SCREENING OF FLAVORING AGENTS FOR USE IN ROOM TEMPERATURE INCUBATED YOGHURT.

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

JUNE 2018
DECLARATION.

I Kinalwa Geoffrey, declare that this report is as a result of my own research carried out at the Makerere University biochemistry laboratory and it has never been submitted to Makerere University or any other institution for a similar academic qualification.

Signature ......................................... Date 27/06/18

SUPervisors' approval.

This is to certify that Kinalwa Geoffrey carried out this research and it is a true record of the work he did under my supervision.

Signature ......................................... Date 27/06/18

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DEDICATION.

I dedicate this work to the almighty God for having given me life to enable me carry out this work, to my beloved parents Ssanyu Allen and Byansi Geoffrey for the unending financial support they gave me whenever I needed it.
ACKNOWLEDGEMENT.

I would like to thank the almighty for the unconditional gift of life and good health he availed me during the execution of the research and throughout the compilation of this work.

Great appreciation to my supervisor Dr. Joel Isanga for his technical advice and guidance during the due process of doing this research.

I extend my sincere gratitude to my parents and relatives for the financial support they gave me to ensure that I successfully finished the work.

I cannot forget to thank my class colleagues who participated in my sensory evaluation panel. I really appreciate the concern they showed me to see that I successfully finish my research.

Lastly I thank the department and Mr. Ambrose Mukisa. The lab technician for availing me the necessary equipment and reagents that I needed to enable me carry out the work.
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LIST OF ABBREVIATIONS

AOAC: Association of Official Analytical Chemist

DNS: Alkaline-3,5- dinitrosalicyclic acid.

STS: Susceptibility to synerisis.

v/v: Volume per volume.

w/v: Weight per volume.

WHC: Water holding capacity.

RTY: Room temperature incubated yoghurt

STY: Standard temperature incubated yoghurt (43°C)
ABSTRACT.

In this study, RTY was prepared using a starter culture that was obtained from a small volume of a commercial yoghurt sample. Another sample incubated at 43°C was used as a control. The yoghurt samples were characterized basing on proximate composition, water holding capacity, syneresis and also subjected the differently flavored yoghurt samples to sensory evaluation using a panel of 20 people so as to screen for the suitability of the different flavors. STY which was used as a control took a shorter time to form (4-5) and contained higher protein content (2.88%), fat (3.48%), moisture (83.16%) and water holding capacity (42.64%) as compared to (2.26%), (2.7%), (81.44%) and (38.33%) respectively as contained in RTY which took quite a long time of about (8-12 hours to form.

However, RTY showed higher carbohydrate (6.7%) and susceptibility to syneresis (69.92%) as compared to the STY (4.66%) and (58.59%) respectively. The fresh milk flavor showed the highest improvement on the appearance, flavor and overall acceptability of the yoghurt according to the panel and it emerged as the best flavor followed by cloud flavor, the plain sample and lastly the mango flavor.
CHAPTER ONE
GENERAL INTRODUCTION

1.1. Introduction

Yoghurt originated among the nomadic tribes of Eastern Europe and Western Asia many centuries ago. However, according to legend, yogurt was first made by the ancient Turkish people in Asia and it is derived from Turkish word Jugurt describing any fermented food with acidic taste (Istikhar et al., 2009).

Yogurt is defined by the Food and Drug Administration (FDA) as a fermented dairy product derived from the fermentation of milk by two species of bacterial cultures, Streptococcus thermophilus and Lactobacillus bulgaricus. (Tamime and Robinson 1999). A wide variety of yogurts are now available around the world, ranging from very-low-fat fruit yogurts to Greek-style yogurt with a fat content around 8 g per 100 g. Yogurt can be made from cows', ewes', goats', or buffalo's milk. (Hassan et al., 1996).

Since yogurt is derived from milk, it provides protein, calcium, and other minerals, and a range of vitamins. Levels of some vitamins, such as vitamin B1 and pantothenic acid, are reduced as they are utilized by the bacterial culture used to produce the yogurt. However, folic acid levels are typically higher than in milk since folic acid is produced by the bacteria. (Trout et-al 1998).

Despite the natural aroma that is contained in the yoghurt due to diacetyl and acetaldehyde produced after fermentation addition of external artificial flavors make yoghurt more attractive and tasty and also create variety of the product due to the enhanced scent and color brought about by some flavors. (Moreno Aznar 2013).

A flavor is a sensory impression of food or other substances and is determined primarily by the chemical senses of taste and smell (Smith et-al 2005). Flavorings are focused on altering the flavors of natural food products such as meat milk, meat, vegetables or creating flavors for food compounds that do not have the desired flavor (Connolly et-al
Flavors may be natural or artificial but it has been suggested that artificial flavors may be safe to consume than natural flavors due to the standards of purity, mixture and consistency that are enforced either by the company or the law. (ISO 133301; 2000).

Currently a number flavors exist on the market such as fresh milk flavor, mango flavor, strawberry flavor straw berry, vanilla, cloud flavor, apple, pineapple flavor etc. that can be added to yoghurt in specific concentrations and add value to the final product.

1.2 Statement of the Problem and Justification.
There has been a success in the preparation of yoghurt by incubating milk containing a starter culture using room temperature conditions. Despite the fact that this room temperature incubated yoghurt contains a natural aroma brought about by the diacetyl and acetaldehyde formed during the process of its fermentation, there is need to screen among the available flavors to select the best flavoring agents so as to create more varieties in which the product can be presented to the market in order to capture the different choices and preferences of consumer.

These flavoring agents can improve color and also introduce more attractive aromas which increase overall acceptability of the product on the market.

1.3. General Objective.
To screen for the best flavor for use in room temperature incubated yoghurt.

1.3.1. Specific Objectives
i) To prepare room temperature bovine incubated yoghurt.
ii) To characterize the room temperature incubated bovine yoghurt basing on the water holding capacity, susceptibility to synerisis, proximate composition and sensory evaluation for flavor screening.
iii) To screen for the best flavor to be used in homemade room temperature incubated bovine yoghurt.
CHAPTER TWO

LITERATURE REVIEW

2.1 History and Origin of Yoghurt.
Yogurt is considered by most regulatory agencies worldwide to be a fermented milk product that provides digested lactose and specifically defined, viable bacterial strains, typically *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Barrantes 1996). It is a source of several essential nutrients, including protein, calcium, potassium, phosphorus, and vitamins B2 and B12, and serves as a vehicle for fortification. Yogurt is an ancient food that has gone by many names over the millennia. It is believed that milk products were incorporated into the human diet around 10000–5000 BC, with the domestication of milk-producing animals (cows, sheep, and goats, as well as yaks, horses, buffalo, and camels) (Bodyfelt.F.1988). However, milk spoiled easily, making it difficult to use. At that time, herdsmen in the Middle East carried milk in bags made of intestinal gut (Tammime 1999). It was discovered that contact with intestinal juices caused the milk to curdle and sour, preserving it and allowing for conservation of a dairy product for extended periods of time.

Some work about the room temperature incubated yoghurt has been carried out before by other people e.g. Shahid Kiyaga in 2010 who prepared the same sample and screened for stabilizers and discovered pectin as the best stabilizer.

2.2 The Biochemistry of Yoghurt
Because yogurt is a milk derivative, it contains many of the same beneficial nutrients as milk: calcium, phosphorus, potassium, riboflavin and vitamin A (Tamime and Robinson 1999). Furthermore, in its traditional form, yogurt is a nutrient-dense food that is rich in high-quality protein, beneficial probiotics and cancer-fighting conjugated linoleic acid. (Van Vliet et-al 1997).

Yogurt production begins with the breakdown of lactose into glucose and galactose, a process catalyzed by β-galactosidase. The glucose produced from this catabolic step then
enters glycolysis, producing pyruvate that then enters lactate fermentation (homolactic fermentation), as it produces only lactic acid molecules (Al-Kadamy et-al 2013).

Initially, *L bulgaricus* possesses weak protease activity which releases peptides from milk protein. Theses stimulate the growth of *S.thermophilus*. (Amanze 2011) *S thermophilus* grows rapidly and produces diacetyl, lactic, acetic and formic acids. The increased acidity then slows the growth of *S. thermophilus* and promotes *L. bulgaricus* which is stimulated by the formate produced in the initial stage. *L. bulgaricus* produces most of the lactic acid and also acetaldehyde which together with diacetyl give the characteristic flavor and an aroma in yoghurt. (Amanze 2011).

The internal structural properties of casein micelles (hydrophobic protein) are disrupted due to acidification of milk (Lee and Lucey, 2010). At isoelectric point (pH 4.6), the net negative charge on casein is reduced due to the protonation of its amino acid residues, decreasing electrostatic repulsion between charged groups, including the phosphoserine residues (Lee and Lucey, 2010). The casein-casein attraction increases through enhanced hydrophobic interactions, creating a structure that allows for the semisolid texture of yogurt (formation of three-dimensional network consisting of clusters and chains of caseins) (Lee and Lucey, 2010).

### 2.3 Flavoring Agents

A flavor is the sensory impression of food or other substance, and is determined primarily by the chemical senses of taste and smell (Barrante 1996). The "trigeminal senses which detect chemical irritants in the mouth and throat, as well as temperature and texture, are also important to the overall gestalt of flavor perception (Smitha et-al 2005). The flavor of the food, as such, can be altered with natural or artificial flavorants which affect these senses. A "flavorant" is defined as a substance that gives another substance flavor, altering the characteristics of the solute, causing it to become sweet, sour, tangy a food's flavor, therefore, can be easily altered by changing its smell while keeping its taste similar (Shankar 2010).
UK Food Law defines a natural flavor as a flavoring substance (or flavoring substances) which is (or are) obtained, by physical, enzymatic, or microbiological processes, from material of vegetable or animal origin which material is either raw or has been subjected to a process normally used in preparing food for human consumption and to no process other than one normally so used (Zahoor T et-al 2002).

The U.S. Code of Federal Regulations describes a natural flavorant as the essential oil, oleoresin, essence, or extractive, protein hydrolysate, distillate, or any product of roasting, heating, or enzymolysis, which contains the flavoring constituents derived from a spice, fruit, or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf, or any other edible portions of a plant, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose primary function in food is flavoring rather than nutritional (Zahoor.T et-al 2002).

Due to the high cost or unavailability of natural flavor extracts, most commercial flavorants are "nature-identical", which means that they are the chemical equivalent of natural flavors, but chemically synthesized rather than being extracted from source materials(Yazici 2004). Identification of components of natural foods, for example a raspberry, may be done using technology such as headspace techniques, so the flavorist can imitate the flavor by using a few of the same chemicals present. (Moreno 2013).

Flavorings are focused on altering the flavors of natural food products such as meats and vegetables, or creating flavor for food products that do not have the desired flavors such as candies and other snacks. Most types of flavorings are focused on scent and taste. Few commercial products exist to stimulate the trigeminal senses, since these are sharp, astringent, and typically unpleasant flavors (Buttris et-al 1997).

Three principal types of flavorings are used in foods, under definitions agreed in the EU and Australia (Shankar et-al 2010);

i) **Natural flavoring substances**: These flavoring substances are obtained from plant or animal raw materials, by physical, microbiological, or enzymatic
processes. They can be either used in their natural state or processed for human consumption, but cannot contain any nature-identical or artificial flavoring substances.

ii) **Natural identical flavoring agents:** These are obtained by synthesis or isolated through chemical processes, which are chemically and organoleptically identical to flavoring substances naturally present in products intended for human consumption. They cannot contain any artificial flavoring substances.

iii) **Artificial flavoring agents:** These are not identified in a natural product intended for human consumption, whether or not the product is processed. These are typically produced by fractional distillation and additional chemical manipulation of naturally sourced chemicals, crude oil, or coal tar. Although they are chemically different, in sensory characteristics they are the same as natural ones.

Most artificial flavors are specific and often complex mixtures of singular naturally occurring flavor compounds combined together to either imitate or enhance a natural flavor. These mixtures are formulated by flavorists to give a food product a unique flavor and to maintain flavor consistency between different product batches or after recipe changes (Shanka et-al 2010).

The list of known flavoring agents includes thousands of molecular compounds, and the flavor chemist (flavorist) can often mix these together to produce many of the common flavors. Many flavorants consist of esters, which are often described as being "sweet" or "fruity" The compounds used to produce artificial flavors are almost identical to those that occur naturally.

It has been suggested that artificial flavors may be safer to consume than natural flavors due to the standards of purity and mixture consistency that are enforced either by the company or by law—Natural flavors, in contrast, may contain impurities from their
sources, while artificial flavors are typically more pure and are required to undergo more testing before being sold for consumption (Shankar et-al 2010).

2.4 Characterization of Yoghurt
The proximate composition of yoghurt could slightly differ from one sample to another basing on the method of preparation, incubation of time range and temperature, type of starter culture used and source of raw material (milk) that is used in the making of yoghurt (Tammime and Robinson 1999). Therefore, it becomes paramount to practically analyze and determine the different components found in a specifically prepared sample yoghurt with comparison to the standard component values.

Yoghurt is characterized as a smooth, viscous, gel with specific taste of sharp acid and green apple flavor (Bodyfelt et al., 1988). Typical plain yoghurt contains 3.5% fat 12.06% total solids, 3.60% protein 18.94% moisture and 4.2% lactose (Malek-et-al 2001). Some yoghurt exhibits a heavy consistency that closely resembles custard of milk pudding. In contrast, (Connolly et al., 1984). The most important textural characteristics of yoghurt are firmness and other ability to retain water. The type of culture is an important factor affecting microstructure and the textural properties of yoghurt (Hussan et al., 1999).

Syneresis or spontaneous whey separation on the surface of set yogurt is regarded as a defect. This problem can be reduced or eliminated by increasing the level of milk solids to 15% (Amatayakul et-al 2001). Most prior art gums and blends have been found to react with the milk protein during the processing procedures resulting in yogurt with whey-off and coarse bodied grainy yogurt. Carrageenan is used in low concentration in most blends to retard such separation (Tamime and Deeth, 1980).

Never the less, the characteristic scent, color and taste of the yoghurt are also key in value assessment of a particular developed yoghurt sample. Screening for the most suitable flavor to be used in the sample is very important in order to bring out the best from the final product.
2.5 Standard Procedure of Yoghurt Preparation

According to literature (Isanga & Zhang, 2009; Tamime and Robinson, 1999), the standard procedure of yoghurt preparation involves the following major steps;

i) **Adjusting milk composition and blend ingredients:** Milk composition is adjusted to achieve the desired fat and solid content. Often dry milk is added to increase the amount of whey protein to provide a desired texture. Ingredients such as stabilizers are added at this time.

ii) **Pasteurizing the milk:** The milk is pasteurized at 85°C for 30 minutes. A high treatment is used to denature the whey protein. This allows the protein to form a more stable gel which prevents separation of water during storage. The high heat treatment also reduces the number of spoilage microorganisms in the milk to provide better environment for the starter culture to grow.

iii) **Homogenization:** The blend is homogenized (2000 to 2500 psi) to mix all ingredients thoroughly and improve yoghurt consistency.

iv) **Cooling milk:** The milk is cooled at 42°C to bring the yoghurt to ideal growth of the starter culture.

v) **Inoculate with starter culture:** The starter culture is mixed into the cooled milk.

vi) **Incubation:** Milk is then incubated at 43-45°C which is the optimum temperature for action of the starter and the sample is incubated between 4-5 hrs.

The sample is then refrigerated at 4°C to stop the fermentation by the starter culture.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Design
Room temperature incubated bovine yoghurt was prepared using a starter culture obtained from use of a small volume of commercial yoghurt on market. Another sample of bovine yoghurt incubated at standard temperature of 43°C was also prepared and used as a control in proximate composition analysis, water holding capacity (WHC) and susceptibility to syneresis (STS).

Another sample of room temperature incubated bovine yoghurt was prepared and different flavors were added but a portion of the plain sample without flavor was used as a control.

A score card for sensory evaluation was designed which was used by a panel of 20 individuals including students and lectures who were provided with different flavored and un flavored samples which they evaluated and scored as per indicated on the score card (Isanga and Zhang 2007).

The study was entirely carried out in Makerere University biochemistry laboratory following the AOAC official methods of analysis.

3.2 Materials
i) Whole cow milk
ii) Commercial yoghurt sample
iii) Flavouring agents
iv) Table sugar.

3.3 Apparatus.
Some of the apparatus used included; testubes, beakers, centrifuge, saucepan, hot plate, conical flasks, distillation apparatus, fume cupboard measuring cylinders, spectrophotometer, separating funnel, plastic bottles, pipettes burettes, fridge, oven, Rota
evaporator, furnace, funnels, weigh balance, disposable tins, thermometer, water bath heating crucibles.

3.4 Reagents

i) Diethyl ether  
ii) Petroleum ether (boiling range 40-60°C)  
iii) Concentrated ammonia solution  
iv) Potassium sulphate  
v) Copper (11) sulphate pentahydrate  
vi) Stock glucose solution  
vii) Sodium potassium tartrate.  
viii) Toshiros indicator  
ix) Concentrated sulphuric acid  
x) Alkaline 3,4-dinitrosalicyclic acid  
xii) Glacial acetic acid  
xiii) Sodium acetate  
xiv) Boric acid

3.5. Preparation of the Yoghurt Samples.

i) 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.  

ii) Whole milk in a saucepan was heated over a medium flame from the hot plate, until the milk started to froth and steam began to rise from the surface. Heating the milk to just below boiling served to kill off any microorganisms that might be present in it, thus creating a conducive environment for the yogurt bacteria to grow by eliminating any competition.  

iii) Sucrose (5.8 % w/v) was added as a sweetener and thoroughly stirred to dissolve.  

iv) The milk was cooled at room temperature to allow the dry skin of cream floating on the surface of the milk to form, which was then, removed (creamed) using a spoon and a sieve.
v) The milk was immediately poured into a clean jug and covered before it was pasteurized for 30 minutes. The pasteurization was carried out by immersing a container with the milk into a bucket containing hot water. The average pasteurization temperature (initial and the final temperatures) was 72°C.

vi) The milk was then cooled to approximately 43°C (look-warm) by immersing a container with the milk into a bucket containing cold water.

vii) Active starter culture (30% v/v) of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was added to the milk, mixed thoroughly by shaking. The starter culture was obtained from commercially-made yoghurt (fresh yogurt).

viii) Room temperature-made yoghurt was prepared by incubating at room temperature for 8-12 hours depending on the prevailing weather conditions. At the end of the incubation period the yoghurt was then transferred to a refrigerator for overnight storage prior to analysis.

ix) On the other hand, laboratory-made yoghurt was prepared by obtaining a portion of milk from step (vii) which was incubated at approximately 43.7°C for about 4 hours and 30 minutes. At the end of the incubation period the yoghurt was then transferred to a refrigerator for overnight storage prior to analysis.

3.6. Proximate Composition Analysis:
Moisture, fat, proteins, and available carbohydrates were determined according to methods described by AOAC, (2000).

3.6.1. Moisture Content:
The yoghurt sample (6 g) was measured into a previously weighed crucible and then dried to constant weight in an oven at 100°C for approximately 4 hours, and then left to cool at room temperature. It was later reweighed. The loss in weight was taken as the moisture content of the yoghurt which was determined by mathematical expression below:

\[ \text{% Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \]
Where: \( W_1 \) = initial weight of empty crucible, \( W_2 \) = Weight of crucible and yoghurt before drying, \( W_3 \) = Weight of crucible and yoghurt after drying

3.6.2. Protein Content
Crude protein was determined by the Kjeldahl method using conversion factor of 6.38 for milk and dairy products. The process was executed in three steps which included digestion of the sample for 7 hours, distillation of the digest and titration using Toshiros indicator (AOAC 2000). Percentage protein was calculated as below:

\[
\% \text{ Protein} = \frac{\% \text{ Nitrogen Content} x \text{Conversion Factor}}{\text{Weight of sample in g}}
\]

Where B is volume (ml) of 0.1M H\(_2\)SO\(_4\) used in titration.

3.6.3. Carbohydrate Content
The carbohydrate content was determined using the Alkaline 3,5-dinitrosalicylic acid(DNS) colorimetric method the absorbance was based on the color intensity of the red brown 3-amino,5-nitro salicylic acid was measured using a spectrophotometer.

The absorbances of different stock glucose dilutions was used to prepare a standard curve which was the used to estimate the carbohydrate concentration in the yoghurt samples.

3.6.4 Susceptibility to Syneresis (STS):
The Susceptibility to Syneresis (STS) of the two yoghurt samples was determined in accordance with Isanga and Zhang (2007) with slight modifications. This involved noting the volume of the whey collected in a measuring cylinder after drainage of 40 ml of yoghurt sample placed on a filter paper on top of a funnel for 5 hours at room temperature. The formula below was used to calculate Susceptibility to Syneresis (STS) of yoghurt:

\[
\text{STS} (\%) = \frac{V_1}{V_2} \times 100
\]
Where: \( V_1 \) = Volume of whey collected after drainage; \( V_2 \) = Volume of yoghurt sample used

### 3.6.5. Water Holding Capacity

Water-holding capacity (WHC) of yoghurt was determined by a method described by Spasenija *et al.*, (2007) based on the principle of centrifugation and wheying off with slight modifications. 20 g of yoghurt was subjected to 20 minutes centrifugation. The water-holding capacity (WHC) was then determined by mathematical expression below:

\[
\text{WHC (\%)} = \left( 1 - \frac{W_1}{W_2} \right) \times 100
\]

Where: \( W_1 \) = Weight of whey after centrifugation \( W_2 \) = Weight of yoghurt sample.

### 3.6.6. Sensory Evaluation

Sensory evaluation of the four yoghurt samples was carried out in accordance with Isanga and Zhang (2007) with slight modifications. A nine-point hedonic scale was used to measure the sensory qualities based on appearance/color, texture/ mouth-feel flavor and overall acceptability of the yoghurt. The samples were flavored with mango flavor, fresh milk flavor, cloud flavor and the plain unflavored sample which was used as a control. They were refrigerated overnight at 4°C and on the following day they were presented in small disposable cups that were labeled with a random three-digit code in order to blind the panel from the real names of the flavors so as to avoid bias while carrying out the exercise. The panel comprised of 20 people including students and lectures together with a score card onto which they were to score as instructed on the card. Water was provided to rinse the mouth in between the tasting process from one sample to another (Isanga and Zhang 2009).
CHAPTER FOUR
RESULTS AND DISCUSSION

From the results in the table 4.0, the yoghurt sample that was incubated at 43°C (the control) contained a higher protein, fat and moisture content as compared to the RTY whereas the former contained higher carbohydrate content compared to the control.

Table 4.0: Proximate composition of the two yoghurt samples

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>RTY</th>
<th>STY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>2.26±0.10</td>
<td>2.88±0.22</td>
</tr>
<tr>
<td>Fat</td>
<td>2.70±0.28</td>
<td>3.48±0.59</td>
</tr>
<tr>
<td>Moisture</td>
<td>81.44±0.34</td>
<td>83.16±0.60</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>6.70±0.06</td>
<td>4.66±0.35</td>
</tr>
</tbody>
</table>

Results are reported as means ± standard deviation. RTY- Room temperature incubated yoghurt, STY- Yoghurt incubated at 43°C.

The lower protein content in the RTY is probably due to prolonged proteolysis of the protein in the yoghurt due to long period of fermentation (11 hours and 23 minutes) as compared to the control which was prepared at standard conditions of 43°C which fermented for only 4hours and 20 minutes. However, the protein concentration was very close to (3.0-4.8g/100) the normal range of good quality yoghurt (Nguyen et-al 2015).

The lower fat content in the room RTY could probably also have been brought about by prolonged break down of the fat by the starter culture to generate energy for growth since they weren’t growing at their optimum temperature as compared to the control which was incubated at its optimum temperature (43.4°C) which led to a quicker growth to the optimum population to ferment the milk into yoghurt in a short time lag. (Tamime and Robinson 1999).

However, the fat content of the two yoghurt samples fell in the range of (2.4-6.8g/100) the normal range of good quality yoghurt. (E. boglio. 2014).
There was a slightly higher moisture content in STY (83.16) as compared to RTY (81.44) but it was not very different from (83.78) and (82.63) the results obtained by a colleague (Shahid Kiyaga) who worked on the same sample in 2010.

The higher carbohydrate content in the room temperature was not very different from 6.3% and 4.44% in the control which were obtained by the Shahid Kiyaga in 2010. However, the carbohydrate concentration of the RTY fell outside the range (5.4-6.6g/100) as observed by Atamamian et-al, 2013.

From table 4.1, RTY showed a higher susceptibility to syneresis and a lower water holding capacity as compared to STY.

**Table 4.1:** Susceptibility to syneresis and water holding capacity of the two yoghurt samples RTY STY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RTY</th>
<th>STY</th>
</tr>
</thead>
<tbody>
<tr>
<td>STS (%)</td>
<td>69.92±0.29</td>
<td>58.59±0.91</td>
</tr>
<tr>
<td>WHC (%)</td>
<td>38.33±0.29</td>
<td>42.64±0.29</td>
</tr>
</tbody>
</table>

Results are reported as means ± standard deviation. **RTY**- Room temperature incubated yoghurt, **STY**- Yoghurt incubated at 43°C, **WHC**- Water holding capacity, **STS**- Susceptibility to syneresis

The higher STS in the RTY sample could be as a result of lower fat content in it than in the control this because high fat content decreased the level of whey protein separation in yoghurt. (Van Vliet 1994). However, STS of the two samples was found to be higher than (47.40%) that was found out by Isanga and Zhang in 2007. This difference could be attributed to the methods of preparation of the yoghurt. RTY showed a lower WHC probably due to prolonged proteolysis as a result of a long fermentation time that led to over destabilization of the casein gel network. This resulted into weak protein matrix that couldn’t hold much water as compared to the control where the proteolysis was less (Tamime and Robinson 1999).
Graph 4.1: Showing susceptibility to syneresis and water holding capacity of the two different yoghurt samples.

As shown in table 4.2, all the sensory scores of the different flavoured yoghurt samples were in the consumer acceptable range of (4-9) score recommended for yoghurt by Karl Ruther 9 point hedonistic scale. (Tamime and Robinson 1999).

Table 4.2: Scores of different Sensory attributes of four flavored yoghurt samples.

<table>
<thead>
<tr>
<th>Flavoring Agent</th>
<th>Sensory Attribute</th>
<th>Appearance/ Color</th>
<th>Texture/Mouth feel</th>
<th>Flavor</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh milk flavor</td>
<td></td>
<td>8.00±1.03</td>
<td>7.38±1.50</td>
<td>8.05±1.43</td>
<td>7.85±1.93</td>
</tr>
<tr>
<td>Mango flavor</td>
<td></td>
<td>5.95±2.14</td>
<td>6.30±1.63</td>
<td>6.05±2.35</td>
<td>5.95±1.40</td>
</tr>
<tr>
<td>Cloud flavor</td>
<td></td>
<td>7.15±1.46</td>
<td>7.55±0.94</td>
<td>7.50±0.95</td>
<td>7.50±1.10</td>
</tr>
<tr>
<td>Plain sample</td>
<td></td>
<td>6.80±1.06</td>
<td>7.05±1.23</td>
<td>7.05±0.69</td>
<td>7.35±1.04</td>
</tr>
</tbody>
</table>

Results are reported as means ± standard deviation

From the graph 4.2, the fresh milk flavor improved the appearance, aroma and overall acceptability of the yoghurt sample as compared to the mango, cloud and plain sample flavors.
Graph 4.2: Showing the mean percentage scores for the sensory attribute of the four differently flavored yoghurt samples.

Despite the fact that the mango flavor introduced a new yellowish color to the yoghurt sample, it scored lowest in the analysis of color and appearance. This was simply because some panelists didn’t appreciate the yellow color in yoghurt. However, some panelists raised a concern about its concentration that it as a bit overwhelming as compared to the other samples even when equal concentrations of 0.5g of flavor per 180 ml of the yoghurt sample were used. This suggests that may be the flavor should be added drop wise until a required concentration is reached since different flavors could possess different strength.

The plain sample natural flavor scored a higher preference compared to some flavors like mango. This indicates that the natural yoghurt flavor could itself compete favorably on market since it can satisfactorily catch consumer’s tastes and preferences.

Generally, the comments from the panel showed that they preferred the yoghurt in its natural color (the milk color) and any flavor that altered this could bias their preference negatively to the sample.
CHAPTER FIVE

GENERAL CONCLUSION

The RTY sample showed low fat, protein, susceptibility to syneresis, moisture and a higher water holding capacity and carbohydrate content as compared to the control.

The flavoring procedure requires excessive care because a little excess could make the yoghurt sample to turn bitter. It is recommended that the flavors are added as drop by drop until the right concentration is attained. This could avoid over shooting.

Basing on the study, the fresh milk flavor was the most preferred followed by cloud flavor, plain yoghurt flavor and lastly the mango flavor.
REFERENCES


ISO 13301:2002 Sensory analysis Methodology General guidance for measuring odor, flavor and taste detection thresholds by a three-alternative forced-choice (3-AFC) procedure.


APPENDIX 1

Score card for sensory evaluation

- You are provided with 4 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.

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>4</th>
<th>Dislike slightly</th>
<th>7</th>
<th>Like moderately</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dislike very much</td>
<td>5</td>
<td>Neither like nor dislike</td>
<td>8</td>
<td>Like very much</td>
</tr>
<tr>
<td>2</td>
<td>Dislike moderately</td>
<td>6</td>
<td>Like slightly</td>
<td>9</td>
<td>Like extremely</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sensory Attribute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Appearance/ color</td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
</tr>
</tbody>
</table>

Comment briefly on the texture and flavor of the four yoghurt samples and make some recommendations where possible.

<table>
<thead>
<tr>
<th>Code</th>
<th>Comment of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Texture/mouth feel</td>
</tr>
<tr>
<td>100</td>
<td>Flavor</td>
</tr>
<tr>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 2

Carbohydrate content

Data for preparation of standard curve.

<table>
<thead>
<tr>
<th>Glucose concentration (g/100)</th>
<th>0.00</th>
<th>0.4</th>
<th>0.8</th>
<th>1.2</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.00</td>
<td>0.018</td>
<td>0.051</td>
<td>0.062</td>
<td>0.072</td>
</tr>
</tbody>
</table>

Percentage carbohydrate = \( \frac{\text{concentration of the sample}}{1.3g} \times 100 \).