

**COMPREHENSIVE ANALYSIS AND QUALITY IMPROVEMENT  
OF “KOTMALE” SET YOGURT**

By

**B.R.C.R. Boraluwa**

**(03/AS/031)**

**This thesis is submitted in partial fulfillment of the requirement for the  
Special Degree of bachelor of Science  
in  
Food science and Technology**

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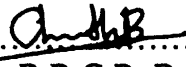
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## DECLARATION

The work described in this thesis was carried out by the Swiss cheese company (pvt) limited, New town, Mulleriyawa and Department of Food science and Technology, Faculty of Applied sciences, Sabaragamuwa University of Sri Lanka under the supervision of Mr.C.P.Samarasekara and Mr.M.C.N.Jayasooriya. The report on this has not been submitted to any other university for another degree.

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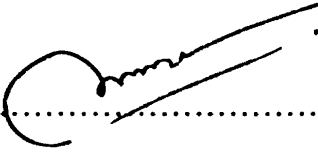
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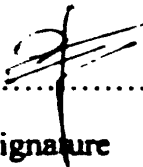
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**Affectionately Dedicated To My Ever Loving Parents And  
Teachers**



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## **Abstract**

Yogurt is a popular dairy product made from concentrated milk fermentation. There are several types of yogurts, such as set, stirred, drinking, frozen, concentrated and flavoured yogurt. Among them the set type yogurt is very popular and common. Quality of a yogurt basically determine according to the flavour, aroma, viscosity, consistency, appearance, freedom from whey separation and long shelf life.

The research was carried out to analyze the quality variation of the set yogurt over the period of one month to diagnose the quality defects of the existing "Kotmale" yogurt and to improve the quality and shelf life of the yogurt. Quality variation was measured by using chemical, microbiological and organoleptic tests. Under chemical tests, pH was measured by using pH meter and acidity was measured by titration method. Coliform, Yeast and Mould counts were counted by using pour plate count technique and organoleptic properties of color, smell and taste were tested everyday over the period of one month.

According to the results obtained from the above microbiological, chemical and organoleptic tests; high acidity, high sour taste and presence of yeast and mould were the predominant quality defects that were significant in the existing set yogurt. Therefore to eliminate the above quality defects, a new yogurt culture was selected with low post acidification and developed a improved yogurt with existing formulae. In third day after manufacturing, nine point hedonic scale sensory analysis was carried out to check the consumer preference. There were more consumer preference in texture and taste for improved yogurt but high overall acceptability for existing "kotmale" set yogurt. Chemical and microbiological tests were carried out to the improved set yogurt in 3<sup>rd</sup>, 14<sup>th</sup>, 22<sup>nd</sup> and 28<sup>th</sup> days after manufacturing. pH of the improved yogurt varied from 4.85 to 4.35 and acidity was varied from 0.79 to 0.99 over the period of 28 days. Coliform and Yeast and Mould counts of the improved yogurt were also conformed to the microbiological limits specified by the Sri Lanka Standard Institute. In 28<sup>th</sup> day after manufacturing another nine point hedonic scale sensory analysis was conducted to check the keeping quality of the improved yogurt. According to the analyzed sensory data there were more consumer preference on texture, sourness and overall acceptability to the improved yogurt over the other market sample of set yogurt and existing "Kotmale" set yogurt.

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# CHAPTER 01

## INTRODUCTION

### 1.1 Background

Dairy industry is one of the largest and wide spread food processing industry in today's world, therefore the dairy products play an important role in our daily lives. Milk is the basic raw material that is used in most of the dairy products. Basically milk is the whole, clean, lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before calving and 5 days after. (Herrington, 2000). Because of its perishable nature, soon after milking milk should be processed to any other value added product or preserved product. Therefore there are various dairy products in the market, such as ice cream, butter, cheese, UHT treated milk products, pasteurized milk products and fermented milk products.

Among them cultured milk products are very popular worldwide. Milk products obtained by the lactic acid fermentation (e.g. yogurt) or a combination of this and yeast fermentation (e.g. kefir) are called fermented or cultured milk products. Cultured milk is the collective name for products such as yoghurt, ymer, kefir, cultured buttermilk, cultured cream and koumiss (a product based on mares' milk). The generic name of cultured milk is derived from the fact that the milk for the product is inoculated with a starter culture which converts part of the lactose to lactic acid. Carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other substances are formed in the conversion process, and these give the products their characteristic fresh taste and aroma (tetra pak, 2000).

Yogurt is a dairy product, which is made by blending fermented milk with various ingredients that provide flavor and color. Although accidentally invented thousands of years ago, yogurt has only recently gained popularity in Sri Lanka. Manufacturers have responded to the growth in the yogurt market by introducing many different types of yogurt including set yogurt, low fat and non fat yogurt, drinking, stirred and frozen yogurt. However many manufacturers strive to maintain their yogurt quality in a high standard level to survive them in the competitive market.

**This research project was carried out in the Swiss cheese company private limited, one of the renowned dairy processing company that produce different kind of dairy products such as Ghee, Cheese, Ice cream, Pasteurized flavored milk, Yogurt, UHT milk and curd under the brand name of “Kotmale”. Set yogurt is one of the major product that they produce and has high consumer demand on it, so as a responsible dairy company in Sri Lanka, they always try to offer high quality products to their valuable customers. Therefore this research was carried out to analyze the all the quality parameters of their existing yogurt and improve the quality of their yogurt in to further extend. Chemical, microbiological and organoleptic tests were done to existing yogurt over the one month of period continuously and analyzed and identified the major quality defects of the existing set yogurt and solutions were provided to that defects, in order to enhance the quality of the “Kotmale” set yogurt.**

### **1.2 General Objective**

- **To improve the quality of the “Kotmale” set yogurt.**

### **1.3 Specific Objectives**

- **To check the quality variation of existing set yogurt over one month of period**
- **To increase the shelf life of set yogurt**
- **To improve the texture of set yogurt**

## **CHAPTER 02**

### **LITERATURE REVIEW**

#### **2.1 Evolution of Yogurt**

It is believed that yogurt originated in Mesopotamia thousands of years ago. Evidence has shown that these people had domesticated goats and sheep around 5000 B.C. the milk from these animals was stored in gourds, and in the warm climate it naturally formed a curd. This curd was an early form of yogurt. Eventually, a process for purposely producing yogurt was developed. While yogurt has been around for many years, it is only recently (within the last 30 – 40 years) that it has become popular. This is due to many factors including the introduction of fruit and other flavoring into yogurt, the convenience of it as a ready – made breakfast food and the image of yogurt as a low fat health food.

Among fermented dairy products Yogurt is very popular whole over the world. There are many definitions for yogurt, however according to the Food and Drug Administrations (FDA) official definition, it can be define as the acidified, coagulated product obtained from milk by fermentation with lactic acid producing bacteria (FDA 2003).

Fermented milks were used by the people of the Eastern Europe and Asia, minor long before the discovery of bacteria. Undoubtedly they observed that milk usually spoiled after it was drawn, but if it were allowed to sour in a controlled manner the resulting product had not only a pleasing flavour but also improved keeping quality. It was not until about 1840 that scientists began to believe that the souring of milk and similar fermentations were caused by the activity of microorganisms; following the work of Pasteur, about 1857, notable progress was made.

The basis of a fermented milk is lactic acid fermentation but special type of bacteria and yeast produce the typical characteristics of the fermented milks used in various parts of the world. There are two principal kinds of fermented milk: 1) the acid type, such as cultured buttermilk and yogurt; and 2) the kefir type, in which a combined acid and gassy fermentation takes place. In the latter case a milk alcoholic fermentation also occurs. (Lampert, 1970)



Manufacturers have responded to the growth in the yogurt market by introducing many different types of yogurt including low fat, non fat, drinking, flavored and frozen. Traditional yogurt is thick and creamy. It is sold plain and in a wide assortment of flavors. These are typically fruit flavors such as strawberry and blueberry, however, newer more unique flavors such as cream pie and chocolate have also been introduced. Some times in some countries cereals and nuts also add to the yogurt. (Lampert, 1970)

The yogurt itself has a generally aldehydic flavor, which is a result of the fermentation process. Since it is made from milk, yogurt is rich in nutrients. It contains protein and vitamins and is a rich source of calcium. In fact, a small container of yogurt contains as much calcium as a third of a pint of milk. In addition to these nutritional characteristics, yogurt is also thought to have additional health benefits. One of the suggested benefits of yogurt is that it acts as a digestive aid. In the body, it is thought that yogurt can encourage the growth of beneficial bacteria in the gut. These organisms help to digest food more efficiently and protect against other harmful organisms. Another health benefit of yogurt is for people that are lactose intolerant. These people have difficulty in digesting milk products however, they can tolerate yogurt. Other than that there are many other purported benefits of yogurt include the reduction of cholesterol, protection against certain cancers, and even boosting the immune system. (Lampert, 1970)

The future of the yogurt manufacturing will focus on the development of new flavors and longer lasting yogurts. The introduction of new flavors will be driven by consumer desire and new developments by flavor manufacturers. The suppliers of the bacterial cultures are conducting research that hints at the development of uniquely flavored yogurts. By varying the types of organisms in the cultures, yogurt is produced much faster and lasts longer than conventional yogurt. (Lampert, 1970)

## **2.2 Types of Yogurt**

Many different types of yogurt are produced worldwide, and these commercial products are classified in to different categories based on the following aspects:

- **Chemical composition (full, semi or low fat)**
- **Flavor (natural, fruit or flavored)**
- **Physical characteristics (set, stirred or drinking yogurt)**

- Miscellaneous post fermentation processing such as heat treatment, fortification with vitamins
- Replacement of the animal fat with vegetable fat

### **2.2.1 Set Yogurt**

This type of yogurt is incubated and cooled in the retail package and is characterized by a firm “jelly” like texture. The coagulum is not broken until it is consumed and therefore the gel firmness is an essential parameter for set yogurt.

For the manufacturing of set yogurt, processed milk base is cooled to 40 – 45 °C and inoculated with starter culture, and optional fruit, flavoring material and / or color is added. The yogurt milk is filled in to retail containers, packed and palletized within 20 - 30 minutes and incubated in cabinets to the required acidity. After obtaining required acidity yogurt pallets are transfer to the cold room to cease fermentation and then dispatch for consumption.

(Texnotext, 1995)

### **2.2.2 Stirred Yogurt**

This type of yogurt is incubated in a tank and the final coagulum is broken by stirring prior to cooling and packaging. The texture of the stirred yogurt will be less firm than a set yogurt. There is usually a slight reformation of the coagulum after the yogurt has been packed. In commercial manufacturing incubation and other processing steps are very similar to the set yogurt, but gel is broken during in tank cooling or prior to being pumped to a cooler.

(Texnotext, 1995)

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### **2.2.3 Drinking Yogurt**

This type of yogurt is very similar to stirred yogurt, having the coagulum broken prior to cooling. In a drinking yogurt the agitation used to break the coagulum is severe. Little if any reformation of the coagulum will reoccur after packing. The basic guidelines for the acidification of the product are in general the same as for stirred yogurt. The fermentation should proceed until the pH reaches 4.2 – 4.0 in order to obtain the best stability.

After fermentation, the coagulum of drinking yogurt is broken down, which is the opposite of stirred yogurt where the coagulum is treated with certain caution. This treatment is carried out to assure a homogeneous product with no gel lumps.

(Texnotext, 1995)

#### **2.2.4 Frozen Yogurt**

Frozen yogurt requires a different recipe to yogurt and usually consist of a thin yogurt blended with a high solid ice cream base mix. Freezing is achieved by pumping through a freezer in a fashion similar to ice cream. The texture of the finished product is mainly influenced by the freezer.

(Texnotext, 1995)

#### **2.2.5 Concentrated Yogurt**

This type of yogurt is inoculated and fermented in the same manner as a stirred yogurt. Following the breaking of the coagulum the yogurt is concentrated by boiling off some of the water, this is often done under vacuum to reduce the temperature required. Heating of low pH yogurt can often lead to protein being totally denatured and producing rough and gritty texture. This is often called strained yogurt due to the fat that is released from the coagulum upon heating used to be strained off in a manner similar to making soft cheese.

(Texnotext, 1995)

#### **2.2.6 Flavored Yogurt**

Yogurt with various flavors and aromas have become very popular. The flavors are usually added at or just prior to filling into cups. Common additives are fruit or berries, usually as a puree or as whole fruit in a syrup. These additives often have as much as 50 % sugar in them, however with the trend towards healthy eating gaining momentum, many manufacturers offer a low sugar and low fat version of their products. Low or no sugar yogurts are often sweetened with saccharin or more commonly aspartame. The use of fruit sugars in the form of concentrated apple juice is sometimes found as a way of avoiding added sugar on the ingredients declaration, this tends to be a marketing ploy and has no real added benefit.

(Texnotext, 1995)

## 2.3 Raw materials for Yogurt

Yogurt is made with a variety of ingredients including milk and milk concentrates as the basic raw material and sweeteners, stabilizers, flavors, colors, bacterial culture and preservative as the optional ingredients.

### 2.3.1 Milk

Milk from different species of mammals (cow, goat, sheep and buffalo) have been used for the production of yogurt and fermented milk for centuries. However the worldwide production of these products is primarily made from cow's milk. The chemical composition of milk varies from day to day due to multitude of factors mainly related to the dairy cow husbandry and, inevitably, the quality of the yogurt will vary.

Milk has a very complex composition. Some of its constituents, such as milk fat, milk sugar and casein are not found elsewhere, either in the body or in nature. Milk is practically the only foodstuff that contains all of the different substances known to be essential for human nutrition. Approximate composition of the milk can be given as follows;

Component	Percentage %
Water	87.29
Protein	3.42
Fat	3.66
Lactose	4.92
Minerals	0.71

**Table 2.1. Average composition of the cow milk**

(Lampert, 1970)

However the milk solid non fat (MSNF) of the milk is not enough to get the required textural properties of the set and stirred yogurt, since the protein content of the milk is not enough to contribute to the MSNF. In such cases, the first step in the manufacture is to raise the level of MSNF in the raw milk. This approach is similar to the traditional process where the milk was heated in an open pan over a fire to evaporate the water or addition of full cream milk powder (FCMP) to the milk to increase the MSNF content of the milk base.

**The quality of the raw milk is very critical factor that effect to the quality of the yogurt. Therefore the protocol of milk handling on reception at the factory is subject to several quality control tests. Firstly immediate quality control tests are carried out. This includes measurement of temperature, pH or titratable acidity, freezing point depression, antibiotic residues and check for smell. Secondly the delayed tests are for proximate chemical composition, total viable counts and somatic cell counts. (Johnson and Alford, 1987)**

### **2.3.2 Sweeteners**

**Sugar or sweetening agents are normally added to the milk base during the manufacturing of yogurt, and they are added to tone down the acidity in the product. However it is important to add the suitable amount of sugars to the milk because higher levels can reduce the rate of acid development by the starter cultures. Therefore the levels of sugar greater than 10 % should not be added to the yogurt mix prior to the incubation. If higher levels of sugar addition are required, then it can be add after the fermentation. Disaccharide sugars such as sucrose or monosaccharide sugars such as glucose can be used alone or in conjunction to produce the sweetness level required. The addition of sugar often improves the “body” and “mouthfeel” of a yogurt. (Robinson and Tamime, 1980)**

### **2.3.3 Permitted Stabilizers**

**Stabilizers are hydrophilic colloids which bind water and consequently increase the viscosity of the yogurt, they also help prevent the separation of whey from the yogurt, a problem known as syneresis. The most beneficial quantity of stabilizers has to be determine by the manufacturer base on their experience. Too much stabilizers cause a rubbery texture to the yogurt, while far too much stabilizers can cause to a hard solid mass. A traditionally produced natural yogurt not required stabilizers to produce a firm, fine gel, however commercially produce yogurt that has to be pumped, stirred, fruited and filled will often break down to a runny liquid without the addition of stabilizers.**

**Gelatin, starch and pectin are the common stabilizers that use in yogurt manufacturing, but in 1975 Food Standard Committee suggested that the weight of the stabilizers that should add to the yogurt mix should not exceed the maximum of 1 % by the weight of the final product. (Robinson and Tamime, 1980)**

### 2.3.4 Permitted Colors and Flavors

It's not essential to add flavors and colors to the yogurt, but in commercial manufacturing many manufacturers add permitted colors such as Egg yellow and permitted flavors such as Vanilla flavor and strawberry flavor to their products to obtain more consumer attraction to their products. (Robinson and Tamime, 1980)

### 2.3.5 Permitted Preservatives

Preservatives are the chemical compounds that use in food products to retard the microbial action and chemical reaction with in the food products during the storage. However chemical preservatives were not used in traditional yogurt manufacturing, but in commercial manufacturing it's use several preservatives, such as ;

- Sulphur dioxide
- Benzoic acid
- Methyl – 4 – hydroxyl benzoate
- Ethyl – 4 – hydroxyl benzoate
- Propyl – 4 – hydroxyl benzoate
- Sorbic acid

However according to the Sri Lanka Standard Institutions (SLSI) specification for fermented milk products, Sodium or Potassium and Calcium salts of sorbic acid is the only preservative that is permitted to use in yogurt manufacturing. (Robinson and Tamime, 1980)

### 2.3.6 Starter Culture

Starter cultures are the defined strains of microorganisms that use in the production of cultured dairy products. *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) are the major bacterial strains that are commonly found in many yogurt cultures. But sometimes other than these strains, there are many other microbial strains such as *Lactobacillus lactis*, *Bifidobacterium*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus rhamnosus* are also find in some commercial yogurt cultures. However the dominated properties of cultures that given to the final product also vary with the microbial strains available in the culture. Acidity, flavor and

textural properties of the yogurt is greatly influence by the culture use in the yogurt manufacturing.

As ST and LB are the common strains found in many cultures, ST grows faster and produces both acid and carbon dioxide. The formate and carbon dioxide produced stimulates LB growth. On the other hand, the proteolytic activity of LB produces stimulatory peptides and amino acids for use by ST. These microorganisms are ultimately responsible for the formation of typical yogurt flavor and texture. The yogurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yogurt mix to approximately 5.0. the lactobacilli are responsible for a further decrease to pH 4.0.

Texture and flavor of the final yogurt is greatly effect by the chosen culture. A firm texture is obtained by aggregation of the casein particles in the milk when pH is lowered by the culture. Certain cultures produce exopolysaccharides (EPS) that contribute to the texture. The typical flavor of yogurt comes from a combination of lactic acid and different carbonyl compounds, such as acetaldehyde and diacetyl.

After the fermentation, cooling of the product below 5 °C reduce the metabolic activity of the starter culture. However, even at such a low temperature, acid development can occur during storage and retailing. This factor call as the “post acidification”. When it’s chose a culture, it’s important to consider about the post acidification factor of the culture, otherwise during the long storage it will give high acidity to the product and cause unpalatable to eat.

(chr.hansen culture specification,2002)

## **2.4 Manufacturing of Yogurt**

Yogurt manufacturing is consist of few steps and every step is very critical to obtain a good quality final product. Therefore the keeping of precision and hygiene throughout the process is very important. The general process of making yogurt includes modifying the composition and pasteurizing milk, fermenting at 43 – 45 °C temperature and cooling to a refrigerate temperature.

### **2.4.1 Milk standardization**

When the milk arrives at the plant, its composition is modified before it is used to make yogurt. This standardization process typically involves reducing the fat content and increasing the total solids. The fat content is reduced by using standardizing clarifier and a separator. However in general practices fat content and the solid non fat (SNF) content of the milk is adjusted by adding certain proportions of skimmed milk, raw milk and milk powder. For yogurt manufacture, the solid content of the milk is increased to 16 % with 1 - 5 % being fat and 11 - 14 % being solid non fat (SNF). Increasing the solids content improves the nutritional value of the yogurt, makes it easier to produce a firmer yogurt and improves the stability of the gel structure. (Robinson and Tamime, 1980)

### **2.4.2 Ingredients Blending**

After standardizing the milk content, other ingredients that are used in yogurt manufacturing should be add to the milk base. In there required amounts of sweeteners, stabilizers and colors are added to the milk base and mix well in the stainless steel mixing vats in high temperature for few minutes. (Robinson and Tamime, 1980)

### **2.4.3 Pasteurization**

After proper mixing milk should be pasteurized. This step has many benefits. First, it will destroy pathogenic bacteria in the milk that may interfere with the controlled fermentation process. Second it will denature the whey proteins in the milk which will give the final yogurt product better body and texture. Third it will not greatly alter the flavor of the milk. Finally, it helps release the compounds of the milk that will stimulate the growth of the starter culture.

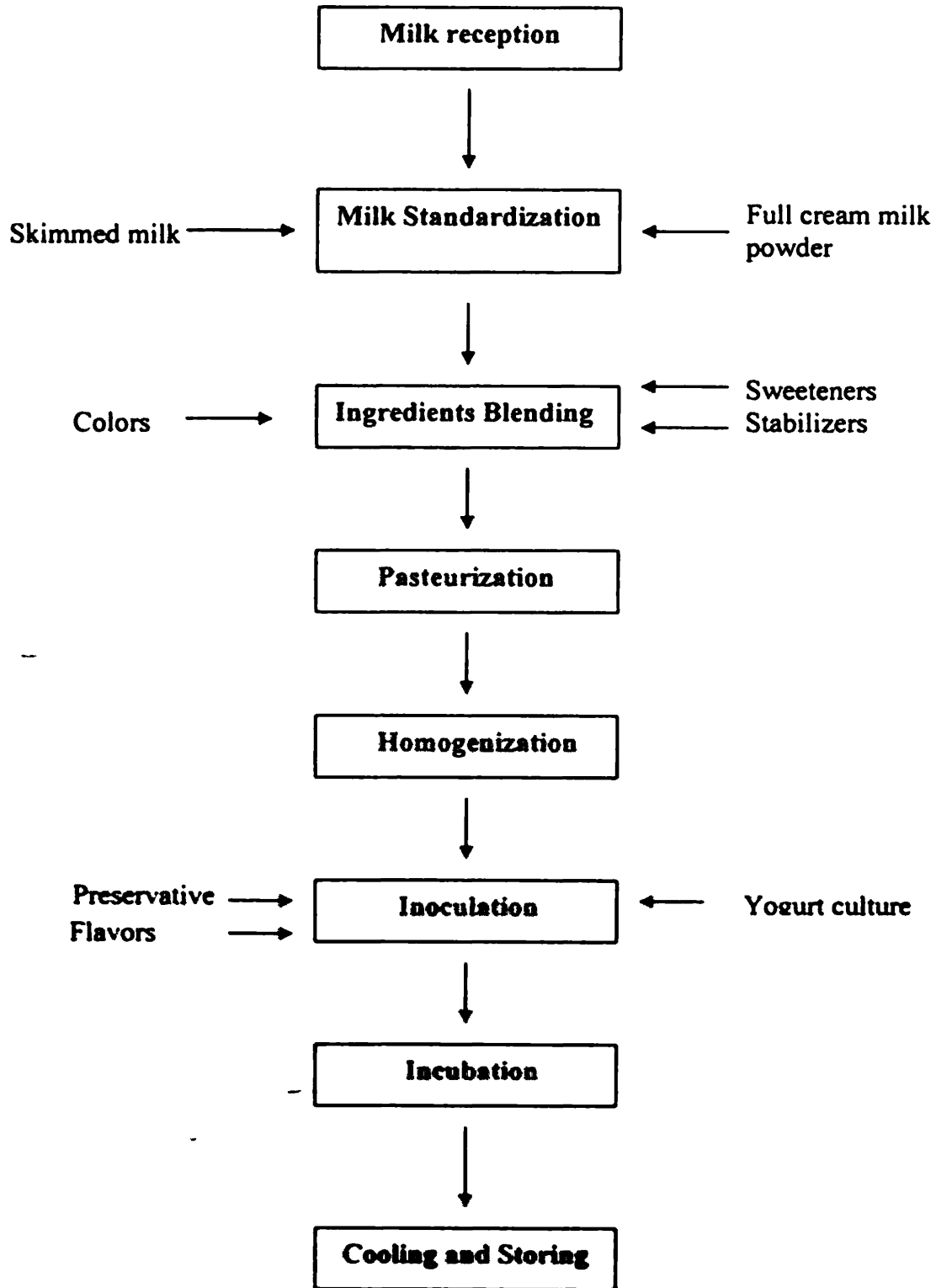
Usually the pasteurization in two methods, batch and continuous. Both of these processes involve heating the milk to a relatively high temperature and holding in there for a set amount of time. In batch processing milk is heated in a large stainless steel vat to about 85 - 90 °C and hold in there for 15 - 20 minutes. (Robinson and Tamime, 1980)

### **2.4.4 Homogenization**

After proper pasteurization mix will pass to the homogenizer for homogenization. Homogenization is a process in which the fat globules in milk are broken up in to smaller.



more consistently dispersed particles. This produce a much smoother and creamier end product. In commercial manufacturing, homogenization has the benefits of giving a uniform product, which will not separate. In a homogenizer, the milk is forced through small openings at a high pressure and fat globules are broken up due to shearing force.



**Figure 2.1 . Set yogurt Manufacturing process**

#### **2.4.5 Inoculation and Incubation**

After pasteurization and homogenization, the yogurt mix will transfer to the cooling vats. In there the mix is cool up to 43 – 45 °C and fill in to the inoculation vats. Inoculation of yogurt culture will be take place with in these inoculation vats and proper amount of yogurt culture and permitted preservatives are added to the yogurt mix. The amount and type of preservative and culture is determine by the manufacturer and keep it as the trade secrets. After proper mixing the yogurt milk is pumped in to the filling machine to fill in to the retail cups.

When the yogurt milk has been filled into the final packages, these must be incubated at the fermentation temperature of around 45 °C in an incubation chamber. It's very important that the packages are not handled in any way during fermentation as any vibration will disturb the curd formation. The temperature in the incubation chamber must be kept constant and the air circulation must be properly controlled. (Robinson and Tamime, 1980)

#### **2.4.6 Cooling and Storing**

With in the fermentation time, due to the lactic acid fermentation the pH of the yogurt mix will drop and casein micelles and whey proteins are coagulated to form a gel structure. After pH of the mix reach to around 4.6 to 4.8 and its complete the pre determined incubation time duration, the yogurt packages should be transfer to the cold room to cease the action of cultured microorganisms. Cooling of the products also involves special care, the products must be cooled as quickly as possible to stop fermentation, but wheying – off in the packages must also be avoided and this requires fairly slow, controlled cooling. Then the final products are store in the cold rooms, under the temperature of around 4 °C until dispatch from the factory. (Robinson and Tamime, 1980)

#### **2.5 Quality control in Yogurt Manufacturing**

The quality of the yogurt can be defined against a wide range of criteria such as the chemical, physical, microbiological, organoleptic and nutritional characteristics. However the quality is basically determine according to the flavor, aroma, viscosity, consistency, appearance, freedom from whey separation and long shelf life. However there are many processing factors that effect to the final product quality.

### **2.5.1 Choice of milk**

Milk intended for yogurt production must be of the highest bacteriological quality. It must have a low content of bacteria and substances which may impede the development of the yogurt culture. The milk must not contain antibiotics, bacteriophages, residues of CIP solution or sterilizing agents. (Texnotext, 1995)

### **2.5.2 Milk standardization**

The fat and dry solid contents of the milk should be standardized in to a standard level. This is achieve by adding skimmed milk, milk concentrates or dry milk powder. (Texnotext, 1995)

### **2.5.3 Milk additives**

Sugar or sweeteners and stabilizers should be add to the yogurt mix to obtain the required amount of flavor and textural properties to the final product. (Texnotext, 1995)

### **2.5.4 Deaeration**

The air content of the milk used to make culture milk products should be as low as possible. Some admixture of air is however unavoidable if the if the MSNF content is increased by addition of milk powder. If this is done, the milk should be deaerated as part of the subsequent processing. (Texnotext, 1995)

### **2.5.5 Homogenization**

The main motives for homogenizing milk intended for cultured milk production are to prevent creaming during the incubation period and to assure uniform distribution of the milk fat. Homogenization also improves the stability and consistency of cultured milks, even those with low fat content. (Texnotext, 1995)

### **2.5.6 Heat treatment**

The milk is heat treated before being inoculated with the starter in order to improve the properties of the milk as a substrate for the bacteria culture, to ensure that the coagulum of

the finished yogurt will be firm and to reduce the risk of whey separation in the end product.

Optimum results are achieved by heat treatment at 90 – 95 °C and a holding time of about 15 minutes. That temperature/ time combination denature about 70 – 80 % of the whey proteins. In particular the beta – lactoglobulin which is principal whey protein, interact with the kappa casein, thereby helping to give the yogurt a stable body. (Texnotext, 1995)

### 2.5.7 Choice and preparation of Culture

It's very important to select the correct culture with specific flavor and viscosity requirements. There are many concentrated, frozen and freeze- dried cultures are available in the market and it's very easy to use this cultures as because they can directly inoculated with milk, hence reduce the risk of contamination. The handling of the starter for production of yogurt demands maximum precision and hygiene. (Texnotext, 1995)

### 2.5.8 Plant design

The coagulum formed during fermentation is sensitive to mechanical treatment. This makes the selection and dimensioning of pipes, valves, pumps, coolers, etc. very important. (Texnotext, 1995)

### 2.6 Nutritional value of yogurt

Nutritional value of yogurt depends upon it is composition, raw materials used, ingredients added and the manufacturing process. This will have effects on carbohydrates, vitamins, proteins, fat and mineral matters. (Johnson and Alford, 1987).

<b>Composition</b>	<b>Non fat yogurt Per 100 g</b>	<b>Low fat yogurt Per 100 g</b>	<b>Whole milk yogurt Per 100 g</b>
<b>Energy</b>	<b>40 Kcal</b>	<b>91 Kcal</b>	<b>119 Kcal</b>
<b>Protein</b>	<b>4.5 g</b>	<b>5 g</b>	<b>5.5 g</b>
<b>Carbohydrates</b>	<b>5.5 g</b>	<b>16 g</b>	<b>18 g</b>
<b>Fat</b>	<b>0.1 g</b>	<b>1 g</b>	<b>3 g</b>
<b>Sodium</b>	<b>0.08 g</b>	<b>0.07 g</b>	<b>0.08 g</b>
<b>Riboflavin</b>	<b>0.23 mg</b>	<b>0.24 mg</b>	<b>0.24 mg</b>

Calcium	150 mg	180 mg	180 mg
Iron	< 1 mg	< 1 mg	< 1 mg
Magnesium	15 mg	18 mg	16 mg
Phosphorus	120 mg	150 mg	150 mg
Potassium	200 mg	230 mg	230 mg
Zinc	< 1 mg	< 1 mg	< 1mg

(Early, 1998)

**Table 2.2. Nutritional significance of standard yogurts**

## **2.6.1 Carbohydrates**

### **2.6.1.1 Available carbohydrates**

The expression “available carbohydrates” is intended to cover all those carbon compounds that can be assimilated by the human body and hence can act as a source of energy for metabolism. In the case of natural yogurt, a number of mono and disaccharides are present in the trace amounts, but lactose remains the dominant sugar in natural yogurt; even after fermentation, the product may contain some 4 -5 g of lactose, so that the lactose content of the end product is little different from normal milk. (Johnson and Alford, 1987)

### **2.6.1.2 Unavailable carbohydrates**

Although yogurt is based entirely on milk, yogurts usually have stabilizers incorporated to reduce whey separation during the distribution. The usage of these stabilizers has been considered in detail elsewhere but it is worth noting that many of them are complex carbohydrates. Thus guar gum, locust bean gum and cellulose derivatives are long chain polysaccharides composed of regular arrangements of monosaccharide units and it is significant, in the present context that the molecules cannot be attack by digestive enzymes in the human body. (Johnson and Alford, 1987)

## **2.6.2 Proteins**

The proteins in milk are in excellent quality in biologically and both the casein and whey proteins are well endowed with essential amino acids. Proteins in yogurt are totally digestible. The fact that the protein content of yogurt is often elevated by concentration or addition of skimmed milk solids, means that it is an even more attractive source of protein

than liquid milk. Consumption of around 200 – 250 ml of yogurt per day can easily provide an individual with the daily requirement of animal protein. (Johnson and Alford, 1987)

### **2.6.3 Lipids**

Although much of the yogurt sold in many countries are produced from skimmed milk, traditional yogurt has 3 – 4 g / 100 g of milk fat. Yogurt traditionally has been lower in fat than whole milk and this partly explains the perception that yogurt has as a lower fat dairy product. The influence of these lipid materials on the consistency and mouthfeel of yogurt has been discussed elsewhere, but it should not be forgotten that lipids are an integral part of a balanced diet. Thus, humans have a double requirement for lipids in that they possess;

- Storage fat composed of saturated fatty acids and serving as a source of energy or as a protection for vital organs.
- Structural fat which, with proteins, forms many of the essential membranes in animal cells, particularly in areas like the brain. (Johnson and Alford, 1987)

### **2.6.4 Vitamins and Minerals**

The relative availability of vitamins in yogurt is much more difficult to assess because, unlike minerals, many vitamins are sensitive to the conditions of processing. The fortification of yogurt with vitamins, such as vitamin A or C, is possible and losses over two weeks in storage are unlikely to exceed 50 %. Yogurt can act as a source of calcium for sufferers of lactose intolerance but, in addition calcium supplied by yogurt may be better absorbed and utilized than calcium made available in other forms. Yogurt contains appreciable quantities of Sodium and Potassium which may not be suitable for feeding babies less than 6 months.

(Tamime and Robinson, 1999)

## **2.7 Compositional and Microbiological standards of yogurt**

### **2.7.1 Compositional standards of yogurt**

The food standard committee report recommended that yogurt should have a minimum fat content of 3.0 % by weight that "partly skimmed" or "reduced fat" yogurt should have a fat content of between 1.0 % to 2.0 % by weight and that "skimmed milk yogurt" or non fat

yogurt should have maximum fat content of 0.3 % by weight. it was further recommended that all yogurt should have a minimum solids non fat (SNF) content of 8.5 % by weight.

(Tamime and Robinson, 1999)

Characteristic	Yogurt	Low fat yogurt	Non fat yogurt
Milk fat, percent by mass	3.0 min	0.5 to 3.0	Less than 0.5
Milk solids not fat, percent by mass, min	8.0	8.0	8.0
Titrateable acidity as lactic acid, percent by mass	0.8 to 1.25	0.8 to 1.25	0.8 to 1.25

**Table 2.3. Compositional requirements for Yogurt**  
(SLSI requirement for yogurt, 1989)

### 2.7.2 Microbiological standard of yogurt

Fermented milks, in general are of low pH value and high lactic acid concentration and are thus a highly selective environment favoring the growth of yeasts and moulds as spoilage microorganisms. For this reason sorbate and benzoate are permitted preservatives in some countries, but their effectiveness may be limited by the emergency of resistant strains. Secondary selective pressure is exerted by oxygen availability which restricts the development of moulds and non – fermentative yeasts, while the addition of added sugars favors the growth of fermentative yeasts. The solute level in some cases is sufficiently high to produce a small, but significant lowering of the water activity level. This is not sufficient to restrict the growth of yeast and moulds, although the behavior of starter microorganisms may be affected. The addition of humectants to lower the water activity level further has, however been investigated as a means of extending the storage life of yogurts.

(Sutherland and Verma, 1994)

Therefore Sri Lanka Standard Institute has specified the recommended microbiological limits for yogurt as follows;

<b>Test organism</b>	<b>Limit</b>
E. coli	Not more than 1 per g
Yeast	Not more than 1000 per g
Moulds	Not more than 1 per g

**Table 2.4. Microbiological limits**  
(SLSI specification for Yogurt, 1996)

## **2.8 Shelf life Evaluation of Yogurt**

Commercially the shelf life of a product may be defined as the number of days after production that the product can be consumed whilst still remaining safe, retaining its quality appeal, and meeting customer expectations. In other words, it should remain microbiologically safe and organoleptically acceptable within its stated shelf life.

To evaluate the shelf life of any product, it's first necessary to identify which characteristics of the ingredients, the process and the storage conditions are responsible for or will have an influence on its shelf life.

The life of the yogurt may be influenced by ;

1. Raw material
2. Production formulation
3. Processing parameters
4. Implementation of GMP
5. Filling and Packaging
6. Storage and Distribution
7. Consumer usage and Handling

Measurement , monitoring and control of the above are of paramount important in the evaluation process. The point at which the product become unacceptable and either organoleptically, chemically or microbiologically, which ever manifests it shelf first, is the ultimate end point which the shelf life can be established. (Dave and Lewis, 1994)



## **2.9 Analyzing of Yogurt**

Milk products such as yogurt are subject to variety of safety testing. Some of these includes tests for microbial quality, degree of pasteurization and various forms of contaminants. In addition to safety tests, the final yogurt is also evaluated to ensure that it meets the specifications set by the manufacturer for characteristics such as pH, rheology, acidity, color, taste and odor. These factors are tested using various laboratory equipment such as pH meters and viscometers and also human panelists.

### **2.9.1 Organoleptic tests**

There are no such a instrument to analyze the organoleptic properties of any food or beverage. Human sensors are the one and only devices that can be use to mesure the sensory properties. There are many sensory properties that check in the organoleptic tests, such as color, smell, taste, acidity or alkalinity, texture, appearance and overall acceptability. These properties are measure via five human sensors; nose, tongue, eyes, ears and skin. However observations gathered through this sensors are very important and essential in quality control in dairy products.

Taste is perceived in mouth, Particularly on the tongue and aroma indirectly by the olfactory organ, during tasting or directly through the nose; flavor is a combination mainly of olfactory- gustatory sensations. Yogurt must have viscous, firm and be coherent enough to be spooned body; smooth, clot less and without fissures texture; and typical acid flavor and taste.

(Panagiotidis and Tzia, 2001)

### **2.9.2 Chemical tests**

#### **2.9.2.1 pH**

pH may be defined as the negative logarithm of the hydrogen ion concentration. The measurement pH is finding increasing use in the dairy industry since it provides in many cases a more meaningful measurement than titratable acidity. Acidity is due to hydrogen ions ( $H^+$ ), alkalinity is due to hydroxyl ions ( $OH^-$ ), and a scale which measures their relative concentration is therefore an index of the intensity of acidity or alkalinity. pH values ranging from 0 – 7 are acid while those ranging from 7 – 14 are alkaline.

pH meter is a device capable of measuring small differences in voltage developed between two electrodes immersed in the sample. The electrical changes are converted in to direct pH readings. The pH meter is standardized by the use of standard buffer solutions which should be carefully handled to prevent contamination. Old solutions or solutions obviously defective, should be discarded. (Herrinton, 2000)

### 2.9.2.2 Acidity

Titrateable acidity and pH measure different things. The titrateable acidity is a measure of the quantity of acid present where the pH is a (numerically inverse) measure of the intensity of that acidity. For example equal quantities of 0.1 N hydrochloric and acetic acids would require equal quantities of 0.1 N sodium hydroxide for neutralization, but would have pH values of 1 and 2.9 respectively, so that the hydrochloric is the more intense acid(more dissociated).

The quantity of acid in milk product is measured by the amount of alkali needed to neutralize it. In order to determine when sufficient alkali has been added, phenolphthalein is used as an indicator. It turns pink when the solution becomes slightly alkaline. By this procedure it can determine how many equivalents of acid are present, but cannot tell what the nature of the acid may be. Therefore the acidity of the milk and milk products usually recorded as the per cent of lactic acid, although there is no evidence at all that the acidity is due to lactic acid.

The acidity due to the casein, phosphate and other milk constituents call as the apparent or the original acidity. The acidity develop due to the activity of microorganisms call as true or real acidity. It's indicate that it's actually due to the lactic acid, and to distinguish it from the apparent acidity due to the normal constitutes of the milk. However in measuring titrateable acidity it is measure the apparent acidity due to the original constitutes of the milk and also real acidity due to lactic acid.

To calculate the percentage of titrateable acidity, move the decimal point one place left to the original titration value. For example, if 7.0 ml of 0.1 N NaOH is used, the titrateable acidity is 0.70 %. However this is true only when 9 mg of sample and exactly 0.1 N NaOH are used. If the volume of the sample and strength of NaOH used are not the same as above, calculate acidity as follows,

$$\text{Titrateable acidity} = \frac{\text{NaOH concentration} * \text{NaOH volume} * 90 / 1000}{\text{Volume of the sample}} * 100$$

(Herrinton, 2000)

### 2.9.3 Microbiological tests

Pour plate method is a common method that is use in direct enumeration of microorganisms in food products. In this method a set of Petri dishes is inoculated with 1 ml aliquots from appropriate dilutions of the food. Some 10 – 15 ml of the molted nutrient agar or other suitable medium, cooled to 45 °C is then added to each of the Petri dishes and mixed carefully with it's aliquot. After the agar has solidified, the plates are incubated at required temperature for a period of time depending upon the incubation conditions. After incubation, plates containing 30 – 300 colonies should be counted from which the number of viable cells per gram of food ca be readily calculated. (Forsythe and Hayes, 1998)

#### 2.9.3.1 Coliform test

The group of coliform microorganisms includes the genera *Escherichia* and *Aerobacter*. The widespread distribution of Coliform in nature, feeding it shelf on available food its habitat, permits a minimal occurrence of these microorganisms in milk, milk products, surface of dairy equipment , air and water supply. A large number of these bacteria indicate poor hygiene and negligence. Proper heat treatment is sufficient to destroy the Coliform bacteria, and its presence in pasteurized milk and milk products serves as index of recontamination or post pasteurization contamination, largely due to poor hygienic practices.

The Coliform bacteria is a gram negative rod, and it is ability to produce acid and gas as products of lactose fermentation is the prime basis of laboratory tests for detecting it's presence in a sample. The medium used contain a bile salt which prevents the growth of the gram – positive organisms.

In colony counting method, the number of Coliform colonies present in the milk, milk products, water, etc can be estimated by feeding these bacteria with media containing a bile salt and lactose as part of the ingredients. This determination is carried out in the Coliform colony count. One milliliter of the diluted sample is inoculated in a Petri dish and grown on violet red bile agar (VRBA), medium which contains lactose, bile salt, peptone, yeast extract, sodium chloride, neutral red, crystal violet agar and water. The Coliform colonies appear purplish red with a zone of precipitated bile adjacent to the colony and colonies having a diameter of 0.5 mm or bigger are counted. The estimated count is reported as “number of Coliform colonies per milliliter”. (Resubal, 1990)

### **2.9.3.2 Yeast and Mould counting test**

Yeast are unicellular, oval to elliptical cell, Gram – positive, non- motile, large in comparison with bacteria and commonly reproduce by budding, appearing like a cactus plant under the microscope.

Moulds are multicellular compose of aggregate branching protoplasmic thread mycelium, producing sexual and asexual spores. Mold colonies appear cottony or wooly, white, cream, green, black or brownish due to pigmentation.

In the dairy industry, the presence of yeast and molds in some dairy products is an index of processing plant sanitation and quality of raw materials. The number of yeast and molds in a measured amount of dairy product can be estimated by feeding these organisms with a medium containing potato infusion, dextrose, agar and water. The acidity is adjusted to pH 3.5 with tartaric acid. At this pH, growth is favorable for the yeast and moulds, but inhibits bacterial growth. (Resubal, 1990)

### **2.10 Sensory evaluation of yogurt**

Consumer sensory evaluation is usually performed towards the end of the product development or reformulation cycle. In foods and consumer products , there are two main approaches to consumer sensory testing, the measurement of preference and the measurement of acceptance. In preference measurement, the consumer panelists has a choice. One product is to be chosen over one or more other products. Preference measurements can be performed directly or indirectly. In the measurement of acceptance

or liking, the consumer panelists rate their liking for the product on a scale. Acceptance measurements can be done on single products and do not require a comparison to other product. Frequently, the most efficient procedure is to determine consumers acceptance scores in a multi product test and then to determine their preferences indirectly from the scores. (Peyram, 1999).

### **2.10.1 Hedonic scaling**

The most common hedonic scale is the 9 point hedonic scale, also known as a degree of liking scale. The 9 point scale is very simple to use and is easy to implement. It has been widely studied and has been shown to be useful in the hedonic assessment of foods, beverages and non food products. However hedonic rating can be affected by changes in the environmental conditions, but the relative order of sample preference was usually not effected. The absolute magnitude of the hedonic score may increase or decrease, but all samples had similar relative changes. (Peyram, 1999).

### **2.10.2 Statistical Aspects of Sensory Evaluation**

In planning sensory experiments, experimental design is of paramount important, because it is need to control or minimize the potential sources of variability associated with the preparation of the test product, measurements and assessment process, including factors such as order effect, carry over effect and assessors fatigue. Choosing a statistical method and statistical package for analyzing data of a sensory evaluation is not an easy task, as there are so many available, but needs to critically determine before embarking of the sensory test.

The hedonic scales used for the collection of consumer liking data are usually ordinal scales with category descriptions of the form, "like extremely " to "dislike extremely". as a general rule of thumb, data collected from a trained sensory panel can be analyzed using parametric methods. conversely, for the analysis of consumer data, it has normally recommended from a statistical point of view that non – parametric methods be used. In practice, where large number of consumers are used to provide the data, parametric analysis of variance is often used. ( Peyram, 1999)

## **CHAPTER 03**

### **MATERIALS AND METHODOLOGY**

#### **3.1 Quality analyzing of existing set yogurt**

The quality variation of the existing set yogurt was measured over the one month of period in daily basis. In there organoleptic, chemical and microbiological variations were measured. Smell, taste and color were measured by organoleptically, pH and acidity were measured through chemical analysis and Direct plate count method was used as the microbiological technique to detect the Coliform, Yeast and Mould count variation of the existing yogurt.

##### **3.1.1 Organoleptic analysis**

Smell, taste and color of the existing “Kotmale” set yogurt was checked by using semi trained five panelists, through out the one month of period in daily basis.

###### **3.1.1.1 Materials**

Existing “kotmale” set yogurt samples

Semi trained sensory panelists

Spoons

###### **3.1.1.2 Method**

A box of yogurt, which containing 36 cups was taken from the production line and kept in the cold room. A one cup was taken out from that box in each day and measured the organoleptic properties by using semi trained sensory panelists and decisions were recorded, over the period of one month.

##### **3.1.2 Chemical analysis**

Under the chemical analysis, pH and acidity variation of the existing yogurt were measured, each day over the period of month.

### **3.1.2.1 Measuring of pH**

pH variation of the existing yogurt was read out from the digital pH meter everyday, over the period of one month.

#### **3.1.2.1.1 Materials**

Existing “Kotmale” set yogurt samples

Digital pH meter (+ / - 0.01)

Buffer solution of pH 4.01

Buffer solution of pH 7.00

Distilled water

Tissue papers

#### **3.1.2.1.2 Method**

Calibrated pH meter was used to measure the pH of the yogurt. A yogurt cup was taken from the stored yogurt box and shaken well before open the lid to break the yogurt structure inside. Then the lid of the yogurt cup was opened and probe of the pH meter was immersed in to the yogurt gel and shake gently. The probe was kept immersed in the yogurt for few minutes until it reached to a definite value. Then the final value was read out and recorded.

### **3.1.2.2 Measuring of acidity**

Acidity is an important parameter of the yogurt quality, hence it was measured by titration method as the percentage of lactic acid concentration.

#### **3.1.2.2.1 Materials**

Existing “Kotmale” set yogurt sample

Pipette - 10 ml

Erlenmeyer flask - 250 ml

Burette - 25 ml

0.1 N NaOH solution

1 % Phenolphthalein indicator

Funnel

Spoon

### 3.1.2.2.2 Method

Yogurt sample was taken out and agitated thoroughly to break the curd structure. Then top cover of the yogurt cup was opened and mixed further by using a spoon to break the curd properly. 9 ml of yogurt mix was pipetted out in to Erlenmeyer flask by using 10 ml pipette. Five drops of the 1 % phenolphthalein indicator were added to the flask and mixed well. Then the mixer was titrated with 0.1 N NaOH , while shaking the flask constantly, until a definite pink color lasting 30 seconds is attained. Number of milliliters of alkali solution that was required in the titration was read out and recorded. Repeated the titration for another two samples from the same cup and recorded the results. Took the average of the results and calculated the acidity of the yogurt by using following formulae;

$$\text{Titrateable acidity} = \frac{\text{NaOH concentration} * \text{NaOH volume} * 90 / 1000}{\text{Volume of the sample}} * 100$$

### 3.1.3 Microbiological analysis

Direct plate count was the method that was used to count the microbial quality of the yogurt. Coliform and yeast and mould were counted by using that method. Before each microbial examination, dilutions of the samples were prepared by using dilution technique.

#### 3.1.3.1 Coliform test

Coliform count of the yogurt was counted by using pour plate technique. In there 1 ml of the sample was inoculated in to the nutrient agar medium and incubated for microbial growth and counted the appeared colonies by using colony illuminator.

##### 3.1.3.1.1 Materials

Yogurt sample

1 ml - Micro pipette



1 ml – Plastic tips  
9ml – Ringer bottles  
Sprit lamp  
Violet Red Bile Agar (VRBA) medium (pH 7.4 at 25 °C)  
Distilled water  
Culture bottles  
Aluminium pan  
Alcohol solution

### **3.1.3.1.2 VRBA medium preparation**

38.5 grams of the VRBA culture powder was suspended in 1 liter of distilled water in the culture bottle. Then the cap of the culture bottle was tightly closed and shaken well to dissolve well. Then it was placed in the water bath for 15 minutes while regular shaking to dissolve the media completely. Then after it was took out from the water bath and allowed to cool down to 45 °C and mixed well before pouring.

### **3.1.3.1.3 Method**

The inoculation was taken place in sterilized inoculation booth, between the two lighted sprit lamps to avoid the cross contamination from the surrounding environment. 1 ml of the sample was extracted to the micropipette and inserted to the sterilized 9ml ringer solution bottle and shake well to prepare the  $10^{-1}$  dilution of the sample. Then, 1 ml from the  $10^{-1}$  dilution was took in to the micropipette and put in to the another 9ml ringer solution bottle and shake well to prepare the  $10^{-2}$  dilution of the sample.

Three sterilized Petri dishes were inoculated with 1 ml per dish of each dilution. Another sterilized Petri dish also inoculated with 1 ml of sterilized ringer's solution as a "control". About 15 milliliters of the molten Violet Red Bile Agar was poured in to each inoculated dishes and mixed well. Inoculated dishes were inverted and incubated at 37 °C for 24 hours. After that period colonies were counted by using colony illuminator and took the average count of all the Coliform colonies on the dishes counted, multiply with the dilution inoculated and reported as "number of Coliforms per mililiter of the sample" by using following formulae;

$$N = \text{sum } C / V * n * d$$

Where,

N = sum of the colonies counted on the two dishes

v = volume of inoculum applied to each dish, in milliliters

d = dilution factor of the first dilution inoculated

n = number of dishes retained

### **3.1.3.2 Yeast and Mould test**

Yeast and mould count of the yogurt was counted by using pour plate technique by using potato dextrose agar as the medium.

#### **3.1.3.2.1 Materials**

Yogurt sample

1 ml - Micro pipette

1 ml – Plastic tips

9ml – Ringer bottles

Spirit lamp

– Potato Dextrose Agar (PDA) medium (pH 5.6 at 25 °C)

Distilled water

Culture bottles

Auto claver

Alcohol solution

#### **3.1.3.2.2 PDA medium preparation**

39 grams of the PDA powder was suspended in 1 liter of distilled water in culture bottle and tightly closed the lid and shaken well to dissolve completely. Then it was autoclaved at 121 °C for 15 minutes and allowed to cool to the room temperature and mixed well before pouring.

### 3.1.3.2.3 Method

The inoculation was taken place in sterilized inoculation booth, between the two lighted sprit lamps to avoid the cross contamination from the surrounding environment. 1 ml of the sample was extracted to the micropipette and inserted to the sterilized 9ml ringer solution bottle and shake well to prepare the  $10^{-1}$  dilution of the sample. Then, 1 ml from the  $10^{-1}$  dilution was took in to the micropipette and put in to the another 9ml ringer solution bottle and shake well to prepare the  $10^{-2}$  dilution of the sample.

Three sterilized Petri dishes were inoculated with 1 ml per dish of each dilution. Another sterilized Petri dish also inoculated with 1 ml of sterilized ringer's solution as a "control". About 15 milliliters of the molten Potato Dextrose Agar was poured in to each inoculated dishes and mixed well. Inoculated dishes were inverted and incubated at  $25^{\circ}\text{C}$  for 3 to 5 days. After that period colonies were counted by using colony illuminator and took the average count of all the yeast and mould colonies on the dishes counted, multiply with the dilution inoculated and reported as "number of yeast and mould per milliliter of the sample" by using following formulae;

$$N = \text{sum } C / V * n * d$$

Where,

N = sum of the colonies counted on the two dishes

v = volume of inoculum applied to each dish, in milliliters

d = dilution factor of the first dilution inoculated

n = number of dishes retained

### 3.2 Problem identification and seeking solutions

All the information gathered through the chemical, microbiological and organoleptic tests were thoroughly investigated. Main quality problems were identified and solutions were identified and selected to improve the quality of the yogurt. Fairly high pH and acidity were the main quality defects that was found in the existing yogurt and high acidic condition provide a favorable condition for yeast and mould growth. Therefore it was seek the solutions for control the acidity of the yogurt in an acceptable level with in the storage period.

### **3.3 Development of improved yogurt**

The yogurt culture has a great influence to the acidity of the yogurt. Therefore to avoid the high acidity problem of the existing yogurt, a new culture was identified with low post aciditification and developed a new yogurt with existing formulae.

#### **3.3.1 Raw materials**

- Fresh milk
- Skimmed milk
- Full cream milk powder
- Yogurt culture
- Refined sugar
- Gelatin
- Permitted colors
- Permitted flavors
- Permitted Preservatives
- Yogurt cups with lids

#### **3.3.2 Procedure**

All the ingredients that was determined according to the recipe was weighted out and weighed amount of skimmed milk and fresh milk was put in to a stainless steel pan and boiled on the water bath, while continuous mixing with the spatula. The temperature of the milk was measured by using a mercury thermometer and at about 60 °C of temperature, the weighted amounts of sugar, gelatin, full cream milk powder and colors were added to the yogurt mix and heating was continue until the temperature of the mix reached to the 90 °C. Then the temperature of the mix was maintained at 90 °C for 15 minutes, while continuous stirring. Then after 15 minutes, the pan was taken out from the heating and allowed to cool to 43 °C, inoculated with new yogurt culture and preservative and Flavors were added to it and mixed thoroughly for few minutes.

Then the yogurt mix was filled in to the yogurt cups and sealed with the lids immediately. Sealed yogurt cups were stacked in a corrugated box and placed in an incubator, under the temperature of 43 – 45 °C for fermentation. The coagulation of milk was monitored by pH

during incubation until pH 4.80 was attained. Finally yogurt box was transferred to cold room to cease the fermentation process and kept their for one month while monitoring chemical, biological and organoleptic variation of the yogurt.

### **3.4 Measuring the quality variation of the improved yogurt**

All the quality tests, that were used to measure the quality variation of existing yogurt was carried out to measure the quality variation of the improved yogurt, in 3<sup>rd</sup> , 14<sup>th</sup> , 22<sup>nd</sup> and 28<sup>th</sup> days after production. pH and acidity was measured as chemical tests and Coliform, Yeast and Mould count of the yogurt were counted by using pour plate technique as microbiological tests. Organoleptic properties of the improved yogurt was analyzed by doing sensory evaluation tests, in 3<sup>rd</sup> and 28<sup>th</sup> days after production.

### **3.5 Sensory analysis**

Two sensory analysis were carried out in 3<sup>rd</sup> and 28<sup>th</sup> days after production for the improved yogurt to check the consumer acceptability by using untrained sensory panel. Gathered data were analyzed by using Friedman test in the MINITAB 14 computerized statistical application.

#### **3.5.1 Materials**

Existing "kotmale" set yogurt samples

Improved set yogurt samples

Market sample of set yogurt

Ballot papers

Untrained human panelists

Pens

Spoons

PERMANENT REFERENCE  
Sabaragamuwa University Library

#### **3.5.2 Method**

Existing "Kotmale" set yogurt samples, improved yogurt samples and one of the market yogurt sample, which has a high consumer demand were subjected to sensory evaluation. There were two sensory evaluations were done to the improved yogurt, in 3<sup>rd</sup> day after manufacturing to analyze the consumer acceptance to the improved yogurt and in 28<sup>th</sup> day

after manufacturing to check the keeping quality of the improved yogurt. The sensory evaluations were done by untrained sensory panelists with use of ballot papers. Each panelist was apart invisible to each other. Three yogurt samples were presented in three identical containers to panelists. The sample containers were coded with 3 digit random numbers and the order of the presenting the samples were also changed to maintain the proper randomness with in the analysis.

The code numbers of the sensory analysis after 3 days of production were;

751 = existing "kotmale" yogurt

517 = improved "Kotmale" yogurt

648 = Market sample

The code numbers of the sensory analysis after 28 days of production were;

751 = Market sample

517 = improved "Kotmale" yogurt

648 = existing "Kotmale" yogurt

Three samples were placed in the sensory booth, before the panelists alone with a ballot paper. Level of preference for each sensory attribute (color, smell, appearance, texture, taste, sourness and overall acceptability) in all three samples were recorded according to the 9 – point hedonic scale. The obtained results were analyzed by Minitab statistical analyzing package with the Friedman test at 5 % significance level.

## CHAPTER 04 RESULTS AND DISCUSSIONS

### 4.1 Organoleptic changers during storage of existing “Kotmale” set Yogurt

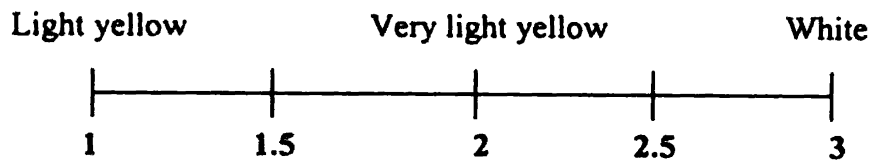
Color, smell and taste of the existing set yogurt were measured by the semi trained sensory panelists and data gathered was recorded according to a scale as follows;

#### 4.1.1 Variation of Color

Typical yogurt has a clear whitish color without adding artificial colors, but in commercial manufacturing artificial colors are added to maintain the color of the yogurt along the shelflife. Color of the existing yogurt changed as follows along with the storage;

Days after production	Color of the yogurt
1	1
2	1
3	1
4	1
5	1
6	1
7	1
8	1
9	1
10	1
11	1
12	1
13	1
14	1
15	1
16	1
17	1
18	1
19	1

20	1
21	1
22	1
23	1
24	1
25	1
26	1
27	1
28	1
29	1
30	1



**Table 4.1 Color variation of the existing yogurt over the period of one month**

In the production of set yogurt; artificial, permitted color was added to the yogurt to enhance the color of the yogurt. During the Storage, color of the yogurt remained unchanged. Usually temperature is the main factor that effect to the degradation of chemical composition of food colors. However the tested yogurts were stored in cold room, under the temperature of 4 ° C. According to the results obtained above, there was no change of color over the period of one month. Therefore it can conclude that, at that temperature chemical composition of the color compounds were not affected by storage temperature, hence color of the yogurt remained unchanged.

#### 4.1.2 Variation of Smell

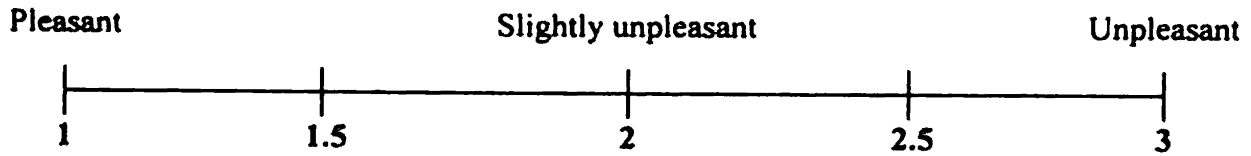
Usually typical yogurt has a very pleasant smell. However during the storage, the smell of the yogurt can be changed in to unpleasant smell, it may be because of chemical changers of milk fat and proteins, driven by microbial actions. If yogurt is attacked by the microorganisms such as yeast, mould and other destructive bacteria, they may contribute to the proteolytic and lipolytic activity which impair the smell of the yogurt. Due to this



chemical and microbiological changes of the yogurt compounds it gives the unpleasant smell to the yogurt with the storage. However the smell variation of the existing yogurt can be recorded according to a scale as follows;

<b>Days after production</b>	<b>Smell variation</b>
1	1
2	1
3	1
4	1
5	1
6	1
7	1
8	1
9	1
10	1.5
11	1.5
12	1.5
13	2
14	2
15	2
16	2
17	2
18	2
19	2
20	2
21	2.5
22	2.5
23	2.5
24	2.5
25	2.5
26	2.5
27	3

28	3
29	3
30	3

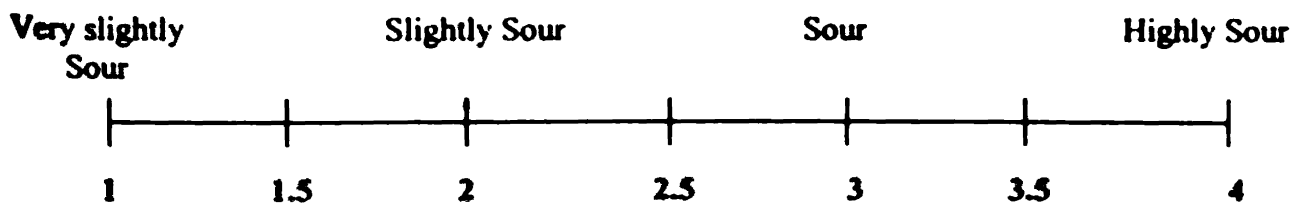


**Table 4.2 Smell variation of the existing yogurt over the period of one month**

After 1 to 9 days of storage pleasant smell of the yogurt remain unchanged. Then after 13 to 26 days of storage there was a slightly unpleasant smell. However after 27 days of storage smell of the yogurt was convert in to very unpleasant level. This variation may be due to slow growth of starter organisms during storage or growth of yeast and mould in the yogurt. Due to their actions on the yogurt, lipolytic and proteolytic changers can be occurred to the yogurt composition and due to end products of that actions many undesirable amino acids and fatty acids can be formed, that impaired the smell of the yogurt.

#### 4.1.3 Variation of Taste

The typical flavor of the yogurt comes from the combination of lactic acid and different carbonyl compounds, such as acetaldehyde and diacetyl. These combination responsible for the typical slightly sour taste of the yogurt. However with the storage, the taste of the yogurt can be changed. The variation of the taste of the existing yogurt can be recorded according to a scale as follows;



<b>Days after production</b>	<b>Taste</b>
1	1
2	1
3	1
4	1.5
5	1.5
6	1.5
7	1.5
8	2
9	2
10	2
11	2
12	2
13	2
14	2
15	2.5
16	2.5
17	2.5
18	2.5
19	2.5
20	3
21	3
22	3
23	3
24	3.5
25	3.5
26	3.5
27	4
28	4
29	4
30	4

**Table 4.3 Taste variation of the existing yogurt over the period of one month**

First three days after the production, there was a characteristic very slightly sour taste of the yogurt, however after 14 days of production, it was gradually changed in to quit unacceptable sour taste and then after 19 days it gave sour taste and finally after 27 day it gave highly unacceptable sour taste. This variation may be due to the slow growth of starter microorganisms and growth of yeast and mould in the yogurt. Due to their actions, proteolytic and lipolytic activities can be initiated in the yogurt which forms many undesirable compounds which impaired the typical taste of the yogurt.

#### 4.2 Chemical changers during the storage of existing “Kotmale” set yogurt

pH and Acidity was the basic chemical parameters that was concerned about the yogurt, during the storage of one month.

##### 4.2.1 Variation of pH

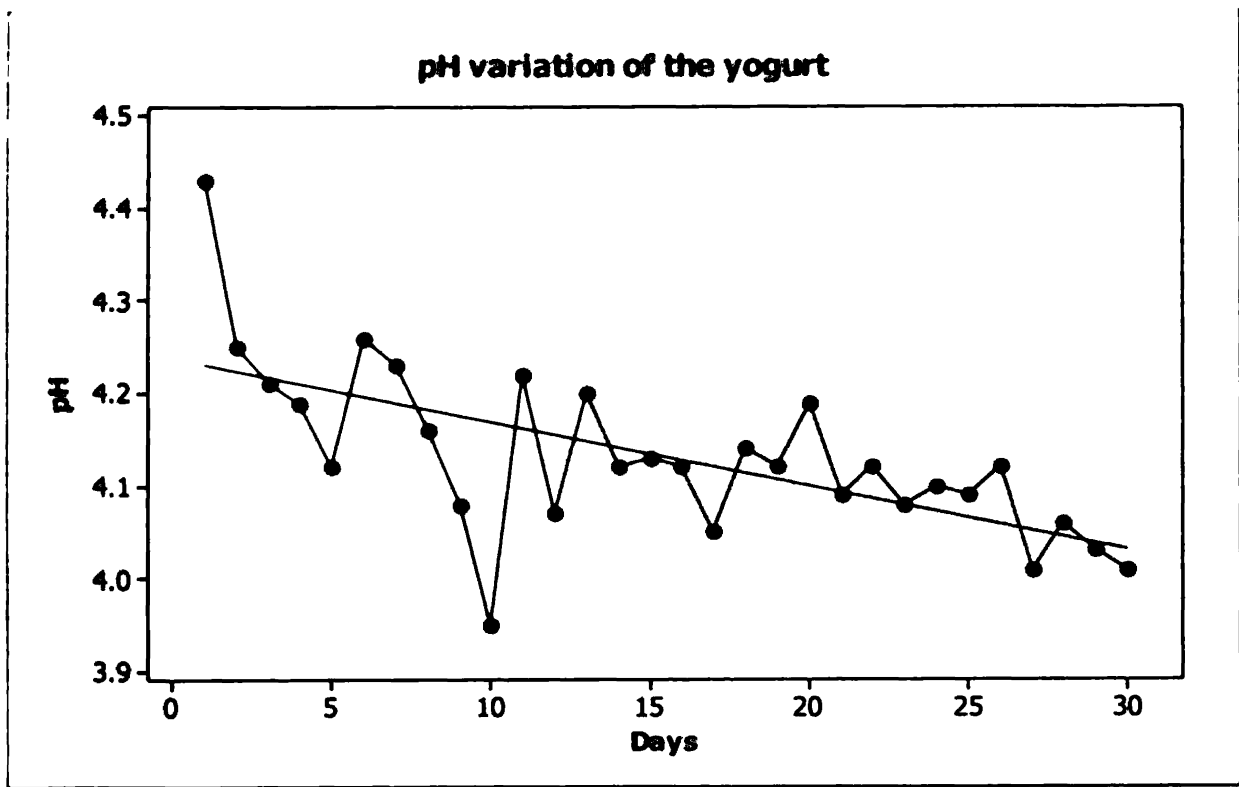
Usually fermentation process of the yogurt making was broken at the pH about 4.8 by cooling them in to the refrigerated temperature. Therefore the initial pH of the yogurt should be around 4.5 to 4.8 . However with the storage pH of a yogurt can be decrease. The pH variation of the existing yogurt can be listed as follows;

Days after production	pH
1	4.43
2	4.25
3	4.21
4	4.19
5	4.12
6	4.26
7	4.23
8	4.16
9	4.08
10	3.95
11	4.22
12	4.07
13	4.20

14	4.12
15	4.13
16	4.12
17	4.05
18	4.14
19	4.12
20	4.19
21	4.09
22	4.12
23	4.08
24	4.10
25	4.09
26	4.12
27	4.01
28	4.06
29	4.03
30	4.01

**Table 4.4 pH variation of the existing yogurt over the period of one month**

pH of the existing yogurt was gradually decrease from 4.43 to 4.01 within one month of period, with slight variations. However in 10<sup>th</sup> day after production there was a outstanding variation of the pH of the yogurt. This may be due to the instrumental error or due to the cup to cup variation. Other than that there was a gradual decrease of the pH. This decrease may be due to the slow growth of starter organisms and growth of yeast and mould in the yogurt. This organisms ferment the remaining lactose in the yogurt in to lactic acid at slower rate and it caused to reduce the pH of the yogurt with the time. pH variation of the yogurt over the period of one month can be graphically illustrate as follows;



**Figure 4.1** pH variation of existing "Kotmale" set yogurt over the period of one month

**4.2.2 Variation of Acidity**

Acidity of the yogurt was measured by titration with alkali solution and expressed as the percentage of lactic acid in yogurt. Acidity variation of the yogurt can be listed as follows:

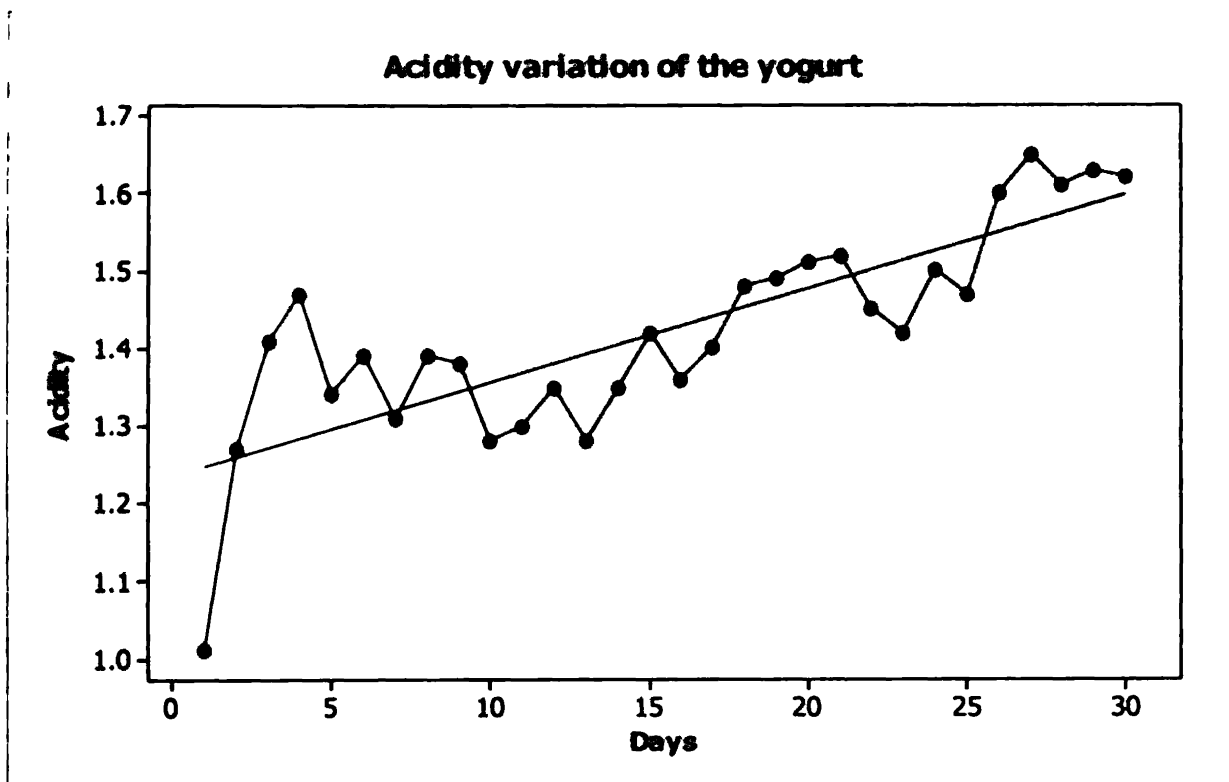
Days after production	Acidity
1	1.01
2	1.27
3	1.41
4	1.47
5	1.34
6	1.39
7	1.31
8	1.39
9	1.38
10	1.28
11	1.30

12	1.35
13	1.28
14	1.35
15	1.42
16	1.36
17	1.40
18	1.48
19	1.49
20	1.51
21	1.52
22	1.45
23	1.42
24	1.50
25	1.47
26	1.60
27	1.65
28	1.61
29	1.63
30	1.62

**Table 4.5 Acidity variation of the existing yogurt over the period of one month**

According to the Sri Lanka Standard Institution's specification for yogurt acidity of a yogurt should be vary between 0.8 to 1.25 . However according to the above data, acidity of the existing yogurt was exceed that limit with in few days of storage. Initial acidity of the yogurt was 1.01 and it changed up to 1.62 after 30 days. Therefore it was a significant variation the acidity of the yogurt. This high acidity may cause to high sourness of the yogurt and also cause to reduce the consumer preference. This high acidity variation may be due to high post acidification of culture, too high inoculation rate or too high storage temperature. Therefore to control the acidity of the yogurt one or more of these factors should be controlled.

**Acidity variation of the existing yogurt can be graphically illustrate as follows;**



**Figure 4.2 Acidity variation of the existing “Kotmale” set yogurt over the period of one month**

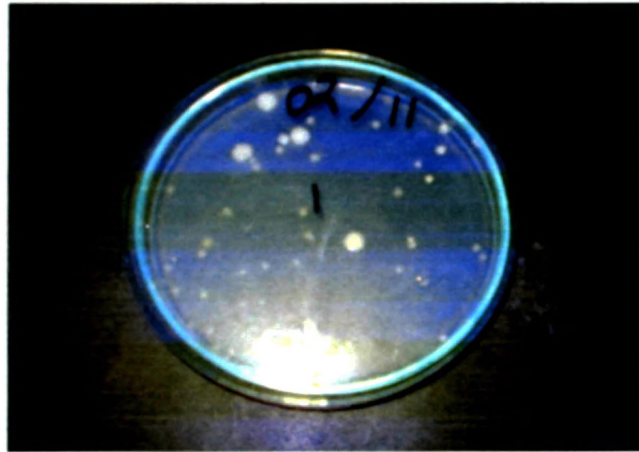
### **4.3 Microbiological changers during the storage of existing “Kotmale” set yogurt**

Coliform, yeast and mould count of the existing yogurt was measured by using pour plate count technique. Microbial count of the product was expressed as “number of microorganisms per one milliliter of the product”.

#### **4.3.1 Variation of the Yeast and Mould count**

Yeast and mould count of the yogurt was counted by inoculating them in PDA medium, allowed to grow and counted by using colony counter. Yeast and mould colonies were appeared in white color, glossy dots, on the surface of the medium. However in this method, it cannot differentiate yeast from the mould. But if the plates are keep at 25 °C for more than 5 days, moulds are form a mycelium and appear as a wool structure; by that it can assume that there is a mould growth in the medium.





**Figure 4.3 Petri dish with Yeast and Mould colonies**

Yeast and mould count variation of the existing yogurt can be listed as follows ;

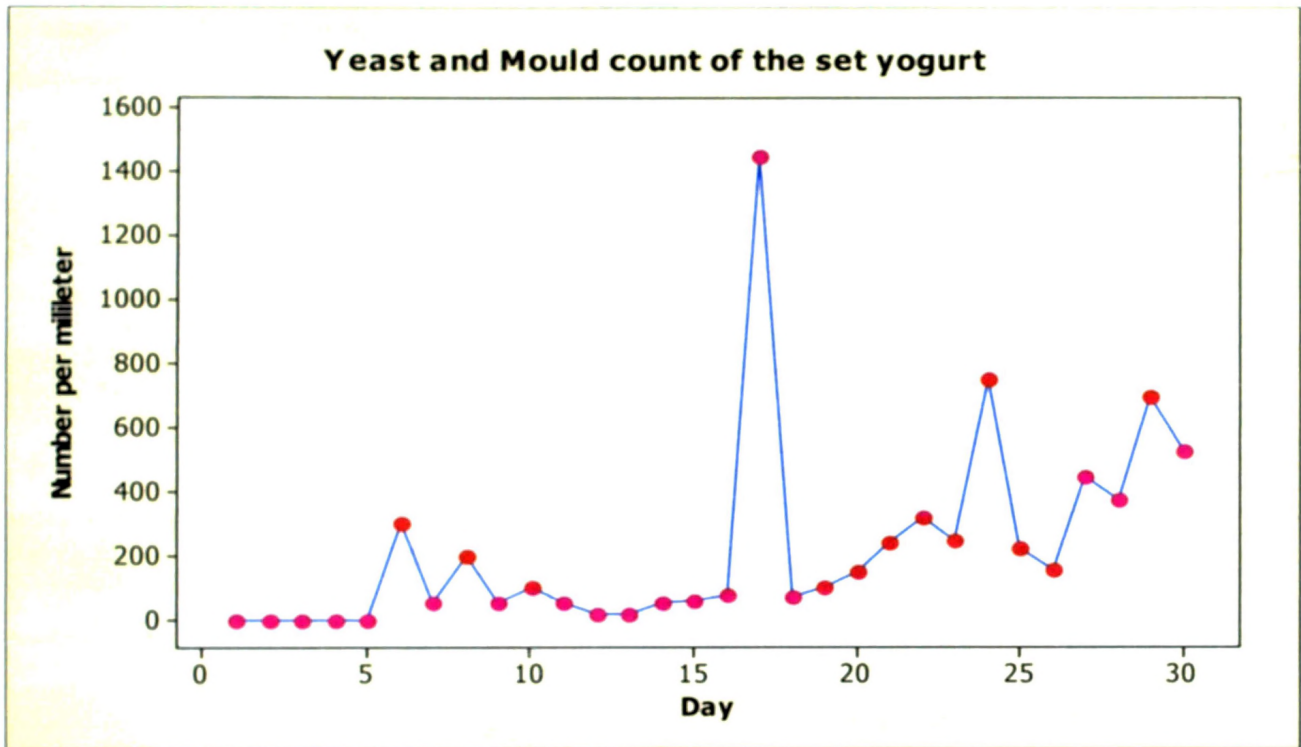
<b>Days after production</b>	<b>Yeast and Mould count (Number on microbes/ ml)</b>
1	Not Observed
2	Not Observed
3	Not Observed
4	Not Observed
5	Not Observed
6	Not Observed
7	Not Observed
8	Not Observed
9	50
10	100
11	50
12	15
13	15
14	50
15	60
16	75
17	1450

18	70
19	100
20	150
21	240
22	320
23	25
24	750
25	225
26	158
27	450
28	375
29	700
30	525

**Table 4.6 Variation of yeast and mould count of the existing yogurt over the period of one month**

Until 8 days after production, yeast and mould count of the yogurt was negative, however after that it was always positive for yeast and mould. High acidity developed in the yogurt may be the main cause for yeast and mould growth, because yeast and moulds are favorable for high acidic conditions. According to the SLSI specifications for yogurt, yeast count should not exceed 1000 per gram and mould count should not exceed 1 per gram. However with this test it cannot identified yeast and mould separately; therefore it's better to control the yeast and mould count in zero level.

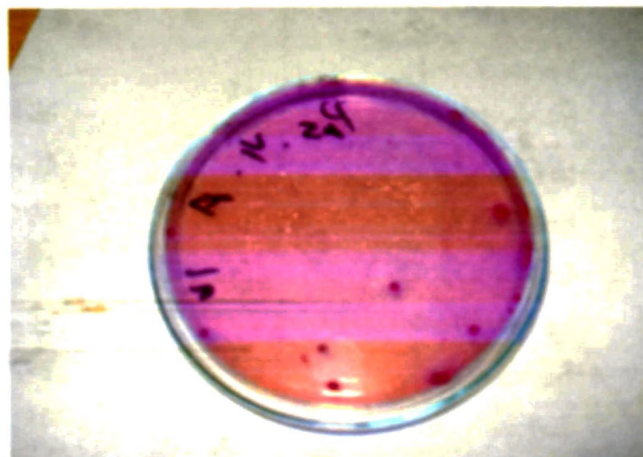
The yeast and mould count variation of the existing yogurt over the period of one month can be graphically illustrate as follows;



**Figure 4.4** Yeast and Mould variation of the existing “Kotmale” set yogurt over the period of one month

#### 4.3.2 Variation of the Coliform count

Coliform count of the yogurt was counted by growing them on the Violet Red Bile Agar medium. After incubation, coliform colonies were appeared in bright purple dots on the surface of the medium.



**Figure 4.5** Petri dish with purple color coliform colonies

Variation of the Coliform count of the existing yogurt over the storage time can be list as follows;

<b>Days after production</b>	<b>Number of coliforms / ml</b>
1	Not Observed
2	Not Observed
3	Not Observed
4	Not Observed
5	Not Observed
6	Not Observed
7	Not Observed
8	Not Observed
9	Not Observed
10	Not Observed
11	Not Observed
12	Not Observed
13	Not Observed
14	Not Observed
15	Not Observed
16	Not Observed
17	Not Observed
18	Not Observed
19	Not Observed
20	Not Observed
21	Not Observed
22	Not Observed
23	Not Observed
24	Not Observed
25	Not Observed
26	Not Observed
27	Not Observed
28	Not Observed
29	Not Observed
30	Not Observed

**Table4.7 Variation of Coliform count of the existing yogurt over the period of one month**

Presence or absence of Coliform in a sample is a parameter of the hygienic conditions of the manufacturing. However along the storage period of the yogurt, it was always negative for the Coliform. It means that there were proper hygienic conditions in the production of yogurt. During the storage period, there is a rare occasion of contaminated with Coliform. Because Coliform bacteria are not transform from the air. So if the cups were sealed properly, there is a minimum chance to contaminate. Negative results for the yogurt means that, yogurts were hygienically safe during manufacturing and storing.

#### **4.4 Comparison of new and old Yogurt cultures**

When considering all the chemical, organoleptic and microbiological parameters of the existing "Kotmale" yogurt, there was a significant effect of the high acidity and yeast and mould count on the quality of the yogurt. Because of high acidity, during the storage its give sour taste to the yogurt. So high sourness cause low consumer acceptance to the yogurt. High acidity also stimulate the yeast and mould growth in the yogurt, because high acidic condition is favorable for yeast and mould growth. Therefore by controlling acidity of the yogurt, it can control the sour taste and the yeast and mould growth of the yogurt.

There are two possible causes that effect to the high acidity of a yogurt;

- Too high storage temperature
- Starter culture with high post acidification during storage.

Yogurts are usually stored in the yogurt cold rooms under the temperature of 4 – 5 °C, as a rule of thumb the temperature of the cold room always maintained around that temperature. Therefore the main possible cause for high acidity should be the starter culture. Therefore to control the acidity of the yogurt it was decided to use a starter culture with low post acidification.

If selected new starter culture has a low post acidification, there are some differences on flavor profile, textural profile and other properties when compare with the existing starter culture. Properties of the new and old cultures can be summarized as follows;

Properties	Old culture	New culture
Flavor intensity	Strong	Mild
Gel firmness	High	High
Mouth thickness	Low	Medium to High
Acidification time	4 hrs – 4 hrs 30 min	4 hrs – 4 hrs 30 min
Post acidification ( Delta pH)	> 0.4	0.3

**Table 4.8 Comparison of new and old starter cultures**

If new starter culture has mild flavor intensity and high mouth thickness over old starter culture, it has a significant low rate of post acidification over the old culture. Therefore to reduce the high acidity during the storage, new starter culture was selected to develop the yogurt.

#### **4.5 Sensory evaluation of the improved yogurt in third day after manufacturing**

The sensory evolution was carried out using the same human subjects serving for three samples at the same time, thus the data generated were depend on each sample observation. Friedman test was selected to analyze the results of sensory attributes. Once samples are seemed to have a statistically significant difference, mean ranks were calculated separately for these attributes, in order to determine the degree of difference and to select the best sample. After treating the data in such a manner outcomes could be able to summarized as appeared in the following table;

Sensory attribute	P - value	Average ranks			Best sample
		751	517	648	
Color	0.027	66.0	66.0	48.0	.....
Appearance	0.107	67.0	62.0	51.0	.....
Smell	0.461	65.0	56.5	58.0	.....
Texture	0.000	50.0	80.0	50.0	517

Taste	0.028	63.0	68.5	48.5	517
Sourness	0.033	71.5	56.0	52.5	751
Overall acceptability	0.007	76.5	55.5	48.0	751

**Table 4.9 Analyzed data of sensory analysis in 3<sup>rd</sup> day after manufacturing**

Coded samples : 751 – Existing “Kotmale” yogurt

517 – Improved yogurt

648 – Market sample of yogurt

When considering the color attribute of the three yogurt samples, p – value of the test was less than  $\alpha$  (  $p = 0.027$ ). Therefore there was a significant difference of color attribute between three samples. According to the average ranks of the samples, existing “Kotmale” yogurt and improved yogurt had equal values. Therefore existing “kotmale” yogurt and improved yogurt had same consumer acceptance for color attribute. (see. app. ii)

When considering appearance of the three samples, p – value of the test was more than  $\alpha$  ( $p = 0.107$ ). Therefore there was no significant difference between three samples, along with the appearance. (see.app. ii)

When considering smell of the three samples, p – value of the test was more than  $\alpha$  ( $p = 0.461$ ). Therefore there was no significant difference between three samples, along with the smell of the three samples. (see.app. ii)

When considering texture of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.000$ ). Therefore there was a significant difference between three samples, along with the texture of the three samples. According to the average ranks of the three samples, improved yogurt had high average rank over other two samples. Therefore improved yogurt was the best sample in texture attribute. (see.app. ii)

When considering taste of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.028$ ). Therefore there was a significant difference between three samples, along with the

taste of the three samples. According to the average ranks of the three samples, improved yogurt had high average rank over other two samples. Therefore improved yogurt was the best sample in taste attribute. (see.app. ii)

When considering sourness of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.033$ ). Therefore there was a significant difference between three samples, along with the sourness of the three samples. According to the average ranks of the three samples, existing “Kotmale” yogurt has high average rank over other two samples. Therefore existing “Kotmale” yogurt was the best sample in sourness. (see.app. ii)

When considering the overall acceptability of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.007$ ). Therefore there was a significant difference between three samples, along with the overall acceptability. According to the average ranks of the three samples, existing “Kotmale” yogurt had high average rank over other two samples. Finally existing “Kotmale” yogurt had high overall acceptability after three days of manufacturing. (see.app. ii)

#### 4.6 Sensory evaluation of the improved yogurt in 28<sup>th</sup> day after manufacturing

The sensory evolution was carried out using the same human subjects serving for three samples at the same time thus the data generated were depend on each sample observation. Friedman test was selected to analyze the results of sensory attributes. Once samples are seemed to have a statistically significant difference, mean ranks were calculated separately for these attributes, in order to determine the degree of difference and to select the best sample. After treating the data in such a manner outcomes could be able to summarized as appeared in the following table;

Sensory attribute	P - value	Average ranks			Best sample
		751	517	648	
Color	0.020	44.5	66.0	69.5	648
Appearance	0.365	54.5	65.5	60.0	.....
Smell	0.330	54.5	66.0	59.5	.....



Texture	0.005	54.0	74.5	51.5	517
Taste	0.000	70.5	68.5	41.0	751
Sourness	0.017	64.0	68.5	47.5	517
Overall acceptability	0.007	62.0	71.0	47.0	517

**Table 4 .10 Analyzed data of sensory analysis in 28<sup>th</sup> day after manufacturing**

Sample codes : 751 – Market sample of yogurt

517 – improved yogurt

648 – existing “Kotmale” yogurt

When considering color of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.020$ ). Therefore there was a significant difference between three samples, along with the color of the three samples. According to the average ranks of the three samples, existing “Kotmale” yogurt ha high average rank over other two samples. Therefore existing “Kotmale” yogurt was the best sample in color attribute. (see.app. iii)

When considering appearance of the three samples, p – value of the test was more than  $\alpha$  ( $p = 0.365$ ). Therefore there was no significant difference between three samples, along with the appearance of the three samples. (see.app. iii)

When considering smell of the three samples, p – value of the test was more than  $\alpha$  ( $p = 0.330$ ). Therefore there was no significant difference between three samples, along with the smell of the three samples. (see.app. iii)

When considering texture of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.005$ ). Therefore there was a significant difference between three samples, along with the texture of the three samples. According to the average ranks of the three samples, improved yogurt had high average rank over other two samples. Therefore improved yogurt was the best sample in texture attribute. (see.app. iii)

When considering taste of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.000$ ). Therefore there was a significant difference between three samples, along with the

taste of the three samples. According to the average ranks of the three samples, tested market yogurt sample had high average rank over other two samples. Therefore market sample was the best sample in taste attribute. (see.app. iii)

When considering sourness of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.017$ ). Therefore there was a significant difference between three samples, along with the sourness of the three samples. According to the average ranks of the three samples, improved yogurt had high average rank over other two samples. Therefore improved yogurt was the best sample in sourness. (see.app. iii)

When considering the overall acceptability of the three samples, p – value of the test was less than  $\alpha$  ( $p = 0.007$ ). Therefore there was a significant difference between three samples, along with the overall acceptability. According to the average ranks of the three samples, improved yogurt had high average rank over other two samples. Finally improved yogurt had high overall acceptability after twenty eight days of manufacturing. (see.app. iii)

#### 4.7 Chemical and Microbiological changers of the improved yogurt

Chemical and microbiological variations of the improved yogurt were measured in 3<sup>rd</sup> , 14<sup>th</sup> , 21<sup>st</sup> and 28<sup>th</sup> days after production. pH and acidity tests were carried out as chemical analysis and coliform, yeast and mould tests were carried out under microbiological analysis. The results obtained can be listed as follows;

Days after production	Chemical variation		Microbiological variation	
	pH	Acidity	Coliform	Yeast and mould
3	4.85	0.79	Not Observed	Not Observed
14	4.76	0.85	Not Observed	Not Observed
21	4.52	0.92	Not Observed	2 cfu / ml
28	4.35	0.99	Not Observed	5 cfu / ml

**Table 4. 11 chemical and microbiological variation of the improved yogurt**

According to the above data, pH of the yogurt along the storage was varied from 4.85 to 4.35 with in 28 days of production. Therefore there was no significant variation of the pH during the Storage and also within the acceptable level. Acidity of the yogurt was changed from 0.79 to 0.99 during the storage of yogurt. However it was varied with in the limits that specified by SLSI specification for yogurt.

When consider the microbiological variation of the yogurt, there was no coliform count was observed during the storage of yogurt. It was a evidence of that improved yogurt has manufactured hygienically. There was no yeast and mould count observed until 21 days after manufacturing. But after 21 days, there was few yeast and mould counts were observed. It may be because of improper pasteurization of milk.

## **CHAPTER 05**

### **CONCLUSION AND RECOMMENDATIONS**

#### **5.1 Conclusion**

High acidity development during the storage was the main factor that effect to limit the shelf life of existing "Kotmale" yogurt. High acidity also contribute to the high sour taste and yeast and mould growth of the yogurt during the storage.

Therefore a new starter culture was introduced, with low post acidification. It had a significant effect on the quality of the yogurt during the storage of yogurt. Because of its low post acidification acidity development of the yogurt during the long storage was very low. Acidity variation of the improved yogurt was within the recommended level, which was specified by SLSI specifications for yogurt and there was a minimum pH variation of the yogurt within the storage period of one month.

According to the analyzed sensory data, there was a high overall acceptability to the existing "Kotmale" yogurt, in the 3<sup>rd</sup> day after prodction but in the 28<sup>th</sup> day after production there was a high overall acceptability to the improved yogurt with new culture over existing "Kotmale " yogurt and other market brand of yogurt.

Therefore it can conclude that improved yogurt with new culture can be stored for about 30 days after production, without any significant chemical, microbiological or organoleptic defects.

#### **5.2 Recommendations**

Storage temperature has a predominant effect on acidity development of a yogurt during storage. Therefore it is recommended to carry out a experiment to study the relationship between storage temperature and acidity development of the yogurt during the storage.

The selected new culture has a mild flavor intensity, because of that it was give a low smell and taste to the yogurt. But there are many starter cultures with high flavor profile and low post acidification. It is recommended to select that kind of culture and develop a

yogurt with that culture and check the chemical, microbiological and organoleptic variation of that yogurt.

Shelf life of a yogurt cannot be extended by only altering the raw materials. Processing conditions and good hygienic practices also effect to the keeping quality of a yogurt. Therefore it is recommended to maintain the proper processing factors,such as milk pasteurization and other hygienic practices in a recommended level during the manufacturing of yogurt.

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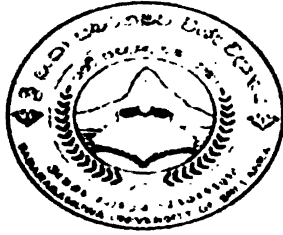
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## APPENDIX I

### Ballot paper used in sensory evaluation test



**Sabaragamuwa University of Sri Lanka  
Faculty of Applied Sciences, BSc. Food Science & Technology  
Department of Food Science & Technology**

### Questionnaire for Sensory Analysis ( Nine Point Hedonic Test)

Name: .....  
Date: .....

Product:  
Time : .....

- Assess the sample individually.
- Indicate how much you preferred each sample after testing.
- Rinse your mouth with water after tasting each sample.
- Give numerical values ranking from Like Extremely to Dislike Extremely.

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

Sensory Aspects	Sample code		
	751	517	648
Color			
Appearance			
Smell			
Texture			
Taste			
Sourness			
Overall Acceptability			

Comments: .....

**Thank you**

.....  
**Signature**



## APPENDIX II

### Friedman test results for improved yogurt in 3<sup>rd</sup> day after production

#### 1. color versus treatment blocked by assessors

##### Test hypothesis

$H_0$  : All the treatment medians are equal according to the color

$H_1$  : At least one treatment median is different from others

##### Data collecting and calculation

$S = 7.20$  DF = 2 P = 0.027  
 $S = 11.08$  DF = 2 P = 0.004 (adjusted for ties)

treat	N	Est	Median	Sum of Ranks
517	30		7.0000	66.0
648	30		6.0000	48.0
751	30		7.0000	66.0

Grand median = 6.6667

##### Decision rule

Reject  $H_0$ , if P value <  $\alpha$  at the significant level of 95 %

##### Statistical decision

In here P value = 0.027 and  $\alpha = 0.05$

So, reject  $H_0$

##### Conclusion

There is a significant statistical difference between three samples according to the color attribute.

#### 2. appearance versus treatment blocked by assessors

##### Test hypothesis

$H_0$  : All the treatment medians are equal according to the appearance

$H_1$  : At least one treatment median is different from others

##### Data collecting and calculation

$S = 4.47$  DF = 2 P = 0.107  
 $S = 5.70$  DF = 2 P = 0.058 (adjusted for ties)

treat	N	Est	Median	Sum of Ranks
517	30		7.0000	62.0
648	30		6.6667	51.0
751	30		7.3333	67.0

Grand median = 7.0000

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.107 and  $\alpha = 0.05$   
So, do not reject  $H_0$

### Conclusion

There is no significant statistical difference between three samples according to the appearance attribute.

## 3. smell versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the smell

$H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 1.55 DF = 2 P = 0.461  
S = 2.19 DF = 2 P = 0.335 (adjusted for ties)

treat	N	Est	Median	Sum of Ranks
517	30		7.0000	56.5
648	30		7.0000	58.0
751	30		7.0000	65.5

Grand median = 7.0000

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.461 and  $\alpha = 0.05$   
So, do not reject  $H_0$

## Conclusion

There is no significant statistical difference between three samples according to the smell attribute.

## 4. texture versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the texture

$H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 20.00 DF = 2 P = 0.000  
S = 22.64 DF = 2 P = 0.000 (adjusted for ties)

treat	N	Est	Median	Sum of Ranks
517	30		8.0000	80.0
648	30		7.0000	50.0
751	30		7.0000	50.0

Grand median = 7.3333

### Decision rule

Reject  $H_0$ , if P value <  $\alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.000 and  $\alpha = 0.05$

So, reject  $H_0$

### Conclusion

There is a significant statistical difference between three samples according to the texture attribute.

## 5. taste versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the taste

$H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 7.12 DF = 2 P = 0.028  
S = 8.06 DF = 2 P = 0.018 (adjusted for ties)

treat	N	Est	Median	Sum of Ranks
517	30		8.0000	68.5
648	30		6.8333	48.5
751	30		7.6667	63.0

Grand median = 7.5000

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.028 and  $\alpha = 0.05$   
So, reject  $H_0$

### Conclusion

There is a significant statistical difference between three samples according to the taste attribute.

## 6. sourness versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the sourness  
 $H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 6.82 DF = 2 P = 0.033  
S = 7.30 DF = 2 P = 0.026 (adjusted for ties)

treat	N	Est Median	Sum of Ranks
517	30	7.000	56.0
648	30	6.000	52.5
751	30	8.000	71.5

Grand median = 7.000

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.033 and  $\alpha = 0.05$   
So, reject  $H_0$

## Conclusion

There is a significant statistical difference between three samples according to the sourness attribute.

## 7. overall acceptability versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the overall acceptability

$H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 14.55 DF = 2 P = 0.001  
S = 16.32 DF = 2 P = 0.000 (adjusted for ties)

treat	N	Est Median	Sum of Ranks
517	30	7.0000	55.5
648	30	6.3333	48.0
751	30	7.6667	76.5

Grand median = 7.0000

### Decision rule

Reject  $H_0$ , if P value <  $\alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.001 and  $\alpha = 0.05$

So, reject  $H_0$

### Conclusion

There is a significant statistical difference between three samples according to the overall acceptability.

## APPENDIX III

### Friedman test results for improved yogurt in 28<sup>th</sup> day after production

#### 1. color versus treatment blocked by assessors

##### Test hypothesis

$H_0$ : All the treatment medians are equal according to the color

$H_1$ : At least one treatment median is different from others

##### Data collecting and calculation

S = 12.22 DF = 2 P = 0.002  
S = 20.08 DF = 2 P = 0.000 (adjusted for ties)

treatme	N	Est Median	Sum of Ranks
517	30	8.0000	66.0
648	30	8.0000	69.5
751	30	7.0000	44.5

Grand median = 7.6667

##### Decision rule

Reject  $H_0$ , if P value <  $\alpha$  at the significant level of 95 %

##### Statistical decision

In here P value = 0.002 and  $\alpha = 0.05$

So, reject  $H_0$

##### Conclusion

There is a significant statistical difference between three samples according to the color attribute.

#### 2. appearance versus treatment blocked by assessors

##### Test hypothesis

$H_0$ : All the treatment medians are equal according to the appearance

$H_1$ : At least one treatment median is different from others

##### Data collecting and calculation

S = 2.02 DF = 2 P = 0.365  
S = 2.49 DF = 2 P = 0.287 (adjusted for ties)

treatme	N	Est Median	Sum of Ranks
517	30	7.6667	65.5
648	30	7.3333	60.0
751	30	7.0000	54.5

Grand median = 7.3333

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.365 and  $\alpha = 0.05$   
So, do not reject  $H_0$

### Conclusion

There is no significant statistical difference between three samples according to the appearance attribute.

## 3. smell versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the smell  
 $H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 2.22 DF = 2 P = 0.330  
S = 2.71 DF = 2 P = 0.257 (adjusted for ties)

treatme	N	Est Median	Sum of Ranks
517	30	7.0000	66.0
648	30	6.8333	59.5
751	30	6.1667	54.5

Grand median = 6.6667

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.330 and  $\alpha = 0.05$   
So, do not reject  $H_0$

## Conclusion

There is no significant statistical difference between three samples according to the smell attribute.

## 4. texture versus treatment blocked by assessors

### Test hypothesis

$H_0$  : All the treatment medians are equal according to the texture

$H_1$  : At least one treatment median is different from others

### Data collecting and calculation

S = 10.62 DF = 2 P = 0.005  
S = 13.27 DF = 2 P = 0.001 (adjusted for ties)

treatme	N	Est Median	Sum of Ranks
517	30	8.0000	74.5
648	30	7.0000	51.5
751	30	7.0000	54.0

Grand median = 7.3333

### Decision rule

Reject  $H_0$  . if P value <  $\alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.005 and  $\alpha = 0.05$

So, reject  $H_0$

## Conclusion

There is a significant statistical difference between three samples according to the texture attribute.

## 5. taste versus treatment blocked by assessors

### Test hypothesis

$H_0$  : All the treatment medians are equal according to the taste

$H_1$  : At least one treatment median is different from others

### Data collecting and calculation

S = 18.12 DF = 2 P = 0.000  
S = 19.59 DF = 2 P = 0.000 (adjusted for ties)



treatme	N	Est	Sum
		Median	of Ranks
517	30	8.000	68.5
648	30	6.000	41.0
751	30	8.000	70.5

Grand median = 7.333

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.000 and  $\alpha = 0.05$   
So, reject  $H_0$

### Conclusion

There is a significant statistical difference between three samples according to the taste attribute.

## 6. sourness versus treatment blocked by assessors

### Test hypothesis

$H_0$ : All the treatment medians are equal according to the sourness  
 $H_1$ : At least one treatment median is different from others

### Data collecting and calculation

S = 8.15 DF = 2 P = 0.017  
S = 8.81 DF = 2 P = 0.012 (adjusted for ties)

treatme	N	Est	Sum
		Median	of Ranks
517	30	8.000	68.5
648	30	5.000	47.5
751	30	7.000	64.0

Grand median = 6.333

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.017 and  $\alpha = 0.05$   
So, reject  $H_0$

## Conclusion

There is a significant statistical difference between three samples according to the sourness attribute.

## 7. overall acceptability versus treatment blocked by assessors

### Test hypothesis

$H_0$  : All the treatment medians are equal according to the overall acceptability

$H_1$  : At least one treatment median is different from others

### Data collecting and calculation

S = 9.80 DF = 2 P = 0.007  
S = 12.12 DF = 2 P = 0.002 (adjusted for ties)

treatme	N	Est Median	Sum of Ranks
517	1	8.0000	71.0
648	30	7.0000	47.0
751	30	8.0000	62.0

Grand median = 7.6667

### Decision rule

Reject  $H_0$ , if P value  $< \alpha$  at the significant level of 95 %

### Statistical decision

In here P value = 0.007 and  $\alpha = 0.05$

So, reject  $H_0$

### Conclusion

There is a significant statistical difference between three samples according to the overall acceptability.

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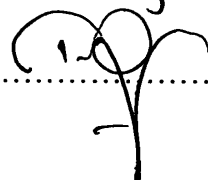
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