MAKERERE UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOCHEMISTRY AND SPORTS SCIENCE

EVALUATION OF THE EFFECT OF HONEY SWEETENER ON YOGHURT SHELF LIFE

BY

MULIIKA BELCHER KELLY

(REG NO.: 19/U/1150)

SUPERVISOR: DR. ISANGA JOEL

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY AND SPORTS SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE DEGREE OF MAKERERE UNIVERSITY

SEPTEMBER, 2022

DECLARATION

I **Muliika Belcher Kelly,** do hereby declare that the research work compiled in this dissertation entitled "Evaluation of the Effect of Honey Sweetener on Yoghurt Shelf Life" was of my personal research efforts under the guidance and supervision of Dr. Isanga Joel of the Department of Biochemistry and Sports Science, College of Natural Sciences, Makerere University.

I further declare that this work has never been submitted to this University or any other institution of higher learning for the award of a degree and/or any other academic qualification.

Signature: ....Kelly..... **Date:** ..28/09/2022...

Muliika Belcher Kelly

(BSc. Candidate, **Reg No.**: 19/U/1150)

i

APPROVAL

This is to certify that the research work compiled in this dissertation entitled "Evaluation of the Effect of Honey Sweetener on Yoghurt Shelf Life" by Mr. **Muliika Belcher Kelly** in partial fulfillment for the award of a Bachelor of Science Degree of Makerere University was based on the results of studies carried out by him in the Biochemistry Laboratory at the College of Natural Sciences, Makerere University, Kampala, Uganda, East Africa under my guidance and supervision.

I further certify that neither the research report nor any part thereof has been submitted previously for consideration of award of a degree and/or any other academic qualification. This work was submitted for examination with my approval as Mr. Muliika Belcher's research Supervisor.

Signature: ..... Date: 28/09/2022

Dr. Isanga Joel
(Lecturer)
Department of Biochemistry and Sports Science,
College of Natural Sciences,
Makerere University
Email: joelisanga@gmail.com

ii

ACKNOWLEDGEMENT

First and above all, I am so thankful to God Almighty Who has blessed me with the gift of life, wisdom and protection and the far He has brought me to be able to complete my research project successfully.

My heartfelt and sincere appreciation goes to my parents Mr. and Mrs. Bugembe and my grandmother for the prayers, financial and material aid, unending support and encouragement. May God richly bless them.

Special thanks to my supervisor Dr. Isanga Joel for supervising and guiding me while I was doing my research and for being a good lecturer and the parental piece of advice he gave me. May God bless him.

Lastly, I am grateful to the Department of Biochemistry and Sports Science for the support it has given me in terms of apparatus and space to use while carrying my our research.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

FOS	Fructooligosaccharides
GOS	Galactooligosaccharides
HSY	Honey Sweetened Yoghurt
LAB	Lactic Acid Bacteria
SSY	Sucrose Sweetened Yoghurt
STS	Susceptibility to Synerisis
TA	Titratable Acidity
WHC	Water Holding Capacity

ABSTRACT

Yoghurt is a fermented dairy product with a short shelf life of about 3 weeks or less and should be stored at low a temperature which has the implication of increasing the cost of storage hence limiting its sustainable consumption in poor communities where electricity is in short supply.

The main purpose of this research was to evaluate the effect of honey sweetener on the shelf life of yoghurt. Honey Sweetened Yoghurt (HSY) was prepared together with Sucrose Sweetened Yoghurt (SSY) as experimental control. The pH, titratable acidity, Susceptibility to Syneresis (STS) and Water Holding Capacity (WHC) were measured under refrigeration and room temperature storage conditions over a period of 26 days for both yoghurt samples. The shelf lives of both honey sweetened yoghurt and sucrose sweetened yoghurt stored at room temperature were also estimated. Finally, sensory evaluation of both yoghurt samples was carried out using the 9-point hedonic scale.

The pH of both SSY and HSY decreased during storage at refrigeration temperature and the decrease was significant ($P<0.05$) for SSY (4.84 to 4.62) compared to that of HSY (4.69 to 4.67) where the decrease was not significant. However, at room temperature storage, there was a significant decrease in pH for both yoghurt samples. There was no significant increase ($P>0.05$) in the titratable acidity of both yoghurt samples at refrigeration temperature but the increase was significant at room temperature with the highest value of 1.53±0.04% for SSY and 1.32±0.13% for HSY. The STS at refrigeration temperature was higher in HSY with the highest value of 67.00±2.65% but at room temperature it was higher in SSY with the highest value of 65.33±0.58%. The WHC decreased at both storage conditions but the decrease was more rapid at room temperature. The lowest WHC was 31.33±1.15% for SSY at room temperature.

The shelf life of SSY at room temperature was 8 days while that of HSY under the same storage conditions was more than 11 days hence, honey had an effect of extending the shelf life of yoghurt for about 3 days under room temperature storage. On the other hand, the yoghurt samples stored at refrigeration temperature did not expire even after the 26 days of storage thus; yoghurt storage at refrigeration temperature remains advantageous and highly recommended even in cases where honey is used as a sweetener.

Upon sensory evaluation, HSY had higher scores for all the sensory attributes tested compared to Sucrose Sweetened Yoghurt and the preference for HSY was also confirmed with the overall acceptability score of 7.00±0.63 for HSY and 6.73±1.62 for SSY. Therefore, in addition to extending shelf life, sweetening yoghurt with honey also enhanced its sensory attributes.

CHAPTER ONE: INTRODUCTION

1.1 Background

Yoghurt is a fermented dairy product produced with the aid of microbial starter cultures. The most commonly used yoghurt starter culture consists of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* mixed in a ratio of 1.1 (Fisberg and Machado, 2015).

Yoghurt contains essential nutrients, highly bioavailable proteins, calcium, potassium, phosphorus, magnesium, riboflavin, vitamin B12 and vitamin A (Fisberg and Machado, 2015). Because the protein, fat, and lactose components undergo partial hydrolysis during fermentation, yoghurt is an easily digestible dairy product (Rasic and Kurmann, 1978).

Amongst diverse fermented milk products, yoghurt is well known for its healthy image and can be suitably utilized as a probiotic carrier (Pandey and Mishra, 2015). Probiotic administration is generally recognized as safe (Van den Nieuwboer *et al*., 2015) and reviewed literature on functional properties of yoghurt and probiotics suggested inclusion of probiotics in yoghurt for augmenting the functionality of plain yoghurt (Sarkar, 2019a).

Yoghurt belongs to the group of dairy products with a short shelf life, which is normally about 3 weeks or less (Mojka 2013). The recommended storage temperature of yoghurt ranges from 4 - 8°C (Pikul, 2004). Improper storage temperature of yoghurt can lead to increased activity the starter culture leading to increased acidification, lowering pH & deterioration of its sensory quality (Cais-Sokoli´nska and Pikul 2001). Improper storage conditions can also lead to a decrease in nutritional value and deterioration of sensory characteristics of yoghurts, as well as to a reduction in content of yogurt bacteria that carry out the process of lactic fermentation (Zareba and Ziarno 2013, Wojtczak *et al*. 2018).

Yoghurt could be sweetened using various sweeteners such as sucrose, high-fructose corn syrup and honey among others (Aryana & Olson, 2017; Popa & Ustunol, 2011). Honey is a natural sweetener containing primarily fructose (38.5%) and glucose (31.3%) (Ustunol and Gandhi, 2001), has anti-oxidative, antibiotic and immune boosting properties (Bansal *et al*., 2005). The antibiotic property of honey could hypothetically contribute to the extended shelf-life of yoghurt by inhibiting the activity of the starter culture hence preventing accumulation of lactic acid after the fermentation process. However

that hypothesis needs to be investigated, proved right or wrong and it forms the basis of my proposed study.

1.2 Problem Statement

Yoghurt is normally stored at refrigeration temperatures of ~4-8 ^{o}C in order to slow down the rate of fermentation and extend its shelf life. The use of electricity/energy consuming refrigerators for yoghurt storage increases the cost of storage hence limiting its sustainable consumption in poor communities where electricity is in short supply. Therefore, there is need to modify the production process so that yoghurt can be stored for longer periods without need of refrigeration.

One way of modifying the production process in order to influence yoghurt storage temperature could be by using honey instead of sucrose to sweeten the yoghurt. Honey is known to be a unique natural sweetener with anti-oxidative, antibiotic and immune boosting properties. By theoretical implication, the antibiotic property of honey is expected to inactivate or inhibit the continued activity of the yoghurt starter culture after fermentation thus preventing the accumulation of lactic acid during storage. This could contribute to the extended shelf-life of yoghurt even when stored at room temperature. Being able to store yoghurt at room temperature without it going bad eliminates the need for refrigeration and reduces cost of storage.

1.3 Objectives

1.3.1 General Objective

The main objective of this study was to evaluate the effect of using honey sweetener on yoghurt shelf life.

1.3.2 Specific Objectives

The specific objectives were to:

1. Determine the effect of storage temperature on Honey Sweetened Yoghurt using parameters such as Water Holding Capacity, Susceptibility to Syneresis, titratable acidity and pH.

2. Estimate the shelf life of honey sweetened yoghurt at refrigeration and room temperature.
3. Carry out sensory evaluation of HSY in comparison with SSY using the 9 point hedonic scale.

1.4 Research Questions

1. Does honey have an effect on the shelf life of yoghurt?
2. What is the optimum concentration of honey that gives the sweetness similar to that of the control yoghurt sample (Sucrose sweetened yoghurt)?

1.5 Significance of the study

The findings of this study will go a long way in providing alternative storage conditions for yoghurt and reducing the cost of storage by using room temperature instead of refrigeration temperature which calls for sustainable electricity supply that may not be readily available in some communities especially in developing countries.

1.6 Justification

The use of honey as a sweetener instead of sucrose in yoghurt does not only extend yoghurt's shelf life but also results into production of a functional food product with antibiotic, anti-oxidative and immune boosting properties. Additionally, the final product would also be suitable for consumption by diabetic patients owing to the hypoglycemic nature of honey which is a natural sugar and thus safe for such individuals.

CHAPTER TWO: LITERATURE REVIEW

2.1 Yoghurt

2.1.1 Definition of yoghurt

Yoghurt is a diary product produced by fermentation of milk using a group of microorganisms known as starter culture which mainly includes *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* (Sanz *et al*. 2005). Yoghurt is easily digested since the milk protein, fat, and lactose components undergo partial hydrolysis during fermentation (Sanchez-Segarra *et al*. 2000).

2.1.2 Yoghurt as a functional food

Functional foods are a new direction in the science that exist at the interface between food and drugs, and this offers great potential for health improvement and prevention of diseases when ingested as part of a balanced diet. Recent societal interest in healthful foods has led to the development of functional dairy products that basically provide health benefits in addition to their fundamental nutrients (Sarkar, 2019b)

With the growing awareness of consumers toward health foods, the basic concepts of nutrition are undergoing significant change. Foods can no longer be evaluated in terms of macronutrient and micronutrient content alone. Consumer's belief that certain foods can show health benefits has resulted in the making up of the term functional foods and consumers are focusing more on the health benefits of functional products, resulting in the growth of global dairy market.

Consumer disposition toward probiotic foods has been stimulated due to well documented evidence of health benefits of probiotic-containing products and consumer demand for natural products (Sarkar, 2018). FAO/WHO (2001) defines probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host.

Probiotics has now become one of the most effective functional foods owing to therapeutic and nutritional features and have been found effective in regulating gastrointestinal flora to prevent proliferation of diverse disease (Sarkar *et al*., 2017). Sanders and Marco (2010) reported better growth of probiotics in food matrices containing bioactive ingredients such as prebiotics, vitamins, minerals, fibers, enzymes, food preservatives and flavors.

2.2 Honey

2.2.1 Definition of honey

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which is collected by bees, transformed by combining with specific substances made by bees, deposited, dehydrated, stored and left in the honey comb to ripen and mature (Codex Standard, 2001).

Microflora of honey is majorly contributed by those originating from pollen, the digestive tracts of honeybees, dust, flowers, dirt and the air and microorganisms capable of surviving the low pH and antimicrobial compounds are present in honey (Tajabadi *et al*., 2013).

2.2.2 Nutritional composition of honey

The nutritional composition of honey is mainly influenced by the floral source (Khalil and Sulaiman, 2010) and external factors such as processing, storage and environmental conditions (Mohan *et al*., 2017). According to Kamal and Klein (2011), honey is a supersaturated solution of sugars, mainly composed of 75% monosaccharides comprising of fructose, glucose; 10-15% disaccharides such as sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, kojibiose and trisaccharides such as maltotriose and melezitose. It also contains minerals, vitamins, enzymes, proteins, free amino acids and polyphenols (Alvarez-Suarez *et al*., 2013).

2.2.3 Therapeutic properties of honey

According to reviewed literature, it is indicated that honey being rich in flavonoids and polyphenols, which act as natural antioxidants and are the two main bioactive molecules present in honey, which play an important role in contributing to human health (Cianciosi *et al*., 2018).

Honey antibacterial activity is due to higher sugar content, higher acidity (Mohan *et al*., 2017) and presence of bacteriostatic and bactericidal factors such as hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal and bee peptides (Israili, 2014). Most prebiotics, such as fructooligosaccharide (FOS), galactooligosaccharide (GOS) or inulin have been documented to support the growth and survival of exogenously administered Bifidobacteria and Lactobacilli inside the gut (Forssten *et al*., 2011). Prebiotics may be defined as non-digestible food ingredients that benefit the host by selectively stimulating the favorable growth and/or activity of one or more indigenous probiotic

bacteria (Roberfroid, 2007). Honey can selectively regulate the gut microbial balance by favoring the growth of probiotic lactobacilli and bifidobacteria due to the presence of oligosaccharides, thus improving the host metabolic function (Mohan *et al*., 2017).

Health benefits confered by honey in humans include; antibacterial, antimutagenic, antiproliferative, hepatoprotective, hypoglycemic, antioxidant (Erejuwa *et al*., 2010), cardiovascular diseases, chemo-preventive activity in multistage carcinogenesis, anti-microbial benefits, improving endothelial function and plasma lipid profile (Alvarez-Suarez *et al.*, 2013), gastrointestinal tract diseases, neurological disorders, fertility disorders (Rao *et al*., 2016), prebiotic properties, human pathogen control and antiviral activity (Miguel *et al*., 2017).

2.2.4 Functionality of honey sweetened yoghurt

Nutritional value of yoghurt is enhanced with the incorporation of honey. Maize yoghurt containing 10% honey had 3.15% protein, 2.73% fat, 20.54% total solids, 0.32%, 4.49% fiber and a total lactic acid bacteria count of $5.3x10^8$ cfu/ml (Nofrianti *et al*., 2013). Average protein content enhanced (3.15 to 4.34%) with increasing honey concentration (0 to 10%) in yoghurt (Krisnaningsih and Yulianti, 2015) due to synthesis of the amino acids, resulting from interaction of components of honey with yoghurt cultures during fermentation (Zhang *et al*., 2011).

Ingestion of soy-yoghurt-honey formula led to a significant reduction in body weight, total fat mass and internal abdominal fat (Konig *et al*., 2015). Reviewed literature indicated that intake of soy-yoghurt-honey formula may be a safe alternative for weight loss and an improvement in the metabolic milieu within a sustained time period, independent of sex, age and diagnosed symptoms of the metabolic syndrome (Koohkan *et al*., 2017).

Recently, it has been reported that honey acts as an encapsulant, comparable to sodium alginate microencapsulation and improved the survivability of two probiotic Bifidobacterium strains in simulated gastrointestinal conditions (Favarin *et al*., 2015).

2.2.5 Growth characteristics of yoghurt cultures in presence of honey

In freshly prepared yoghurt, the viable count (cfu/ml) of *Streptococcus thermophilus* increased (32×10^6 to 40×10) with the addition of 5% black cumin bee honey but decreased (32×10^6 to 30×10^6) with increasing concentration of honey (10 to 15%). However, viability of *Lactobacillus delbrueckii* subsp.

bulgaricus decreased (55 x 10^6 to 34 x 10^6) in yoghurt containing 5% honey, which further decreased (20 x 10^6 to 21 x 10^6) at higher concentration of honey. Higher total mesophilic aerobic bacteria counts (8.27 vs 4.39 log cfu/g) were encountered in yoghurt containing 7 per cent honey in contrast to those without honey at 28 day of cold storage. Significant higher counts of *L. delbrueckii* subsp. *bulgaricus* were noted in yoghurt containing 7% honey but yoghurt with 3% and 5% honey induced lower counts than those in control (Bakr *et al*., 2017). On the contrary, Mercan and Akin (2016) reported that yoghurt with 7% honey had the lowest *S. thermophilus* counts in comparison to those with 3% and 5% honey.

A higher viable population (cfu/g) of both *L. delbrueckii* subsp. *bulgaricus* (19 x 10^5 vs. 5 x 10^5) and *S. thermophilus* (22 x 10^5 vs 10 x 10^5) were observed in yoghurt made from cow milk containing honey employing ABT cultures (combination of *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *S. thermophilus*) than classical yoghurt cultures during 15 days of storage at 4°C (Hamad *et al*., 2016).

However, lower viable counts (cfu/ml) of *L. bulgaricus* (25 x 10^5) than *S. thermophilus* (27 x 10^5) were encountered in freshly prepared ABT yoghurt made from mixed cow milk and coconut milk (50:50) containing 5% honey (Ismail *et al*., 2017).

No effect on the growth and survival of starter streptococci in camel and cow milks containing 5% honey was observed during production and subsequent refrigerated storage of fermented ABT milks at 4°C up to 5 weeks (Varga *et al*., 2014).

Recently, a decline in counts of yoghurt cultures and *L. acidophilus* La-05 was encountered during 21 day of refrigerated storage but decrement was less in yoghurt containing honey (Machado *et al*., 2017). Attempts were also made to evaluate growth behavior of yoghurt cultures in the presence of certain additives along with honey. It was reported that counts of lactic acid bacteria in freshly prepared yoghurt containing 1% whey protein concentrate (3.0 x 10^{11} ±6 4 x 10^9) increased with the increase in extent of incorporation of honey from 2% (7.0 x 10^{11} ±6 9 x 10^9) to 4% (1.1$10^{12}$ ±6 10^{10}) (Glusac *et al*., 2015).

It has been reported as well for the viable population (log cfu/ml) of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in freshly prepared yoghurt to be 1.38 x 10^7 and 1.59 x 10^7 respectively. An abatement in counts (2.14 x 10^8 and 2.17 x 10^8 , respectively) were noted with the inclusion of goji berries (*Lycium barbarum*) but further addition of honey induced lower values (1.70 x 10^7 and 1.30 x 10^8 , respectively) (Rotar *et al*., 2015). Therefore starter cultures may be inhibited or retarded due to

bacteriostatic/bactericidal effect of honey and thus suitability of starter cultures must be evaluated prior to its application.

Since yoghurt is known to have a short shelf life which is approximately less than 3 weeks (Mojka, 2013), this research will focus on assessing the effect of using honey as a sweetener instead of sucrose on the shelf life of yoghurt.

Titratable acidity and pH have important roles in lactose fermentation and acceptability, as well as in the shelf life of yogurt (Kavas *et al*., 2003).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Research Design

The research was mainly focused on determining the effect of using honey sweetener on the shelf life of yoghurt. Honey sweetened yoghurt was prepared together with a control yoghurt (sucrose sweetened) in the laboratory. The two yoghurt samples were further grouped into two batches with one batch stored at room temperature and another batch stored at refrigeration temperature. Each batch consisted of one sample of honey sweetened yoghurt and one sample of sucrose sweetened yoghurt. During the storage period, the titratable acidity, pH, Susceptibility to Syneresis (STS) and Water Holding Capacity (WHC) of each sample were measured over a period of 26 days.

Another batch of honey sweetened and sucrose sweetened yoghurt was freshly prepared and then subjected to sensory evaluation using the 9- point hedonic scale to evaluate the different sensory attributes of both yoghurt samples.

The investigation was carried out at the Biochemistry laboratory at the College of Natural Sciences of Makerere University from 29th May 2022 to 4th September 2022.

3.2 Materials

- Milk
- Sucrose
- Honey
- Yoghurt starter culture
- NaOH solution
- Phenolphthalein indicator
- Heat source
- Falcon tubes
- Refrigerator
- Four 1 liter jerry cans
- Centrifuge
- Clean water source

3.3 Preparation of yoghurt

3.3.1 Preparation of Sucrose Sweetened Yoghurt (Control)

a) The apparatus used in yoghurt production were first washed thoroughly using hot water with soap and finally rinsed to remove dirt and other competing microorganisms that might overpower the starter culture.
b) 500ml of milk were boiled in a source pan with the aid of a hotplate in order to kill whatever unsavory microbes that maybe lurking in the milk and to ensure that there was no remnant bacteria, pathogens, molds or spores thus creating a sterile environment for the yoghurt bacteria. Heating the milk also created thicker yoghurt by changing the protein structure.
c) 7% (w/v) sucrose was be added to the heated milk as a sweetener.
d) After adding the sweetener (sucrose), the milk was then cooled to room temperature to allow the cream to form which was removed using the sieve.
e) The milk was transferred into a one liter jerry can for pasteurization at 85^{o}C for 30 minutes using a water bath set at 85^{o}C.
f) The milk was then cooled to about 45^{0}C under tap water.
g) 30% (w/v) of active culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was added to the milk and mixed thoroughly by stirring. A commercial yoghurt starter culture was used.
h) The milk was incubated at 43^{o}C in a water bath for about 5 hours.
i) After the yoghurt was formed, it was transferred to a refrigerator set at 4^{0}C for storage prior to analysis for refrigeration-stored sample or just left to stand at room temperature for the room temperature-stored yoghurt.

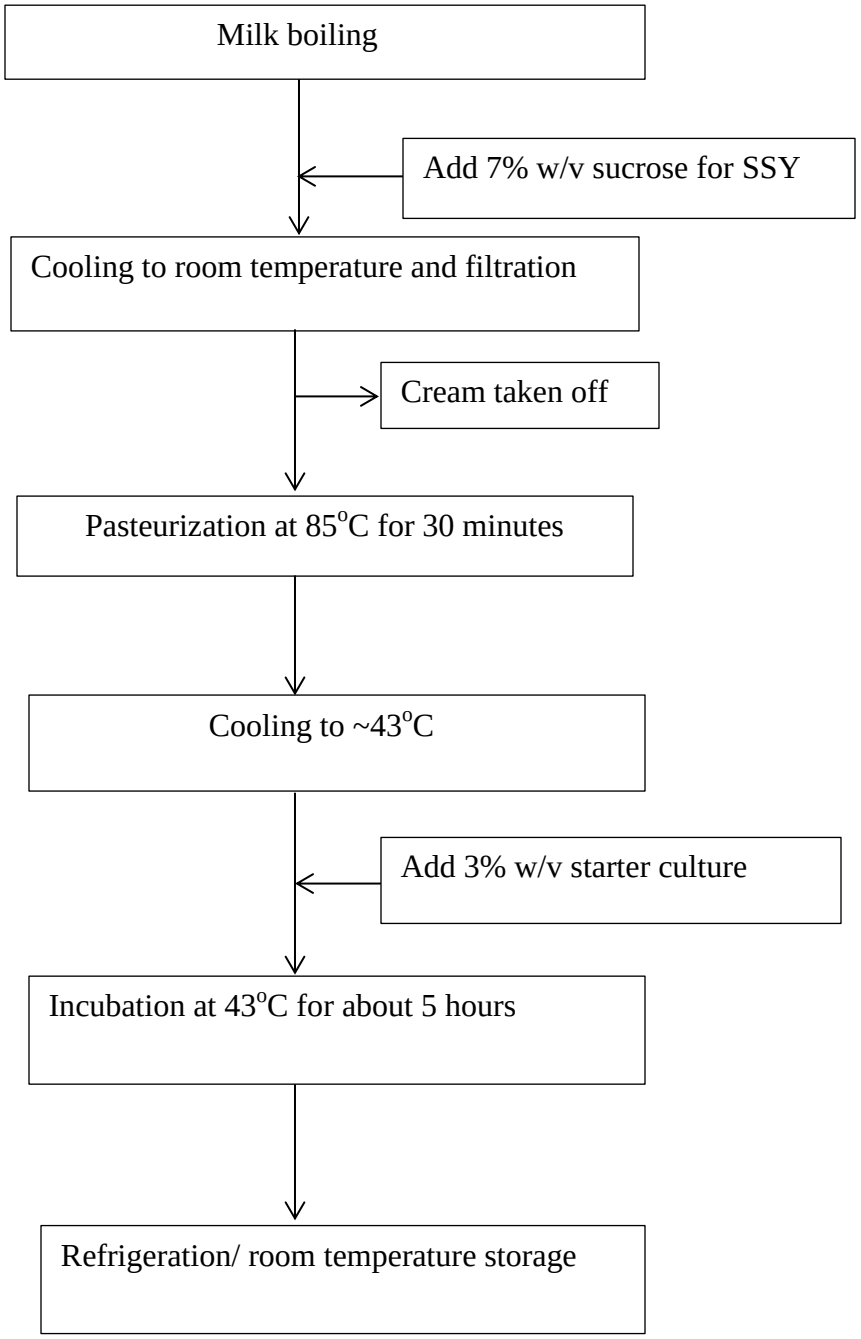


Figure 3.1: A flow chart summarizing the preparation of sucrose sweetened yoghurt

3.3.2 Preparation of honey sweetened yoghurt

a) The apparatus used in yoghurt production were first washed thoroughly using hot water with soap and finally rinsed to remove dirt and other competing microorganisms that might overpower the starter culture.

b) 500ml of milk in a saucepan were boiled using a hotplate in order to kill off any microorganisms that might be present in it, thus creating a sterile environment.
c) The milk was cooled to room temperature to allow the cream floating on the surface of the milk to form, which was filtered off using a sieve.
d) The milk was the transferred to a one liter jerry can for pasteurization at 85^{o}C for 30 minutes using a water bath set at this temperature.
e) The milk was cooled to about 45^{o}C under tap water.
f) 30% (w/v) of active starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was added to the milk, mixed thoroughly by shaking. A commercial yoghurt starter culture was used.
g) The mixture was then incubate at 43^{o}C using a water bath set at this temperature for 5 hours.
h) 10% (v/v) of honey was added, stirred thoroughly and transferred to the refrigerator for storage prior to analysis for the refrigeration-stored sample or just left to stand at room temperature for the room temperature-stored yoghurt.

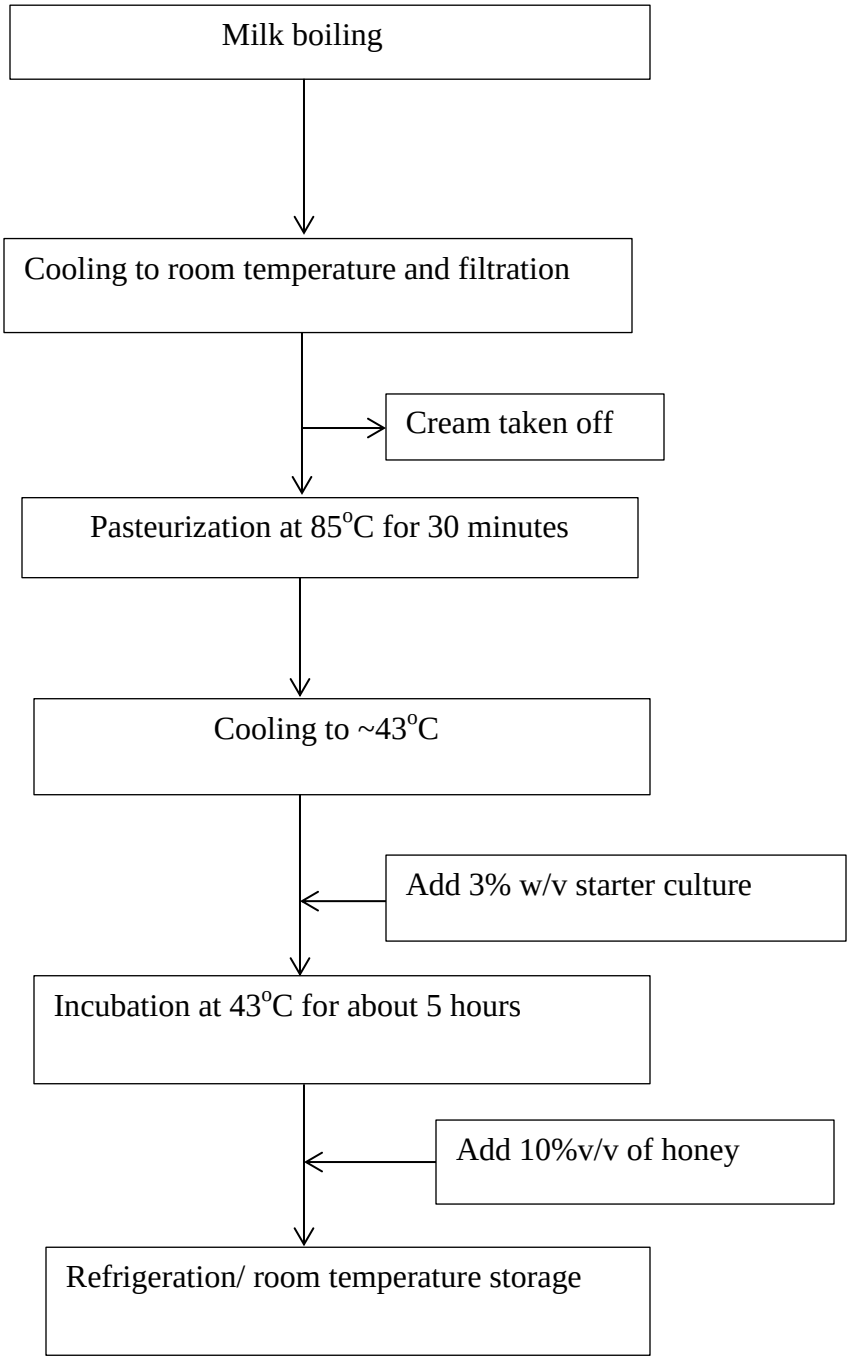


Figure 3.2: A flow chart summarizing the preparation of honey sweetened yoghurt

3.4 Water Holding Capacity (WHC)

Water holding capacity measures the amount of water absorbed in the protein structure of the yogurt.

This was determined by centrifuging 10ml of a yoghurt sample at 3,000rpm for 15 minutes using a centrifuge (Spasenija *et al*., 2007). The WHC was then calculated using the mathematical formula below;

$$\%WHC = (1 - \frac{v1}{v2})X100$$

Where: V_1 = volume of whey after centrifugation V_2 = volume of yoghurt sample.

Figure 3.3: Centrifugation and whey separation

3.5 Susceptibility to Syneresis (STS)

This was determined by measuring the volume of the whey collected in a measuring cylinder after the drainage of 10ml of yoghurt sample placed on a filter paper on top of a funnel for 2 hours at room temperature (Isanga and Zhang, 2007). The STS was then calculated with the formula as follows;

$$\%\ STS = \frac{V1}{V2}X100$$

Where: V_1 = Volume of whey collected after drainage and V_2 = Volume of yoghurt sample used.

Figure 3.4: Drainage of yoghurt samples through filter paper.

3.6 Determination of pH values

The pH of yoghurt samples was measured using a pH meter (Akalın *et al*., 2012).

3.7 Determination of Titratable Acidity

Titratable acidity is an approximation of the total acidity in a substance. The titratable acidity of the yoghurt samples was determined by measuring how much base (0.1N NaOH) was required to neutralize lactic acid in 10ml of yoghurt using phenolphthalein indicator (Parmar, 2003).

The titratable acidity was then calculated using the formula below;

$$\text{Titratable acidity} = \frac{Volume\ of\ titrant\ x\ N\ x\ 90}{Weight\ of\ sample\ X\ 100}\ x\ 100$$

Where N = Normality of titrant and 90 is the equivalent weight for lactic acid.

Figure 3.5: Color of yoghurt sample before and after titration

3.8 Shelf life determination

The shelf life of the honey sweetened yoghurt and sucrose sweetened yoghurt stored at room and refrigeration temperatures was determined by estimating the period for which the pH and titratable

acidity were still within the working ranges of 4.4-4.8 and 0.7-1.2% respectively. Any sample whose pH or titratable acidity was not within those working ranges were considered expired.

3.9 Sensory Evaluation

The yoghurt samples (sucrose sweetened and honey sweetened) were subjected to sensory evaluation in order to determine the preference of each type of yoghurt. Sensory evaluation of the yoghurt samples was carried out using a nine-point hedonic scale to measure the sensory attributes based on appearance, texture (mouth-feel), flavor and overall acceptability of the yoghurt samples by a group of 11 people and these were students from my class who were first briefed before carrying out the sensory evaluation (Isanga and Zhang, 2007).

Each individual was given about 20ml from each sample in a small cup coded with a 3-digit code and the individuals rinsed their mouth with water before testing each sample. The samples were scored using the following opinions; 1 for dislike extremely, 2 for dislike very much, 3 for dislike moderately, 4 for dislike slightly, 5 for neither like nor dislike, 6 for like slightly, 7 for like moderately, 8 for like very much and 9 for like extremely.

3.10 Statistical Data Analysis

All analytical determinations were performed in triplicates and values for the different parameters were expressed as the mean ±standard deviation.

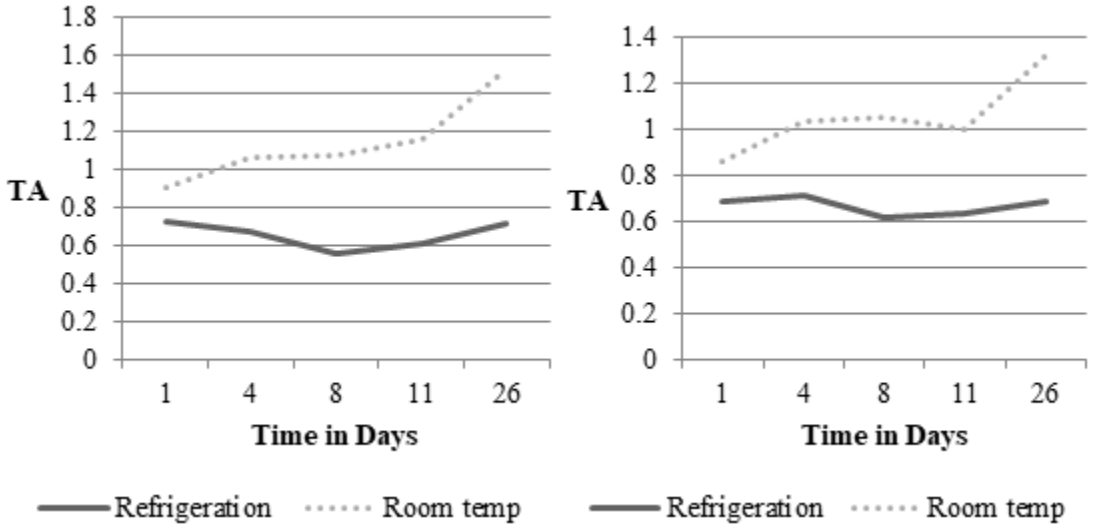
The data was analyzed statistically using the one way ANOVA to obtain the p-value using Microsoft Excel 2016. P values ≤ 0.05 were considered significant.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Honey sweetened yoghurt preparation

Unlike during the preparation of SSY (figure 3.1), in the case of honey sweetened yoghurt preparation (figure 3.2); the sweetener (honey) was added after the incubation step. This was so because if honey had been added before incubation, it would have inhibited the yoghurt starter culture before gel formation due to its antimicrobial activity (Chen *et al*., 2000).

4.2 Titratable Acidity



(a) **(b)**

Figure 4.1: Titratable acidity at different storage temperatures in SSY and HSY. **(a)** Variation of titratable acidity in SSY at different storage temperatures. **(b)** Variation of titratable acidity in HSY at different storage temperatures.

As seen in figures 4.1(a) and 4.1(b), there was a significant increase ($P<0.05$) in the titratable acidity of both yoghurt samples at room temperature storage. The titratable acidity was higher in samples stored at room temperature. The significant increase in titratable acidity at room temperature was due to rapid fermentation of the lactose sugar into lactic acid by the yoghurt starter cultures. However, the lower titratable acidity during refrigeration storage could be attributed to the decreased activity of the yoghurt starter cultures at the lower temperatures (Jankowska and Reps 2013). Therefore, storage temperature significantly affected the titratable acidity in both yoghurt samples.

Table 4.1: Variation of yoghurt titratable acidity during refrigeration storage

Time in Days	SSY (%)	HSY (%)
1	0.73±0.01 [a]	0.69±0.04 [a]
4	0.67±0.04 [a]	0.71±0.02 [a]
8	0.56±0.03 [a]	0.62±0.03 [b]
11	0.61±0.02 [a]	0.64±0.04 [a]
26	0.72±0.02 [a]	0.69±0.06 [a]

The results indicated in the table above are expressed as means ± Standard deviation. The Mean values having similar superscripts within the same row are not significantly different (P>0.05).

From table 4.1, there was no significant (P>0.05) difference in the titratable acidity during refrigeration temperature storage between the yoghurt samples except on day 8. There was an initial decrease in the titratable acidity which then increased with increasing storage time. The initial decrease could be explained by the rapid reduction in the rate of lactic acid production from the fermentation of the lactose sugar in milk by the yoghurt starter cultures due to lower refrigeration temperatures in comparison to the previous higher incubation temperatures at which the yoghurt was prepared.

On the other hand, the increase could be attributed to the continued post acidification by the yoghurt starter cultures after adaptation to the low refrigeration temperatures. This observation is consistent with reports from several authors who reported that the titratable acidity of yoghurt stored under refrigeration conditions increases significantly with increasing storage time (Singh & Muthukumarappan, 2008; Hassan & Amjad, 2010).

Table 4.2: Variation of yoghurt titratable acidity during room temperature storage

Time in Days	SSY (%)	HSY (%)
1	0.91±0.04 [a]	0.86±0.02 [a]
4	1.06±0.02 [a]	1.04±0.03 [a]
8	1.08±0.03 [a]	1.05±0.09 [a]
11	1.16±0.14 [a]	1.00±0.04 [a]
26	1.53±0.04 [a]	1.32±0.13 [a]

The results indicated in the table above are expressed as means ± Standard deviation. The Mean values having similar superscripts within the same row are not significantly different (P>0.05).

From the table 4.2, the titratable acidity generally increased with increasing time in both yoghurt samples. This could be attributed to the fact that the fermentation and subsequent lactic acid production

rate remained the same since the storage temperature was equivalent to the incubation temperature used during yoghurt preparation. However, the titratable acidity was lower in honey sweetened yoghurt throughout the storage period and this was probably because of the reduced rate of lactic acid production by the yoghurt starter culture due to inhibition by honey which has antimicrobial properties (Chen *et al*., 2000).

4.3 pH

As observed in figures 4.2 (a) and 4.2 (b), there was a more rapid decrease in pH at room temperature compared to that during refrigeration storage in both yoghurt samples. The rapid decrease at room temperature was probably due to faster production of lactic acid from fermentation of lactose by the yoghurt starter cultures compared to the slower production of lactic acid at refrigeration temperature due to reduced activity of the yoghurt starter cultures (Jankowska and Reps 2013). Therefore, storage temperature had a substantial effect on the pH in both yoghurt samples.

From figure 4.2 (c), the pH of both yoghurt samples initially increased and then decreased for the rest of the storage period. The initial increase can probably be accounted for by certain milk salts such as calcium phosphate which exists partly in soluble form and partly in colloidal phase associated with casein micelles. Changes in temperature modify the equilibrium between the soluble and colloidal phases. Moderate heating for example during pasteurization decreases the solubility of Calcium phosphate due to formation of the colloidal calcium phosphate which is accompanied with a decrease in pH. However, subsequent cooling and storage at low temperature perhaps restores the original equilibrium by increasing both the solubility of calcium phosphate and the yoghurt pH. Therefore calcium phosphate probably influences the pH of yoghurt through the buffering action (Lucey and Horne, 2009).

The decrease in pH during the rest of the storage period was probably due to the continued slow production of lactic acid and other organic acids after the starter culture adaptation to the low refrigeration temperatures However, the decrease in pH was lower in the Honey sweetened yoghurt compared to that in the sucrose sweetened yoghurt. This is probably because in addition to the low temperatures, the honey further reduced the activity of the yoghurt starter cultures due to its

antimicrobial activity thus the rate at which lactic acid was produced from the fermentation of the lactose by the starter cultures was much slower in honey sweetened yoghurt than in sucrose sweetened yoghurt.

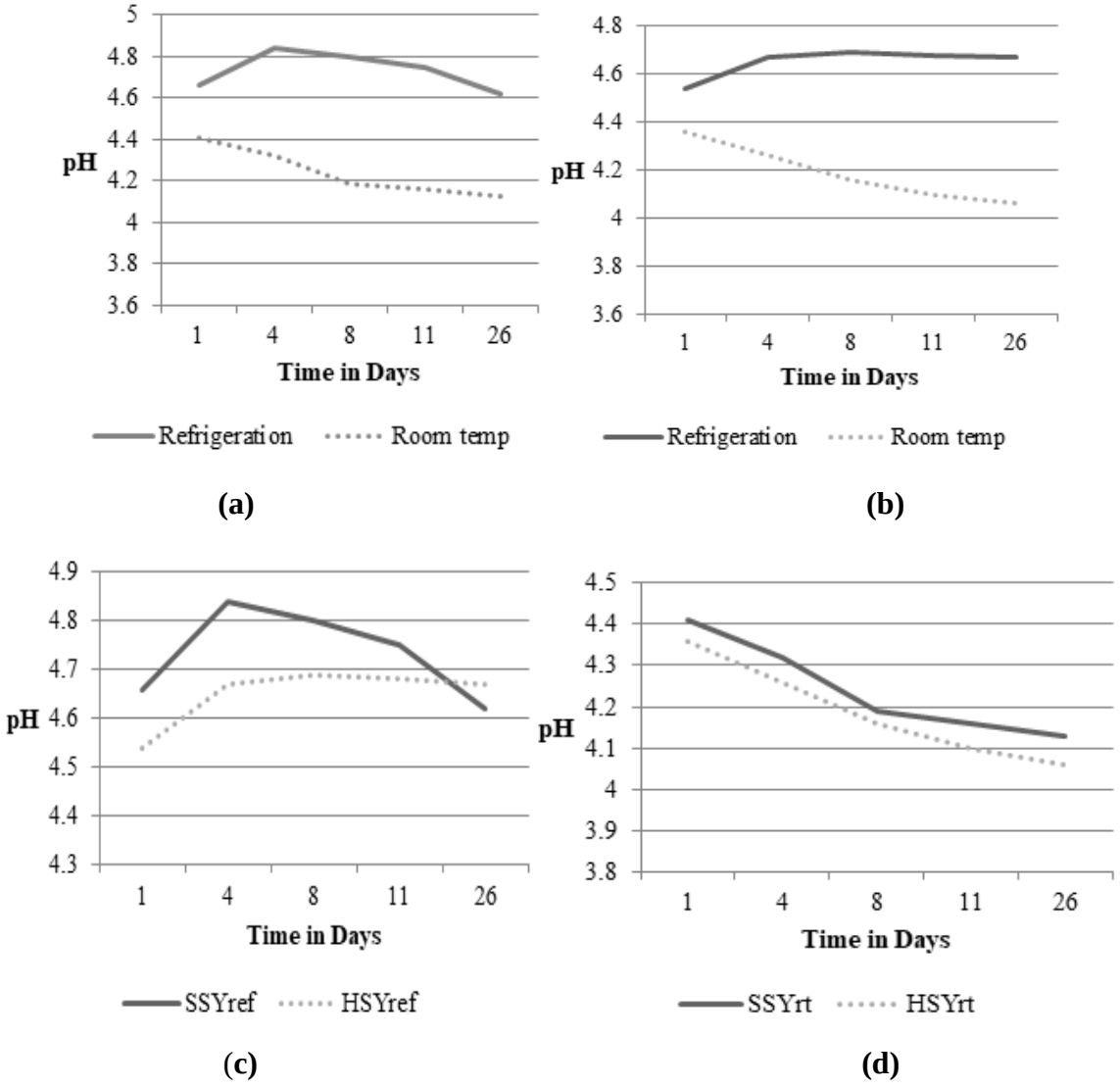


Figure 4.2: Variation of pH at different storage temperatures in HSY and SSY. **(a)** A graph showing variation of pH in SSY at different storage temperatures. **(b)** A graph showing variation of pH in HSY at different storage temperatures. **(c)** A graph showing variation of pH in SSY and HSY at refrigeration temperature. **(d)** A graph showing variation of pH in SSY and HSY at room temperature.

From figure 4.2(c), the pH of honey sweetened yoghurt was generally lower than that of sucrose sweetened yoghurt. This could be attributed to the presence of organic acids in honey such as lactic,

malic, pyroglutamic, formic, citric, acetic, butyric and gluconic acid which is the major acid in honey and a flavor enhancer (Resurreccion, 1995).

As observed in figure 4.2(d) above, the pH decreased with increasing storage time in both yoghurt samples during room temperature storage. This was probably due to the formation lactic acid as well as other organic acids such as formic acid from the fermentation of the lactose sugar in milk by the yoghurt starter cultures. The continuous formation of lactic acid by the yoghurt starter cultures was responsible for the decrease in yoghurt pH during storage and this decrease was more rapid compared to that during refrigeration storage. However, the pH of honey sweetened yoghurt was lower than that of sucrose sweetened yoghurt and this was probably because honey contains organic acids such as lactic, malic, pyroglutamic, formic, citric, acetic, butyric and gluconic acid which is the major acid in honey and a flavor enhancer (Resurreccion, 1995).

4.3 Susceptibility to Syneresis

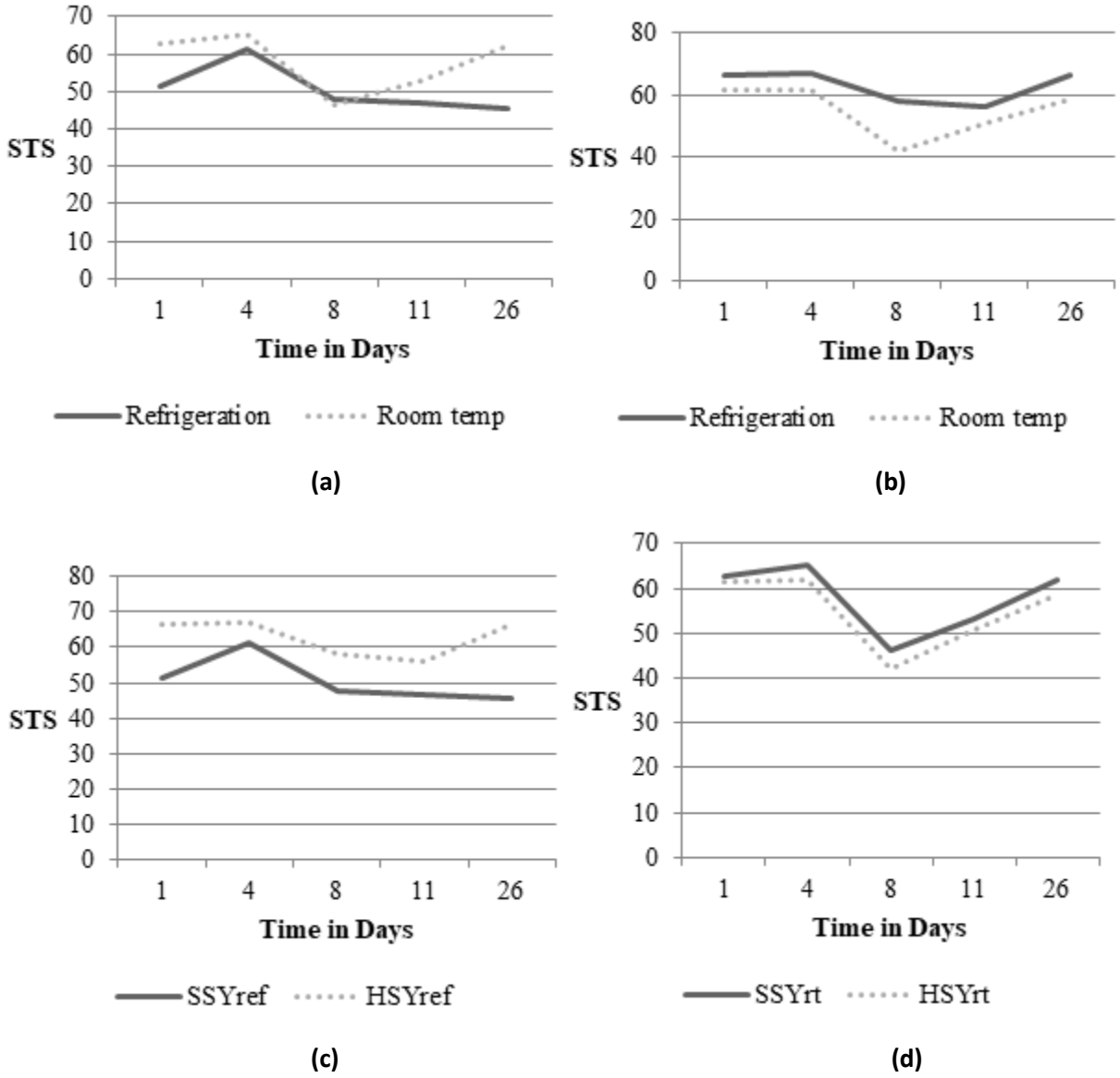


Figure 4.3: Variation of STS at different storage temperatures in SSY and HSY. (a) A graph showing variation of STS in SSY at different storage temperatures. (b) A graph showing variation of STS in HSY at different storage temperatures. (c) A graph showing variation of STS in SSY and HSY at refrigeration temperature. (d) A graph showing variation of STS in SSY and HSY at room temperature.

From figures 4.3(a) and 4.3(b), there was no clear relationship between the STS and storage temperature. This implied that storage temperature had no significant influence on the STS of the yoghurt samples.

From figure 4.3(c), it can be observed that the STS was higher in honey sweetened yoghurt throughout the storage period at refrigeration temperature. This can be explained by the increased acidity of honey in the honey sweetened yoghurt at refrigeration temperature (Kędzierska-Matysek, 2016) making it more acidic than the sucrose sweetened yoghurt which causes the denaturation of the casein protein in yoghurt that loses the capacity to attract/ hold the whey which is easily expelled and is observed in the yoghurt.

As seen in figure 4.3(d), the STS was lower in honey sweetened yoghurt throughout the storage period. The lower STS in the yoghurt with honey was probably due to the high osmolarity of honey which attracts water to the yoghurt-forming casein micelles which reduces liquid (whey) release to the surrounding. Conversely, the higher STS in sucrose sweetened yoghurt could be due to the three dimensional protein networks becoming denser thus gradually lose the ability to attract the whey which is easily expelled and becomes visible in the yoghurt (Bezerra *et al*., 2012). The results obtained were consistent with those earlier reported by Tamires *et al* (2017).

4.4 Water Holding Capacity

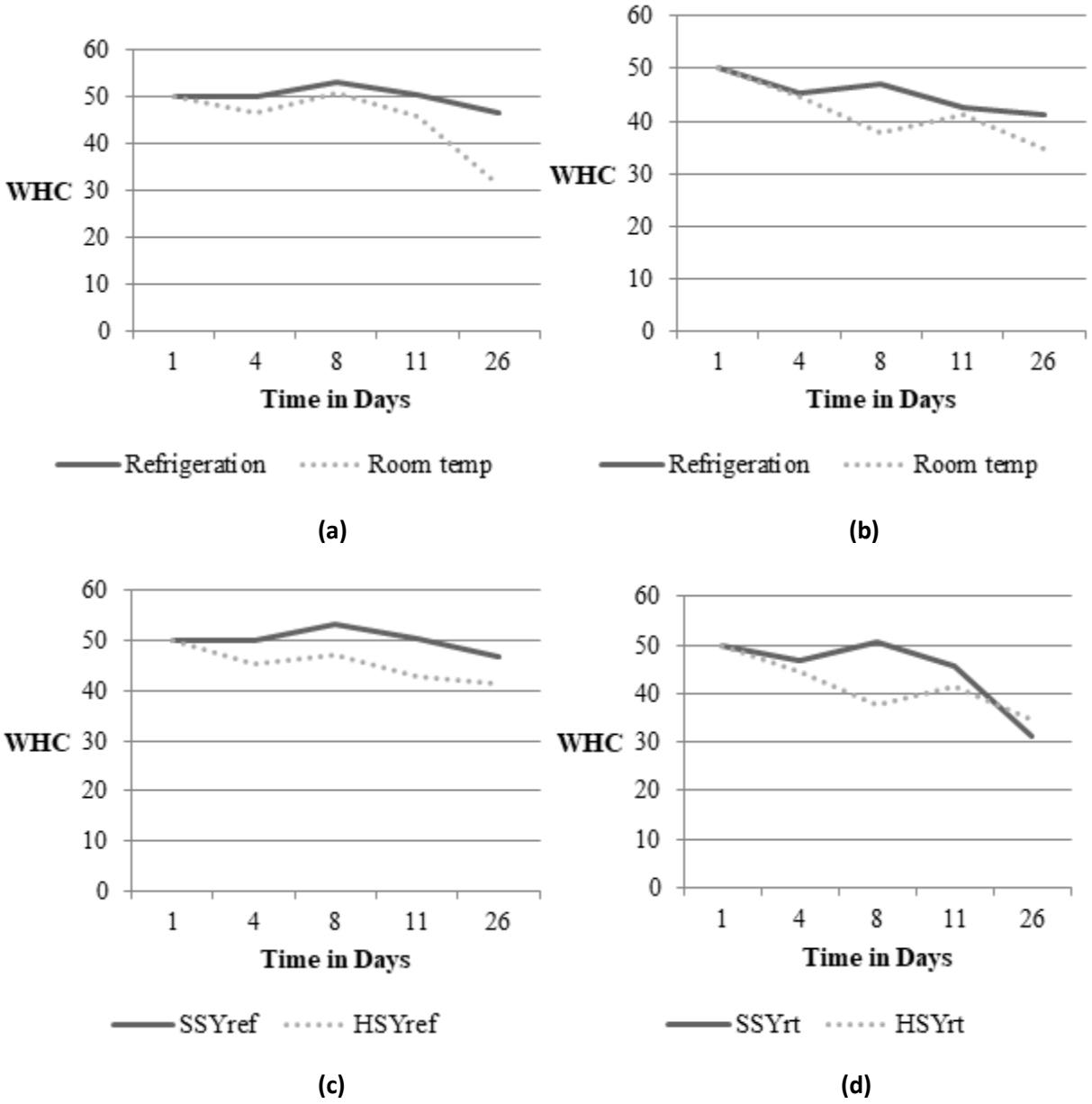


Figure 4.4: Variation of WHC at different storage temperatures in SSY and HSY. **(a)** A graph showing variation of WHC in SSY at different storage temperatures. **(b)** A graph showing variation of WHC in HSY at different storage temperatures. **(c)** A graph showing variation of WHC in SSY and HSY at refrigeration temperature. **(d)** A graph showing variation of WHC in SSY and HSY at room temperature.

From figures 4.4(a) and 4.4(b), the WHC in both yoghurt samples was lower at room temperature storage. This may be associated with the faster fermentation of the yoghurt starter cultures at room temperature which results into increase in acidity during storage that increases water release (syneresis) in the yoghurt because of possible protein denaturation due to pH decrease up to the isoelectric point.

This causes destabilization of the casein micelles and subsequent loss liquid (Bezerra *et al*., 2012) leading to low WHC at room temperature.

From the figure 4.4(c) above, there was a decrease in the Water Holding Capacity (WHC) during refrigeration storage though the decrease was slight. This behavior is probably associated with syneresis as the increasing release of the liquid phase in yoghurt is concurrent with smaller WHC in yoghurt over time. It may also be associated with the slower metabolic rate of the yoghurt starter culture at refrigeration temperature which results into a slight increase in acidity during storage that slightly increases water release (syneresis) in the yoghurt because of possible protein denaturation due to pH decrease up to the isoelectric point. This causes destabilization of the casein micelles and subsequent loss liquid (Bezerra *et al*., 2012) thus a decrease in WHC.

The findings of this study were similar to those of Coskun and Karabulut (Coskun and Karabulut, 2019) who reported that the WHC decreased during storage. However the findings were contrary to those reported by Sert et al (Sert *et al*., 2011) whose values for WHC of yoghurt with honey increased during storage. The difference between these two studies could be attributed to the differences in the starter cultures and the production methods used.

From the figure 4.4(d), there was a decrease in the WHC which was more rapid compared to that during refrigeration temperature storage. This is probably due to rapid fermentation of lactose by the yoghurt starter culture at room temperature which results into increase in acidity during storage and therefore increased water release (syneresis) in the yoghurt because of possible protein denaturation due to rapid pH decrease up to the isoelectric point. This causes destabilization of the casein micelles and subsequent loss of liquid (Bezerra *et al*., 2012) thus a decrease in WHC.

4.5 Shelf life estimation

For room temperature-stored yoghurt, the shelf life of honey sweetened yoghurt was estimated to be 11 days while that of sucrose sweetened yoghurt was estimated to be 8 days. This implies that honey extended the shelf life of room temperature-stored yoghurt by 3 days.

Titratable acidity and pH were the main parameters used in the estimation of shelf life since they have important roles in lactose fermentation and acceptability, as well as in the shelf life of yoghurt (Kavas *et al.*, 2003).

The shelf life of sucrose-sweetened yoghurt was estimated to be 8 days because there was growth of molds and yeast which were indicators of spoilage at a pH of 4.19 and the yoghurt had a bad odor. Yeast and mold are the principal agents of microbial spoilage of yogurt. In fresh yoghurt products, yeast and mold may be present due to contamination in the processing operations, from the packaging materials and/or the filling operations (MacBean, 2010). Yeast and molds are less affected by low pH and may cause spoilage of yoghurt during storage (Al-Ashmawy & Ibrahim, 2009).

On the other hand, the shelf life of honey-sweetened yoghurt was estimated to be about 11 days because there was a rapid increase in the titratable acidity and a significant decrease in the pH of the yoghurt sample and it as well had a bad odor after 11 days. However there was no growth of molds and yeast in the honey-sweetened yoghurt during the storage period. This could be due to the low water activity of honey owing its high sugar concentration and such conditions did not favor the growth of yeast and molds. Honey also contains hydrogen peroxide which kills the yeast and molds (Crandal, 2007).

The yoghurt samples stored at refrigeration temperature did not expire for the 26 days of storage since their ti-3tratable acidity and pH values remained within the acceptable ranges of 0.7-1.2% and 4.3-4.8 respectively (Choi *et al*., 2016).

4.6 Sensory Evaluation

From the table 4.3, honey sweetened yoghurt had higher sensory scores for all the sensory attributes compared to sucrose sweetened yoghurt and this therefore implied that honey sweetened yoghurt was more accepted by the panelists compared to sucrose sweetened yoghurt. This could be because of the sweet taste of honey, which was preferable to most panelists. Addition of honey not only improved yoghurt flavor, but also body and texture. The results of this study were in agreement with those earlier reported by Riazi and Ziar, 2012 who also obtained higher scores in yoghurt containing honey compared to that without honey. However the results obtained were contradictory to those obtained by Popa and

Ustunol, 2011 who found that sucrose sweetened yoghurt was more preferred over honey sweetened yoghurt.

Table 4.3: Scores for different sensory attributes of the two yoghurt samples

Sample	**Sensory Attribute**			
	Appearance/Color	**Texture/mouth feel**	**Flavor**	**Overall Acceptability**
SSY	6.55±1.44[a]	6.73±1.68 [a]	6.82±1.83 [a]	6.73±1.62 [a]
HSY	7.00±0.89 [a]	6.82±0.87 [a]	7.55±0.93 [a]	7.00±0.63 [a]

The results indicated in the table above are expressed as means ± Standard deviation. The Mean values having similar superscripts within the same column are not significantly different (P>0.05).

Various comments were obtained from the panelists in regards to improvement of the quality of the product for example homogenization of the sample in order to improve the texture of the yoghurt. The panelists also recommended that the honey sweetened yoghurt should be made more viscous by addition of stabilizers.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Honey Sweetened Yoghurt was successfully prepared together with Sucrose Sweetened Yoghurt as the experimental control. The pH, titratable acidity, Water Holding Capacity and Susceptibility to Syneresis were determined for each yoghurt sample under refrigeration and room temperature storage conditions for a period of 26 days. Generally, the storage temperature significantly affected the pH, titratable acidity and WHC but had no clear effect on the STS.

The pH of both SSY and HSY decreased during storage at refrigeration temperature and the decrease was significant ($P<0.05$) for SSY (4.84 to 4.62) compared to that of HSY (4.69 to 4.67) where the decrease was not significant. However, at room temperature storage, there was a significant decrease in pH for both yoghurt samples. There was no significant increase ($P>0.05$) in the titratable acidity of both yoghurt samples at refrigeration temperature but the increase was significant at room temperature with the highest value of 1.53±0.04% for SSY and 1.32±0.13% for HSY. The STS at refrigeration temperature was higher in HSY with the highest value of 67.00±2.65% but at room temperature it was higher in SSY with the highest value of 65.33±0.58%. The WHC decreased at both storage conditions but the decrease was more rapid at room temperature. The lowest WHC was 31.33±1.15% for SSY at room temperature.

The shelf life of SSY at room temperature was 8 days while that of HSY under the same storage conditions was more than 11 days hence, honey had an effect of extending the shelf life of yoghurt for about 3 days under room temperature storage. On the other hand, the yoghurt samples stored at refrigeration temperature did not expire even after the 26 days of storage thus; yoghurt storage at refrigeration temperature remains advantageous and highly recommended even in cases where honey is used as a sweetener.

Upon sensory evaluation, HSY had higher scores for all the sensory attributes tested compared to sucrose sweetened yoghurt and the preference for HSY was also confirmed with the overall acceptability score of 7.00±0.63 for HSY and 6.73±1.62 for SSY. Therefore, in addition to extending shelf life, sweetening yoghurt with honey also enhanced its sensory attributes.

5.2 RECOMMENDATIONS

- A suitable stabilizer should be identified and added to honey sweetened yoghurt in order to increase its viscosity.
- Honey sweetened yoghurt should still be stored at low temperature to further extend its shelf life.

REFERENCES

Akalin, A.S., Gonc, S., Unal, G., Fenderya, S. (2007). Effects of fructoooligosaccharide and whey protein concentrate on the viability of starter culture in reduced-fat probiotic yoghurt during storage. J. Food Sci., 72, 222-227.

Al-Ashmawy, M. A. M. and J. I. Ibrahim. (2009) Influence of potassium sorbate on the growth of yeasts and moulds in yogurt. Int. J. Dairy Tech., 62: 224-227.

Alvarez-Suarez, J.M., Giampieri, F. and Battino, M. (2013), "Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases", Current Medicinal Chemistry, Vol. 20 No. 5, pp. 621-638.

Arvanitoyannis, I., and Krystallis, A. (2006), "An empirical examination of the determinants of honey consumption in Romania", International Journal of Food Science and Technology, Vol. 41 No. 10, pp. 1164-1176.

Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. Journal of Dairy Science, 100(12), 9987- 10013.

Bakr, I.A., Mohamed, T.H., Tammam, A.A. and El-Gazzar, F.E. (2017), "Characteristics of yoghurt fortified with black cumin honey", Assiut Journal of Agricultural Sciences, Vol. 48, pp. 67-79.

Choi, Y.J., Jin, H.Y., Yang, H.S. *et al.* (2016) Quality and storage characteristics of yogurt containing *Lacobacillus sakei* ALI033 and cinnamon ethanol extract. *J Anim Sci Technol* **58**, 16.

Cianciosi, D., Forbes-Hernandez, T.Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P.P., Zhang, J., Bravo, L.L., Martinez, F.S., Agudo, T.P., Quiles, J.L., Giampieri, F. and Battino, M. (2018), "Phenolic compounds in honey and their associated health benefits: a review", Molecules, Vol. 23 No. 9, p. 2322

Kędzierska-Matysek, M., Florek, M., Wolanciuk, A. *et al.* (2016)Effect of freezing and room temperatures storage for 18 months on quality of raw rapeseed honey (*Brassica napus*). *J Food Sci Technol* **53**, 3349–3355.

Codex Standard (2001), “Codex standard for honey”, Adopted in 1981. Revisions 1987 and 2001, pp. 12-1981.

Coskun, F., and Karabulut Dirican, L. (2019). Effects of pine honey on the physicochemical, microbiological and sensory properties of probiotic yoghurt. Food Science and Technology, 39(suppl 2), 616–625

Crandall, G. (2007, June 30). Honey and Mold Growth. Retrieved September 21, 2022, from http://eziniearticles.com/?Honey-and-Mold-Growth&id=627811

Erejuwa, O.O., Gurtu, S., Sulaiman, S.A., Wahab, M.S.A., Sirajudeen, K.N.S., Salleh, M.S.M. 2010. Hypoglycemic and antioxidant effects of honey supplementation in streptozotocininduced diabetic rats. Int. J. for Vitamin and Nutrition Res., 80, 74–82.

FAO/WHO (2001), Regulatory and Clinical Aspects of Dairy Probiotics, Food and Agriculture Organization of the United Nations, World Health Organization Cordoba, Argentina.

Favarin, L., Laureano-Melo, R. and Luchese, R.H. (2015), “Survival of free and microencapsulated bifidobacterium: effect of honey addition”, Journal of Microencapsulation, Vol. 32 No. 4, pp. 329-335.

Fisberg, M., & Machado, R. (2015). History of yogurt and current patterns of consumption. Nutrition Reviews, 73(1), 4–7

Forssten, S.D., Lahtinen, S.J. and Ouwehand, A.C. (2011), “The intestinal microbiota and probiotics”, in Malago, J.J., Konikx, J.F.J.G. and Marinsek-Loger, R. (Eds), Probiotic Bacteria and Enteric infections- Cryoprotection by Probiotic Bacteria, Springer Science, New York, NY, pp. 41-63.

Glusac, J., Stijepic, M., Durdevic-Milosevic, D., Milanovic, S., Kanuric, K. and Vukic, V. (2015), “Growth and viability of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus in traditional yoghurt enriched by honey and whey protein concentrate”, Iranian Journal of Veterinary Research, Vol. 16 No. 3, pp. 249-254.

Hamad, M.N.F., Ismail, M.M., El-Kadi, S.M.L. and Zidan, M.S. (2016), “Chemical composition, microbial properties and sensory evaluation of bio-yoghurt made from admixture of cow and

coconut milk and honey", International Journal of Scientific Research and Management, Vol. 5, pp. 246-260.

Hassan, A. and I. Amjad (2010). Nutritional evaluation of yogurt prepared by different starter cultures and their physicochemical analysis during storage. Afr. J. of Biotechnol. 9: 2913-2917.

Isanga J. and G Zhang. (2007) Preliminary investigation of the production and characterization of peanut milk based stirred yoghurt. Int. J. Dairy science. Pages 207-216.

Ismail, M.M., Hamad, M.F., El-Kadi, S.L. and Zidan, M.S. (2017), "Utilization ABT culture, coconut milk, and honey in bio-yoghurt manufacture", International Journal of Current Microbiology and Applied Sciences, Vol. 4, pp. 1-9.

Israili, Z.H. (2014), "Antimicrobial properties of honey", American Journal of Therapeutics, Vol. 21 No. 4, pp. 304-323

Jan Mei, S., Mohd Nordin, M.S. and Norrakiah, A.S. (2010), "Fructooligosaccharides in honey and effects of honey on growth of Bifidobacterium longum BB 536", Food Research International, Vol. 17, pp. 557-561

Jankowska, A., Reps, A. (2013). Factors affecting the shelf-life of yoghurt during storage [Czynniki decyduj ˛ace o trwałosci ´ jogurtu podczas przechowywania]. Prz. Mlecz. 11, 2–5 [in Polish].

Kadri, S.M., Zaluski, R. and Orsi, R.D.O. (2017), "Nutritional and mineral contents of honey extracted by centrifugation and pressed processes", Food Chemistry, Vol. 218, pp. 237-241.

Kamal, M.A. and Klein, P. (2011), "Determination of sugars in honey by liquid chromatography", Saudi Journal of Biological Sciences, Vol. 18 No. 1, pp. 17-21.

Kavas, G., H. Uysal, S. Kiliç, N. Akbulut, and H. Kesenkas. (2003). Some properties of yoghurts produced from goat milk and cowgoat milk mixtures by different fortification methods. Pak. J. Biol. Sci. 6:1936–1939

Kędzierska-Matysek, M., Florek, M., Wolanciuk, A. *et al.* (2016)Effect of freezing and room temperatures storage for 18 months on quality of raw rapeseed honey (*Brassica napus*). *J Food Sci Technol* **53**, 3349–3355.

Khalil, M.I. and Sulaiman, S.A. (2010), "The potential role of honey and its polyphenols in preventing heart diseases: a review", African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM, Vol. 7 No. 4, pp. 315-321.

Konig, D., Zdzieblik, D., Deibert, P., Berg, A., Gollhofer, A. and Buchert, M. (2015), "Internal fat and cardiometabolic risk factors following a meal- replacement regimen vs. comprehensive lifestyle changes in obese subjects", Nutrients, Vol. 7, pp. 9825-9833.

Koohkan, S., McCarthy, D.H. and Berg, A. (2017), "The effect of a soy-yoghurt-honey product on excess weight and related health risk factors - a review", Journal of Nutritional Health and Food Science, Vol. 2, pp. 1-10.

Krisnaningsih, A.T.N. and Yulianti, D.L. (2015), "Improving the quality of the yoghurt with the addition of honey", Proceeding International Seminar, Improving Tropical Animal Production for Food Security, Universitas Halu Oleo, Kendari, 3-5 November, pp. 205-211.

Lee, W.J. & Lucey, J.A.. (2010). Formation and Physical Properties of Yogurt. Asian-Australasian Journal of Animal Sciences. 23. 10.5713/ajas.2010.r.05.

Lucey J.A., Horney D.S. (2009) Milk Salts: Technological Significance P. L. H. McSweeney, P. F Fox (Eds), Advanced Diary Chemistry. Vol. 3: Lactose, Water, Salts and Minor Constituents (3rd ed), Springer Science + Business Media, New York NY pp351-384.

MacBean, R. D. Packaging and the Shelf Life of Yogurt. (2010)In: Food packaging and shelf life-A practical guide. (Ed. G. L. Robertson). CRC Press, Taylor and Francis Group, Boca Raton, FL. 143-156.

Machado, T.A.D.G., de Oliveira, M.E.G., Campos, M.I.F., de Assis, P.O.A., de Souza, E.L., Madruga, M. S., Pacheco, M.T.B., Pintado, M.M.E. and do Egypto Queiroga, R.C.R. (2017), "Impact of honey on quality characteristics of goat yogurt containing probiotic Lactobacillus acidophilus", Lwt – Lwt, Vol. 80, pp. 221-229.

Manyi-Loh, C.E., Clarke, A.M. and Ndip, R.N. (2011), “An overview of honey: Therapeutic properties and contribution in nutrition and human health”, African Journal of Microbiology Research, Vol. 5, pp. 844-852

Mercan, E. and Akin, N. (2016), “Effect of different levels of pine honey addition on physicochemical, microbiological and sensory properties of set-type yoghurt”, International Journal of Dairy Technology, Vol. 69, pp. 8.

Miguel, M.G., Antunes, M.D. and Faleiro, M.L. (2017), “Honey as a complementary medicine”, Integrative Medicine Insights, Vol. 12, pp. 1-15.

Mohan, A., Quek, S.Y., Gutierrez-Maddox, N., Gao, Y. and Shu, Q. (2017), “Effect of honey in improving the gut microbial balance”, Food Quality and Safety, Vol. 1 No. 2, pp. 107-115

Mojka, K. (2013). Charakterystyka mlecznych napojów fermentowanych. Characteristics of fermented milk drinks. Probl. Hig. Epidemiol., 94(4), 722–729 [in Polish].

Mouhoubi-Tafinine, Z., Ouchemoukh, S. and Tamendjari, A. (2016), “Antioxydant activity of some algerian honey and propolis”, Industrial Crops and Products, Vol. 88, pp. 85-90.

Nofrianti, R., Azima, F. and Eliyasmi, R. (2013), “Effect of addition of honey yoghurt corn quality”, Journal of Food Technology Applications, Vol. 2, pp. 62-67.

Pandey, S.M. and Mishra, H.N. (2015), “Optimization of the prebiotic and probiotic concentration and incubation temperature for the preparation of synbiotic soy yogurt using response surface methodology”, LWT - Food Science and Technology, Vol. 62, pp. 458-467.

Parmar, R. (2003). Incorporation of acid whey powders in probiotic yoghurt. M. Sc. Thesis, Major in Biological Sciences, Specialization in Dairy, South Dakota State University, U.S.A.

Pikul, J. (2004). Factors affecting the shelf life and milk product. Part II. Dairy products with short, intermediate and long shelf life [Czynniki wpływaj ¸ace natrwało´s´c mleka i produktów mlecznych. Cz¸e´s´c II. Produkty mleczne o krótkim, ´srednim orz długim okresie trwało´sci]. Chłodnictwo XXXIX, 10, 41–47 [in Polish]

Poorani, A., Subramonian, B.S., Elango, A., Pughazhenthi, T.R., Dhanalakshmi, B. (2012). Synbiotic bifido milk powder in weaned piglets: Effect on growth, hematology and intestinal histology. Egyptian J. Dairy Sci., 40, 91-97

Popa, D., & Ustunol, Z. (2011). Sensory attributes of low-fat strawberry yoghurt as influenced by honey from different floral sources, sucrose and high-fructose corn sweetener. International Journal of Dairy Technology, 64(3), 451-454.

Ranzato, E., Martinotti, S. and Burlando, B. (2013), "Honey exposure stimulates wound repair of human dermal fibroblasts", Burns and Trauma, Vol. 1 No. 1, pp. 32-38.

Rao, P.V., Krishnan, K.T., Salleh, N. and Gan, S.H. (2016), "Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review", Revista Brasileira de Farmacognosia, Vol. 26 No. 5, pp. 657-664

Rasic J and Kurmann J A (1978) Yoghurt: Scientific Grounds, Technology Manufacture and Preparation. Copenhagen, Denmark: Technical Dairy Publishers House

Resurreccion, A. (1995) Effect of Enhancement of the Basic Tastes and Desirable Flavors by Honey. Unpublished manuscript. Dept. of Food Science, Universityof Georgia.Athens, Georgia.

Riazi, A., Ziar, H. 2012. Effect of honey and starter culture on growth, acidification, sensory properties and bifidobacteria cell counts in fermented skimmed milk. African J. of Microbiology Res. 6, 486-498.

Roberfroid, M. (2007),"Prebiotics: the concept revisited", The Journal of Nutrition, Vol. 137, pp. 830-837

Rotar, A.M., Vodnar, D.C., Bunghez, F., Catunescu, G.M., Pop, C.R., Jimborean, M. and Semeniuc, C.A. (2015), "Effect of goji berries and honey on lactic acid bacteria viability and shelf life stability of yoghurt", Notulae Botanicae Horti Agrobotanici Cluj-Napoca, Vol. 43, pp. 196-203.

Sanchez-Segarra P J, Garcia-Martinez M, Gordillo-Otero M J, Diaz-Valverde A, Amaro-Lopez M A and Moreno-Rojas R (2000) Influence of the addition of fruit on mineral content of yoghurts: nutritional assessment. *Food Chemistry* **70** 85– 89.

Sanders, M.E. and Marco, M.L. (2010), "Food formats for effective delivery of probiotics", Annual Review of Food Science and Technology, Vol. 1, pp. 65-85

Sanz M L, Polemis N, Morales V, Corzo N, Drakoularakou A, Gibson G R and Rastall R A (2005) In vitro investigation into the potential prebiotic activity of honey oligosaccharides. Journal of Agricultural and Food Chemistry 53 2914–2921.

Sarkar, S. (2011), "Probiotics, prebiotics and synbiotics for infant feeding – a review", Journal of Microbial and Biochemical Technology, Vol. S1, p. 004.

Sarkar, S. (2018), "Whether viable and dead probiotic are equally efficacious?", Nutrition and Food Science, Vol. 48 No. 2, pp. 285-300.

Sarkar, S. (2019a), "Potentiality of probiotic yoghurt as a functional food – a review", Nutrition and Food Science, Vol. 49 No. 2, pp. 182-202.

Sarkar, S. (2019b), "Synbiotic fruit yoghurt with enhanced functionality", International Journal of Food Science, Nutrition and Dietetics, Vol. 8, pp. 1-2.

Sarkar, S., Sur, A., Das, K. and Boral, J. (2017), "Safety of probiotics for preterm infants – a review", Nutrition and Food Toxicology, Vol. 1, pp. 219-227

Sert, D., Akin, N., & Dertli, E. (2011). Effects of sunflower honey on the physisochemical, microbial and sensory characteristics in set type yoghurt during refrigerated storage. International Journal of Dairy Technology, 64(1), 99-107.

Shamala, T. R., Shri Jyothi, Y., Saibaba, P. (2000). Stimulatory effect of honey on multiplication of lactic acid bacteria under in vitro and in vivo conditions. Letters in Applied Microbiology, 30: 453–455.

Singh, G. and K. Muthukumarappan. (2008). Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. LWT. 41: 1145-1152.

Spasenija, D., M.C. Milanovi, S.U. Mirjana, D. I. Mirela and G.D. Katarina (2007). *Physico-chemical properties of probiotic yoghurt produced with transglutaminase* APTEFF, 38, 1-190.

Tajabadi, N., Mardan, M., Saari, N., Mustafa, S., Bahreini, R. and Abdul Manap, M.Y. (2013), "Identification of Lactobacillus plantarum, lactobacillus entosus and Lactobacillus fermentum from honey stomach of honeybee", Brazilian Journal of Microbiology, Vol. 44 No. 3, pp. 717-722.

Tamires Alcântara Dourado Gomes Machado, Maria Elieidy Gomes de Oliveira, Maria Isabel Ferreira Campos, Paloma Oliveira Antonino de Assis, Evandro Leite de Souza, Marta Suely Madruga, Maria Teresa Bertoldo Pacheco, Maria Manuela Estevez Pintado, Rita de Cássia Ramos do Egypto Queiroga (2017). Impact of honey on quality characteristics of goat yogurt containing probiotic Lactobacillus acidophilus, LWT, Volume 80,Pages 221-229,

Van den Nieuwboer, M., Brummer, R.J., Guarner, F., Morelli, L., Cabana, M. and Claassen, E. (2015), "Safety of probiotics and synbiotics in children under 18 years of age", Beneficial Microbes, Vol. 6 No. 5, pp. 615-630.

Varga, L. (2006). Effect of acacia (Robinia pseudo-acacia L.) honey on the characteristic microflora of yogurt during refrigerated storage. International Journal of Food Microbiology, 108(2), 272-275

Varga, L., Sule, J. and Nagy, P. (2014), "Viability of culture organisms in honey-enriched acidophilusbifidus-thermophilus (ABT)-type fermented camel milk", Journal of Dairy Science, Vol. 97 No. 11, pp. 6814-6818.

Wojtczak, A., Ziarno, M., Czarniak, K. (2018). The influenceof technological factors on the survivability Lb. acido-philus and Lb. casei [Wpływ czynników technologicznychna prze˙zywalno´sci Lb. acidophilus i Lb. Casei]. Przem.Spo˙z., 72(8), 42–45 [in Polish].

Zareba, D., Ziarno, M. (2013). Viability of lactic acid bacteria and bifidobacteria in the commercially-available yoghurts [Prze˙zywalno´s´c bakterii kwasu mlekowego i bifidobakteriiw jogurtach handlowych]. Prz. Mlecz., 9, 14–20 [in Polish]

Zhang, S., Liu, L., Su, Y., L., H., Sun, Q., Liang, X. and Lv, J. (2011), "Antioxidative activity of lactic acid bacteria in yoghurt", African Journal of Microbiology Research, Vol. 5, pp. 5194-5201.

APPENDICES

Appendix 1: Score Card

Sensory evaluation of yoghurt using the 9-point hedonic scale by 11 panelists

Name:……. Type of sample: *Yoghurt*

Sensory attributes to evaluate: *Appearance/Color, Texture (Mouth-feel) and overall acceptability.*

Instructions

- You are provided with 2 coded samples of yoghurt for sensory evaluation.
- From the **nine statements** below, carefully choose the phrase which best describes your attitude towards the sample whose code matches that on the score card and fill it in the space provided in the table below.
- Remember to **rinse** your mouth with water before testing another sample.

1 Dislike extremely	**4 Dislike slightly**	**7 Like moderately**
2 Dislike very much	**5 Neither like nor dislike**	**8 Like very much**
3 Dislike moderately	**6 Like slightly**	**9 Like extremely**

Samples	**Sensory Attribute**			
	Appearance/Color	Texture/Mouth-feel	Flavor	Overall acceptability
954				
946				

Thanks for your co-operation.

Key

954 – Sucrose sweetened yoghurt

946 – Honey sweetened yoghurt

Appendix 2: Sensory evaluation scores

Score values obtained during sensory evaluation of yoghurt using the 9-point hedonic scale by 11 panelists.

	Sucrose Sweetened Yoghurt				Honey Sweetened Yoghurt			
No.	**Appearance**	**texture**	**flavor**	**overall acceptability**	**Appearance**	**texture**	**flavor**	**Overall acceptability**
1	5	7	8	7	7	6	6	6
2	8	7	8	7	8	8	8	8
3	7	7	6	7	8	7	8	7
4	3	2	2	2	6	7	9	8
5	7	8	8	8	7	7	6	7
6	7	8	6	7	8	7	7	7
7	6	7	7	8	8	7	7	7
8	7	8	8	7	6	5	8	7
9	7	6	8	7	6	8	8	7
10	8	7	6	7	7	6	8	6
11	7	7	8	7	6	7	8	7

Appendix 3: Work Plan

The research was carried out according to the following schedule.

Date	**Activity**
May 2022	Proposal development and proposal writing.
June 2022	Yoghurt production.
July to August 2022	Data collection and data analysis.
September 2022	Report writing and final Report submission.