KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI
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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

DETERMINATION OF SHELF LIFE OF HONEY SHAKE MILK DRINK

BY

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MSC. FOOD QUALITY MANAGEMENT

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Food has undergone several transformations with the advancement in technology and with the present emphasis on food quality and safety, the shelf life and quality of food products are highlighted issue.

This study sought to determine the quality and shelf life of Honey Shake Milk Drink (HSMD) during storage. Three different flavours (Vanilla, Strawberry and Chocolate) of HSMD were randomly selected and subjected to microbiological, physicochemical and sensory analyses using standard procedures. Results showed that all the freshly prepared HSMD samples were safe for consumption with <30 cfu/g aerobic count, no *Salmonella* spp. and *Staphylococcus aureus* detected, pH of 6.98, milk fat content of 2.08%, non-fat content of 15.64% and Brix-TC content of 17.10. The products had overall acceptability score of 5-7 on a 9-point hedonic scale with the chocolate HSMD the most acceptable by consumers. The products exhibited a reduction in pH, fat content and Brix over time with a more profound and significantly different reduction ($p < 0.05$) in the products stored under ambient conditions as opposed to those stored under refrigeration conditions. The pH decreased from 6.98 to 3.32 under ambient storage conditions and from 6.98 to 5.68 under refrigeration conditions whereas the milk fat content reduced from 2.08% (w/w) to 0.48% (w/w) and from 2.08 to 1.84% (w/w) under ambient and refrigeration storage conditions, respectively for storage period of 35 days.

The Brix content reduced from 17.0 to 15.6 and from 17.0 to 12.2 under ambient and refrigeration storage conditions respectively over 35 days of storage. The ultimate shelf life of the product was estimated to be 7 days when stored under ambient conditions and 52 days when stored under refrigeration conditions. The results suggest that the HSMD products will be best stored at refrigeration temperature (≤ 5°C).

CHAPTER ONE

INTRODUCTION

1.1 Background

Milk due to its components like protein, fat, lactose, water and minerals is often considered as a complete meal hence has become part of human diet for thousands of years. It can be converted into different forms of products while in others cases used as ingredient for other food products (Feary, 2010). According to Tijani (2014), the average Ghanaian diet as at 2001, consisted of about 5% animal based and the rest comprised of starchy staples which resulted in high protein deficiency. A great solution to this national problem is to consider milk and milk sources as part of our daily diet. Milk due to its components like carbohydrates, proteins and fats provides great source of nutrients and able to support microbial growth and development. Milk in its raw form is highly perishable and provides great source of nutrients for human and microbial growth and metabolism (Udayathilaka, 2003).

Honey Shake milk drink (HSMD) is a delicious milk beverage produced and registered in Ghana by SS Brothers Company Limited in Accra, Ghana. It is produced from purified water, spray-dried whole milk powder which is imported from Ireland, uses pure natural honey as sweetener and comes in three different flavours. Standard operating procedures are adhered to make sure quality and safe products are being produced from the pasteurization plant.

In the pasteurization process, the target is to remove all non-spore forming pathogens in the milk and eliminate commonly associated spoilage microbes thereby ensuring quality of food product under the right refrigeration storage (Udayathilaka, 2003).

Over the years, incorporation of milk powders into the production of various food products have increased immensely hence the importance of the functionalities of milk powders. Though the reconstitution properties of these milk powders can be inconsistent and uncertain sometimes, many foods and dairy products manufacturers used them as a major ingredient (Macbean, 2009).

The time at which the quality of beverages decreases and becomes unacceptable from the time of production is described as "shelf life". Determining the shelf life of a product is very essential because it takes into account the quality of the product and is expressed in terms of flavour, nutritional value or appearance as well as its safety through microbial analysis (Leo, 2015).

1.2 Significance of Study

Honey Shake milk drink beverage is consumed widely in the city of Accra due to its nutritive value hence irregular identification of off-flavour and sour taste by some clients. The research seeks to identify and estimate the proper storing condition of the milk beverage and estimates its shelf life through varying temperatures to improve quality of the product.

1.3 Aim of the study

The main objective of the study is to assess the effect of temperatures on the quality and shelf life of Honey shake milk drink (HSMD).

1.3.1 Specific Objectives

The specific objectives include:

1. To determine the qualitative changes of HSMD with varying temperature.
2. To determine the shelf life of HSMD.

CHAPTER TWO

LITERATURE REVIEW

2.1 Whole Milk Powder

Technological processes like roller drying, freeze-drying, or spray-drying processes enable the production of milk powders by evaporating water from a pasteurized and homogenized liquid milk. In milk powder production spray drying determines the type of milk powder to be produced considering its specifications and properties (Rebaka, 2013). Milk powder in its dry form possess all the appealing properties of milk, hence an important ingredient for manufacturing varieties of food products. One variety of milk powder known as whole milk powder consists mainly of whey proteins (almost 4%), caseins (almost 20%), milk fat (almost 26%), and lactose (almost 38%). The particles of milk powder consist of a continuous mass of formless lactose and other low-molar-mass components in which fat globules and proteins are embedded. The shelf life of whole milk powders and its dry based milk products is influenced by the chemical reaction of the milk fat and lactose present. Some of these dry milk based products include infant formula and instant powders for coffee, cocoa, and chocolate-flavoured beverages (Macbean, 2009).

Milk powder can be considered a natural ingredient, nutritious and cost effective and beneficial in its functionality having a shelf life of about six months. It has a particularly variety range of applications in the confectionery industry, namely for chocolate, toffee and caramel confections, ice cream, milk beverages, toppings and icings, recipe dishes, infant formula, breakfast cereals, soups and sauces, bakery products and coffee whiteners (Pavon, 2003).

The consumption of soft drinks, fruit drinks and flavoured tea has created a decrease in calcium intake through milk beverages (Crane *et al.*, 2000). Children and adolescents have increased their consumption of soft drinks, fruit drinks and fruit-flavored drinks at the expense of milk. In the United States, consumers refuse to drink milk due to health challenges like lactose intolerance (Pavon, 2003).

Dairy products are highly perishable and as such converting milk into milk powders can increase its shelf life and enables it to be stored for quite a long time without substantial loss of quality, even at ambient temperatures. Not only are dairy-based powders used for recombination or reconstitution, but they can also be exploited for their intrinsic functional properties for application as a food ingredient in several "value-added foods" such as confectionery, bakery, and meat products (Sharma *et al.*, 2012). Foods are usually considered to be of lower quality when prepared from powdered ingredients than fresh or frozen ingredients and products. In current times like this great emphasis on adding value to powders, and therefore, an inclusive effort from dairy plant and powder producers, ingredient people, marketing experts is requisite to identify the means to add more value. Consumers of milk powders will be willing to pay more if they perceive high functionality and quality, as well as multifunctional properties of these powders (Sharma *et al.*, 2012). Dairy powders are usually used due to their convenience in applications for transportation, handling, processing, and for product formulations. Some of the physical and functional properties of dairy powders include powder structure, particle size distribution, powder density, bulk density, particle density, occluded air, interstitial air, flowability, rehydration (wettability, sinkability, dispersibility, solubility, hygroscopicity, heat stability, emulsifying

properties, glass transition temperature, water activity, stickiness, caking, and even scorched particles(Sharma *et al.*, 2012).

Drying in milk usually means water in the liquid form has been evaporated and the final product obtained is in the solid form. Water content of milk powder usually ranges between 2.6% and 5% (Macbean, 2009), where there is usually no microbial growth at such low water content. This thereby extends the shelf life of milk and also reduces the weight and volume making it easy to transport and store (Macbean, 2009).

According to Singh (2004), the unique property of milk powders as being heat stable enables them to be used in hot beverages, custards, white sauce mixes, bakery items, and, most importantly, the manufacture of recombined milk products (such as evaporated milk). Milk powder used for making recombined evaporated milk (REM), that must withstand sterilization requires adequate heat stability, otherwise the protein precipitates during or shortly after sterilization. The heat stability property of milk is mainly a function of its protein stability (Singh, 2004), and may be affected by protein content. Protein standardization can be used to achieve more consistent protein content in dairy products and perhaps improve heat stability. Heat stability is influenced by the type of nonfat dry milk (NDM) powder (low- or medium-heat powder) and standardization material (permeate powder or edible lactose powder) (Sharma *et al.*, 2012)

2.2 Honey

Natural honey is a sweet, highly nutritional flavourful liquid food full of immense health benefits. Natural Honey is produced by honey-bees as blossom honey by secreting nectars of flowers, and honeydew honey (forest honey) by secreting the exudates of plant sucking

insects (Aphids). Natural honey is widely consumed by all age groups, and its use cut across barriers of culture and ethnicity. The use of honey is accepted by all religious and cultural beliefs (Ajibola *et al.,* 2012). Honey can be mono-floral or multi-floral depending on the floral source, thus honey can be mono-floral that is honey produced from nectar of one predominant plant species.

According to Gupta and Sharma (2009), colour is one of the contributing factors of the appearance of honey. The colours of natural honey has been classified under seven categories and can be determined using the calorimetric method. These include, water white, extra white, white, extra light amber, light amber, amber and dark amber (Gupta and Sharma 2009)

Honey is a natural product also known for its therapeutic properties. Honey is still used in folk medicine particularly where conventional and modern therapeutic agents fail. In current time, medical professions have rediscovered the use of honey as therapeutic substance and acceptance as an antibacterial treatment of gastroenteritis ulcers, bed sores, and other surface infection. It is therefore not surprising that research findings on its antibacterial activities have been highlighted (Aurongzeb and Azim, 2011). Natural honey is not only known for its medicinal uses, but also useful as food sweetener, complete food and natural beauty agent too (Ajibola *et al*., 2012).

The basic components of honey are fructose and glucose but also contains fructo-oligosaccharides and many amino acids, vitamins, minerals and enzymes. The plants on which the bee feeds also determine the variations in honey. Almost all natural honey provides synergistic antioxidant effects due to the presence of flavonoids, phenolic acids, ascorbic acid, tocopherols, catalase, superoxide dismutase, reduced glutathione, Millard

reaction products and peptides. Most of the above compounds work together to provide a synergistic antioxidant effect (Eteraf-Oskouei & Najafi, 2013).

Bee keepers and consumers mostly refer to the aroma and flavour of honey as the most important characteristics. Ghazali and Sin (1986) determined effects of storage of honey at room temperature 28±2^{o}C and at 50±2^{o}C and reported that as the storage period increases so also the darkening of honey increased.

Honey is most widely consumed in its unpreserved state, that is, liquid, crystallized or in the comb. In these forms, it is taken as medicine, eaten as food or incorporated as an ingredient in various food recipes. In confectionery production, honey is still included in many traditional products, which are consumed locally in considerable quantities and also exported. In gelatinous or gum product, honey can be used as flavouring agent. In industrial sector, some honey milk products exist such as pasteurized and homogenized sweetened with honey for long time storage e.g. yoghourt with honey (Olaitan, *et al.*, 2007).

The additional benefit of consuming honey as a source of energy over the commonly used artificial sugar is that, the sugar constituents of natural honey are present as monosaccharides while artificial sugars undergo processes of digestion to breakdown sugar components into smaller forms prior to their absorption, these sugar molecules in natural honey are in pre-digested forms, and can be directly absorbed into the human system (Ajibola *et al.*, 2012). The stability, texture and shelf life is usually influences by its pH, which is also influenced by conditions during extraction and storage. pH is a helpful index to determine possibility of microbial growth due to presence of various organic acids like pyruvic acid, maleic acid, citric acid and gluconic acid in balance equilibrium with lactones, esters and inorganic ions

like sulphate, phosphate and chloride. The darkness in honey is reported to be closely proportional to the pH value of the honey (Kamboj, 2017).

2.2.1 Chemical Composition of natural Honey

The basic components of honey are nearly the same for all honey samples but differ according to the plant species on which the bees forage. Basically, natural honey is a sticky and viscous solution with a content of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants. Each of these constituents is known to give the distinctive nutritional or medicinal properties with its unique blend accounting for the varied and different applications of natural honeys (Buba *et al.,* 2013)

According to Aurongzeb and Azim (2011), natural honey in addition to sugar contains water. Sugar make up about 95-99% of honey dry matter. Majority of these simple sugars are D-fructose (38.2%) and D-glucose (31.3%), which represents 85-95% of total sugars. These are readily absorbed by the body when consumed. Other sugars include disaccharides such as maltose, sucrose, and isomaltose. Few oligosaccharides are also present (Aurongzeb & Azim, 2011).

2.2.2 Antimicrobial Properties of Honey

Studies have shown that the antimicrobial activities of honey are due to its physiochemical properties such as high osmotic pressure, high viscosity, low water activity and high content of reducing sugars (Libonatti *et al.,* 2014). It has been suggested that the clinical significance of honey can be attributed to growth inhibition of various bacteria strains. This property is due to the hydrogen peroxide which is produced by the action of glucose-oxidase in the

honey. Hydrogen peroxide and gluconic acid are produced when there is a breakdown of bee glucose through the honey-glucosidase secreted by the hypo pharyngeal gland. The glucosidase in honey is usually inactive due to the little water present and low pH, the enzymes are activated when honey is diluted with water (Libonatti *et al.*, 2014).

Due to its antimicrobial properties, some physicians advise their patients to consume natural honey to improve healing of surgical scars and other medical treatments as well as wound infections (Cortés *et al.*, 2011)

Several studies have shown that consumption of natural honey can improve defenses against oxidative stress due to the presence of natural phenolic compound called flavonoids. Flavonoids are phytochemicals that exert antioxidant properties by decreasing or removing reactive oxygen species (ROS), thus reducing the risk of pathologies and damages caused by free radicals (Cortés *et al.*, 2011).

Sharma *et al.*, (2012), reported lost in inhibition of bacteria growth after exposing a thin film of honey to sunlight. This confirms the investigation that exposure of honey in 1-2 mm thick to sunlight for 15 minutes will lead to complete loss of non-osmotic activity. The more honey is exposed to heat or light the greater its loss of microbial inhibition (Sharma *et al.*, 2012).

2.2.3 Medicinal Aspect of Honey

Several studies have showed the anti-inflammatory, anticarcinogenic, anti atherogenic and wound healing properties of honey due to its good source of bioactive compounds that exerts these features (Kamboj, 2017). Studies on adult rat according to Ajibola *et al.*, (2012), showed significant enhancement in blood profiles after feeding with floral honey, study showed significant improvement of haemoglobin concentration which can be accounted to the iron constituent in natural honey, increased erythrocyte counts and elevated haematocrit

in the honey eaters hence can improve conditions of anaemia in humans. In another lab analysis, enhanced haematology and immune response in rats fed 10% honeydew honey supplemented diet was documented. The researcher noted increased lymphocyte count and higher neutrophil phagocytosis in experimental natural honey-fed rats than the control (Ajibola *et al.*, 2012).

In recent days' intestinal tract infections are common throughout the world affecting people of all ages. According to Singh *et al.,* (2012), pure honey has bactericidal properties that fight against many entero-pathogenic organisms that causes these infections including those of the *Salmonella* and *Shigella* species, and *E. coli*. In vitro studies show that inhibition of *Helicobacter pylori* isolates which cause gastritis using a 20% solution of honey during the experiment. Even isolates that exhibited a resistance to other antimicrobial agents were susceptible. A clinical study using the administration of a bland diet and 30 mL of honey three times a day was found to be an effective remedy against *H. pylori* in 66% of patients and offered relief to a further 17%, while anaemia was corrected in more than 50% of the patients conducted (Singh *et al*., 2012).

2.2.3 Microbes found in honey

Microbes that survive in honey are able to withstand the concentrated sugar, acidity levels and other antimicrobial properties of honey. The primary sources of microbial contamination in honey are likely to include pollen, the digestive tracts of honeybees, dirt, dust, air and flowers. Microbes found in honeycomb are principally bacteria and yeast and come from the bees, the raw materials (nectar) or from external sources (Olaitan *et al*., 2007). This can lead to various diseases such as botulism. Total plate counts from honey samples vary from zero to tens of thousands per gram for no apparent reason. Majority of honey samples contain

detectable levels of yeasts. Although yeast counts could be below 100 colony forming units per gram (cfu/g), yeasts can grow in honey to very huge numbers. Practicing good standards in the honey production industry control yeast growth. Bacterial spores are commonly found in honey especially those in the Bacillus genus. Low concentration of *C. botulinum* have been reported in honey samples (Singh *et al*., 2012).

Secondary sources of microbial contamination in honey include activities of humans, equipment used during processing, containers for storage, wind and dust. A study shows how yeasts were recovered from equipment in honey houses. Contaminated equipment can also introduce yeast cells into clean honey. Possible routes of microbial transmission into extracted honey would include air and also food handlers through skin infections and improper hygienic practices (Olaitan *et al*., 2007).

2.3 Flavours and Fragrances

The raw materials used for flavour and fragrance can be categorized into two; thus natural and synthetic. Over the years production of synthetic raw materials has increased intensively using development of synthetic chemistry techniques, natural raw materials are also used in the process, as synthetic materials cannot reproduce the sensorial features of natural raw materials readily (Yin, 2017).

A food's unique flavour and aroma are as a result of a complex construct of hundreds of individual constituent components interacting with each other to produce a recognizable taste and aroma. Thus, food quality would be affected if one or more flavour constituents are altered or diminished. A reduction in food quality which affects taste and aroma may result from the oxidation of aroma components due to the ingress of oxygen, or loss of

specific aroma through the packaging material of the said food to the environment (Willige, 2002).

Sensory quality of foods is sometimes lost through absorption of these aromas in their plastic packaging. The change is usually certain when only certain components of these complex aroma mixtures are absorbed hence overall loss of odour or change in aroma of the food product. However, in the beverage industry investigations about loss of flavour compounds for sensory quality are insufficient. This is because various factors influence loss of flavour and aroma which includes storage temperature and type of packaging material used. Appropriate use of polymers especially packaging materials with a very low absorption will help maintain normal levels of aromas and flavours in the food industry below human sensory detection (Van Willige, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Freshly prepared Honey Shake Milk Drink (HSMD) of three flavors was sampled from the production unit using a stratified sampling plan. The three flavors (Vanilla, Strawberry and Chocolate) served as the subgroups (strata) under which 12 bottles each were randomly selected to produce a total sample size of 36. The samples were transported on ice to the microbiology laboratory of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, KNUST where they were stored at refrigeration temperature (0-4)^{o}C until the inception of analysis.

3.2 Laboratory Analyses

The analyses conducted on the product included both microbiology and physicochemical analyses with emphasis on standard test required by the regulatory body, Food and Drugs Authority of Ghana specification for milk drinks.

3.2.1Microbial Analysis

The microbial analysis included total aerobic count, *Salmonella* and *Staphylococcus aureus* assay.

3.2.1.1 Media preparation

The reagents used in the study were agars for the culture and isolation of microbes as well as biochemical assay. The agars used were products of OXOID Laboratories (Basingstoke Hampshire, England). They included Plate Count Agar used for the total viable count;

Mannitol Salt Agar (MSA) for *staphylococcus aureus* and Rappaport Vassiliads broth (RVB), Brilliant Green Agar (BGA) and Xylose Lysine deoxycholate agar (XLD) for *Salmonella.*

3.2.1.2 Preparation of Plate Count Agar

Plate Count Agar (Nutrient agar) was prepared by suspending 23.5 g in 1000 ml (1 liter) distilled water and heated to boil to dissolve completely. It was sterilized at 121°C for 15 min in sealed bottle. The sterilized agar was left to cool at 50°C before pouring into sterile Petri plates. The prepared plates were allowed to set and incubated for 24 h at 37^0C prior to use for sterility validation.

3.2.1.3 Preparation of Mannitol Salt Agar

Agar powder of Mannitol salt (111 g) was suspended in 1 L of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 min. The prepared plates were validated after 24 h of preparation for sterility prior to use.

3.2.1.4 Preparation of Brilliant Green and XLD agars

The agar was prepared by dissolving 38.5g of agar powder in one liter of distilled water and heated in a water bath at 100°C for 20 min to dissolve. The agar was allowed to cool and poured into sterile Petri dishes to set after which sterility checks were conducted after 24-h incubation at 37^0C.

3.2.1.5 Assessment of the microbial quality and safety of product.

The procedures described below were used to test for presence (qualitative) and enumerate (quantitative) the microorganisms in the samples. Colonies on selected plates were counted using a colony counter.

3.2.1.6 Diluent preparation and serial dilution

The diluent used in this study was buffered peptone water from Biolab which was prepared according to the manufacturer's instruction on label (10g of agar was dissolved in a liter of distilled water and stirred to dissolve completely and then dispensed into appropriate containers. It was sterilized at 121^0C for 15 min and allowed to cool).

The stock dilution was prepared by dissolving 10 ml of sample in 90ml of sterile diluent and shaken for 30s. The subsequent dilutions were prepared by adding 1ml aliquot of the stock solution in 9ml of sterile diluent in succession to obtain a six-fold serial dilution. The dilutions were then inoculated unto the respective media.

3.2.1.7 Total Viable Count (TVC)

Total Viable Counts were enumerated by spread plate method and grown on Plate Count Agar (PCA). A volume of 100 µL of the dilutions were inoculated unto Petri dishes of plate count agar and spread evenly in triplicates. The inoculated plates were incubated at 37°C for 24 h and 48 h after which all white spot or spread were counted and recorded as total viable count using the colony counter.

3.2.1.8 Determination of Staphylococcus species

Staphylococcus species were enumerated by spread plate method and grown on Mannitol Salt Agar (MSA). An inoculum volume of 100µL was inoculated unto the agar plate and incubated at 35 ºC for 24 h. After incubation yellow colonies were counted and recorded as *Staphylococcus* counts using the colony counter.

3.2.1.9 Determination of Salmonella

The assay for Salmonella adopted a 3 level assay from pre-enrichment, enrichment through to plating. The pre-enrichment stage was carried out by inoculating 1ml of the stock dilution (10^{-1}) into 9 ml of 1% peptone solution and incubating for 24 h at 37°C. The next level of enrichment followed with inoculating 0.1 ml of the pre-enriched culture in 9ml of RVB and incubating under same conditions as the first stage. The final plating stage was carried out by streaking the enriched culture on BGA and XLD and incubating under same conditions. The presence of red colonies on BGA and pink with black spots on XLD was recorded as positive for *Salmonella.*

3.2.2 Physicochemical Analysis

The physicochemical analysis included pH, milk fat, milk non-fat and Brix-TC assays. These were conducted following the AOAC protocols.

3.2.2.1 pH Determination

The pH of the milk beverage HSMD was determined using a pH meter (Metller Tolledo, Ohio, US). The readings were taken in triplicates and the mean calculated for each product variant.

3.2.2.2 Determination of Milk Fat

The known mass of the sample was weighed into a flask and 5 ml of ammonia added and shaken for a minute to denature proteins present. This was followed by 45 ml of Petroleum ether and shaken for 3 min with intermittent release of pressure from gas buildup. About 5 ml of ethanol was used as emulsifier with intermittent shaking for 2 min. The solvent phase was collected and evaporated in pre-weighed flasks in a hot air oven at 80 °C for 15 min.

The flasks were cooled in desiccators and reweighed for final weights. The Milk fat content was calculated using the formula:

$$\text{Milk fat} = \frac{(\text{wt. of flask + oil}) - (\text{wt. of flask})}{\text{wt. of sample}} \times 100$$

wt = weight

3.2.2.3 Determination of Milk non-fat

The milk non-fat was determined by first estimating the total solids of the products. This was carried out by weighing a known mass of sample into pre-weighed Petri dishes and drying them at 105 °C for 4 h. The dishes with dried samples were reweighed and the total solids calculated from the formula:

$$\text{Total Solids} = \frac{(\text{wt. of dish + dried sample}) - (\text{wt. of dish})}{\text{wt. of sample}} \times 100$$

The milk non-fat was determined from:

$$\text{Milk non-fat} = \text{Total Solids} - \text{Milk Fat}$$

3.2.2.4 Determination of Brix-TC

The Brix content of the HSMD was determined using a digital refractometer (Reichert AR200). The drink was dropped unto the sample reader and the Brix-TC read from the display. Readings were carried out in triplicates and the mean taken.

3.3 Shelf life study

The Shelf life of the product was determined using the microbial, sensory and physicochemical parameters as indicator factors.

3.3.1 Sample preparation for shelf life study

The samples were stored under two conditions; ambient (25°C±1) and refrigeration (5^0C±1) temperatures for four (4) weeks. The sampling interval was set at a 5 days interval to give a 7-point sampling.

3.3.2 Microbial and physicochemical analyses in shelf life study

The microbial analysis for the shelf life study consisted of the total aerobic count as no *Salmonella* and *S. aureus* were detected in the samples. The tests were conducted as described above in section 3.2 under their various headings and specifications with no modifications.

The physicochemical analysis of the experiment featured some sensory parameters such as appearance, color and smell alongside pH, milk fat and non-fat as well as Brix.

The sensory assessment was carried out by the personnel at the laboratory with the assistance of three untrained panelists during each sample section thus a total panel number of 10. The assessment was done with the aid of a sensory assessment form (Appendix 1). The assessment was done using a nine-point hedonic scale from 'dislike extremely' to 'like extremely' in ascending order from 1-9.

During the sensory analyses tests were coded randomly to hide the identity of the products from the panelists to avoid any misjudgment or partiality based on intended preference.

3.4 Statistical Analysis of Data

The data obtained from the microbiological and physicochemical analyses were analyzed using the GraphPad Prism 5.0 software using Two-way ANOVA with Bonferroni Post Hoc analysis at 95% confidence interval. The data from the Sensory analysis was analyzed using

the IBM SPSS 16.0 software using a One-way ANOVA with Tukey descriptive at 95% confidence interval.

The shelf life prediction was done using the Statgraphic Centurion XV software (Rockville, USA) by simple and multiple regression analysis of the data at 80% confidence interval as by the Polhemus model.

CHAPTER FOUR

RESULTS

4.1 Quality Assessment of Honey Shake Milk Drink

The quality assessment of the Honey Shake Milk Drink (HSMD) was determined using standard parameters for Food and Drugs Authority of Ghana for both microbiological and physicochemical analysis.

4.1.1 Microbiological Quality Assessment of HSMD

The safety and quality of the Honey shake milk drink was evaluated on the merit of the microbial dynamics both qualitatively and quantitatively. The quantitative assay via the total aerobic count did establish a high microbial quality and good safety index of the product (within 24 h of production time) with microbial loads falling <30 cfu/g for all flavors (vanilla, strawberry and chocolate) as against the acceptable limit of 1.0×10^4cfu/g (Table 1). The qualitative assay focusing on *Staphylococcus aureus* and *Salmonella typhi* also showed the absence of both target organisms in all the flavors produced (within 24 h of production time).

The assessment on both qualitative and quantitative assays establishes the fresh HSMD as a safe product for consumption microbiologically.

4.1.2 Physicochemical Quality Assessment of HSMD

The physicochemical parameters analysed include pH, milk fat, milk non-fat and Brix. The pH of the HSMD prior to experiment was 6.81± 0.01. The milk fat content of the drinks was

determined to be 2.08%± 0.00 and the non-fat content was 15.64%±0.02. The Brix-TC content of the drinks was determined to be 17.10± 0.15.

Table 1: Microbial quality of freshly prepared HSMD prior to shelf life study

Product	Test	Results (cfu/ml)	Specification(FDA Ghana) (cfu/ml)	Inference
Vanilla	Total aerobic count	<30	1.0×10^4	safe
	Salmonella typhi	None detected	0	safe
	Staphylococcus aureus	None detected	0	safe
Strawberry	Total aerobic count	<30	1.0×10^4	safe
	Salmonella typhi	None detected	0	safe
	Staphylococcus aureus	None detected	0	safe
Chocolate	Total aerobic count	<30	1.0×10^4	safe
	Salmonella typhi	None detected	0	safe
	Staphylococcus aureus	None detected	0	Safe

cfu - Colony Forming Unit

4.2 Sensory Analysis on HSMD

The sensory analysis on the parameters of taste, aftertaste, color, smell, appearance and overall acceptability showed no significant difference ($p > 0.05$) in the responses of the panel on the merit of the nine (9) hedonic scale score (Table 2). Strawberry was scored as the most preferred on the account of color, smell and appearance with a mean score of 6.9, 6.9 and 6.4 respectively whereas chocolate was most preferred on the account of taste, aftertaste and overall acceptability with mean score of 6.7, 6.0 and 7.0, respectively.

The chocolate flavor on the merit of overall acceptability score was implicated as the most preferred product (Figure 1; Table 2).

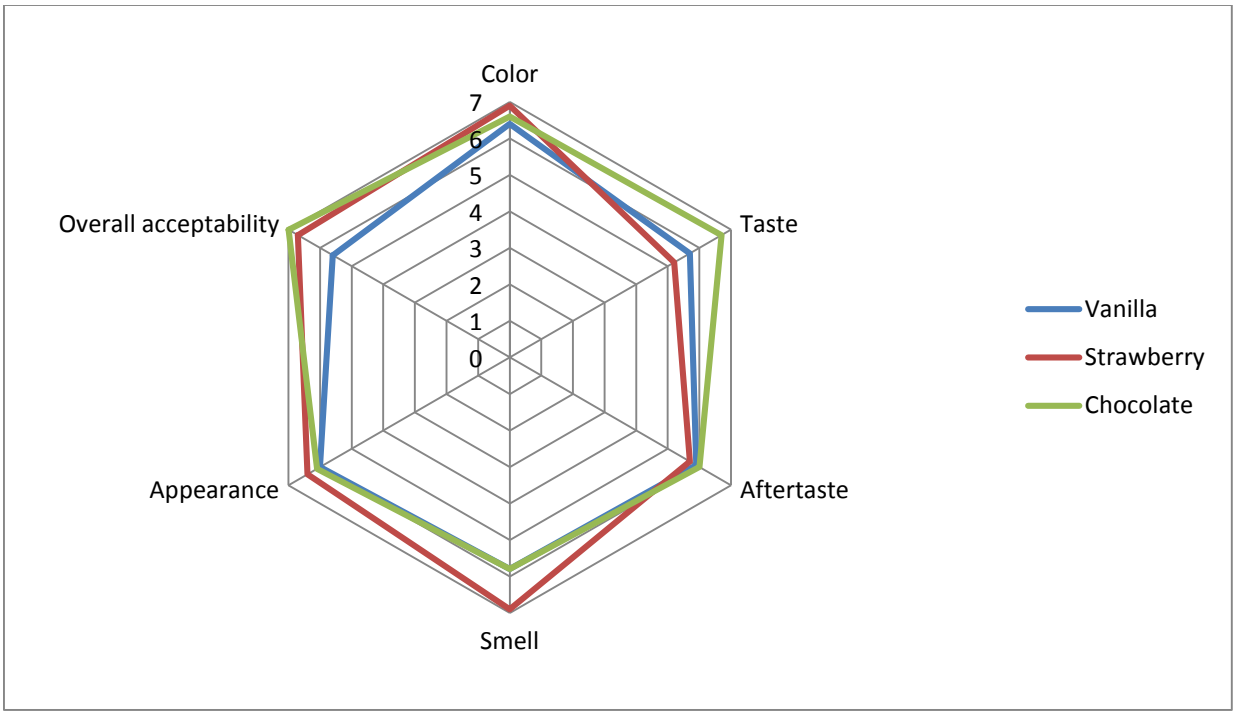

Figure 1: Sensory analysis on three flavors of Honey Shake Milk Drink

4.3 Shelf life determination of HSMD

The shelf life of the product was determined using Physicochemical, microbiological and sensory indicators.

4.3.1 Preliminary assessment of Product Reaction to Temperature changes over time

The preliminary analysis of the products in selecting the best flavor to be used for the shelf life study focused on pH. The preliminary shelf life study carried out on the three flavors showed no significant difference ($p > 0.05$; Table3) in the reactions of the three flavors to pH changes over time at ambient and refrigeration conditions(Figure 2).

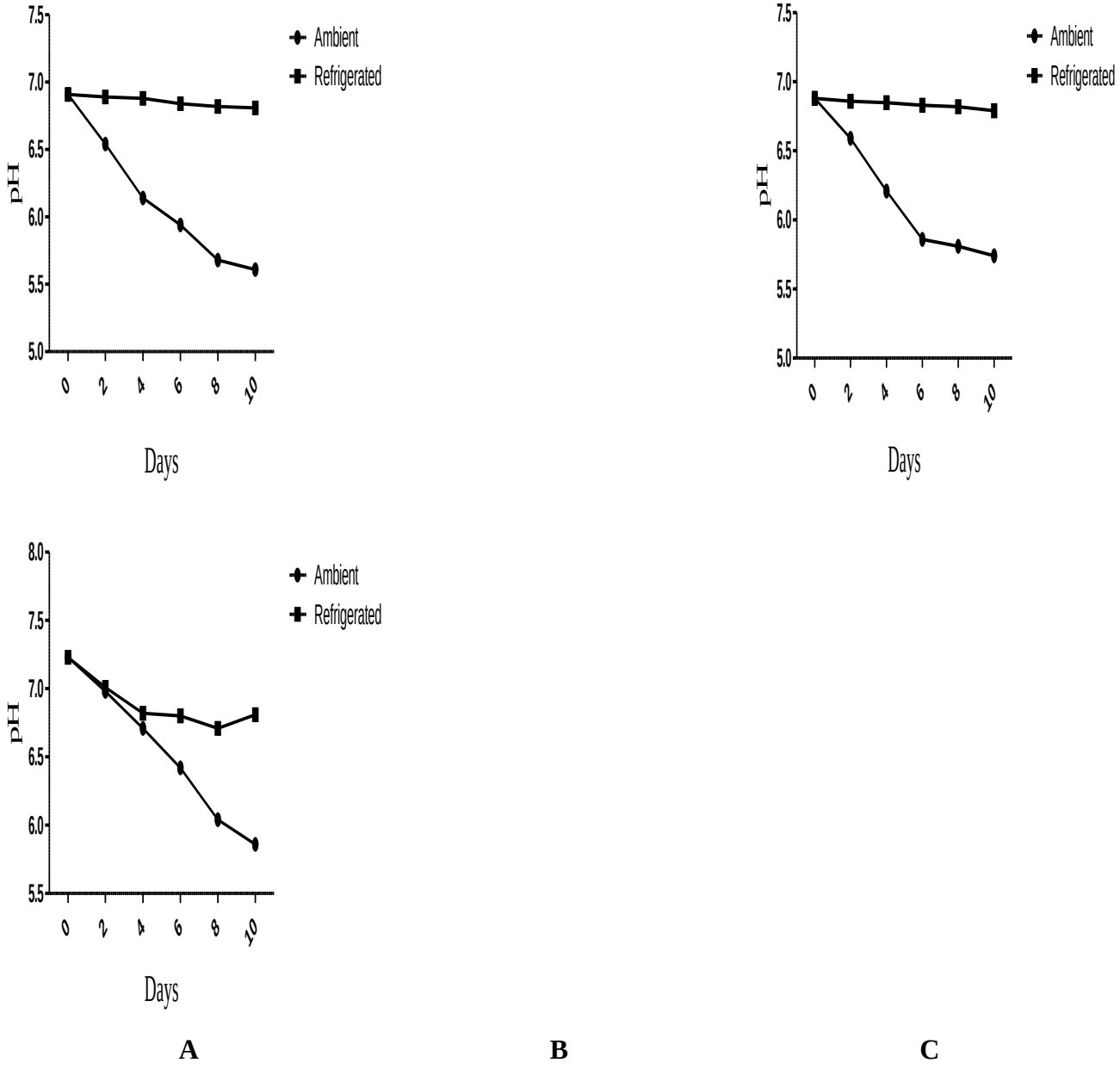

Figure 2: Change in pH over time in the preliminary study on (A) Vanilla, (B) Strawberry and (C) Chocolate flavors of HSMD stored under ambient and refrigeration conditions

The chocolate flavor was selected to be used as the model product in the main shelf life study on the basis that no significant difference exists in the reactions of the three products over

time and chocolate scored the highest acceptability of 7.0, making it the most preferred flavor.

4.3.2 Determination of Shelf life using Physicochemical Parameters

The shelf life study was run for a period of 35 days comprising 7 sample sections with each section featuring total aerobic count, pH, milk fat and non-fat and Brix-TC. The tests were mainly microbiological, physicochemical and sensory analysis.

4.3.2.1 pH in Shelf Life Determination

The pH showed a downward progression over the experimental period with a gradual decline of averagely 0.5 units per sampling interval for the ambient temperature and a shallow decline of averagely 0.1 units for the refrigeration temperature. The statistical analysis showed significant differences ($p<0.05$; Table 4) between the pH of the ambient and refrigerated samples. The analysis of the pH trend across the samples over the study period showed a constant decline in pH with each sample session for both refrigerated and non-refrigerated samples. This produced a negative gradient plot which declines towards a more acidic region though it is more profound in the non-refrigerated samples with pH dropping from 7.02 to 3.32 for ambient temperature and 7.02 to 5.56 for refrigeration temperature (Figure 3).

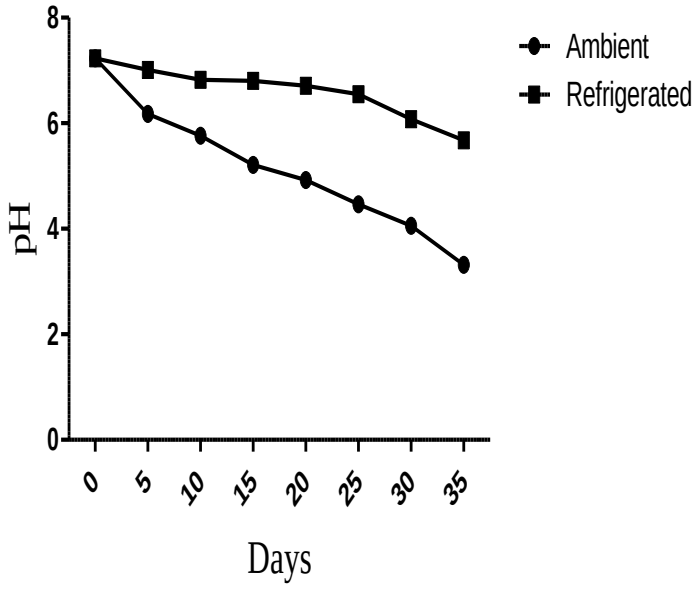

Figure 3: pH of refrigerated and non-refrigerated (ambient) samples of Honey Shake Milk Drink.

Table 2: Statistical Analysis of pH of Honey Shake Milk Drink Stored under different conditions

Source of variation	% of total variation	P value
Column factor	3.50	0.0012
Row factor	89.26	0.0274
Residual	4	

Column factor indicates the interaction between the different modes of storage (refrigerated and non-refrigerated) whereas the row factor indicates the interaction between the different days of storage.

The model obtained for the ambient temperature of storage was the Square root-Y squared-X model: $Y = (a + b*X^2)^2$, which gave the equation: $Days = (8.08911 - 0.153301*6.5^2)^2$ to obtain a Best Before date of 3 days after production (Fig. 4).

The model obtained for the samples stored under refrigeration temperature was the Squared-Y reciprocal-X model: Y = sqrt(a + b/X), which gave the formula Days = sqrt(-5429.97 + 38704.5/6.5) to obtain a Best Before date of 26 days after production.

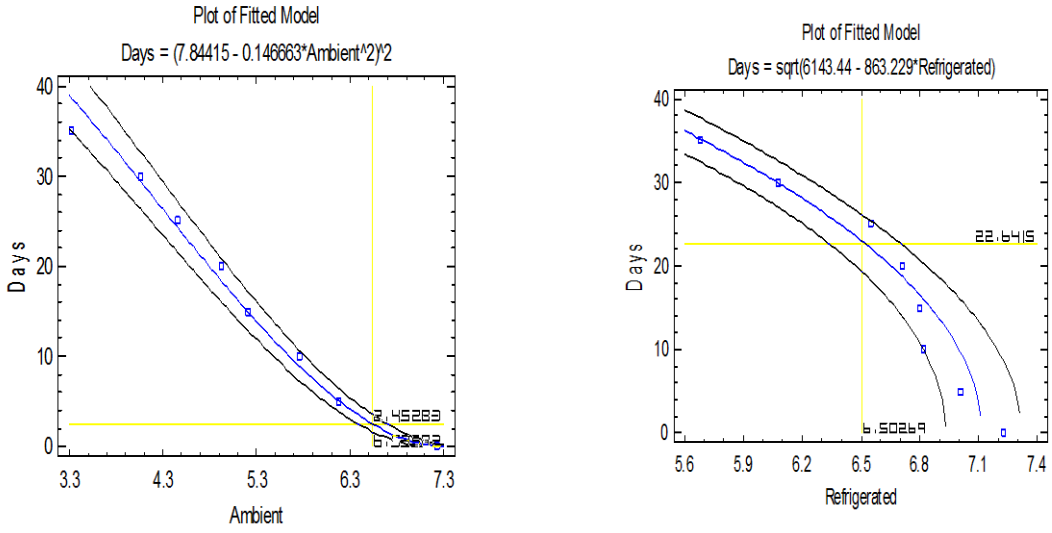

Figure 4: Shelf life of Honey Shake Milk Drink under ambient and refrigeration storage conditions using pH as predictor

4.2.2 Milk Fat assessment of HSMD

The determination of Shelf life using the milk fat as predictor showed similar trends as observed with the pH. The samples stored under ambient conditions showed a steep decline in milk fat content over time as opposed to the gradual decline observed with the samples stored under refrigeration conditions (Figure 5).

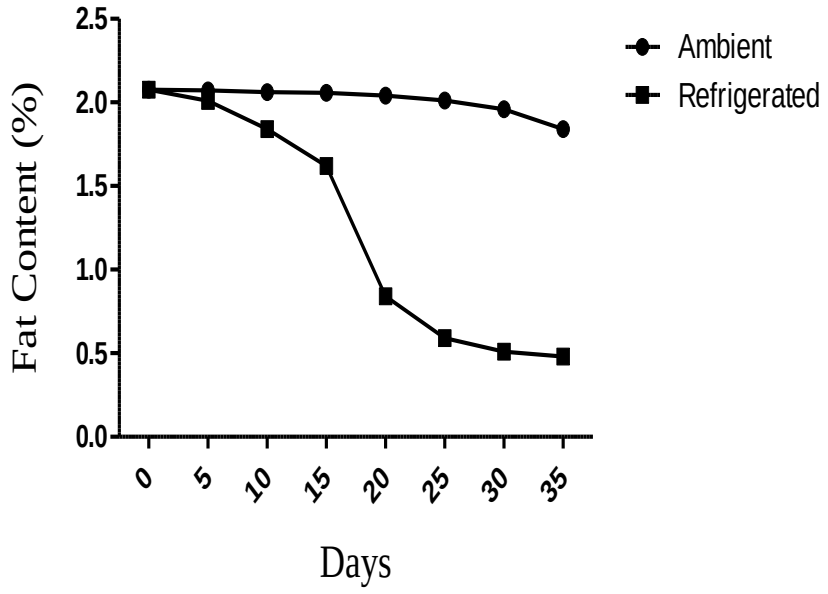

Figure 5: Fat Content of refrigerated and non-refrigerated (ambient) samples of Honey Shake Milk Drink over 35 days of storage

The statistics showed significant difference ($p<0.05$) in the fat content of the product stored under refrigeration and ambient conditions.

The model obtained for the samples stored at ambient temperature was the Squared-Y reciprocal-X model: Y = sqrt(a + b/X), which gave the formula Days = sqrt(-270.033 + 624.1/1.5) to obtain a Best Before date of 12 days after production.

The analysis also gave a model Double-squared: $Y = \text{sqrt}(a + b*X^2)$ for the samples stored at refrigeration temperature to obtain the equation: $\text{Days} = \text{sqrt}(5957.99 - 1357.93*1.5^2)$, thus giving a Best Before date of 54 days after production.

4.2.2 Total aerobic count

The total aerobic count of the beverage drink HSMD showed a trend of increase in microbial load with increasing time for test samples stored under both ambient and refrigeration

temperatures but a gradual increase was observed with the refrigerated samples and a sharper rise in microbial population with time was observed for the samples stored under ambient conditions (Figure 6).

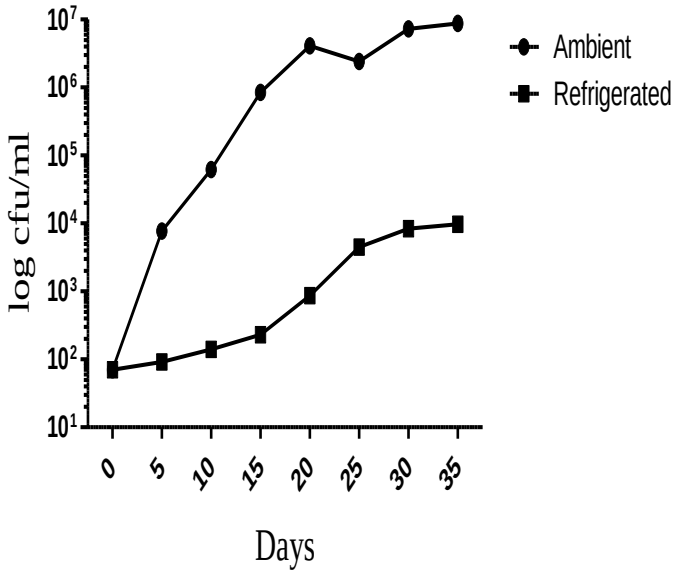

Figure 6: Total aerobic count on HSMD stored under ambient and refrigeration conditions.

The computation produced the model Square root-Y reciprocal-X model: $Y = (a + b/X)^2$, which produced the equation $Days = (4.31798 - 302.663/1.0\times10^4)^2$ giving a Best Before date of 18 days after production for the samples stored at ambient temperature.

The model Squared-Y square root-X: Y = sqrt(a + b*sqrt(X)) was obtained for the samples stored at refrigeration temperature giving the equation: Days = sqrt(-34.0254 + 11.3732*sqrt(1.0×10^4)) and a Best Before date of 33 days after its day of production.

The statistical analysis of the results showed a significant difference ($p < 0.05$; Table 5) between the aerobic count of the product stored under ambient and refrigeration conditions

at 95% confidence level but no significant difference in the progress in microbial loads over the days of storage.

Table 3: Statistical analysis of Total Aerobic Count of HSMD stored under different conditions

Sources of variation	% of total variation	P value
Column factor	25.62	0.043
Row factor	35.65	0.489
Residual	7	

Column factor indicates the interaction between the different modes of storage (refrigerated and non-refrigerated) whereas the row factor indicates the interaction between various day of storage in the study.

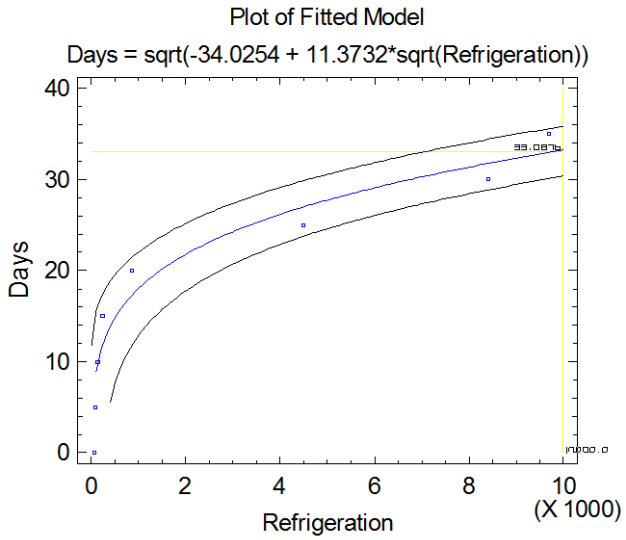

Figure 7: Shelf life of HSMD store at refrigeration temperature for 35 days using Total Aerobic Count

4.2.3 Shelf life using sensory parameters

The sensory parameters included color, smell, appearance and overall acceptability for the study. The results showed a decline of score sharply after 5 days of storage under ambient conditions with the panelists not liking the product (score 1.1). The refrigerated samples

however did not show any significant decline in score over time with the panelists liking the product at the end of the study period with a score of 6.8.

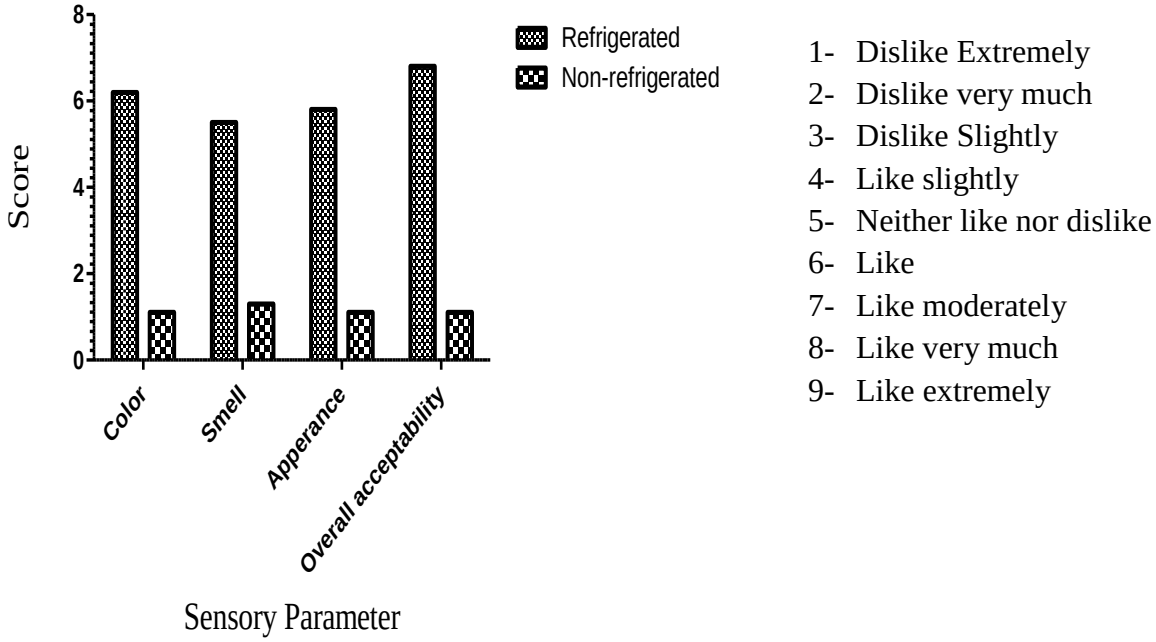

Figure 8: Sensory responses from panelists on HSMD after 35 days of storage under refrigeration and ambient conditions.

The statistical analysis showed a significant difference ($p < 0.05$) between the scores obtained for samples stored under the varying conditions (refrigeration and ambient) but again there was no significant difference ($p > 0.05$; Table 6) in the scores over the days of storage.

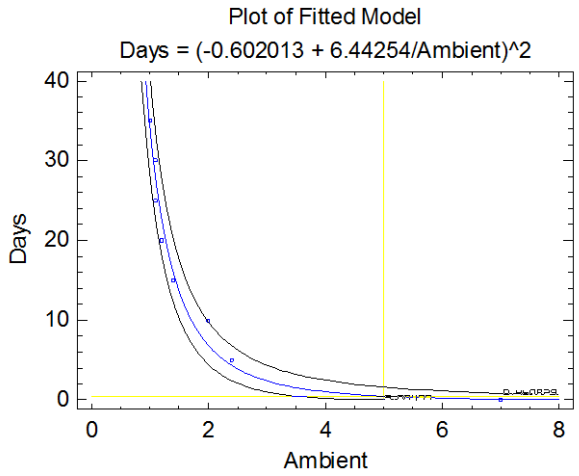

Figure 9: Shelf life of HSMD stored at ambient temperature for 35days using Sensory parameter

The estimation predicted the Reciprocal-X model: Y = a + b/X to obtain the equation: Days = -428.255 + 3085.78/5.0 to obtain a Best Before date of 12 hours after production for the samples stored at ambient conditions and the model Square root-Y reciprocal-X model: Y = $(a + b/X)^2$ was obtained for the samples stored at refrigeration temperature to give the equation Days = $(-0.602013 + 6.44254/5.0)^2$ and a Best Before date of 189 days after production.

Table 4: Statistical analysis of sensory parameter of HSMD after 35 days storage period

Source of variations	% of total variation	P value
Column factor	98.72	0.0006
Row factor	0.76	0.6484
Residual	3	

Column factor indicates the interaction between the different modes of storage (refrigerated and non-refrigerated) whereas the row factor indicates the interaction between the days of storage for the study.

4.2.4 Overall Shelf Life Determination

The shelf life study using all three indicators (physicochemical-pH and fat, microbiological-TAC and sensory-acceptability) was determined using a multiple regression analysis of the linear order (Figure 10).

The model obtained was a linear model of the order Days = 64.4851 - 8.48605*6.5-4.13826*1.5 + 4.85078E-8*1.0×10^4 + 0.776311*5.0 for the samples stored under ambient conditions to give a Best Before date of 7 days after production.

A linear model was also obtained for the samples stored under refrigeration conditions of the order Days = -8.9219 - 40.5927*6.5 + 120.134*1.5 + 0.000315914*1.0×10^4 + 7.40715*5.0 to obtain a Best Before date of 52 days after production.

The analysis revealed the interaction between all three parameters considered to be significant recording a p-value of 0.0344, but pH being the most important factor in determining the shelf life.

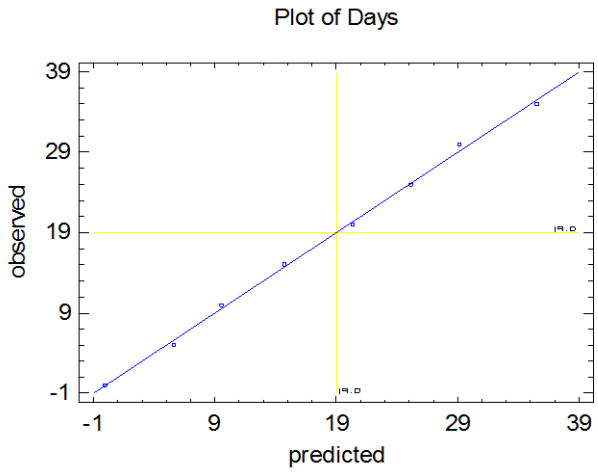

Figure 10: Shelf life of HSMD using all indicators of prediction after 35 days of storage

CHAPTER FIVE

DISCUSSION

5.1 Quality and Shelf life of Honey Shake Milk Drink

The Honey Shake Milk Drink (HSMD) analysis conducted prior to the shelf life study was marked for good quality both physiochemically and microbiologically. The analysis proved negative results for Coliforms, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* in the microbial assessment. These microbes are of great essence in the food industry because they have been recorded to bring about many health related issues in developing countries (Grauke *et al.*, 2002).

Strict standard operating procedures are implemented in the production of the beverage drink hence not surprising and indication of absence of these pathogens. Also good manufacturing practices are observed to ensure safe products are being manufactured from the factory plant. The production line ensures that raw materials are under safe and quality state before it is use hence the whole milk powder used for production has the required certification for safe foods according to the regulatory standards, the use of a well filtered system to provide quality water for manufacturing that are usually tested before its use, also a certified body provides for additional flavours used for the processes. These precautions are necessary because there can be least contamination and growth of microorganisms since milk is a rich source of medium to support its life (Udayathilaka, 2003). Lactose serves as substrate for some class of coliforms and this sugar predominant in milk helps the metabolise them to produce lactic acid which is essential in fermented food products and disadvantage to non-fermented products like Honey shake milk drink. These group of microbes makes an

undesirable end products and diverse the original flavour of the said product hence effective ways of ensuring their absence is very essential (Adu-Gyamfi *et al.,* 2009).

The health risks associated with these microbes in the food include diarrhea, fever and general uneasiness. These are classified as potential hazards and as such the reference or safe limits for such organisms by international standards such as Codex Alimentarius and ISO to be nil or zero(0) cfu/g or ml.

The quality assay conducted on samples of HSMD fell within the acceptable limits with zero (0) recording of these pathogens thus qualifying the product as safe and good for consumption. This was achieved through careful selection of quality of the raw materials and also the effectiveness of the processing technology. Thus pasteurization process employed at the factory effective at reducing and/or eliminating non-spore forming microbes, reflecting in the aerobic count recorded to be <30 cfu/g in the light of the reference standard and safe limit of 1.0×10^4 cfu/g.

The pH and milk fat results showed the beverage was of good quality and safe for consumption. The pH was determined to be 6.81 which fell within acceptable standards of 6.5-7.5 making it an ideal product. The milk fat content recorded 2.077% w/w which also fell within the acceptable standard of <2.0%.

The sensory analysis conducted on HSMD showed positive responses from the panelists indicating an appreciable effect on the consumer acceptance and patronage, hence consumer protection and security be ensured by creating a quality product on the market with documented shelf life analysis. The statistical analysis conducted on assay showed no significant difference in the scores of sensory analysis of the three flavors on all test parameters which is an indication of closeness of the formulations in quality and sensory

attributes. The mean score of the products on all parameters was within 5-7 score which is indicative of the "like" region on the hedonic scale used. This shows the potential consumer preference for the product. The overall acceptability scores were also within 5-7 indicative of a good market acceptability of the product and a consumption on a large scale by the general public.

The preliminary assessment conducted on the samples with regard to biochemical changes using pH as indicator showed no significant difference in the pH trend over time in all the three flavours. The pH over the time declined to the more acidic region on the pH scale. This can be attributed to the utilization of the sugars (lactose) in the milk drink by the microorganisms in a fermentative order to produce a breakdown of lactose into lactic acid and other metabolite resulting in the reduction in pH (Liu *et al.,* 2011). The responses were similar in all the three flavours because the base components for the production of beverage are equal thus consisting of same ingredients in same proportions with the exception of flavour, that differentiates these samples. Since these form the biochemical processes and largely depend on them hence similar reactions and response. This statistical and empirical information informed the selection of the chocolate flavor of the milk beverage as the model product in the shelf life study since it recorded the highest acceptability score and also most preferred and consumed product, even though there was no significant difference in the acceptability of the chocolate and other flavors thus it can serve as a representative and model product.

The shelf life determination of the analysis using pH as indicator recorded an inverse proportion to storage thus a trend of decreasing pH was observed with increasing storage time and indication of increase microbial load. The increase in microbial population can be

attributed to couple of interrelated biochemical reactions supporting its growth both in size and numbers. The degradation of sugars in these biochemical processes occur under anaerobic conditions (particularly as observed in fermentative metabolism) which leads to production of acids as its byproducts. The presence of these acids have resulted in lowering of pH of the medium. The statistics showed a significant difference in the pH trend between the products stored under ambient and refrigeration conditions with the decline being more sharp and profound in the product stored under ambient conditions as opposed to the one stored under refrigeration conditions. The above can be due to degradation of sugars and production of its acids in the samples stored under ambient conditions as compared to those under refrigeration conditions as seen in the Brix results. The biochemical reactions resulting in the production of the organic acids just like all typical reactions is directly proportional to temperature thus with an increase in temperature resulting in a consequent increase in rate of reaction till an optimum temperature for the system understudy is reached, and the inverse holding with a decrease in temperature resulting in a decrease in the rate of reaction (Jiménez-Colmenero *et al.,* 2001). The refrigeration temperature of 5^{o}C lies below the optimum temperature for the mesophilic microorganisms which is 30^{o}C± 2 thus the rate of reaction is reduced under refrigeration conditions resulting in lower organic acid production and shallow decline in pH (Jiménez-Colmenero *et al.,* 2001). The samples stored under ambient conditions for the analysis on the other hand had the ideal temperature for microbial proliferation and metabolism thus production of more organic acids due to continuous breakdown of sugar resulting in the sharp decline in pH (Gillooly *et al.,* 2001). This explains the reason for a shorter shelf life in samples stored under ambient conditions compared to the products stored under refrigeration conditions.

During the milk fat analysis, it was observed that there was a significant difference between samples stored under ambient and refrigeration conditions. The decrease in fat content per time can be explained to the fact that microorganisms due to increased growth and numbers hence are being utilized in the system. Like sugars, fats serve as substrate for microorganisms to sustain growth and provide energy by producing lipases to break them down into triglycerides. The biochemical processes of the enzymes activities are influenced by temperature thus the lower the temperature the slower the activities of the enzyme in the degradation process. The fat analysis showed how important it is to support microbial growth as there was optimal degradation of fat component especially in ambient temperature thereby affect the quality of the test samples giving it a shorter shelf life.

The profile showed for microbial analysis of the sample showed an increase in microbial loads with increase in storage time. This is an expected result because medium is milk which is rich nutrient for microbial growth under favourable pH. The results showed significant difference between the two observed temperatures under which samples were stored. This can be accounted for because microbes survive under different temperatures and can be classified into mesophilic, thermophilic and psychrophilic (Gillooly *et al.*, 2001). The standard microorganisms under study in the milk beverage are mesophilic hence able to grow and multiply under ambient storage conditions as demonstrated in a logarithmetric growth pattern and rate. Refrigeration below 5^{o}C reduces activities of the type of microorganisms by slowing its rate of metabolism. The shelf life of the analysed sample was shorter under ambient conditions as rate of metabolism in microorganisms was faster, attaining the maximum limit of 1.0×10^{4} cfu/g rendering the product unsafe for consumption.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study showed that the freshly produced Honey Shake Milk Drink (HSMD) for all the three variants (vanilla, strawberry and chocolate) were safe for consumption on both microbiological and physicochemical properties analyzed.

The chocolate HSMD had the highest overall acceptability score of 7 on the 9-point hedonic scale indicating a strong positive consumer acceptability.

The decline in pH, fat content and Brix over time was sharper for ambient condition as compared to refrigerated samples while statistically different for both storage conditions.
The ultimate shelf life of the product was estimated to be 7 days when stored under ambient conditions and 52 days when stored under refrigeration conditions. The study therefore concludes that the best temperatures for the storage of HSMD beverage is 5^{o}C or lower (refrigeration).

6.2 Recommendation

In view of the findings of this study, since the product has no added preservatives further analysis had to be conducted where preservatives are added to product and experiments conducted to study effects on shelf life of the product without changing its physicochemical and sensory properties.

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APPENDIX

Appendix 1: Sensory evaluation form

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
KUMASI

(GRADUATE STUDIES)

Sensory Evaluation of Honey Shake Milk Drink

Panelist No:. …….
Date: ……./……./2018

INSTRUCTIONS:

i. Please observe the sample and indicate your observation on the sheet provided.
ii. Observe all samples from left to right
iii. Please add any additional comment at the bottom of the sheet.

You have been presented with ten cups of Honey shake with varying percentages of preservatives. You are to kindly evaluate the **taste, color, smell/flavor appearance, aftertaste and overall acceptability.**

Please, evaluate the samples in the nine point hedonic scale produced below where 1-dislike extremely to 9-like extremely.

Inspect the cups in the order presented below and indicate by writing the appropriate number in the spaces provided showing how much you like or dislike each sample.

Key

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

Sensory evaluation table

Sample	Taste	Color	Smell /flavor	Aftertaste	Appearance	Overall acceptability
001						
002						
003						

Please, leave a comment:

……
……
……
……
……

Appendix 2: Statistical Analysis of pH of Honey Shake Milk Drink after storage

Parameter

Table Analyzed pH of Chocolate

Two-way ANOVA

Source of Variation	% of total variation	P value
Column Factor	40.58	0.0012
Row Factor	49.21	0.0274

Source of Variation	P value summary	Significant?
Column Factor	**	Yes
Row Factor	*	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	1	8.614	8.614	27.82
Row Factor	7	10.45	1.492	4.820
Residual	7	2.167	0.3096	

Number of missing values	0

Bonferroni posttests

Ambient vs Refrigerated

Row Factor	Ambient	Refrigerated	Difference	95% CI of diff.
Day 0	7.230	7.230	0.0	-3.034 to 3.034
Day 5	6.180	7.010	0.8300	-2.204 to 3.864
Day 10	5.760	6.820	1.060	-1.974 to 4.094
Day 15	5.210	6.800	1.590	-1.444 to 4.624
Day 20	4.920	6.710	1.790	-1.244 to 4.824
Day 25	4.460	6.550	2.090	-0.9437 to 5.124
Day 30	4.060	6.080	2.020	-1.014 to 5.054
Day 35	3.320	5.680	2.360	-0.6737 to 5.394

Row Factor	Difference	t	P value	Summary
Day 0	0.0	0.0	P > 0.05	ns
Day 5	0.8300	1.055	P > 0.05	ns
Day 10	1.060	1.347	P > 0.05	ns
Day 15	1.590	2.021	P > 0.05	ns

Day 20	1.790	2.275	P > 0.05	ns
Day 25	2.090	2.656	P > 0.05	ns
Day 30	2.020	2.567	P > 0.05	ns
Day 35	2.360	2.999	P > 0.05	ns

Appendix 3: Statistical Analysis of Milk Fat of Honey Shake Milk Drink after storage

Parameter				
Table Analyzed	Milk Fat			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	40.10	0.0120		
Row Factor	35.08	0.3296		
Source of Variation	P value summary	Significant?		
Column Factor	*	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	1	2.366	2.366	11.31
Row Factor	7	2.070	0.2957	1.414
Residual	7	1.464	0.2092	
Number of missing values	0			
Bonferroni posttests				
Ambient vs Refrigerated				

Row Factor	Ambient	Refrigerated	Difference	95% CI of diff.
Day 0	2.076	2.076	0.0	-2.493 to 2.493
Day 5	2.071	2.010	-0.06100	-2.554 to 2.432
Day 10	2.062	1.840	-0.2220	-2.715 to 2.271
Day 15	2.057	1.620	-0.4370	-2.930 to 2.056
Day 20	2.041	0.8400	-1.201	-3.694 to 1.292
Day 25	2.012	0.5900	-1.422	-3.915 to 1.071
Day 30	1.960	0.5100	-1.450	-3.943 to 1.043
Day 35	1.840	0.4800	-1.360	-3.853 to 1.133

Row Factor	Difference	t	P value	Summary
Day 0	0.0	0.0	P > 0.05	ns
Day 5	-0.06100	0.09432	P > 0.05	ns
Day 10	-0.2220	0.3432	P > 0.05	ns
Day 15	-0.4370	0.6757	P > 0.05	ns
Day 20	-1.201	1.857	P > 0.05	ns
Day 25	-1.422	2.199	P > 0.05	ns
Day 30	-1.450	2.242	P > 0.05	ns

Appendix 4: Statistical Analysis of Aerobic Count of Honey Shake Milk Drink

Parameter	
Table Analyzed	TAC

Two-way ANOVA

Source of Variation	% of total variation	P value
Column Factor	28.90	0.0483
Row Factor	35.63	0.4978

Source of Variation	P value summary	Significant?
Column Factor	*	Yes
Row Factor	ns	No

Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	1	3.450e+013	3.450e+013	5.703
Row Factor	7	4.253e+013	6.076e+012	1.004
Residual	7	4.235e+013	6.050e+012	

Number of missing values 0

Bonferroni posttests

Ambient vs Refrigerated

Row Factor	Ambient	Refrigerated	Difference	95% CI of diff.
Day 0	70.00	70.00	0.0	-1.341e+007 to 1.341e+007
Day 5	7700	92.00	-7608	-1.342e+007 to 1.340e+007
Day 10	62000	140.0	-61860	-1.347e+007 to 1.335e+007
Day 15	850000	230.0	-849770	-1.426e+007 to 1.256e+007
Day 20	4.100e+006	870.0	-4.099e+006	-1.751e+007 to 9.311e+006
Day 25	2.400e+006	4500	-2.396e+006	-1.581e+007 to 1.101e+007
Day 30	7.300e+006	8400	-7.292e+006	-2.070e+007 to 6.119e+006
Day 35	8.800e+006	9700	-8.790e+006	-2.220e+007 to 4.620e+006

Row Factor	Difference	t	P value	Summary
Day 0	0.0	0.0	P > 0.05	ns
Day 5	-7608	0.002187	P > 0.05	ns
Day 10	-61860	0.01778	P > 0.05	ns
Day 15	-849770	0.2443	P > 0.05	ns

Day 20	-4.099e+006	1.178	P > 0.05	ns
Day 25	-2.396e+006	0.6887	P > 0.05	ns
Day 30	-7.292e+006	2.096	P > 0.05	ns
Day 35	-8.790e+006	2.527	P > 0.05	ns

Appendix 5: Statistical Analysis of Sensory Analysis of Honey Shake Milk Drink

```
ONEWAY Taste Aftertaste Color Smell Appearance OA BY Sample
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05).
```

Oneway

Notes

Output Created		01-Jan-2009 17:44:24
Comments		
Input	Data	C:\Users\user\Documents\Sensory B.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	100
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY Taste Aftertaste Color Smell Appearance OA BY Sample /MISSING ANALYSIS /POSTHOC=TUKEY ALPHA(0.05).
Resources	Processor Time	00:00:00.546
	Elapsed Time	00:00:00.577

[DataSet1] C:\Users\user\Documents\Sensory B.sav

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Taste	Between Groups	11.667	2	5.833	1.461	.250
	Within Groups	107.800	27	3.993		
	Total	119.467	29			
Aftertaste	Between Groups	.467	2	.233	.076	.927
	Within Groups	83.000	27	3.074		
	Total	83.467	29			
Color	Between Groups	1.267	2	.633	.209	.812
	Within Groups	81.700	27	3.026		
	Total	82.967	29			
Smell	Between Groups	8.067	2	4.033	1.812	.183
	Within Groups	60.100	27	2.226		
	Total	68.167	29			
Appearance	Between Groups	.867	2	.433	.109	.897
	Within Groups	107.300	27	3.974		
	Total	108.167	29			
OA	Between Groups	10.867	2	5.433	1.869	.174
	Within Groups	78.500	27	2.907		
	Total	89.367	29			

Post Hoc Tests
Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Taste	Vanilla	Strawberry	.50000	.89360	.842	-1.7156	2.7156
		Chocolate	-1.00000	.89360	.511	-3.2156	1.2156
	Strawberry	Vanilla	-.50000	.89360	.842	-2.7156	1.7156
		Chocolate	-1.50000	.89360	.232	-3.7156	.7156
	Chocolate	Vanilla	1.00000	.89360	.511	-1.2156	3.2156
		Strawberry	1.50000	.89360	.232	-.7156	3.7156
Aftertaste	Vanilla	Strawberry	-.10000	.78410	.991	-2.0441	1.8441
		Chocolate	.20000	.78410	.965	-1.7441	2.1441
	Strawberry	Vanilla	.10000	.78410	.991	-1.8441	2.0441
		Chocolate	.30000	.78410	.923	-1.6441	2.2441
	Chocolate	Vanilla	-.20000	.78410	.965	-2.1441	1.7441
		Strawberry	-.30000	.78410	.923	-2.2441	1.6441
Color	Vanilla	Strawberry	-.50000	.77794	.798	-2.4288	1.4288
		Chocolate	-.20000	.77794	.964	-2.1288	1.7288
	Strawberry	Vanilla	.50000	.77794	.798	-1.4288	2.4288
		Chocolate	.30000	.77794	.922	-1.6288	2.2288
	Chocolate	Vanilla	.20000	.77794	.964	-1.7288	2.1288
		Strawberry	-.30000	.77794	.922	-2.2288	1.6288
Smell	Vanilla	Strawberry	-1.10000	.66722	.243	-2.7543	.5543
		Chocolate	.00000	.66722	1.000	-1.6543	1.6543
	Strawberry	Vanilla	1.10000	.66722	.243	-.5543	2.7543
		Chocolate	1.10000	.66722	.243	-.5543	2.7543
	Chocolate	Vanilla	.00000	.66722	1.000	-1.6543	1.6543
		Strawberry	-1.10000	.66722	.243	-2.7543	.5543
Appearance	Vanilla	Strawberry	-.40000	.89152	.895	-2.6105	1.8105
		Chocolate	-.10000	.89152	.993	-2.3105	2.1105

	Strawberry	Vanilla	.40000	.89152	.895	-1.8105	2.6105
		Chocolate	.30000	.89152	.940	-1.9105	2.5105
	Chocolate	Vanilla	.10000	.89152	.993	-2.1105	2.3105
		Strawberry	-.30000	.89152	.940	-2.5105	1.9105
OA	Vanilla	Strawberry	-1.10000	.76255	.334	-2.9907	.7907
		Chocolate	-1.40000	.76255	.177	-3.2907	.4907
	Strawberry	Vanilla	1.10000	.76255	.334	-.7907	2.9907
		Chocolate	-.30000	.76255	.918	-2.1907	1.5907
	Chocolate	Vanilla	1.40000	.76255	.177	-.4907	3.2907
		Strawberry	.30000	.76255	.918	-1.5907	2.1907

Homogeneous Subsets

Taste

Tukey HSD

Sample	N	Subset for alpha = 0.05
		1
Strawberry	10	5.2000
Vanilla	10	5.7000
Chocolate	10	6.7000
Sig.		.232

Means for groups in homogeneous subsets are displayed.

Color

Tukey HSD

Sample	N	Subset for alpha = 0.05
		1
Vanilla	10	6.4000
Chocolate	10	6.6000
Strawberry	10	6.9000
Sig.		.798

Means for groups in homogeneous subsets are displayed.

Aftertaste

Tukey HSD

Sample	N	Subset for alpha = 0.05
		1
Chocolate	10	5.7000
Vanilla	10	5.9000
Strawberry	10	6.0000
Sig.		.923

Means for groups in homogeneous subsets are displayed.

Smell

Tukey HSD

Sample	N	Subset for alpha = 0.05
		1
Vanilla	10	5.8000
Chocolate	10	5.8000
Strawberry	10	6.9000
Sig.		.243

Means for groups in homogeneous subsets are displayed.

Appearance

Tukey HSD

Sample	N	Subset for alpha = 0.05
		1
Vanilla	10	6.0000
Chocolate	10	6.1000
Strawberry	10	6.4000
Sig.		.895

Means for groups in homogeneous subsets are displayed.

OA

Tukey HSD

Sample	N	Subset for alpha = 0.05
		1
Vanilla	10	5.6000
Strawberry	10	6.7000
Chocolate	10	7.0000
Sig.		.177

Means for groups in homogeneous subsets are displayed.

Appendix 6:

Days	Ambient	Refrigerated
0	7.23±0.01	7.23±0.01
5	6.18±0.01	7.01±0.01
10	5.76±0.01	6.82±0.01
15	5.21±0.01	6.80±0.01
20	4.92±0.01	6.71±0.01
25	4.46±0.01	6.55±0.01
30	4.06±0.00	6.08±0.00
35	3.32±0.00	5.68±0.00

Days	Ambient	Refrigerated
0	7.23±0.01	7.23±0.01
5	6.18±0.01	7.01±0.01
10	5.76±0.01	6.82±0.01
15	5.21±0.01	6.80±0.01
20	4.92±0.01	6.71±0.01
25	4.46±0.01	6.55±0.01
30	4.06±0.00	6.08±0.00
35	3.32±0.00	5.68±0.00