotic yogurts and may not know that this could be a way to differentiate them. This shows that manufacturers and marketers of yogurts should inform the public of this difference and this could be done using visual advertisements e.g. posters and TV commercials.

The results however prove the hypothesis which states that 'That there is a significant difference between probiotic and natural yogurt in terms of texture, taste, appearance, and overall quality and this difference is easily noticeable'. This is because the results show that the various yogurt

samples are different in texture, taste, appearance and overall quality however consumers do not know that these differences can be used to differentiate between probiotic and natural yogurts.

Cross examination was done using a Chi square test in SPSS to determine if there was a relationship between people's preference (brand and yogurt preference) and the sensory evaluation carried out. The test was to determine if the same person that preferred a particular brand (expensive or non-expensive) or yogurt (probiotic or non-probiotic) also preferred that brand in the blind sensory evaluation. The result showed that there was no relationship between the preference of a participant in the consumer analysis and their preference in the sensory evaluation. This proves that the participants could not tell the difference between the different yogurt types and that their choices was purely psychological i.e. their choices could have been influenced by what they'd seen, heard, or generally perceived of a particular brand or yogurt type. Probiotic yogurts should be promoted more by manufacturers and food regulatory bodies and it should also be packaged properly in such a way that it attracts consumers.

4.3. NUTRITIONAL ANALYSIS

From the result in Table 3.4, the probiotic yogurts have a lower pH than the non-probiotic ones. This finding is different from the results of Hussain et al. and Olugbuyiro et al. (Hussain, Attiq-ur-Rahman, et al. 2009; Olugbuyiro, Oseh 2011) which showed probiotic products having a higher pH than non-probiotic products. This could be as a result of the varying composition of the products and shelf life. The result also shows that there is no difference between the expensive and non-expensive yogurts pH. The results are however in line with the FDA specifications for the pH of yoghurt (4.6 or lower) (Olugbuyiro, Oseh 2011). The moisture content (MC) of sample B and D (probiotic) was 87.5 and 87.2 respectively, while sample A and C (non-probiotic) had a moisture content of 86.5 and 81.4 respectively. This shows that the non-probiotic yogurts had a higher thickness than the probiotic yogurts. The total solids (TS) results showed that the probiotic samples B and D had lower TS (12.5 and 12.8 respectively) than the non-probiotic samples A and C with TS 13.6 and 18.6 respectively. This is expected as samples B and D were rated to have lower texture than samples A and C. According to table 3.6, there

was however no difference between the MC and TS of A, B and D. Only sample C had a significant difference when compared to the rest of the samples.

For the fat content (FC), according to the result in Table 3.4, sample A contains 0.98g of fat and sample B, C and D contain 0.54g, 5.08g and 3.32g of fat respectively. According to the labeling on the pack of the samples, sample A and B were labelled 'low-fat' yogurt while sample C and D were labelled just 'yogurt'. This is in accordance with the specifications of USDA 2001 and the UK Dairy Council 2015 which states that a yogurt with less than 0.5 fat content be labelled non-fat, yogurt with fat content ranging from 0.5-2.0 be labelled low-fat and yogurt with fat content of 3.25 and above be labelled yogurt (Olugbuyiro, Oseh 2011; The Dairy Council, 2015). The USDA standard was used because there are no European standards for yogurts available. According to table 3.6, there was a significant difference between the expensive (C and D) and non-expensive (A and B) brands. This is expected as samples C and D are whole yogurts while samples A and B are low-fat yogurts.

However there was a significant difference in the result of the fat content when analyzed and the result put on the label of the yogurt samples. This was also the case for the reference material used to validate the methodology used to determine the fat content. This suggests that the methodology used (Soxtec) to calculate the fat content was probably inaccurate. A more accurate method such as 'Gerber' could be used in the future.

For the protein content according to table 3.4, sample A, B, C, and D are 4.6g, 4.5g, 3.3g, and 3.8g respectively. This shows the protein content for the non-expensive brand of yogurt (A and B) to be higher than that of the expensive brand (C and D). However there was a significant difference in the result of the protein content when analysed and the result put on the label of the yogurt samples. According to table 3.6, there was a significant difference between the expensive (C and D) and non-expensive (A and B) brands. This results differs from that of Cano-Sancho et al. which showed no significant difference between the different brands of products (Cano-Sancho, Perelló et al. 2015). This could be as a result of the fat content as the samples A and B are low fat while samples C and D are whole yogurts. The energy content of the various yogurt samples after laboratory analysis also differed from that of the labelling and according to table 3.6, there was a significant difference between the expensive (C and D) and non-expensive (A

and B) brands. This could also be as a result of the fat content as the samples A and B are low fat while samples C and D are whole yogurts.

According to Table 3.4, the calcium and sodium content of the samples when analysed were different from the label. According to Table 3.6, there was a significant difference in the calcium content of all the samples when compared to each other. For sodium content, there was a significant difference between the expensive (C and D) and non-expensive (A and B) brands. This results differs from that of Cano-Sancho et al. which showed no significant difference between the different brands of products (Cano-Sancho, Perelló et al. 2015). However, a reason the results differ from that of Cano-Sancho et al. could be as a result of the different fat contents of the samples.

The reason behind the differences between the label value and the lab values of the nutrient composition of the products could be as a result of different methodologies used to determine the nutrient compositions. It could also be due to the drying of the yogurt as some of the nutritional compositions might be lost during the process of drying as in the case of the fat content. Another reason could be from the manufacturing process. Most of the labels used for the yogurt products have been manufactured a lot longer than the batch of yogurts put in the package hence that particular batch could have a nutrient content which is in contrast to the label.

4.4. MICROBIOLOGICAL ANALYSIS

Total viable count: According to Table 3.7, Sample A had the most microbial count (1.27×10^{-8} cfu/ml), while Sample D had the least count (1.33×10^{-6} cfu/ml) and this could be as a result of the dilution factor used as 10^{-6} seemed to be too high. Comparing between the non-probiotic and probiotic yogurt samples, Sample A and C (non-probiotic) had more count (1.27×10^{-8} and 1.17×10^{-7} cfu/ml respectively) than the probiotic samples B and D (2.67×10^{-7} and 1.33 cfu/ml respectively). Comparing the expensive and non-expensive brands, Sample A and B (non-expensive) had more counts (1.27×10^{-8} and 2.67×10^{-7} cfu/ml respectively) than the expensive brands C and D (1.17×10^{-7} and 1.33×10^{-6} cfu/ml respectively). However the above results may not contain the entire levels of organisms present in the samples because the analysis was done

aerobically and organisms like *Bifidobacterium* would not grow in aerobic conditions and as such might be unaccounted for. According to the National Yogurt Association standards of 2013, for refrigerated yogurt, the total viable count at the time of manufacture must be at least 10^8 CFU per gram. In the case of frozen yogurt, the total viable count at the time of manufacture must be at least 10^7 CFU per gram (National Yogurt Association, 2013). Although the TVC for this research was not done at the time of the manufacturing of the products, the results show that the products analyzed conform to the National Yogurt Association standards at the time of testing at least for the total viable organisms present.

According to the labelling on the yogurt samples analyzed, the following organisms were expected to be present in the yogurt samples;

- Sample A (non-expensive non-probiotic): Not specified. However, generally manufacturing processes would suggest *Lactobacillus bulgaricus* and *Streptococcus thermophiles* was most likely.
- Sample B (non-expensive probiotic): *Bifidobacterium*, *Lactobacillus acidophilus*, and *Streptococcus thermophiles*.
- Sample C (expensive non-probiotic): Not specified. However, generally manufacturing processes would suggest *Lactobacillus bulgaricus* and *Streptococcus thermophiles* was most likely.
- Sample D (expensive probiotic): *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*.

MRS Growth: Only samples A and D showed growth on this agar while Samples B and C did not grow even after incubation for 72 hours. This is unexpected as *Lactobacillus* is expected to grow on all the samples since *Lactobacillus* is also used as a starter culture. This could be as a result of the *Lactobacillus* originally present in the yogurt dying due to the shelf life. *Lactobacilli* are generally characterized as gram-positive, non-spore forming, non-motile rods or coccobacilli. Biochemical tests and gram staining confirmed the presence of *Lactobacillus* spp. in samples A and D

M17 Growth: This agar favors the growth of *Streptococcus* while inhibiting the growth of *Lactobacillus*. All the yogurt samples showed growth on the M17 agar plates. This is expected as

streptococcus is a starter organism which is added to both probiotic and non-probiotic yogurt. Biochemical tests and gram staining carried out confirmed the presence of *Streptococcus* spp. in all the samples. However, *Lactobacillus* spp. was also found growing on M17 agar from samples C and D. This could be as a result of the agar favoring the growth of some species of *Lactobacillus*.

Modified MRS Growth: Only samples A and D showed growth on this agar while Samples B and C did not grow even after incubation for 72 hours. This could be as a result of two major reasons;

1. *Bifidobacterium* originally present in Sample B as a result of it's being probiotic had low shelf life and died as a result of the storage and manufacturing processes.
2. The technique used in the isolation of the *Bifidobacterium* specie from the yogurt was wrong. The organisms were not incubated anaerobically which is the usual incubation environment for *Bifidobacterium*. This was as a result of lack of anaerobic facilities during the experiment.

Another reason could be because of the dilution factor used (10^{-6}) as might have been too high for the enumeration of *Bifidobacterium* however, the extensive dilutions carried out before the 10^{-6} dilution was used makes this unlikely.

However, Sample C showing no growth is not surprising as this modified MRS agar should only support the growth of *Bifidobacterium* and Sample C is a non-probiotic yogurt. Sample D showed growth on the agar and this is expected as it is a probiotic yogurt and *Bifidobacterium* was stated as present on the label. Bifidobacteria are generally characterised as gram-positive, non-spore forming, often branched rods, non-motile, catalase-negative anaerobes (Charteris, Kelly et al. 1997). Gram staining and biochemical tests carried out confirmed the presence of *Lactobacillus* spp.

Sample A showed growth on this agar and this is unexpected because it is a non-probiotic yogurt and *Bifidobacterium* is generally only used in probiotic yogurt (as a probiotic organism). This could be a false positive as although modified MRS agar should inhibit the growth of *lactobacillus*, *lactobacillus* could have managed to grow. Another reason could be that there

could have been cross contamination from the manufacturing process or that the wrong yogurt was packed into specific pots e.g. Non-probiotic yogurts were packed into probiotic packages.

This analysis however could not tell if there was any *Bifidobacterium* present due to some reasons. Firstly, *Bifidobacterium* species are strict anaerobes (Mombelli, Gismondo 2000) and the test was carried out aerobically due to the unavailability of anaerobic equipment at the time of testing. Secondly, *Bifidobacterium* selective medium was not used to culture the samples and this media is selective to *Bifidobacterium*. Modified MRS Agar was used instead and this media is not officially vetted although it had previously been used successfully for culturing of *Bifidobacterium* spp. (http://www.researchgate.net/post/Can_I_get_Bifidobacteria_to_grow_in_MRS).

Probiotic organisms present could not be identified for certain as the identification was not to the species level. *Lactobacillus* was confirmed in the samples A and D but it was not determined if it was *Lactobacillus acidophilus* or *bulgaricus*. If the probiotic organisms present in the samples at the time of manufacture were no longer present as at the time of the analysis, it shows that the samples were no longer probiotic as at the time they were purchased. This could be as a result of low shelf life and their viability not maintained throughout storage. According to Kailasapathy et al. the efficacy of the probiotic added depend on viability being maintained throughout storage, inoculum level, product's shelf life and survival of the organism in the gut environment (Kailasapathy, Harmstorf et al. 2008). The viability of probiotics in yogurts depend on some factors such as; the yogurt starter cultures used and the interaction between the species present, strain of the probiotic used, culture conditions, availability of nutrients, oxygen content, sugar concentration, pH of the yogurt (post-acidification during storage) etc. (Kailasapathy, Harmstorf et al. 2008; Dave, Shah 1997). Any of the factors mentioned above could have affected the viability of the probiotic organisms present in the samples.

Other organisms are however suspected in the samples. *Bacillus* spp. is suspected in all the samples. This is because of some of the organism's isolated testing positive for catalase test. The organisms were also motile and spore forming (Mahon, Lehman et al. 2014). If this organism (*Bacillus*) is present in the samples, it could be as a result of contamination either during the

analysis or during the manufacture of the products. The presence of *bacillus* indicates soil or fecal contamination of the samples. Some strains of this organism causes infections and diseases in the host.

In summary, *Lactobacillus* and *Streptococcus* were confirmed in all the samples but *Bifidobacteria* was not for several reasons stated above. However only the genus was confirmed and not the particular specie of the organisms due to the level of analysis carried out on the yogurt samples. It is therefore advised that further tests, e.g. PCR should be carried out to identify the specie level of the organisms.

4.5. CONCLUSION AND RECOMMENDATION

The aim of this research was to compare probiotic and standard yogurts based on branding (premium and basic brands), microbiological analysis, nutritional analysis, consumer preference and sensory analysis and this have been achieved.

The research partially proves the hypothesis that 'there is a significant difference (microbiological, nutritional and sensory quality) between premium and basic brands of yogurts and also probiotic and non-probiotic yogurts' but it depends on the individual product and not necessarily on the quality, nutritional or sensory properties as those could differ depending on the individual product. Also the microbiological property of the yogurt product is what defines the product as either probiotic or non-probiotic however, there is no significant difference between premium and basic yogurts in terms of microbiological, nutritional or sensory quality.

The research also partially proves the hypothesis that 'there is a significant difference between probiotic and natural yogurt or basic and premium yogurts in terms of texture, taste, appearance, and overall quality and this difference is easily noticeable'. This is because the research indicates that although there are slight differences in sensory characteristics of probiotic and non-probiotic yogurts, people can rarely spot the difference between expensive/non-expensive brands and probiotic/non-probiotic products. This shows that most preference for either category is mostly psychological. The research also shows that there was no association between the consumer preferences and the sensory evaluation tests. This shows that consumer choices are mostly influenced by external and psychological factors rather than quality or sensory properties of the yogurt products.

The research also shows that a lot of the participants had little or no idea about the meaning of probiotics and that some of the reasons that influences their choice of yogurt includes cost, taste, and availability, and this nullifies the hypothesis which states that 'consumer's understand the meaning of probiotic and its significance and that this influences their yogurt choices'. It is recommended that better awareness of the importance of probiotics should be done to enlighten the consumers as most are unaware. This could be done by posters, television and radio advertisement, and online awareness ads. Probiotic yogurts should also be sold at reasonable prices to promote sales.

The results of the nutritional composition of the products nullify the hypothesis that the addition of probiotic cultures influences the nutritional composition of the products. This is because the results show that each product has varying nutritional compositions irrespective of the probiotic cultures and brands of the yogurt. This suggests that the nutritional composition of yogurts depends on the manufacturing process rather than on the live cultures present or on the price of the yogurt product. The results however nullifies the hypothesis that 'the fat content of a yogurt product affect the choices and preference of consumers' as it shows that there is no relationship between the consumer preference/choice of the products and the fat content of the products as the participants showed no preference for either the low-fat or the whole yogurt samples.

This research nullifies the hypothesis which states that 'the commonly available yogurts in retail stores contain the stated organisms and nutritional composition (written on the label) in the product and in the appropriate amount' as the research shows that the nutritional content of the yogurt product on analysis differ from that on the label. However, the yogurts contained the said organisms with the exception of *Bifidobacterium* as this organism was not confirmed. Most of the products conformed to the specified standards stated by the National Dairy Association for total viable count. However, some (sample D) of the live cultures including the probiotic ones were no longer present as at the time of consumption and this could be as a result of the organisms not having the capacity to survive over the product shelf life or inadequate storage facilities. It is however recommended that the yogurt products be stored at 2-4^{0}C (35-45 days), -18 to -23^{0}C (1 month), or -29^{0}C (3-5 months) although it is not guaranteed that freezing would not affect the probiotic cultures present in the yogurts. Yogurts should not be stored for more than 5 months as this could result in degradation of the product (WFLO Commodity Storage

Manual 2008). It is also recommended that the cultures used for production have a longer shelf life to ensure that the organisms are still present at the time of consumption so as to have the necessary effect on the consumer.

Yogurt samples should also be adequately pasteurized to remove all pathogens and this should be done before fermentation to prevent the killing of the live cultures. Food handlers should also be health and safety conscious to prevent the risk of contamination of the products.

4.6. LIMITATIONS TO THIS RESEARCH

This research has a few limitations. The first is the limited number yogurt products used in this research. The results should only be interpreted within the specific frame of its sample size, product (yogurts) size and geographic region as generalization to a broader public remain to be validated. Another limitation is that there is limited availability of other researches that combines results for both nutritional and microbiological composition of probiotic and natural yogurt and compares them with the sensory evaluation and consumer preference. There is also limited research that combines the results of sensory properties and consumer preferences as most perform one or the other and this results in having very limited research to compare findings with and. However this makes this research novel one and also a strength as this emphasizes the importance of this research.

Another limitation is the inability to identify the organisms to specie level. This could be done by using PCR techniques, Genotyping and use of other selective and differential mediums to identify the organisms to their specie levels (Charteris, Kelly et al. 1997; Yeung, Sanders et al. 2002; Pyar, Liong et al. 2014), however this is beyond the scope of this research.

4.7. FURTHER STUDY

Further studies should be done in comparing premium and basic brands of yogurt in terms of quality to test the hypothesis that there is no significant difference in terms of quality. This is because very limited research has been done both in this study and by other researchers and as

such no claim can be made. The studies should be done on a wider scale so as to render the result authentic.

Further studies should also be done with participants testing the ability to differentiate between probiotic and non-probiotic yogurts based on thickness, and quality as this would provide a more definite result and tell if this could be a definite way of differentiating them. The test should be done on a wider scale and with different yogurt products both commercially and laboratory manufactured.

REFERENCES

Abidin, S.Z., Effendi Raja Ahmad, Azmeer Raja Ahmad, Ibrahim, R. & Idris, M.Z. 2014, "A Semantic Approach in Perception for Packaging in the SME's Food Industries in Malaysia: A Case Study of Malaysia Food Product Branding in United Kingdom", *Procedia - Social and Behavioural Sciences*, vol. 115, no. 0, pp. 115-130.

Abrams, K.M., Evans, C. & Duff, B.R.L. 2015, "Ignorance is Bliss. How Parents of Preschool Children Make Sense of Front-of-Package Visuals and Claims on Food", *Appetite*, vol. 87, no. 0, pp. 20-29.

Adolfsson, O., Meydani, S.N. & Russell, R.M. 2004, "Yogurt and Gut Function", *The American Journal of Clinical Nutrition*, vol. 80, pp. 245-256.

Ailawadi, K.L. & Keller, K.L. 2004, "Understanding Retail Branding: Conceptual Insights and Research Priorities", *Journal of Retailing*, vol. 80, no. 4, pp. 331-342.

Ajmal, S. & Ahmed, N. 2009, "Probiotic Potential of Lactobacillus Strains in Human Infections", *African Journal of Microbiology Research*, vol. 3, no. 12, pp. 851-855.

Allgeyer, L.C., Miller, M.J. & Lee, S. 2010, "Sensory and Microbiological Quality of Yogurt Drinks With Prebiotics and Probiotics", *Journal of dairy science*, vol. 93, no. 10, pp. 4471-4479.

Arslanagić, M., Peštek, A. & Kadić-Maglajlić, S. 2013, "Perceptions of Healthy Food Packaging Information: Do Men and Women Perceive Differently?", *Procedia - Social and Behavioural Sciences*, vol. 109, no. 0, pp. 78-82.

Ashraf, R. & Shah, P.N. 2011, "Selective and Differential Enumerations of *Lactobacillus Delbrueckii* Subsp. *Bulgaricus*, *Streptococcus Thermophilus*, *Lactobacillus Acidophilus*, *Lactobacillus Casei* and *Bifidobacterium* Spp. in Yogurt- A Review", *International journal of food microbiology*, vol. 149, pp. 194-195-208.

Aureli, P., Capurso, L., Castellazzi, A.M., Clerici, M., Giovannini, M., Morelli, L., Poli, A., Pregliasco, F., Salvini, F. & Zuccotti, G.V. 2011, "Probiotics and Health: An Evidence-Based Review", *Pharmacological Research,* vol. 63, no. 5, pp. 366-376.

Auttarapoag, D. 2011, "Package Design Expert System Based on Relation between Packaging and Perception of Customer", *Procedia Engineering,* vol. 32, no. 0, pp. 307-314.

Babalis, A., Ntintakis, I., Chaidas, D. & Makris, A. 2013, "Design and Development of Innovative Packaging for Agricultural Products", *Procedia Technology,* vol. 8, no. 0, pp. 575-579.

Bajaj, J. S., M.B.B.S., M.D., M.S., Saeian, K., M.D., M.S., Christensen, K.M., P.A.-C., Hafeezullah, M., M.B.B.S., Varma, R.R., M.D., Franco, J., M.D., Pleuss, J.A., R.D., Krakower, G., Ph.D., Hoffmann, R.G., Ph.D. & Binion, D.G., M.D. 2008, "Probiotic Yogurt for the Treatment of Minimal Hepatic Encephalopathy", *American Journal of Gastroenterology,* vol. 2008, no. 103, pp. 1707-1715.

Bhardwaj, A., Puniya, M., Sangu, K.P.S., Kumar, S. & Dhewa, T. 2012, "Isolation and Biochemical Characterization of Lactobacillus species Isolated from Dahi", *A Journal of Dairy Science and Technology,* vol. 1, no. 2, pp. 1-2-14.

Boyland, E.J. & Halford, J.C.G. 2013, "Television Advertising and Branding. Effects on Eating Behaviour and Food Preferences in Children", *Appetite,* vol. 62, no. 0, pp. 236-241.

Cano-Sancho, G., Perelló, G., Nadal, M. & Domingo, J.L. 2015, "Comparison of the Nutritional Composition and the Concentrations of Various Contaminants in Branded and Private Label Yogurts", *Journal of Food Composition and Analysis*, vol. 42, pp. 71-77.

Chandan, R.C., Charles, H., White, A.K. & Hui, Y.H. 2006, *Manufacturing Yogurts and fermented milk*, First Edition edn, Blackwell Publishing, Blackwell Publishing Professional 2121 State Avenue, Ames, Iowa 50014, USA.

Charteris, W.P., Kelly, P.M., Morelli, L. & Collins, J.K. 1997, "Selective Detection, Enumeration and Identification of Potentially Probiotic Lactobacillus and Bifidobacterium

Species in Mixed Bacterial Populations", *International Journal of Food Microbiology*, vol. 35, no. 1, pp. 1-27.

Colby, S.E., Johnson, L., Scheett, A. & Hoverson, B. 2010, "Nutrition Marketing on Food Labels", *Journal of Nutrition Education and Behaviour*, vol. 42, no. 2, pp. 92-98.

Courvalin, P. 2006, "Antibiotic Resistance: The Pros and Cons of Probiotics", *Digestive and Liver Disease*, vol. 38, Supplement 2, no. 0, pp. S261-S265.

Cruz, A.G., Cadena, R.S., Faria, J.A.F., Bolini, H.M.A., Dantas, C., Ferreira, M.M.C. & Deliza, R. 2012, "PARAFAC: Adjustment for Modelling Consumer Study Covering Probiotic and Conventional Yogurt", *Food Research International*, vol. 45, no. 1, pp. 211-215.

Cultures for Health 2015, *What is Yogurt*. Available: http://www.culturesforhealth.com/what-is-yogurt-history [2015, August].

Czinkota, M., Kaufmann, H.R. & Basile, G. 2014, "The Relationship Between Legitimacy, Reputation, Sustainability and Branding for Companies and Their Supply Chains", *Industrial Marketing Management*, vol. 43, no. 1, pp. 91-101.

Dave, R.I. & Shah, N.P. 1997, "Viability of Yoghurt and Probiotic Bacteria in Yoghurts Made From Commercial Starter Cultures", *International Dairy Journal*, vol. 7, no. 1, pp. 31-41.

Davidson, R.H., Duncan, S.E., Hackney, C.R., Eigel, W.N. & Boling, J.W. 2000, "Probiotic Culture Survival and Implications in Fermented Frozen Yogurt Characteristics", *Journal of dairy science*, vol. 83, no. 4, pp. 666-673.

Del Piano, M., Morelli, L., Strozzi, G.P., Allesina, S., Barba, M., Deidda, F., Lorenzini, P., Ballaré, M., Montino, F., Orsello, M., Sartori, M., Garello, E., Carmagnola, S., Pagliarulo, M. & Capurso, L. 2006, "Probiotics: from Research to Consumer", *Digestive and Liver Disease*, vol. 38, Supplement 2, no. 0, pp. S248-S255.

Editorial 2004, "Retail Branding and Consumer Loyalty: An Overview", *Journal of Retailing*, vol. 80, pp. xi-x-xii.

Ezema, C. 2013, "Probiotics in Animal Production: A Review", *Journal of Veterinary Medicine and Animal Health*, vol. 5, no. 11, pp. 308-316.

Flávia, C.A., Buriti, Tania, Y., Okazaki, João, H.A., Alegro, Susana, M.I. & Saad 2007, "Effect of a Probiotic Mixed Culture on Texture Profile and Sensory Performance of Minas Fresh Cheese in Comparison with the Traditional Products", *Nutrition of Latin American archives*, vol. 57, no. 2, pp. 179-180-185.

Fluka Analytical 2015, 2015-last update, *18502 Oxidase Reagent acc. Gordon-McLeod* [Homepage of sigma-aldrich.com], [Online]. Available: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Fluka/Datasheet/18502dat.pdf [2015, April 3].

Forman, J., Halford, J.C.G., Summe, H., MacDougall, M. & Keller, K.L. 2009, "Food Branding Influences Ad Libitum Intake Differently in Children Depending on Weight Status. Results of a Pilot Study", *Appetite*, vol. 53, no. 1, pp. 76-83.

Fuller, R. 1989, "Probiotics in Man and Animals", *Journal of Applied Bacteriology*, vol. 66, no. 5, pp. 365-378.

Gibson, G.R. & Fuller, R. 2000, "Aspects of In Vitro and In Vivo Research Approaches Directed Toward Identifying Probiotics and Prebiotics for Human Use", *The Journal of Nutrition*, vol. 130, no. 2, pp. 391S-395S.

Gismondo, M.R., Drago, L. & Lombardi, A. 1999, "Review of Probiotics Available to Modify Gastrointestinal Flora", *International journal of antimicrobial agents*, vol. 12, no. 4, pp. 287-292.

Google Images (https://www.google.co.uk/search?q=yogurt&newwindow=1&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiloJKko9jKAhXDuw8KHarJAIcQ_AUIBygB), 2014.

Granato, D., Branco, G.F., Cruz, A.G., Faria, José de Assis Fonseca & Shah, N.P. 2010, "Probiotic Dairy Products as Functional Foods", *Comprehensive Reviews in Food Science and Food Safety*, vol. 9, pp. 455-456-470.

Guarner, F., Perdigon, G., Corthier, G., Salminen, S., Koletzko, B. & Morelli, L. 2005, "Should Yoghurt Cultures be Considered Probiotic?", *British Journal of Nutrition*, vol. 2005, no. 93, pp. 783-786.

Gueimonde, M., Delgado, S., Mayo, B., Ruas-Madiedo, P., Margolles, A. & de los Reyes-Gavilán, C.G. 2004, "Viability and Diversity of Probiotic Lactobacillus and Bifidobacterium Populations Included in Commercial Fermented Milks", *Food Research International*, vol. 37, no. 9, pp. 839-850.

Gueimonde, M. & Salminen, S. 2006, "New Methods for Selecting and Evaluating Probiotics", *Digestive and Liver Disease*, vol. 38, Supplement 2, no. 0, pp. S242-S247.

Hale, C.M.D. 2013, August 2013-last update, *Microbiology; Gram positive Streptococcus species* [Homepage of PathologyOutlines.com, Inc.], [Online]. Available: http://www.pathologyoutlines.com/topic/microbiologystreptococci.html [2015, 09/20].

Hamlin, R.P., Lindsay, S. & Insch, A. 2012, "Retailer Branding of Consumer Sales Promotions. A Major Development in Food Marketing?", *Appetite*, vol. 58, no. 1, pp. 256-264.

Han, X., lee, F.L., Zhang, L. & Guo, M.R. 2012, "Chemical Composition of Water Buffalo Milk and its Low-Fat Symbiotic Yogurt Development", *Journal of Functional Foods in Health and Disease*, vol. 2, no. 4, pp. 86-106.

Hebden, L., King, L., Kelly, B., Chapman, K. & Innes-Hughes, C. 2011, "A Menagerie of Promotional Characters: Promoting Food to Children through Food Packaging", *Journal of Nutrition Education and Behaviour*, vol. 43, no. 5, pp. 349-355.

Hekmat, S. & Reid, G. 2006, "Sensory Properties of Probiotic Yogurt is Comparable to Standard Yogurt", *Nutrition Research*, vol. 26, no. 4, pp. 163-166.

Holzapfel, W.H., Haberer, P., Geisen. R., Bjorkroth. J. & Schillinger. U., 2001, "Taxonomy and Important Features of Probiotic Microorganisms in Food and Nutrition", *The American Journal of Clinical Nutrition,* no. 73, pp. 365-373.

Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U. & Huis in't Veld, J.H.J. 1998, "Overview of Gut Flora and Probiotics", *International Journal of Food Microbiology,* vol. 41, no. 2, pp. 85-101.

Holzapfel, W.H. & Schillinger, U. 2002, "Introduction to Pre- and Probiotics", *Food Research International,* vol. 35, no. 2–3, pp. 109-116.

Hussain, I., Attiq-ur-Rahman, & Atkinson, N. 2009, "Quality Comparison of Probiotic and Natural Yogurt", *Pakistan Journal of Nutrition,* vol. 8, no. 1, pp. 9-12.

Illupapalayam, V.V., Smith, S.C. & Gamlath, S. 2014, "Consumer Acceptability and Antioxidant Potential of Probiotic-Yogurt with Spices", *LWT - Food Science and Technology,* vol. 55, no. 1, pp. 255-262.

Jamal, A., Peattie, S. & Peattie, K. 2012, "Ethnic Minority Consumers' Responses to Sales Promotions in the Packaged Food Market", *Journal of Retailing and Consumer Services,* vol. 19, no. 1, pp. 98-108.

Kailasapathy, K., Harmstorf, I. & Phillips, M. 2008, "Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in Stirred Fruit Yogurts", *LWT - Food Science and Technology*, vol. 41, no. 7, pp. 1317-1322.

Kaur, I.P., Chopra, K. & Saini, A. 2002, "Probiotics: Potential Pharmaceutical Applications", *European Journal of Pharmaceutical Sciences,* vol. 15, no. 1, pp. 1-9.

Keller, K.L., Kuilema, L.G., Lee, N., Yoon, J., Mascaro, B., Combes, A., Deutsch, B., Sorte, K. & Halford, J.C.G. 2012, "The Impact of Food Branding on Children's Eating Behaviour and Obesity", *Physiology & Behaviour*, vol. 106, no. 3, pp. 379-386.

Klaenhammer, T.R. & Kullen, M.J. 1999, "Selection and Design of Probiotics", *International Journal of Food Microbiology*, vol. 50, no. 1–2, pp. 45-57.

Koenig-Lewis, N., Palmer, A., Dermody, J. & Urbye, A. 2014, "Consumers' Evaluations of Ecological Packaging – Rational and Emotional Approaches", *Journal of Environmental Psychology*, vol. 37, no. 0, pp. 94-105.

Kopp-Hoolihan, L. 2001, "Prophylactic and Therapeutic Uses of Probiotics: A Review", *Journal of the American Dietetic Association*, vol. 101, no. 2, pp. 229-241.

Kuo, C., Wang, S.S.W., Lu, C., Hu, H., Kuo, F., Weng, B., Wu, C., Liu, C., Tsai, P., Lee, T., Chen, L., Cheng, K., Chang, L. & Wu, D. 2013, "Long-Term Use of Probiotic-Containing Yogurts is a Safe Way to Prevent Helicobacter pylori Based on a Mongolian Gerbil's Model", *Biochemistry Research International*, vol. 2013, no. 594561, pp. 1-7.

Kurniawan, I. & Simadibrata, M. 2011, "The Role of Probiotics to Improve Gut Health in the Elderly", *Med J Indones*, vol. 20, no. 3, pp. 226-231.

Lamoureux, L., Roy, D. & Gauthier, S.F. 2002, "Production of Oligosaccharides in Yogurt Containing Bifidobacteria and Yogurt Cultures", *Journal of Dairy Science*, vol. 85, no. 5, pp. 1058-1069.

Lilly D.M., Stillwell, R. 1965, "Probiotics. Growth Promoting Factors Produced by Micro-Organisms", *Science*, no. 147, pp. 747-748.

Liu, W.J., Chen, Y.F., Kwok, L.Y., Li, M.H., Sun, T., Sun, C.L., Wang, X.N., Dan, T., Menghebilige, Zhang, H.P. & Sun, T.S. 2013, "Preliminary Selection for Potential Probiotic Bifidobacterium Isolated from Subjects of Different Chinese Ethnic Groups and Evaluation of their Fermentation and Storage Characteristics in Bovine Milk", *Journal of Dairy Science*, vol. 96, no. 11, pp. 6807-6817.

Mahalik, N.P. & Nambiar, A.N. 2010, "Trends in Food Packaging and Manufacturing Systems and Technology", *Trends in Food Science & Technology*, vol. 21, no. 3, pp. 117-128.

Mahon, Lehman & Manuselis 2014, "Gram Positive Bacilli" in *Textbook of Diagnostic Microbiology*, 4th Edition edn, Saunders, USA, pp. 369-370-373.

Mani-López, E., Palou, E. & López-Malo, A. 2014, "Probiotic Viability and Storage Stability of Yogurts and Fermented Milks Prepared with Several Mixtures of Lactic Acid Bacteria", *Journal of Dairy Science,* vol. 97, no. 5, pp. 2578-2590.

Méndez, J.L., Oubiña, J. & Rubio, N. 2008, "Expert Quality Evaluation and Price of Store vs. Manufacturer Brands: An Analysis of the Spanish Mass Market", *Journal of Retailing and Consumer Services,* vol. 15, no. 3, pp. 144-155.

Moayyedi, P., Ford, A.C., Talley, N.J., Cremonini, F., Foxx-Orenstein, A.E., Brandt, L.J. & Quigley, E.M.M. 2008, "The Efficacy of Probiotics in the Treatment of Irritable Bowel Syndrome: A Systematic Review", *group.bmj.com,* vol. 59, pp. 325-332.

Mohd Yusof, Y.L. & Wan Jusoh, W.J. 2014, "Islamic Branding: The Understanding and Perception", *Procedia - Social and Behavioural Sciences,* vol. 130, no. 0, pp. 179-185.

Mombelli, B. & Gismondo, M.R. 2000, "The use of Probiotics in Medical Practice", *International Journal of Antimicrobial Agents,* vol. 16, no. 4, pp. 531-536.

Myers, D. 2007, "Probiotics", *Journal of Exotic Pet Medicine,* vol. 16, no. 3, pp. 195-197.

National Yogurt Association 2013, *Live and Active Culture Yogurt Seal Program Procedures and Guidelines*, Procedures and Guidelines edn, McLean, VA.

Nielsen, S.S. 2010, *Food Analysis Laboratory Manual,* Second Edition edn, Purdue University, West Lafayette, IN, USA.

Nomoto, K. 2005, "Prevention of Infections by Probiotics", *Journal of Bioscience and Bioengineering,* vol. 100, no. 6, pp. 583-592.

O'Brien, J., Crittenden, R., Ouwehand, A.C. & Salminen, S. 1999, "Safety Evaluation of Probiotics", *Trends in Food Science & Technology,* vol. 10, no. 12, pp. 418-424.

Olugbuyiro, J.A.O. & Oseh, J.E. 2011, "Physio-chemical and Sensory Evaluation of Market Yoghurt in Nigeria", *Pakistan Journal of Nutrition,* vol. 10, no. 10, pp. 914-918.

Pishva, E., Hassannia, N., Fazeli, M.R., Havaee, A., Jamalifer, H., Hossein, M.P., Shojaee, H. & Akbar, M. 2014, "Antibacterial Effect of Authochlorous Lactobacillus Strains Isolated from Traditional Yogurts", *Pakistan Journal of Nutrition,* vol. 8, no. 8, pp. 1132-1137.

Prescott, S.L. & Björkstén, B. 2007, "Probiotics for the Prevention or Treatment of Allergic Diseases", *Journal of Allergy and Clinical Immunology*, vol. 120, no. 2, pp. 255-262.

Pyar, H. & Peh, K.K. 2013, "Characterization and Identification of Lactobacillus Acidophilus using Biolog Rapid Identification System", *International Journal of Pharmacy and Pharmaceutical Sciences,* vol. 6, no. 1, pp. 189-193.

Pyar, H., Liong, M., Masazurah, A.R., Lew, L. & Peh, K.K. 2014, "Study of Genotypic Characteristics of Probiotics *Lactobacillus spp* using PCR", *Asian Pacific Journal of Tropical Disease,* vol. 4, no. 3, pp. 225.

Quigley, E.M. 2011, "Gut Microbiota and the Role of Probiotics in Therapy", *Current Opinion in Pharmacology,* vol. 11, no. 6, pp. 593-603.

Ranathunga, M.T.N. & Rathnayaka, R. M. U. S. K. 2013, "Comparison of Physio-chemical and Sensory Properties of Probiotic and Natural Yoghurt", *Journal of Biological and Food Science,* vol. 2, no. 1, pp. 1-6.

Reid, G. 2008, "Probiotics and Prebiotics – Progress and challenges", *International Dairy Journal,* vol. 18, no. 10–11, pp. 969-975.

ResearchGate 2014-2015, *Can I get Bifidobacteria to grow in MRS?*.

Saarela, M., Mogensen, G., Fondén, R., Mättö, J. & Mattila-Sandholm, T. 2000, "Probiotic Bacteria: Safety, Functional and Technological Properties", *Journal of Biotechnology,* vol. 84, no. 3, pp. 197-215.

Salminen, S., von Wright, A., Morelli, L., Marteau, P., Brassart, D., de Vos, W.M., Fondén, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S. & Mattila-Sandholm, T. 1998, "Demonstration of Safety of Probiotics — A Review", *International Journal of Food Microbiology,* vol. 44, no. 1–2, pp. 93-106.

Saxelin, M., Grenov, B., Svensson, U., Fondén, R., Reniero, R. & Mattila-Sandholm, T. 1999, "The Technology of Probiotics", *Trends in Food Science & Technology,* vol. 10, no. 12, pp. 387-392.

Schillinger, U. 1999, "Isolation and Identification of Lactobacilli from Novel-Type Probiotic and Mild Yoghurts and their Stability during Refrigerated Storage", *International Journal of Food Microbiology*, vol. 47, no. 1–2, pp. 79-87.

Schrezenmeir, J. & Michael de Vrese 2001, "Probiotics, Prebiotics, and Synbiotics—Approaching a Definition", *The American Journal of Clinical Nutrition,* vol. 73, pp. 361-364.

Seo, S. & Jang, S. 2013, "The Roles of Brand Equity and Branding Strategy: A Study of Restaurant Food Crises", *International Journal of Hospitality Management,* vol. 34, no. 0, pp. 192-201.

Serafeimidou, A., Zlatanos, S., Laskaridis, K. & Sagredos, A. 2012, "Chemical Characteristics, Fatty Acid Composition and Conjugated Linoleic Acid (CLA)", vol. 134, pp. 1839-1846.

Shah, N.P. & Lankaputhra, W.E.V. 1997, "Improving Viability of *Lactobacillus acidophilus* and *Bifidobacterium spp*. in Yogurt", *International Dairy Journal,* vol. 7, no. 5, pp. 349-356.

Soukoulis, C., Panagiotidis, P., Koureli, R. & Tzia, C. 2007, "Industrial Yogurt Manufacture: Monitoring of Fermentation Process and Improvement of Final Product Quality", *Journal of Dairy Science*, no. 90, pp. 2641-2654.

Sullivan, A. & Nord, C.E. 2002, "The Place of Probiotics in Human Intestinal Infections", *International Journal of Antimicrobial Agents,* vol. 20, pp. 313-319.

Sun, S., Yang, K., He, X., Tian, J., Ma, B. & Jiang, L. 2009, "Probiotics in Patients with Severe Acute Pancreatitis: A Meta-Analysis", *Langenbecks Arch Surg,* , no. 394, pp. 171-177.

Tannock, G.W. 1999, "Identification of Lactobacilli and Bifidobacteria", *Current Issues in Molecular Biology*, vol. 1, no. 1, pp. 53-54-64.

The Dairy Council 2015, 1/2015-last update, *Yogurt Facts sheet* [Homepage of The Dairy council], [Online]. Available: http://www.milk.co.uk/consumers/resources/resource.aspx?intResourceID=150 [2015, 09/14].

University of Guelph 2013, 20/02-last update, *Yogurt Production* [Homepage of Efficacy Finders], [Online]. Available: http://wiki.zero-emissions.at/index.php?title=Yogurt_production [2015, 09/09].

Van Loo, E.J., Diem, M.N.H., Pieniak, Z. & Verbeke, W. 2013, "Consumer Attitudes, Knowledge, and Consumption of Organic Yogurt", *Journal of Dairy Science*, vol. 96, no. 4, pp. 2118-2129.

Velasco, C., Salgado-Montejo, A., Marmolejo-Ramos, F. & Spence, C. 2014, "Predictive Packaging Design: Tasting Shapes, Typefaces, Names, and Sounds", *Food Quality and Preference*, vol. 34, no. 0, pp. 88-95.

WFLO Commodity Storage Manual 2008, "Yogurt and Yogurt Products".

Wikström, F., Williams, H., Verghese, K. & Clune, S. 2014, "The Influence of Packaging Attributes on Consumer Behaviour in Food-Packaging Life Cycle Assessment Studies - A Neglected Topic", *Journal of Cleaner Production,* vol. 73, no. 0, pp. 100-108.

Yeung, P.S.M., Sanders, M.E., Kitts, C.L., Cano, R. & Tong, P.S. 2002, "Species-Specific Identification of Commercial Probiotic Strains", *Journal of Dairy Science,* vol. 85, no. 5, pp. 1039-1051.

APPENDICES

APPENDIX A:

BAHSS 240 Full Review Approval

27th March 2015

Carol Anne Wallace/Ebojie Onoguese Obehi

School of Sport, Tourism and the Outdoors

University of Central Lancashire

Dear Carol/Ebojie,

Re: BAHSS Ethics Committee Application Unique Reference Number: BAHSS 240

The BAHSS ethics committee has granted approval of your proposal application 'Yogurts: A comparison of Probiotic and Standard yogurt based on branding (premium and basic brands), consumer preference, sensory evaluation, microbiological analysis, and nutritional analysis'. Approval is granted up to the end of project date* or for 5 years from the date of this letter, whichever is the longer.

It is your responsibility to ensure that

• The project is carried out in line with the information provided in the forms you have submitted

• You regularly re-consider the ethical issues that may be raised in generating and analyzing your data

• Any proposed amendments/changes to the project are raised with, and approved, by Committee

• You notify roffice@uclan.ac.uk if the end date changes or the project does not start

• Serious adverse events that occur from the project are reported to Committee

• A closure report is submitted to complete the ethics governance procedures (Existing paperwork can be used for this purposes e.g. funder's end of grant report; abstract for student award or NRES final report. If none of these are available use e-Ethics Closure Report Proforma).

Yours sincerely,

Colin Morrell

Deputy Vice chair

BAHSS Ethics Committee

* for research degree students this will be the final lapse date

NB - Ethical approval is contingent on any health and safety checklists having been completed, and necessary approvals as a result of gained.

APPENDIX B:

Research Program Approval

Date: 28th January 2015

Ebojie Onoguese Obehi

Email: OOEbojie@uclan.ac.uk

Dear Ebojie,

RESEARCH PROGRAMME APPROVAL FOR THE AWARD OF RESEARCH DEGREE OF THE UNIVERSITY OF CENTRAL LANCASHIRE

I am pleased to inform you that the School of SSTO has approved your application for Research Program Approval on a FULL time basis for the degree of MSc (by Research)

RECOMMENDATION: The research design, hypotheses and statistical analysis of each dataset is further clarified prior to data collection.

Title of Program of Research

A comparison of Probiotic and Standard yogurt based on branding (premium and basic brands), consumer preference, sensory evaluation, microbiological analysis, and nutritional analysis.

Supervisors

Director of Studies: Professor Carol Wallace

School of SSTO

Second Supervisor 1: Professor Nicola Lowe

School of SSTO

Second Supervisor 2: Dr Brigit Ramsingh

School of SSTO

Program Start Date and Duration

The expected program length is 12 months (full-time) with effect from 1st October 2014, subject to conditions specified in the University Regulations.

The expected date for submission of your final thesis is 30th September 2015.

Ethical Approval of your Project

Your application for RPA has been approved. However, please note that until you have gained ethical clearance (where you answer "No" to all questions on the Ethics checklist and clearance is confirmed by the ethics committee) or ethical approval (where you answer "Yes" to any question on the Ethics checklist and submit an application for full ethical approval which is subsequently approved by the ethics committee) you are not permitted to do any data collection or fieldwork, or participant surveys. To do so will mean you are uninsured, in breach of the Code of Conduct for Research, and liable for disciplinary action.

Examination Arrangements

a) The arrangements for examining you on your program of work.

b) The external and internal examiners to be appointed.

These arrangements should be submitted no later than 4 months before you propose to submit your thesis for examination. Please note that you will not be able to submit your thesis until examination arrangements have been approved.

Please feel free to contact me about any aspect of your research program or with any other queries you may have.

Yours sincerely

Clare Wiggans

Senior Administrative Officer (Research)

Research Student Registry

Harris Building room HB104

Copies: Nicola Lowe

Carol Wallace

Brigit Ramsingh

Sarah Hobbs

APPENDIX C:

Participant Information Sheet

PARTICIPANT INFORMATION SHEET

TITLE OF RESEARCH PROJECT: Yogurts: A comparison of Probiotic and Standard yogurt based on branding (premium and basic brands), consumer preference, sensory evaluation, microbiological analysis, and nutritional analysis.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?

The primary aim of the research is to compare probiotic and standard (natural or non- probiotic) yogurts on the basis of brands (basic and premium brands), microbiological analysis, nutritional analysis, sensory evaluation and consumer preference. The study is intended to run for one year and only persons who are 18 and above will be able to participate in the study.

Why have I been invited to participate?

You are been invited to take part in the consumer preference testing and sensory evaluation. The study intends to use Uclan students and staff members only. You will be required to fill out a questionnaire for both the consumer and sensory evaluations. On the consumer preference questionnaire, you will be asked some questions about your frequency of yogurt consumption, preferred type of yogurt, and the like. For the sensory evaluation, you will be asked to taste four samples of yogurt and evaluate them based on taste, appearance, texture, and overall quality. You will also be asked to try to identify each of the samples based on the taste i.e. probiotic, non-probiotic, premium and basic brands.

Do I have to participate?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason however if you decide to withdraw after your data has been entered into the computer, it will be impossible to withdraw your data as all information given are anonymous and as such it will be impossible to identify which belongs to you. As a Uclan student or staff, choosing to participate or not participate in the research will not affect your grades, jobs, etc.

What are the benefits or risks of taking part?

There is no benefit or risk to participants involved in this research as all yogurts are commercially available in supermarkets. However, only lactose tolerant individuals are able to participate due to the nature of the sample (yogurt). The yogurt samples will be purchased fresh from the supermarkets in and around Preston. All the information provided by you will be stored safely on the school database that only I and my director of studies have access to for 5 years from the end of the study following the university regulations.

What will happen to the result of the study?

This research is funded by the university and the results of the study will be used in my thesis for the attainment of an MSc by research in Nutrition and Food Science. I am conducting this research as a student of Uclan from the School of Sports Tourism and Outdoors.

Contact information

For any query or information, please feel free to contact any of the following numbers: Ebojie Obehi (research student) – ooebojie@uclan.ac.uk, Prof Carol Wallace (Director of studies) – CAWallace@uclan.ac.uk. Should you have any concerns about the way in which the study has been conducted, you should contact University Officer for Ethics (email address OfficerforEthics@uclan.ac.uk).

Thank you.

20/01/2015

APPENDIX D:

Consent Form

CONSENT FORM

Title of the project:

Yogurts: A comparison of Probiotic and Standard yogurt based on branding (premium and basic brands), consumer preference, sensory evaluation, microbiological analysis, and nutritional analysis.

Details of the Researcher:

Ebojie Onoguese Obehi (MSc by research student in Nutrition and Food Science)

OOEbojie@uclan.ac.uk

Instruction: Please read the following statements and initial the boxes to indicate your agreement

Please initial box

I confirm that I have read and understand the information sheet, dated …….. for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason. ☐

I agree to take part in the above study. ☐

I understand that it will not be possible to withdraw my data from the study after data has been entered into the computer ☐

Name of Participant	Date	Signature

Name of Researcher	Date	Signature

APPENDIX E:

Poster

University of Central Lancashire

YOGURT! YOGURT!! YOGURT!!!

Do you like yogurt? If so then I think you will be interested in this.




TITLE OF RESEARCH PROJECT: Yogurts: A comparison of Probiotic and Standard yogurt based on branding (premium and basic brands), consumer preference, sensory evaluation, microbiological analysis, and nutritional analysis.

Hi yogurt lovers, I am an MSc student working on yogurts for my research. I need participants for a sensory evaluation. You will be required to evaluate 4 yogurt samples based on taste, texture, appearance, and overall quality. You will also be asked to fill a questionnaire on your yogurt preference. The entire evaluation will not take more than 15 minutes of your time. The date, time and venue is

Date: 08/05/2015 and 15/05/2015

Time: 10am

Venue: Scholar Bar, Foster Building

If you are interested then you will be given an information sheet and consent form. You have to be 18 and above to take part and only lactose tolerant individuals can participate due to the nature of the sample (yogurt). For more information, please contact me on ooebojie@uclan.ac.uk

Thank you.

Obehi Ebojie

MSc by research student

APPENDIX F:

Consumer Preference Questionnaire

University of Central Lancashire

Consumer Preference

Note: This questions are to know your understanding of yogurts and probiotics. They are also to know your preferred choice in yogurt.

Participant Information

Sex- (a) Male (b) Female

Age- (a) 18-25 (b) 26-35 (c) 36 and above

Questions

Instructions: For the questions below, pick one of the options

1. How often do you consume yogurt?

(a) Daily (b) Weekly (c) Twice in a month (d) Rarely (e) Never

2. Do you look at the labelling to determine the type (Probiotic or Non- probiotic) of yogurt before you buy?

(a) Yes (b) No (c) Sometimes

3. Which type of yogurt do you prefer?

(a) Probiotic (b) Non-probiotic (c) Indifferent

4. For question 3 above, what is the reason for your answer?

5. Which brand of yogurt do you prefer?

(a) Premium/Luxury brand (Expensive) (b) Basic/Regular brand (Non- expensive)

(c) Indifferent

6. For question 5 above, what is the reason for your answer?

Instructions: For the question below, answer based on your understanding.

7. What do you understand by the term ‘probiotics’?

APPENDIX G:

Sensory Evaluation Questionnaire

Sensory Evaluation Questionnaire

Note: This questions are to know your opinion on the yogurt samples (4) given. Of the four (4) samples given, two (2) are probiotic, two (2) are non-probiotic, two (2) are expensive (premium) brands, and two (2) are non-expensive (basic) brands.

Questions

Instructions: For the questions below, choose from the numbers 1-5 the one that best fit.

Yogurt samples		**1(very poor)**	**2 (poor)**	**3 (fair)**	**4 (good)**	**5 (very good)**
1 (a)	How is the texture (thickness) of Sample A					
(b)	Sample B					
(c)	Sample C					
(d)	Sample D					

Yogurt samples		**1 (very poor)**	**2 (poor)**	**3 (fair)**	**4 (good)**	**5 (very good)**
2 (a)	How is the taste of Sample A					
(b)	Sample B					
(c)	Sample C					
(d)	Sample D					

Yogurt samples		**1(very poor)**	**2(poor)**	**3(fair)**	**4(good)**	**5(very good)**
3 (a)	How is the appearance of Sample A					
(b)	Sample B					
(c)	Sample C					
(d)	Sample D					

Yogurt samples		**1(very poor)**	**2(poor)**	**3(fair)**	**4(good)**	**5(very good)**
4 (a)	How is the overall quality of Sample A					
(b)	Sample B					
(c)	Sample C					
(d)	Sample D					

Instructions: For the questions below, choose (by ticking) two of the options that best fit.

5. Which of the samples do you think are probiotic?

(a) Sample A (b) Sample B (c) Sample C (d) Sample D (e) I don't know

6. Which of the samples do you think are non-probiotic?

(a) Sample A (b) Sample B (c) Sample C (d) Sample D (e) I don't know

7. Which of the samples do you think are the expensive (premium) brands?

(a) Sample A (b) Sample B (c) Sample C (d) Sample D (e) I don’t know

8. Which of the samples do you think are the non-expensive (basic) brands?

(a) Sample A (b) Sample B (c) Sample C (d) Sample D (e) I don’t know

APPENDIX H:

BIOCHEMICAL AND GRAM STAINING TESTS RESULTS

Below shows a list of tables (Tables H.1-H.6) representing the series of biochemical and gram stain done on the organisms in the various agars.

Key

A1a- 1st colony of the first plate of sample A

A1b- 2nd colony of the first plate of sample A

A2a- 1st colony of the second plate of sample A

A2b- 2nd colony of the second plate of sample A

A3a- 1st colony of the third plate of sample A

A3b- 2nd colony of the third plate of sample A

B1a- 1st colony of the first plate of sample B

B1b- 2nd colony of the first plate of sample B

B2a- 1st colony of the second plate of sample B

B2b- 2nd colony of the second plate of sample B

B3a- 1st colony of the third plate of sample B

B3b- 2nd colony of the third plate of sample B

C1a- 1st colony of the first plate of sample C

C1b- 2nd colony of the first plate of sample C

C2a- 1st colony of the second plate of sample C

C2b- 2nd colony of the second plate of sample C

C3a- 1st colony of the third plate of sample C

C3b- 2nd colony of the third plate of sample C

D1a- 1st colony of the first plate of sample D

D1b- 2nd colony of the first plate of sample D

D2a- 1st colony of the second plate of sample D

D2b- 2nd colony of the second plate of sample D

D3a- 1st colony of the third plate of sample D

D3b- 2nd colony of the third plate of sample D

BIOCHEMICAL TESTS

Table H.1 shows the biochemical tests of organisms grown on MRS agar

Sample	Oxidase	Catalase	Starch hydrolysis	Motility	MRS broth	Glucose	Sucrose	Fructose	Lactose
$A1_a$	-	-	-	-	+	+	+	+	-
$A1_b$	-	-	-	-	+	+	+	+	+
$A2_a$	-	-	-	-	+	+	+	+	+
$A2_b$	-	-	-	-	+	+	-	+	+
$A3_a$	-	-	-	-	+	+	+	+	+
$A3_b$	-	-	-	-	+	+	+	+	+
$D1_a$	-	+	-	+	+	+	+	+	+
$D1_b$	-	+	-	+	+	+	+	+	+

+ = Positive, - = Negative.

Table H.2 shows the biochemical tests of organisms grown on modified MRS agar.

Sample	Oxidase	Catalase	Starch hydrolysis	Motility	MRS broth	Glucose	Sucrose	Fructose	Lactose
$A1_a$	-	-	-	-	+	+	-	+	-
$A1_b$	-	-	-	-	+	+	+	+	+
$A2_a$	-	-	-	-	+	+	-	-	-
$A2_b$	-	-	-	-	+	+	-	+	-
$A3_a$	-	-	-	-	+	-	-	-	-

$A3_b$	-	-	-	+	+	+	+	+	+
$D1_a$	-	+	-	+	+	+	+	+	+
$D1_b$	-	+	-	+	+	+	+	+	+

+ = Positive, - = Negative

Table H.3 shows the biochemical tests of organisms grown on M17 agar.

Sample	**Oxidase**	**Catalase**	**Starch hydrolysis**	**Motility**	**MRS broth**	**Glucose**	**Sucrose**	**Fructose**	**Lactose**
$A1_a$	-	+	+	+	+	+	+	+	+
$A1_b$	-	+	+	+	+	+	+	+	+
$A2_a$	-	-	-	-	+	-	-	-	-
$A2_b$	-	-	-	-	+	-	-	-	-
$A3_a$	-	-	-	-	+	-	-	+	+
$A3_b$	-	+	+	+	+	+	+	+	+
$B1_a$	-	-	-	-	+	-	-	-	-
$B1_b$	-	+	+	+	+	+	+	+	+
$B2_a$	-	+	+	+	+	+	+	+	+
$B2_b$	-	+	+	+	+	+	+	+	+
$B3_a$	-	+	+	+	+	+	+	+	+
$B3_b$	-	+	+	+	+	+	+	+	+
$C1_a$	-	+	+	+	+	+	+	+	+
$C1_b$	-	+	+	+	+	+	+	+	+

$C2_a$	-	-	+	+	+	+	+	+	+
$C2_b$	-	-	+	+	+	+	+	+	+
$C3_a$	-	-	-	-	+	-	-	-	-
$C3_b$	-	-	-	-	+	-	-	-	-
$D1_a$	-	-	-	-	+	-	-	-	-
$D1_b$	-	+	+	+	+	+	+	+	+
$D2_a$	-	-	-	-	+	-	-	-	-
$D2_b$	-	-	-	-	+	-	-	-	-
$D3_a$	-	-	-	-	+	-	-	-	-
$D3_b$	-	+	+	+	+	+	+	+	+

+ = Positive, - = Negative

GRAM STAIN

Table H.4 shows the gram stain results of organisms grown on MRS agar

Sample	Gram reaction	Appearance
$A1_a$	+	Rods
$A1_b$	+	Rods
$A2_a$	+	Rods
$A2_b$	+	Rods
$A3_a$	+	Rods

$A3_b$	+	Rods
$D1_a$	+	Cocci
$D1_b$	+	Cocci

+ = Positive, - = Negative

Table H.5 shows the gram stain results of organisms grown on modified MRS agar

Sample	**Gram reaction**	**Appearance**
$A1_a$	+	Rods
$A1_b$	+	Rods
$A2_a$	+	Rods
$A2_b$	+	Rods
$A3_a$	+	Rods
$A3_b$	+	Rods
$D1_a$	+	Rods
$D1_b$	+	Cocci

+ = Positive, - = Negative

Table H.6 shows the gram stain results of organisms grown on M17 agar

Sample	**Gram reaction**	**Appearance**
$A1_a$	+	Cocci
$A1_b$	+	Cocci
$A2_a$	+	Cocci

$A2_b$	+	Cocci
$A3_a$	+	Cocci
$A3_b$	+	Cocci
$B1_a$	+	Cocci
$B1_b$	+	Cocci
$B2_a$	+	Cocci
$B2_b$	+	Cocci
$B3_a$	+	Cocci
$B3_b$	+	Cocci
$C1_a$	+	Cocci
$C1_b$	+	Cocci
$C2_a$	+	Cocci
$C2_b$	+	Not defined
$C3_a$	+	Rods
$C3_b$	+	Rods
$D1_a$	+	Rods
$D1_b$	+	Cocci
$D2_a$	+	Rods
$D2_b$	+	Rods
$D3_a$	+	Rods
$D3_b$	+	Cocci

+ = Positive, - = Negative