COMPARATIVE STUDY OF QUALITY CHARACTERISTICS, SHELF LIFE AND SENSORY EVALUATION OF STIRRED AND SET YOGHURT INCORPORATED WITH CHOCOLATE

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DECLARATION

The work describe in this thesis was carried out by me at Lucky Lanka Dairies Company and the Department of Food Science & Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, under the supervision of Mr. D.M.J.N. Danasekara and Mrs. P.S. Perera. The report on this has not been submitted to another university for another degree.

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Affectionately Dedicated

To My Beloved Parents

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ABSTRACT

Yoghurt is a very popular flavourful and healthful dairy product in all over the world. The classifications are as follows: set type, stirred type, drinking type and frozen type. Flavoured yoghurt contains fruit and berries, vanilla, honey, coffee essences, colourings, sucrose or aspartame and sugar free dietary sweeteners.

This study was carried out to formulate chocolate layered set yoghurt and chocolate incorporated stirred yoghurt and to compare the quality characteristics, shelf life and sensory evaluation of both stirred and set yoghurt initially, the best formula was developed for the chocolate that were taken to incorporate into both set and stirred yoghurt. The effect of different percentage of liquid chocolate on the quality of set and stirred yoghurt production was investigated. Liquid chocolate was added to cultured milk and stirred gel to give chocolate levels of 5, 10, 15 and 20%. The experimental yoghurts were compared with control yoghurt produced without incorporation of chocolate.

Fat, total soluble solids, viscosity, whey separation were determined in the experimental yoghurts on 1st day. Total solids, protein, ash were measured on 2nd day after manufacture. pH, Yeast and Mould count, Coliform count was determined after 1, 7, 14, 21 and 28 days. Sensory properties of the yoghurts were evaluated during storage.

Fat, total soluble solids, total solids, viscosity, whey separation were increased in both set and stirred yoghurt with the increase of chocolate percentage. pH was increased in both set and stirred yoghurt with the increase of chocolate percentage. But with the time being pH was decreased in both yoghurt types. Viscosity level was high in set yoghurt than stirred yoghurt. On the other hand, amount of whey separation was high in stirred yoghurt than set yoghurt. According to the sensory analysis data, the best sample for the set yoghurt was 10% chocolate added product and the best sample for the stirred yoghurt was 15% chocolate added product. Both yoghurt types were microbiologically safe. Coliform, Yeast and mould content were under SLSI standard level. But Coliform count was somewhat high in stirred yoghurt than set yoghurt.

CONTENTS

CHAPTER 03: MATERIALS AND METHODALOGY

CHAPTER 04: RESULTS AND DISCUSSION

LIST OF ABREVIATIONS

DM	Dry Matter
EU	European Union
FAO	Food and Agricultural Organization
Hrs	hours
MSNF	Milk Solid Non Fat
SLSI	Sri Lanka Standard Institute
SLS	Sri Lankan Standard
SNF	Solid Non Fat
WHO	World Health Organization
w/w	weight to weight

LIST OF FIGURES

LIST OF TABLES

CHAPTER 01

INTRODUCTION

1.1 Background:

Yoghurt is an acidified, coagulated product obtains from milk by fermentation with lactic acid producing bacteria (Ralph Early, 1998).There has been a phenomenal increase in the production of fermented milks in developed countries. Yoghurt is a very popular flavourful and healthful dairy product in all over the world. Its production and consumption is growing continuously due to its therapeutic properties beside its high nutritive value (Karagul *et al.*, 2004).

Yoghurt is usually classified according to its physical state in the retail container and its shelf life. These characteristics are influenced by manufacturing process, raw materials and ingredients added. The classifications are as follows: set type, stirred type, drinking type and frozen type (Hui,1993).Flavoured yoghurt contains fruit and berries, vanilla, honey, coffee essences, colorings, sucrose or aspartame and sugar free dietary sweeteners (Teknotext, 1995).

The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose by maldigesters, decreased risk of cancer; lower blood cholesterol, improved immune responses and help the body assimilate protein, calcium and iron (Perdigeon *et al.*, 1998).

In effort to offer variety and competition in the market, new researches are currently in progress on the use of several flavours and other ingredients in yoghurt industry. Chocolate are incorporated in ice cream industry and doing many researches in that field. The chocolate, which has several significant flavour notes, is involved in binding, the balance of flavor would be affected because only certain components react and lose their flavor intensity. Changing the balance of the flavour profile of chocolate can produce a preferable flavour (Welty *et al.*, 2001).

Chocolate comprises a number of raw and processed foods that originate from the seed of the tropical *cacao* tree. It is a common ingredient in many kinds of confections such as chocolate bars, candy, ice cream, cookies, cakes, pies, chocolate mousse, and other desserts. It is one of the most popular (or at least recognizable) flavors in the world. There has been disagreement in the EU about the definition of chocolate, but chocolate is any product made primarily of cocoa solids and cocoa fat. Different flavours can be obtained by varying the time and temperature when roasting the beans, and by varying the relative quantities of the cocoa solids and cocoa fat, and of course by adding non-chocolate ingredients (wikipedia encyclopedia).

By going through all the facts the main objectives of the present study was to formulation and development of chocolate incorporated stirred and set yoghurt. And then compare the quality characteristics, (pH, viscosity and whey separation) chemical composition of yoghurts (Total solids(%), fat(%), protein(%), lactose(%) and ash(%) and organoleptic properties of yoghurt samples (Colour and appearance, body and texture, taste and aroma and general acceptability).

1.2 Objectives

1.2.1 Overall objective

To compare the quality characteristics, shelf life and sensory evaluation of both stirred and set yoghurt that are incorporated with chocolate.

1.2.2 Specific objectives

01. To develop and formulate the chocolate.
02. Development of chocolate layered set yoghurt.
03. Development of stirred yoghurt that are incorporated with chocolate.
04. Determination of chemical composition, physical and chemical characteristics yoghurts.
05. Determination of shelf life of both set and stirred yoghurt.
06. Selection of the best milk and chocolate combination for higher sensory appeal, product quality characteristics in the final product of both set and stirred yoghurt.

CHAPTER 02

LITERETURE REVIEW

2.1 Historical background of yoghurt

Yoghurt is believed to have originated from the Balkans and the Eastern Mediterranean countries and traditionally would have been made with whole milk obtain from cows, goat and sheep depending on availability. The production of sour milk soon became and establishes pattern of preservation and one such product become known as yogurt from the Turkish word "Jugurt"; numerous variants of this word have appeared over the years and a selection of alternatives is shown in table 2.1 (Tamime and Robinson, 1999).

On the southern slope of Mt.Elbrus, micro-organisms preferring relatively high temperatures, 40-45 °C, came together in a milk pitcher that probably belonged to a Turkish nomad, and the result was what the Turks called "Yogurut". Some sources say that this name was introduced in the 8th century and that it was changed in the 11th century to its present form, yoghurt.

Table 2.1- Selection of yoghurt and yoghurt-like products that have been identified in the Middle East and elsewhere.

Country	Traditional name
Turkey	Jugurt / Eyran/
Balkans	Yaurt/Naja
Lebanon and some Arab countries	Levan/Laban
Egypt	Zababy
Iraq	Roba
India	Dahi/Dadhi
Greece	Yiaourti
Italy	Cieddu
Hungary	Tarho
Finland	Viili
Scandinavia	Filmjolk
Nepal	Shosim
Mongolia	Tarag
Rest of the world	Yoghurt/Yogurt/Yaort/Yourt/etc.

Source: Kosikowski and Mistry (1997).

2.2 Classification of yoghurt

The consistency, flavour and aroma vary from one district to another. In some areas yoghurt is produced in the form of a highly viscous liquid, whereas in other countries it is in the form of a softer gel. Yoghurt is also produced in frozen form as a dessert, or as a drink. The flavour and aroma of yoghurt differ from those of other acidified products, and the volatile aromatic substances include small quantities of acetic acid and acetaldehyde (Teknotext, 1995).

All types of yoghurt are usually classified into four categories based on the physical characteristics of the product. This approach is illustrated in Table 2.2.

Table 2.2- Proposed scheme for the classification of all yoghurt products.

Category	Physical states	Yoghurt Products
I	Liquid/Viscous	Yoghurt
II	Semi solid	Concentrated
III	Solid	Frozen
IV	Powder	Dried

Source: Tamime and Robinson (1999).

Yoghurt is typically classified as follows:

- Set type-incubated and cooled in the package,
- Stirred type-incubated in tanks and cooled before packing,
- Drinking type-similar to stirred type, but the coagulum is "broken down" to a liquid before being packed,
- Frozen type-incubated in tanks and frozen like ice cream,
- Concentrated-incubated in tanks, concentrated and cooled before being packed. This type is sometimes called strained yoghurt (Teknotext, 1995).

Yoghurt are sub divided in to different grouping based on fat content, physical nature, flavours, post fermentation processing:

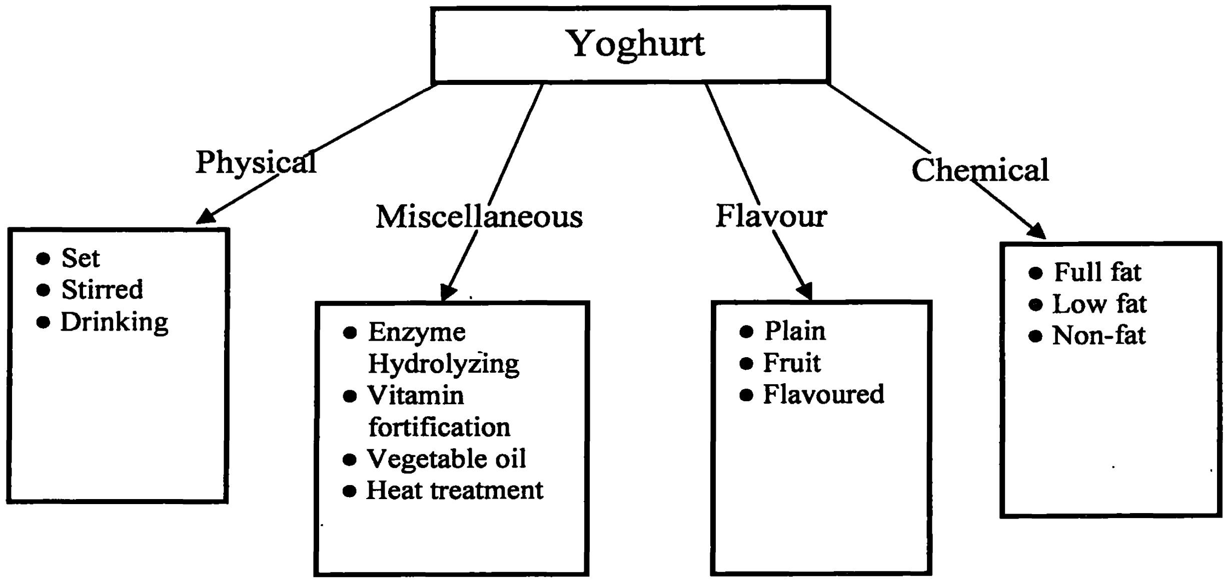

Figure 2.1- Scheme for the classification of yoghurt. Source: Tamime and Robinson (1999).

2.3 Types of yoghurt

2.3.1 Set yoghurt

Set yoghurt should have a glossy appearance without excessive whey. The texture is smooth and almost junket-like, giving a clean cut when spooned (Man and Jones, 2000). Set yoghurt is filled into the retail container after the inoculation stage of manufacture. Any colour/flavour addition will also be injected into the retail container immediately before the inoculated milk in order to aid agitation and the retail container will then be incubated at a suitable temperature. The coagulum produced is undisturbed and the resultant gel is in the form of a semi-solid mass, hence the term 'set' (Ralph Early, 1998).

2.3.2 Stirred yoghurt

The texture of the stirred yoghurt should be smooth with glossy appearance, thick and almost pourable, but must not be grainy or nodulated. (Man and Jones, 2000)
Stirred yoghurt is inoculated and incubated in a fermentation vessel (or churn) and the coagulum is broken during the cooling and packaging stages. Times and temperatures of incubation will apply as for set yoghurt manufacture method. The coagulum is removed from the fermentation vessel and cooled or cooled in place once the required pH is obtained (Ralph Early, 1998).

2.3.3 Flavoured yoghurt

Yoghurt with various flavouring and aroma additives is very popular, although the trend back towards natural yoghurt is clearly discernible on some markets. Common additives are fruit and berries in syrup, processed or as a puree. The proportion of fruit usually about 15%, of which about 50% is sugar.

The fruit is mixed with the yoghurt before or in conjunction with packing it can also be placed in the bottom of the pack before the latter is filled with yoghurt. Alternatively, the fruit can be separately packed in a "twin cup" integrated with the basic cup.

Sometimes yoghurt is also flavoured with vanilla, honey, coffee essences, etc. Colouring and sugar in the form of sucrose, glucose, or aspartame, a sugar-free diet sweetener, are often added together with the flavouring. The additives increase the DM content of the finished yoghurt. (Teknotext, 1995).

2.3.4 Frozen yoghurt

Frozen yoghurt can be manufactured in two ways. Either yoghurt is mixed with ice cream mix or a yoghurt mix is fermented, before further processing. Frozen yoghurt can be divided into soft-served and hard frozen types. The mix intended for soft-served yoghurt differs somewhat from that of the hard frozen type (Teknotext, 1995).

Frozen yoghurt varies widely in nature. As originally conceived, the product can be prepared from conventional set or stirred yoghurt, although and elevated level of sugar and stabilizers is required to maintain the coagulum during freezing and storage and small quantity of cream may be added to improve "mouth feel". It is also possible to replace the milk solids with whey protein concentrate. The yoghurt is then either frozen in a blast freezer to at least –20 °C, or frozen with aeration in an ice cream freezer (Erickson and Hung, 1997).

2.3.5 Dried yoghurt

The primary objective of manufacturing yoghurt in powder form is to store the product in a stable and readily utilizable state. Traditionally, natural/plain yoghurt, which is low in fat, is concentrated, shaped into flat rolls and sun dried. But for large-scale manufacture for this purpose either freeze-drying or spray drying may be used. The dried yoghurt is normally utilized by the desert dwellers in the preparation of food dishes (Tamime and Robinson, 1999).

2.3.6 Pasteurized/long life yoghurt

Heat treatment helps to prolong the shelf life of the product. Set-type yoghurt can be heat treated in the retail container and some examples are 75 °C for 5-10min, 58 °C for 5min, 85 °C for 35min.Heat-treated or pasteurized yoghurt is intended to be stable at room temperature for periods of three months. In that the application of heat inactivates the starter culture bacteria and their enzymes. As well as other contaminants. e.g.yeasts and mould. Two main problems have been associated with the manufacture of pasteurized yoghurt. First, a reduction in viscosity and whey syneresis may occur and second there may be loss of flavour (Tamime and Robinson, 1999).

2.3.7 Concentrated/strained yoghurt

While the yoghurt was hanging in the animal skin some of the liquid phase would have been absorbed into the skin, while some of the whey that had seeped through the skin would have been lost by evaporation. In this way concentration of the product took place and the new product was referred to as concentrated/strained yoghurt. This latter product would have had a better better keeping quality than normal yoghurt, mainly as a result of the higher concentration of lactic acid. The different methods available to manufacture strained yoghurt in large volumes are as follows;

- Cloth bag or the "Berge" system
- Mechanical separators
- Ultra filtration
- Product formulation

These yogurts are fermented by mesophilic starter cultures in conjunction, in some cases with yeast (Tamime and Robinson, 1999).

2.3.8 Drinking yoghurt

Drinking yoghurt is based on the stirred manufacture process, except that lower solids are used, the coagulum is broken down before filling into retail containers and fruit juice may be used instead of fruit concentrate. Low viscosity drinkable yoghurt, normally with a low fat content, is popular in many countries (Ralph Early, 1998).

Drinking yoghurt is essentially stirred yoghurt has total solids content not exceeding 11% and which has undergone homogenization to further reduce viscosity. Flavouring and colouring are invariably added and one type is slightly carbonated. Heat treatment may be applied to extend the storage life.

2.3.9 Carbonated yoghurt

Carbonated yoghurt can be manufactured in either a liquid or a dry form. The former type is, in effect, a carbonated, flavoured drinking yoghurt, while the dry mix gradually releases carbon dioxide (CO_2) when the powder is reconstituted with water. Additionally this type of yoghurt is less acidic and has pH around 7 (Tamime and Robinson, 1999).

2.3.10 Therapeutic yoghurt

The overall nutritive value of yoghrt is well established. But special types of yoghurt are often manufactured for dietetic/ therapeutic purposes. For example, the low lactose yoghurt is beneficial for lactose intolerance patient. The addition of different vitamins to yoghurt improves its nutritive value. The low calorie yoghurt are attractive to diet conscious consumer, cholesterol free yoghurt could also be beneficial for certain coronary conditions, and "The yoghurt tablet is especially developed, sugarless confectionary product for patients who suffer from diabetes.

2.4 Compositional standards of yoghurt

The food standard committee report recommended that yoghurt should have minimum fat content of 3.5% by weight that 'partly skimmed' or 'reduced fat' yoghurt should have fat content of between 1.0 to2.0% by weight and that 'skimmed milk yoghurt' or non fat yoghurt should have maximum fat content of 0.3% by weight. It was further recommended that 'all yoghurt should have a minimum solids not fat (SNF) content of 8.5% by weight (Tamime and Robinson, 1999).

Table 2.3 Specification for fermented milk products (yoghurt)

SL No.	Characteristics	Yoghurt	Low fat yog.	Non fat yog.	Method of test
i)	Milk fat, % by mass	3.0 min	0.5 to 3.0	Less than 0.5	SLS 735 part 1
ii)	Milk solid not fat, % by mass	8.0 min	8.0 min	8.0 min	SLS 735 part 5
iii)	Titratable acidity as lactic acid, % by mass	0.8 to 1.25	0.8 to 1.25	0.8 to 1.25	SLS 735 part 2

Source: Sri Lanka Standard 824: Part 2: (1989).

2.5 Nutritional value of yoghurt

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

Table 2.4 - Natural significance of standard yoghurt

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

Source: Ralph Early (1998).

2.5.1 Carbohydrate

2.5.1.1 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 yoghurt, a number of mono and disaccharides are present in trace amount, but lactose remains the dominant sugar in natural yoghurt; even after fermentation, the product may contain some 4-5g 100g-1 lactose, so that the lactose content of the end product is little different from normal milk.

2.5.1.2 Unavailable carbohydrates

Although natural yoghurt is based entirely on milk, stirred fruit yoghurts usually have stabilisers 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 attacked by digestive enzymes in the human body.

2.5.2 Protein

The proteins in milk are of excellent quality biologically and both the casein and whey proteins are well endowed with essential amino acids. Proteins in yoghurt are totally digestible. The fact that the protein content of yoghurt 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 200-250ml of yoghurt per day can easily provide an individual with the minimum daily requirement of animal protein (15g).

2.5.3 Lipids

Although much of the yoghurt sold in industrialized countries is produced from skimmed milk, traditional yoghurt has always contained some 3-4g 100g-1 milk fat. Yoghurt traditionally has been lower in fat than whole milk and this partly explains the perception that yoghurt has as lower fat dairy product. The influence of these lipid materials on the consistency and mouth feel of yoghurt 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.

2.5.4 Vitamins and Minerals

The relative availability of vitamins in yoghurt is much more difficult to assess because, unlike minerals, many vitamins are sensitive to the conditions of processing. The fortification of yoghurt with vitamins, such as vitamins A or C, is possible and losses over two weeks in storage are unlikely to exceed 50%. Yoghurt can act as a source of calcium for sufferers of lactose intolerance but, in addition, calcium supplied by yoghurt may be better absorbed and utilized than calcium made available in other forms. Yoghurt contains appreciable quantities of sodium and potassium which may not be suitable for feeding babies less than 6 months (Tamime and Robinson, 1999).

2.6 The manufacture of yoghurt

The manufacturing process for yoghurt is by no means uniform. Processes will vary from one manufacturer to another. Compositional values vary considerably, as do yoghurt types, which thereby affect the principles of manufacture. Numerous factors must be carefully controlled during the manufacture process in order to produce a high quality yoghurt with the required taste, aroma, viscosity, appearance, consistency and shelf-life (Ralph Early, 1998). These include:

- Choice of milk;
- Milk standardisation;
- Milk additives;
- Deaeration;
- Homogenisation;
- Heat treatment;
- Choice of culture;
- Culture preparation;
- Plant design. (Teknotext, 1995).

2.6.1 Ingredients

2.6.1.1 Milk ingredients

The main ingredient of yoghurt is milk or ingredients derived from milk (whole milk) and the most commonly used milk based ingredients are:

- Whole milk.
- Skimmed milk.
- Concentrated skimmed milk.
- Skimmed milk powder.
- Cream.
- Milk protein concentrates and isolates.

In fat-free or low-fat formulations, fresh skimmed milk or concentrated skimmed milk is the main ingredients. Solid-not-fat content can be adjusted by the addition respectively, of skimmed milk powder or water.

All ingredients used in the formulation of the milk base must be of high quality. Total bacterial count must be of unacceptably low level and contain no pathogenic or food spoilage micro organisms.

Ingredients are required to be free of antibiotics, bacteriopage, residues of cleaning solutions or sterilizing agents and any other substances, which will be inhibitory to the growth of starter culture micro organisms (Ralph Early, 1998).

I Constituent of milk

Table 2.5 - Constituent of milk

Constituent	Percentage
Water	87.3% (Range of 85.5 – 88.7)
Milk fat	3.9% (Range of 2.4-5.5)
SNF	8.8% (Range of 9-10)
Protein	3.25%
Lactose	4.6%
Minerals	0.65%
Vitamins	A, C, D, Thiamin, Riboflavin and other
Acids	0.18% (citrate, formate, Acetate, Oxalate)
Enzymes	Peroxidase, Catalase, Phospatase, Lipase
Gases	Oxygen, Nitrogen

Source: Dairy processing Hand Book (1995).

2.6.1.2 Sweeteners

Sweetening compounds are normally added during the manufacture of fruit/ fruited yoghurt and in some instances for the production of 'sweet' natural yoghurt. The main reason for addition of sweeteners is to subdue the level of acidity produced, particularly when high acid /low sugar content fruits.

The level of incorporation of sweetening agents will depend upon factors such as,

- The type of sweetening compound used.
- Consumer preference.
- Type of fruit used.
- Inhibitory effects on starter microorganisms.
- Legal aspects.
- Economic considerations.

Sucrose and glucose are the most common sweeteners added although many other sweetening compounds such as maltose, galactose and fructose may be used. Non-calorific sweeteners are used in low calorie formulations, for example, aspartame, cyclamate and saccharine. Artificial sweeteners are added because they have significantly greater sweetness than sucrose, i.e. cyclamate is 30-80 times sweeter, saccharine 240-350 times sweeter. (Ralph Early, 1998).Cyclamate has been banned in many countries as an additive, due to possible toxic effects. The use of such sweeteners in the food industry is therefore restricted (Tamime and Robinson, 1999).

2.6.1.3 Stabilisers

0.5% by weight of final product, except for the addition of gelatin, starches and pectin. The most commonly used stabilisers and thickeners are modified or natural starches, alginates, agar, carrageenan, edible gums, pectins and celluloses. Their main functions are to:

- Maintain viscosity during processing and to improve viscosity of final product.
- Influence structure and texture.
- Help to prevent serum separation.
- Influence creaminess and mouth feel.

The type of stabiliser used and the rate at which it should be added must be determined by each manufacturer. There are recommendations with regard to addition levels, for example, the 1975 Food Standard Committee Report suggests a total maximum addition when the total maximum may be raised to 1.0% by weight of final product.

2.6.1.4 Preservatives

A number of individual preservatives are used in yoghurt, either directly into the milk itself prior to fermentation or, more usually, via the fruit preparation. The most common include potassium sorbate, sodium benzoate, and sulphur dioxide. Sorbic acid added as its potassium salt is selective against mainly yeasts and moulds and does not significantly affect the starter culture organisms. Sulphur dioxide is some times added to the fruit preparation in order to help to preserve it. There are controls in many countries with reference to amounts of preservative which are allowed in yoghurt.

Sulphur dioxide	60 mg/kg
Benzoic acid	120 mg/kg
Methyl 4-hydroxybenzoate	120 mg/kg
Ethyl 4-hydroxybenzoate	120 mg/kg
Propyl 4-hydroxybenzoate	120 mg/kg
Sorbic acid	300 mg/kg (Ralph Early, 1998).

2.6.1.5 Yoghurt culture

Many have their own standards of identity for yoghurt with regard to composition as well as starter bacteria. Most countries and codex regulations define yoghurt as the product obtained by fermenting milk with a culture that includes *Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*. Some countries permit additional lactic acid bacteria (Elmer and James, 2005).

The general function of any starter culture should be to produce sufficient lactic acid in as short a time as possible to ferment milk from pH 6.4-6.7 to pH 3.8-4.2 to give acceptable texture, viscosity and flavour in the final product.

Starter cultures can be loosely divided into mesophilic and thermophilic, depending on the temperature growth characteristics. Although the term 'thermophilic' should be reserved for microorganisms whose optimum growth lies between 55-70°C, it tends to be employed within the dairy industry to describe cultures that are most active between 35-45°C. This description then provides a useful distinction between yoghurt and cheese cultures, which have optimum growth ranges between 20-35°C and are describe as 'mesophilic'. Strictly speaking mesophilic bacteria have an optimum growth range of 20-45°C (Ralph Early, 1998). *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* are thermophilic organisms and grow best at approximately 45°C but not above 50°C. They are typically added in a 1:1 ratio (Elmer and James, 2001).

Most commercial manufacture involves the use of *Lactobacillus delbrueckii, Lactobacillus bulgaricus, Streptococcus salivarius, Streptococcus thermophilus* and *Bifidobacterium* species. Other microorganisms that have been used in yoghurt manufacture include *Lactobacillus lactis, Lactobacillus acidophilus, Lactobacillus helveticus*, etc (Ralph Early, 1998).

2.6.1.6 Flavouring and Colouring ingredients

Flavouring agents are divided into three categories depending their source:

- Natural flavours and flavouring substances (botanical origin).
- Nature-identical flavouring substances (botanical origin).
- Artificial/synthetic substances (chemical origin).

These compounds are also used during the manufacture of flavoured (set or stirred), drinking, frozen and possibly, dried yoghurt. List of permitted compounds varies from one country to another.

Different food products, including alcoholic drinks, have been used to flavour yoghurt and some examples of these are:

- Sweet products (honey, maple syrup, butterscotch),
- Nuts (coconut, hazel, brazil, walnut),
- Cereals (muesli),
- Vegetables (cucumber, tomato, celery)
- Miscellaneous (coffee, spices, paprika, vanilla).

Colour is added to fruit and flavoured yoghurts to make the products more attractive. The active agents may be naturally derived, nature identical, caramel or artificial. The list of colours which may be used as food additives differ from one country to another. However, the FAO/WHO (1990) have offered some guidance about which colour compounds should be permitted and at what concentrations in yoghurt, assuming that the agents come entirely from the fruit/flavouring ingredients (see table 2.5) (Tamime and Robinson, 1999).

Table 2.6 Permitted food colouring matter arising exclusive from flavouring substances as a result of carry-over

Name of colour	Maximum level (mg kg-1)
Indigotine	6
Brilliant black PN	12
Sunset yellow FCF	12
Tartrazine	18
Cochineal	20
Carminic acid	20
Erythrosine	27
Red 2G	30
Ponceau	48
Caramel	150
Brilliant blue FCF	200

Source: Data complied from FAO/WHO (1990).

2.6.2 Chocolate Manufacturing Process

2.6.2.1 Chocolate and their history

Chocolate comprises a number of raw and processed foods that originate from the seed of the tropical *cacao* tree. It is a common ingredient in many kinds of confections such as chocolate bars, candy, ice cream, cookies, cakes, pies, chocolate mousse, and other desserts. It is one of the most popular flavours in the world.

Chocolate was created by the Mesoamerican civilization, from cacao beans, and cultivated by pre-Columbian civilizations such as the Maya and Aztec, who used it as a basic component in a variety of sauces and beverages. The cocoa beans were ground and mixed with water to produce a variety of beverages, both sweet and bitter, which were reserved for only the highest noblemen and clerics of the Mesoamerican world. Chocolate is made from the fermented, roasted, and ground beans taken from the pod of the tropical cacao tree, *Theobroma cacao*, which was native to Central America and Mexico, but is now cultivated throughout the tropics. The beans have an intensely flavoured bitter taste. The resulting products are known as "chocolate" or, in some parts of the world, cocoa.

2.6.2.2 Varieties

The three main varieties of *cacao* beans used in chocolate are *Criollo, Forastero* and *Trinitario.*

2.6.2.3 Harvesting

First, the pods, containing cacao beans, are harvested. The beans, together with their surrounding pulp, are removed from the pod and left in piles or bins to ferment for three to seven days. The beans must then be quickly dried to prevent mold growth; weather permitting this is done by spreading the beans out in the sun.

2.6.2.4 Sourcing raw cocoa beans and blending for flavour

In order to produce a consistent chocolate, commercial chocolate manufacturers reserve most cocoa bean crops on the world market well in advance of their actual availability. This ensures the manufacturer of a steady supply of cocoa beans. Distributors who supply small chocolate factories purchase the remaining beans

A variety of cocoa beans are blended by the chocolatier to a specific formula they have developed in order to make their final product unique. Blending also insures that the manufacturer delivers to their customers, the same product year in and year out. There is a movement within the small luxury chocolate manufacturers to produce chocolates by "cru" or vintage. This means that each chocolate they produce has its own distinct flavour and aroma – these can vary crop to crop. Vintage chocolates satisfy the chocolatier's or pastry chef's need for special occasion chocolates and desserts.

2.6.2.5 Roasting and Grinding the Cocoa Beans

After blending the cocoa beans are roasted and ground. Roasting brings out the chocolate flavour and aroma associated with chocolate and is accomplished much in the same way that coffee beans are roasted. Grinding the roasted beans to a fine pulp in very important as it is the first step in producing an exceptionally smooth product. The pulp, or chocolate liquor as it is called in the industry, is then melted. Extra cocoa butter is added to increase the delicacy of the chocolate mass. The pulp naturally contains about 45% cocoa butter. Fine quality chocolate can range for 60 - 70% cocoa butter, so this addition of cocoa butter is an integral part of the process.

2.6.2.6 Refining the cocoa paste with other ingredients

Sugar, vanilla and possibly dry milk powder for milk chocolate are added to the melted cocoa liquor and then emulsified in a refining process called conching. In most cases, a small amount of soy lecithin is added to help with emulsification. Conching is the secret to fine quality chocolate. The mixture is agitated in a folding or wave motion by machine for at least a number of hours and sometimes up to days until all the particles of the chocolate mixture have been made smaller. The result is a smooth texture that literally melts in your mouth.

You can test the importance of conching yourself by starting with two different quality chocolates. For this test, use products that are very divergent in price so the difference in texture will be more obvious. Take a small piece of chocolate and let it melt on your tongue, using your tongue to rub the mixture on your upper palette. You will be able to discern a "grittiness" in the less quality product. The expensive product, especially if it is a European brand, should be much smoother. That is the result of conching for long periods of time.

2.6.2.7 Tempering the chocolate for storage and distribution

The conched mixture is then cooled slowly to about 90 degrees Fahrenheit while it is still in movement. This "tempers" the product giving the chocolate a sheen and crisp bite. To explain further, the crystalline structure of the cocoa mass is broken when chocolate is melted over 90 degrees F. In order the re-establishment the crystals in their normal order, the mass must be heated to around 100-110 degrees F and then cooled back down to 90 degrees F. Keeping the crystals moving while the chocolate mass cools enhances the process and helps insure the original structure is regained. Once tempering has been accomplished, the chocolate is molded into bars and shipped to customers (Minifie, 1997).

2.6.3 Yoghurt processing.

The essential steps in the manufacture of fresh stirred and set yoghurt are outlined in Figure 2.2. The pre-incubation stages are common to both yoghurt types.

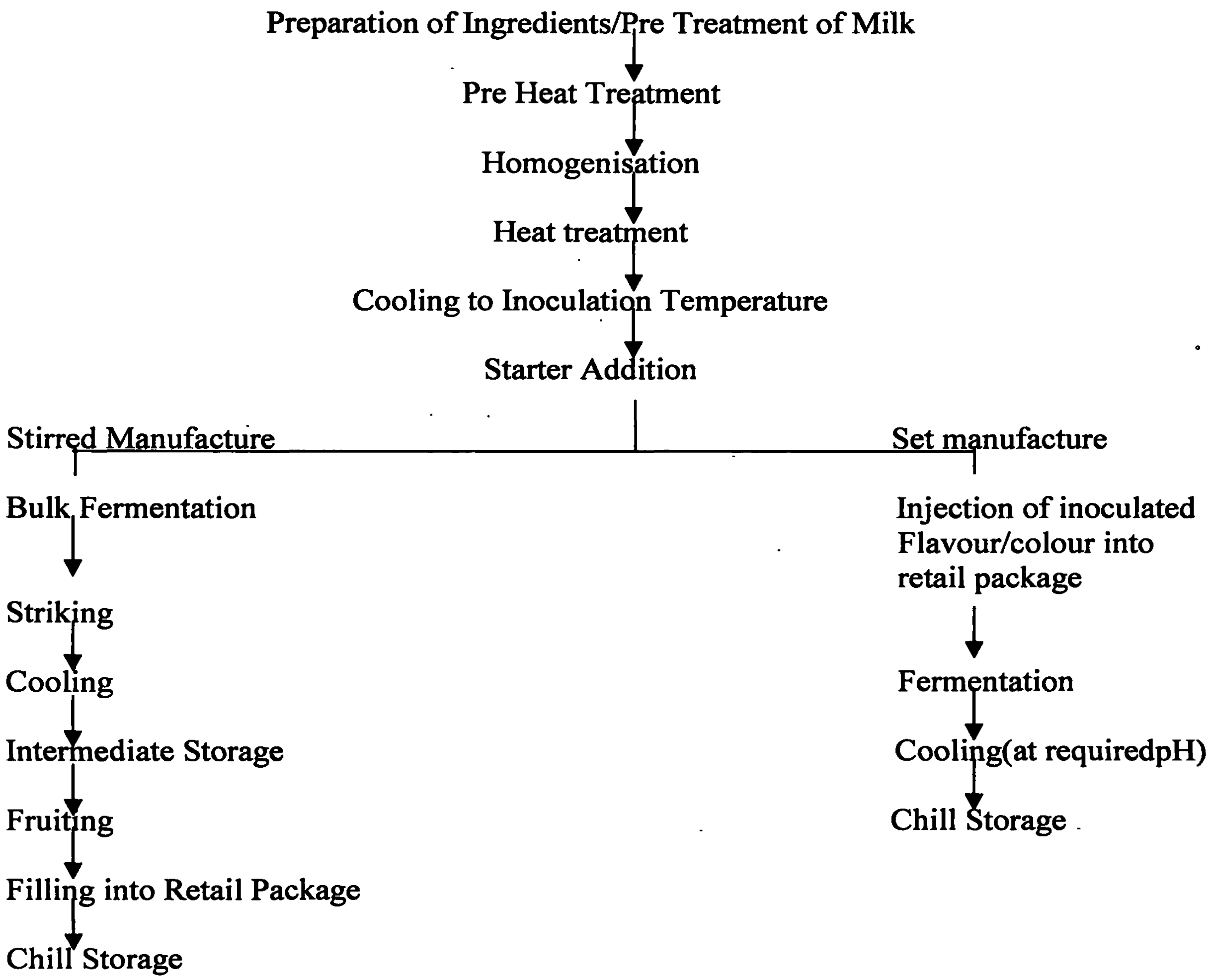

Figure 2.2 Steps in the production of yoghurt. Source: Ralph Early (1998).

2.6.3.1 Preparation of ingredients

The first stage of yoghurt manufacture is to combine all the ingredients, which are included in the formulation of the base material.

I Milk standardisation

Solid not fat, fat and total solids levels will vary dependant upon the type of yoghurt to be manufactured, as will be the inclusion of other ingredients such as sugar, skimmed milk powder, water and cream (Ralph Early, 1998).The fat and dry solids contents of the milk are normally standardized according to the FAO/WHO code and principles described below.

Fat

Yoghurt should have minimum fat content of 3.5% by weight that 'partly skimmed' or 'reduced fat' yoghurt should have fat content of between 1.0 to2.0% by weight and that 'skimmed milk yoghurt' or non fat yoghurt should have maximum fat content of 0.3% by weight (Tamime and Robinson, 1999).

Dry matter (DM) content

According to the FAO/WHO code and principles the minimum MSNF is 8.2%. An increase in the total DM content, particularly the proportion of casein and whey proteins, will result in a firmer yoghurt coagulum, and the tendency to whey separation will then be reduced.
The most common ways to standardize the DM content are:

- Evaporation (10-20 % of the milk volume is normally evaporated).
- Addition of skim milk powder, usually up to 3%.

Addition of milk concentrate (Teknotext, 1995).
Sugar may be added as liquid but care must be taken not to add too much sugar to the pre-fermented white base since this can result in dehydration of the starter organisms. Dry ingredients (milk powders, stabilizers, sugar) require sufficient time for rehydration and deaeration (Ralph Early, 1998).

2.6.3.2 Emulsification

The homogenization process was invented by Gaulin in 1899 who described it as "fixer a composition des liquids" (Tamime and Robinson, 1999). It is necessary to prevent the separation of fat during fermentation and at later stages of storage and transportation. Yoghurt milk is an oil-in-water emulsion and the function of the homogenizer in this respect is to reduce the size of the discontinuous phase, i.e. fat, in order to assist the creation of a stable emulsion. The diameter of fat globules in milk ranges from 1 to 20 microns. The function of homogenization is to reduce the average diameter of fat globules to less than 2 microns (Ralph Early, 1998).

Homogenisers may be equipped with one homogenizing device or two connected in series, hence the names single-stage homogenization and two-stage homogenization.

Effect of homogenization:

The effect of homogenization on the physical structure of milk has many advantages:

- Smaller fat globules leading to no cream-line formation,
- Whiter and more appetizing colour,
- Reduced sensitivity to fat oxidation,
- More full-bodied flavour, better mouth feel,
- Better stability of cultured milk products.

However, homogenization also has certain disadvantages:

- Homogenized milk can not be efficiently separated.
- Somewhat increased sensitivity to light-sunlight and fluorescent tubes-can result in "Sunlight flavour".
- Reduced heat stability (Teknotext, 1995).

2.6.3.3 Heat treatment

The objectives of this stage of the process are to:

- Eliminate vegetative food poisoning microorganisms.
- Eliminate or reduce to acceptable levels, food spoilage microorganisms.
- Reduce the total microbiological population to a level, which will not compromise the growth of the starter culture microorganisms.
- Denature the whey proteins in order to improve the texture of the final product and to assist in the prevention of whey separation at any subsequent time during shelf life.
- Hydrate stabilizers, which require heat.

These objectives are usually achieved in the heating section of a plate heat exchanger, or tubular heat exchanger where the temperature can be raised to 80 ^{0}C minimum. Functional properties of whey proteins become more apparent after heating milk and they begin to become denatured above 60 ^{0}C. (Ralph Early, 1998).Optimum results are achieved by heat treatment at 90-95 ^{0}C and a holding time of about 5 minutes. That temperature/time combination denatures about 70-80% of the whey proteins. In particular the ß-lactoglobulin, which is the principal whey protein, interacts with the k-casein, thereby helping to give the yoghurt a stable "body" (Teknotext, 1995).

2.6.3.4 Pre fermentation cooling

After heat treatment the milk is required to be cooled to a suitable temperature for inoculation. In most cases this will be carried out in the regenerative section of a plate heat exchanger. Inoculation temperature for short set method will approximately to 42°C. For short set incubation it is critical to achieve an accurate inoculation temperature since too high a temperature can inhibit and ultimately kill starter culture microorganisms and too low a temperature will result in unnecessary extension of fermentation time (Ralph Early, 1998).

2.6.3.5 Starter addition

As has already been indicated, there are a number of different forms of starter culture and to some extent the method of addition is influenced by the form of the culture. Bulk starter, made in fermentation tank, will be added to the milk via a dosed injection at levels approximating to 2% v/v. Then effective agitation can take place. It is ensure the even distribution of microorganisms throughout the milk. Freeze dried and deep frozen starters will be added aseptically into the fermentation vessel, often once the vessel has been part filled and then effective agitation can take place as the vessel is filled. In all cases, good agitation is required to ensure even distribution of microorganisms throughout the milk (Ralph Early, 1998).

Culture laboratories now use advanced techniques to produce customized yoghurt cultures to satisfy specific flavour and viscosity requirements. Some examples of end-product properties that can be achieved are:

- High viscosity with low acetaldehyde content and a fairly high final pH.
- Low viscosity and medium acetaldehyde content, suitable for drinking yoghurt, etc.

The handling of the starter for production of yoghurt demands maximum precision and hygiene (Teknotext, 1995).

2.6.3.6 Fermentation

In modern automated plants, stirred type and set type yoghurts are often produced concurrently. In the manufacture of stirred and liquid/drinking type yoghurt, bulk incubation is carried out in large, hot water-jacketed incubation tanks (e.g. 5000-10 000 l capacity). With reference to the manufacture of set yoghurt type, incubation takes place inside the retail container. The temperature of incubation is dependent upon the starter organisms used and the proposed length of incubation. The incubation of retail containers in set type yoghurt takes place within a warm air incubation room.

As has already been indicated, the short set method of incubation of yoghurt milk, using traditional starter organisms such as *Strptococcus salivarious* ssp. *Thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, will require the incubation temperature to be approx. 42 °C, which will provide optimum temperature environment for the microorganisms to metabolise synergistically. There should be no agitation during incubation. The yoghurt curd or "coagulum" begins to form as more lactic acid is produced and the iso-electric point of casein (pH 4.6-4.7) is approached (Ralph Early, 1998).

The formation of acetaldehyde and other aromatic compounds by the starter organisms takes place during fermentation and the final levels are dependent upon the presence of specific enzymes which are able to catalyse the formation of carbonyl compounds from the different milk constituents (Tamime and Robinson, 1999).

2.6.3.7 Striking

This stage applies only to stirred and liquid/drinking type yoghurt and is essentially the operation of breaking the warm gel/curd and reincorporation of the whey. Slow speed paddle agitation (e.g. 2-4 rpm) for approx. 5-10 min is usually sufficient to obtain a homogeneous mix. Agitation also tends to inhibit the culture activity and slows the rate of acidity development (Ralph Early, 1998).

2.6.3.8 Post fermentation cooling

Cooling of the coagulum commences directly after the fermented yoghurt reaches the desired acidity. The desired acidity will be dependent upon type of yoghurt being produced, method of cooling, time taken to empty fermentation vessel and desired final acidity. This will take place at approximately pH 4.5 - 4.6.

Cooling is achieved in stirred/liquid yoghurt by pumping the yoghurt via a gentle action positive displacement pump, through a plate or tubular cooler in order to achieve a temperature which is low enough to retard starter culture activity.
Cooling temperatures vary and are dependent upon the:

- Composition of yoghurt and its inherent ability to withstand cold mechanical handling;
- Filling capacity of the process;
- Duration of intermediate storage;
- Efficiency of refrigeration plant;
- Cooling capability post-fill, i.e. chill room temperature, air circulation, etc.

Recommends primary cooling to 24 ° C, followed by secondary cooling at 7-10 °C for the first 5-6h with final cooling at 1-2 ° C for the remainder of the cooling period.

On cooling, the curd becomes much firmer and if the yoghurt is formulated correctly, surface whey will be re-absorbed after 24h chilled storage (Ralph Early, 1998).

2.6.3.9 Intermediate storage of stirred yoghurt

Intermediate storage is often necessary due to yoghurt production rate and filling rate incompatibility. Storage times are shorter than 24 hours and ideally much less than this, nominally, a few hours. Ideally a temperature of 8-10 ° C is optimal, depending upon storage time. Intermediate storage should be as short as possible since physical changes take place that can affect final yoghurt quality. The product may release whey that is difficult to re-incorporate, resulting in loss of yield (Ralph Early, 1998).

2.6.3.10 Packaging

Primary packaging will include glass, polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyvinyl chloride, polyvinylidene chloride, plastic sachets and paper cartons. The majority of containers used are manufactured from polystyrene or polypropylene. Plastic containers will either be formed via an injection moulding process or via a thermoforming process which is commonly known as a form-fill-seal operation. A dual head filling machine will be required. Layered yoghurt products will require multi head filling.

Aluminium foil is widely used to seal yoghurt containers. Due to the acidity of yoghurt and the requirement for the foil to be heat sealed to the plastic container, the aluminium foil is normally coated with a layer of plastic.

Individual packages may be further collated into packs of four, six, twelve, etc. and the most popular secondary packaging is either cardboard in the form of an outer sleeve or tray or semi-rigid plastic crates (Tamime and Robinson, 1993).

2.6.3.11 Chill storage

Yoghurt, which has not been subjected to any form of heat treatment such as pasteurization, sterilization or UHT processes, needs to be kept cold until its reaches the customer. This includes the majority of yoghurts which will have a shelf life of approximately 15-20 days. Temperature variation will affect texture, viscosity, syneresis as well as improving the environment for potential food spoilage and food poisoning microorganisms. Exposure to higher temperatures than recommended below can increase biochemical reactions such as fat oxidation, hydration of protein constituents in yoghurt and slight dehydration of exposed yoghurt surface. Chill storage should be 2-5 ° C, with no rise above 10 ° C at intermediary stages in distribution (Teknotext, 1995).

2.6.3.12 Distribution

Quality assurance principles should extend to monitoring food products throughout the distribution chain. Although the final yoghurt is likely to be stored for only a short period of time prior to distribution to customer's premises, any identified hazards such as rodent/insect infestation, exposure to temperature increase, potential for physical damage, etc., need to be monitored and preventative action taken where appropriate (Ralph Early, 1998).

Yoghurt can be subjected to textural stress during transportation. Set yoghurt is particularly sensitive to transportation and poor formulation or processing can result in broken curd structure and excessive wheying off. Fluctuating temperatures during distribution can adversely affect the coagulum stability reducing viscosity and encouraging syneresis. Any significant fluctuation in temperature may also result in the continuation of fermentation by starter culture microorganisms, which will affect quality in an adverse manner. During the first 24-48 hrs. of cold storage, improvements in the physical characteristics take place, mainly as a result of hydration and/or stabilisation of the casein micelles. If practically possible, it would therefore be an advantage to retain yoghurt in chill storage for at least 24 hrs. before commencing distribution (Teknotext, 1995).

2.6 Spoilage of yoghurt

Yoghurt, in general is of low pH value and high lactic acid concentration and are thus a highly selective environment favouring for the growth of spoilage microorganisms.
Yeast and moulds as spoilage microorganism. Secondary selective pressure is excreted by oxygen availability, which restricts the development of moulds and non-fermentative yeasts, while the addition of added sugars favours the growth of fermentative yeasts.

Yeasts are most important spoilage organisms and are most commonly associated with fermentation leading to gas production. Such spoilage is readily recognized by 'doming' of foil lids and even burst containers. Genera such as *Kluyveromyces marxianus* and *Saccharomyces* species are most commonly involved in fermentative spoilage. How ever permit sufficient air to enter the pack to support the growth of oxidative yeast in the bulk of the yoghurt. In such circumstance oxidative yeast predominate near the pack walls and fermentative yeast in the center of the pack. Wide ranges of oxidative yeast have been isolated from yoghurt including species of *Candida, Pichia, Rhodotorula,* etc (Garbutt, 1997).

Mould growth at the yoghurt/air interface leads to development of visible mycelial 'mats' or 'buttons' .A wide range of species have been isolated including *Alternaria, Aspergillus, Mucor, Penicillium, etc* (Garbutt, 1997).

Coliform bacteria are not caused for spoiling of yoghurt. They can't survive for much longer than 24 hours due to the low pH. Their initial presence in the yoghurt gives an indication of pre process contaminations.

Table 2.7- Microbiologically requirements for yoghurt

SL No.	Test organisms	Limit	Method of test
i)	E-coli	Not more than 1 per g	Appendix A
ii)	Yeast	Not more than 1000 per g	Appendix A
iii)	Moulds	Not more than 1 per g	Appendix A

Source: Sri Lanka Standard 824: Part 2: (1989).

Quick spoilage of yoghurt is mainly due to,

- Raw materials.
- Product formulation.
- Unsatisfactory processing parameters.
- Implementing good manufacturing practices.
- Filling and packaging.
- Storage and distribution.
- Consumer usage and handling.

2.8 Sensory evaluation of yoghurt

2.8.1 Sensory Evaluation: A Brief Review

Sensory analysis is the identification, scientific measurements, analysis and interpretation of the properties (attributes) of a product as they are perceived through the five senses of sight, smell, taste, touch and hearing. Sensory analysis answers questions of quality under three main headings-discrimination, description and preference. In discrimination questions aim to find out whether or not a difference exist between two or more products. In description, questions aim to describe and measure any difference that is found to exist between products. In preference, hedonic questions aim to identify liking or acceptability (Roland *et al.*, 2000).

The organoleptic qualities of yoghurt are traditionally assessed by sensory evaluation of flavour, body, texture, finish and overall acceptability by experienced judges or trained consumer panelists. However sensory analysis is subjective and although at present the best index of consumer acceptability, they provide data that are difficult to evaluate scientifically. Therefore instrumental analysis of yoghurt is carried out hence it enables objective, faster and less expensive assessment of quality, usually to complement sensory evaluation (Blazquez *et al.*, 2006).

2.8.2. Statistical Aspects of Sensory Evaluation

In planning sensory experiments, experimental design is of paramount importance, 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 assessor's 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 on 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'. 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 numbers of consumers are used to provide the data, parametric analysis of variance is often used (Roland *et al.*, 2000).

CHAPTER 3.0

MATERIALS AND METHODALOGY

3.1 Liquid chocolate making

Materials

Hot plate	Cooking chocolate bars (50% wgt.)
Stainless steel container	Raw milk (20% wgt.)
Spoon	Sugar (15% wgt.)
Glassware	Vanilla
Electric balance (accuracy of 0.1mg)	Butter (15% wgt.)

Method

At first all the compounds that are taken to make chocolate were measured by the electric balance. Raw milk was placed in the stainless steel container and boiled by the hot plate. Then sugar was added and stirred well. As well cooking chocolate bars, butter and vanilla was added and stirred well. The mixture was heated just under the boiling temperature and poured in to a separate container.

3.2. Formulation of chocolate layered set yoghurt

Materials

Digital pH meter (pH scan2, Singapore)	Starter culture
Thermometer	Liquid chocolate
Incubator	Sugar (White Refined)
Heater	Gelatin (200 bloom)
Raw milk	Preservatives
Refrigerator	

Method

In this process there was not any cream separation and homogenization. The raw milk was dissolved with sugar. Gelatin was dissolved in warm water (at 60 ^{0}C) and it was added while stirring. The mixture was heated at 90 ^{0}C for 15 min, while stirring. The mixture was cooled to about 42^{0}C and well mixed fresh culture, egg yellow, preservatives and liquid chocolate were added and uniformly mixed. Then the mixture was poured into the clean sterile yoghurt cups. The yoghurt was incubated at 42 ^{0}C for 4-5 hours in incubator. After set it was transferred to refrigerator to storing.

3.2.1Formulation of recipes to set yoghurt

Following proportions was separated to formulate the chocolate layered set yoghurt.

Table 3.1 Four recipes that were used to prepare final set yoghurt which gained for sensory analysis

Ingredients	Sample code 174	Sample code 910	Sample code 526	Sample code 839
Milk	1000 ml	1000ml	1000 ml	1000 ml
Sugar	125 g	125 g	125 g	125 g
Chocolate	200 g	150 g	100 g	50 g
Gelatin	10g	10 g	10 g	10 g
Culture	80 g	80 g	80 g	80 g
Yog :Choc. %	80 : 20	85 : 15	90 : 10	95 : 05

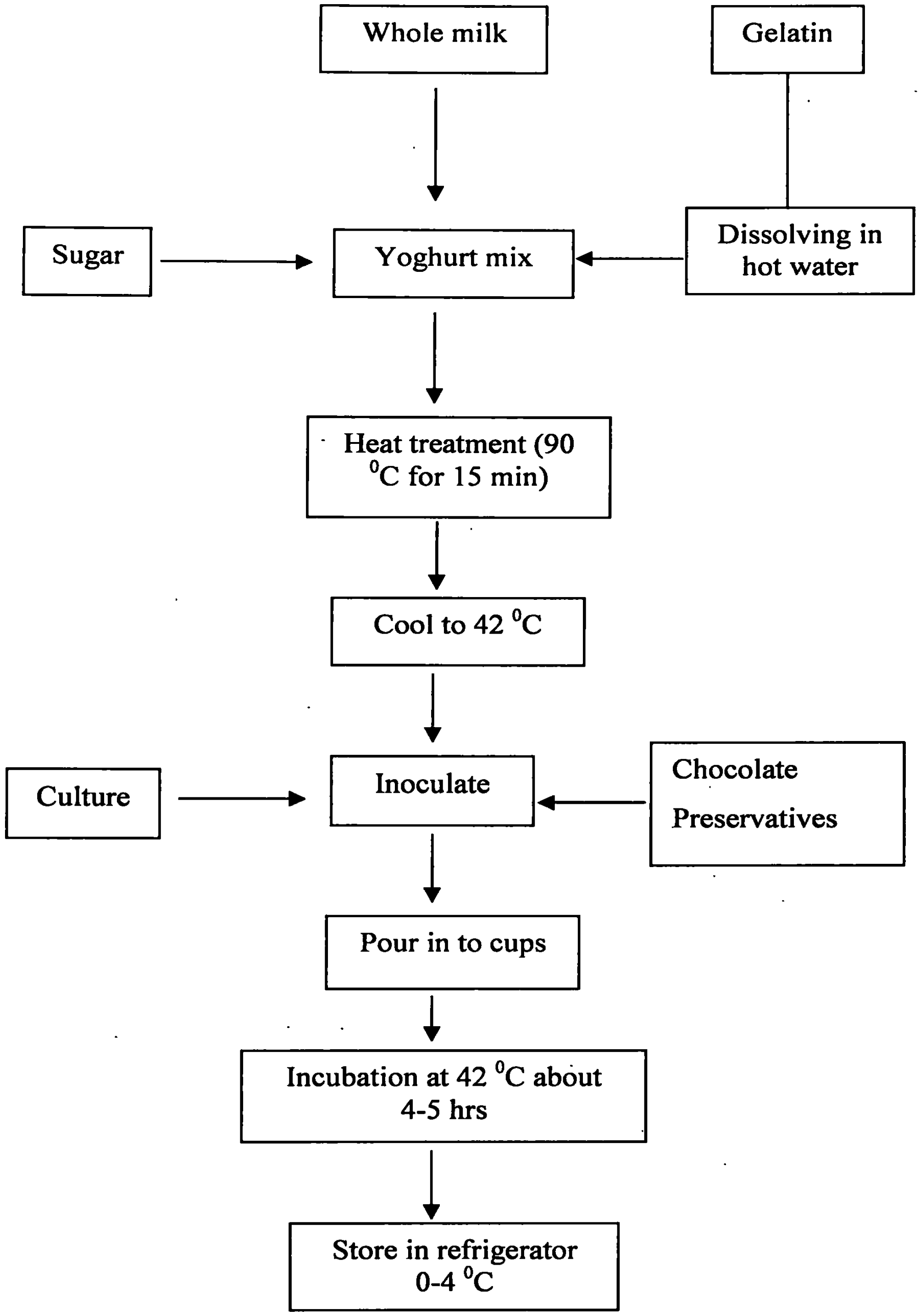

Figure 3.1 - Set yoghurt technology.

3.3 Formulation of stirred yoghurt incorporated with chocolate

Materials

Digital pH meter (pH scan2, Singapore)
Thermometer
Incubator
Heater
Raw milk
Refrigerator
Starter culture
Liquid chocolate
Sugar (White Refined)
Preservatives
Gelatin (200 bloom)

Method

In this process there was not any cream separation and homogenization. The raw milk was dissolved with sugar. Gelatin was dissolved in warm water (at 60 ^{0}C) and it was added while stirring. The mixture was heated at 90 ^{0}C for 15 min, while stirring. The mixture was cooled to about 42^{0}C and well mixed fresh culture and preservatives were added and uniformly mixed. Then the mixture was poured into the bulk container. The bulk container yoghurt was incubated at 42 ^{0}C for 3-4 hours in incubator. After it was getting set, it was transferred to room temperature condition. Then the gel was broken by stirring and liquid chocolate was added to it. Then the mixture was poured into the clean sterile yoghurt cups and transferred to cold storage.

3.3.1 Formulation of recipes

Following proportions was separated to formulate the chocolate incorporated stirred yoghurt.

Table 3.2 Four recipes that were used to prepare final stirred yoghurt which gained for sensory analysis

Ingredients	Sample code 174	Sample code 910	Sample code 526	Sample code 839
Milk	1000 ml	1000ml	1000 ml	1000 ml
Sugar	125 g	125 g	125 g	125 g
Chocolate	200 g	150 g	100 g	50 g
Gelatin	10g	10 g	10 g	10 g
Culture	80 g	80 g	80 g	80 g
Yog. : Choc. %	80 : 20	85 : 15	90 : 10	95 : 05

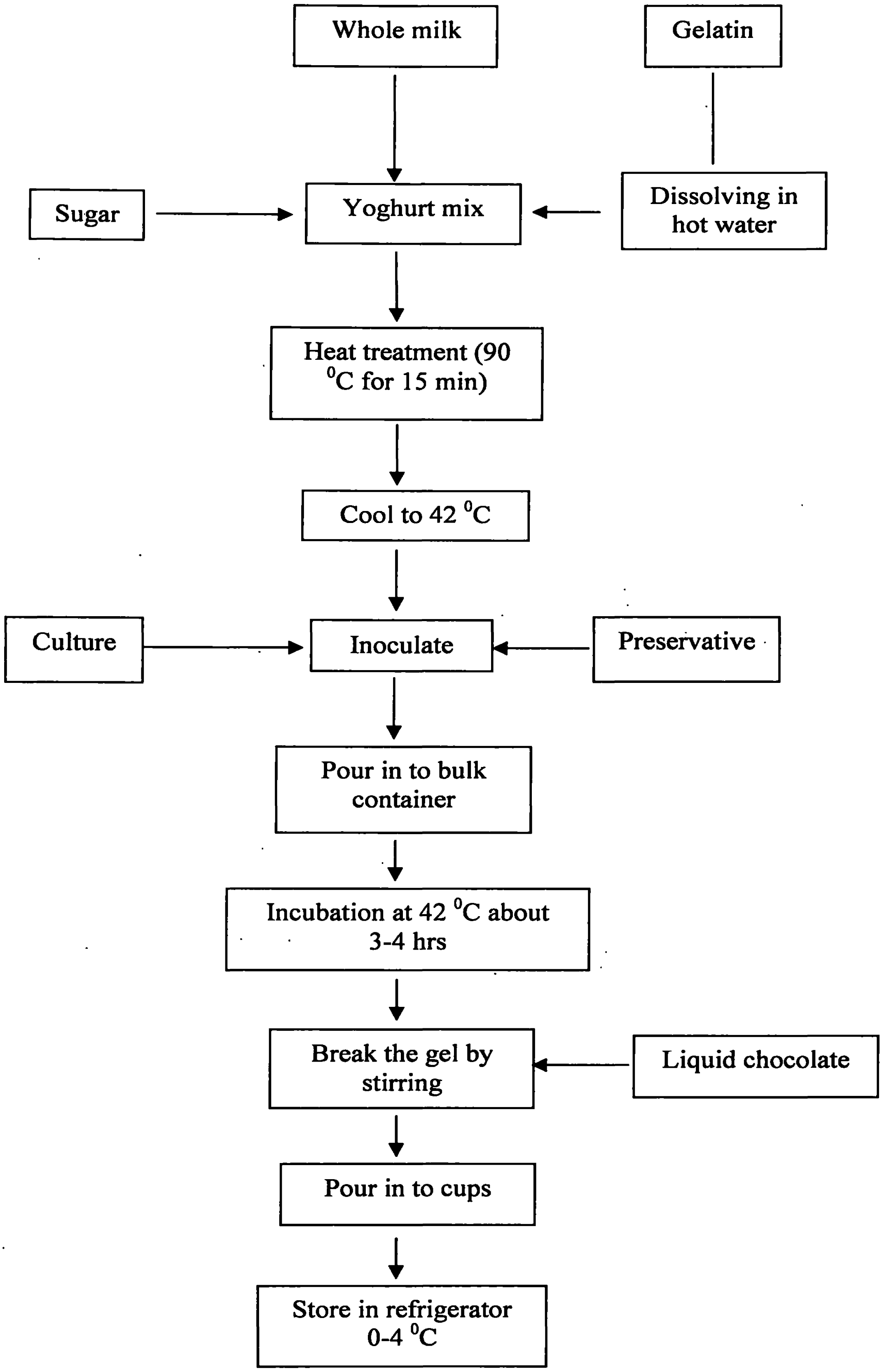

Figure 3.2 - Stirred yoghurt technology.

Sampling:

150 samples of yoghurt for each chocolate percentage levels were collected randomly with sterilized conditions and analyzed for physico-chemical, microbiological and organoleptic characteristics.

3.4 Determination of chemical composition of both yoghurt types.

3.4.1 Total solid

Materials

Flat dish (25 mm Ht and 75mm diameter/ Noncorrodable)
Electric balance (accuracy of 0.1mg)
Drying oven (102°C)
Desiccators
Quartz sand or sea sand (passing through 500μm sieve and retained 180 μm sieve)

Method

25g of sand was placed in the dish and transfered it to the oven dry for about 2 hrs at 105°C. It was transfered to the desiccator and allowed to cool for room temperature. Weigh it to the nearest 0.1mg. (M_0)

3-4 g of yogurt was weighted in the dish (with dried sand) (M_1) and transfered it to the oven dry for about 2 hrs at 105°C. It was transferred to the desiccator and allow cooling to room temperature. Weigh it to the nearest 0.1mg. (M_2) (SLS 735 part 1, 1986)

M_0 = Wt of Crucible + Sand 25g

M_1= Wt of Crucible + Sand 25g +Yogurt

M_2= Wt of Crucible + Sand 25g + dried yogurt

$$\text{Total Solids} = \frac{M_2 - M_0 \times 100}{M_1 - M_0}$$

SNF = TS – Fat %

3.4.2 Fat

Materials

Milk pipette (10.98ml)
Butyrometer & Lock stopper
Lock key
Tilt measure (1ml & 10ml)
Protected stand,
Centrifuge (± 50 rev/min and vertical-loading type),
Water bath
Thermometer

Method

10ml of sulfuric acid was measured into butyrometer using a safety measure. Gently inverted the bottle containing the test sample transferred 10.98ml of it into the butyrometer using a milk pipette (prevent mixing with the acid).1ml of amyl alcohol was added to it. The butyrometer securely stoppered without disturbing the content and thoroughly mixed it. The butyrometer was placed in the centrifuger and operate it for 5 min and the butyrometer was placed in the water bath and take the reading (fat column). In sulfuric acid protein is completely dissolved. The fat content is expressed in grams of fat per 100g of yoghurt. (SLS 735 part 1, 1986)

3.4.3. Ash

Materials

Flat-bottomed dish of stainless steel or porcelain
Oven (100 ± 2^0 C)
Muffle furnace ($550 \pm 10\ ^0$C)
Electric balance (accuracy of 0.1mg)
Desiccators
Water bath

Sample preparation

Container was heated to 40 ^{0}C temperature in water bath for 30 minutes. Then the container was opened and transferred all material adhering to the lid. (Mixed thoroughly with a spatula)

Method

The dish was dried in the oven, cooled in the desicator and weighted. It was weighted to the nearest milligram, about 2g to 3g of the prepared sample in the above dish. Heated gently on a flame until the charring is complete. Then it was transferred to the muffle furnace and heated till a grey ash results. Cooled in the desiccator and weighted. Repeated the process of heating, cooling and weighing at 30 min. intervals until the difference between two successive weighings does not exceed 1mg. (SLS 735 part 8, 1990)

$$\text{Total ash, percent by mass} = \frac{M_2 - M_0 \times 100\%}{M_1 . M_0}$$

M_0 – is the mass, in grams, of empty dish
M_1 – is the mass, in grams, of dish with sample
M_2 – is the mass, in grams, of dish with ash.

3.4.4 Protein

Materials

Micro Kjeldhal digestion unit (Tecator 1007)
Distilling unit (Kjeltec system-Tetcator)
Micro Kjedhal digestion glass tubes
Burette (100ml)
Conical flasks (250ml)
Concentrated Sulfuric acid
Catalyst mixture (Kjeltabs)
40% Sodium hydroxide solution
4% boric acid
Mixed indicator (Bromocresol green/Methyl red)
0.1 N Hydrochloric acid

Method

3.4.4.1 Sample digestion

2g of sample was weighted and transferred in to kjeldahl digestion tube. 12 ml of conc. H_2SO_4 and catalyst mixture was added to it. The tube was revolved gently to wash down any particle adhering to the walls of the tube and placed on the digestion unit. It was positioned inside the safety flow. This unit was kept 45 min. in digestion unit under 400 ^{0}C. (until all the carbon has been oxidized and contents if the tubes is colorless or pale straw colour) The digestion tube was taken out and cooled for about 15 min. and 75 ml of tap water was added. Blank digestion was carried out without the sample.

3.4.4.2 Distillation

Distillation unit was set up.25 ml of 4% Boric acid was Pipetted and added 4 drops of the mixed indicator in to a clean conical flask and placed it under the condenser. The digestion tube was exposed to the steam. When sample got boiled, added 50ml of 40% NaOH and immediately closed the stopcock. Distilled for about 5 min. until no more gas formation inside the boric acid beaker where boric acid change its color from pink to green. (SLS 735 part 7, 1989)

3.4.4..3 Titration

The content of the receiver flask was titrated against 0.1 N Hidrocloric acid and recorded the required volume.

$$\% \text{ Nitrogen} = \frac{\text{Burette reading} * \text{Normality of HCl} * 14.01}{\text{Weight of food (g)} * 10}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} * \text{Conversion factor (6.38)}$$

3.4.5 Total Soluble Solid

Materials

Refractometer (Stago hand refractometer) (Brix range 0-32%)

Distilled water

Tissue paper

Glass rod

Method

Refractometer was properly cleaned and put the drop of distilled water into the surface. Then drop of well mixed sample was placed on to the surface of refractometer. The cover was closed and got the reading. Repeated the same procedure to the other samples too.

3.5 Determination of physical and chemical characteristics of yoghurts

3.5.1 Determination of pH

Materials

Digital pH meter (pH scan2, Singapore)
Spoon
Distilled water

Method

The pH meter was calibrated using pH 7 & 4 buffer solutions at 30°C according to instructions in the label of buffer solutions. The samples of the four percentage of chocolate incorporated (both set and stirred) were taken separately and mixed thoroughly by a spoon, and standard for 2-3 minutes at 29°C. Before getting the readings the pH probe was dipped in distilled water and then the pH of the yoghurt was measured by dipping the pH probe in the yoghurt. Readings were recorded.

3.5.2 Viscosity (Rotational viscometer):

Materials

Brookfield dial reading viscometer (RV type)
Yoghurt samples
Thermometer
Beaker (100 ml) 3.5 inches diameter

Method

The guard leg was mounted on the Viscometer. With no spindle on, the Viscometer was lowered by turning the black knob on the right side of the clamp and centred the viscometer over the test material. Trapping air bubbles were avoided under the disk spindles. For viscosity measurement, the most appropriate spindle chosen as number 4 with the speed of 20 rpm (Harte et al, 2006).

The spindle was immersed on a diagonal path across the surface of the fluid. The spindle was slowly dragged across the fluid surface and brought the spindle to the upright position. Then the spindle was threaded onto the Viscometer. Attaching the spindle correctly was very important. The Viscometer coupling screw was pushed up on gently, avoiding any side to side movements. It was held it securely while screwing the spindle on. The Viscometer was lowered and centred the spindle in the test material until the meniscus is in the middle of the immersion mark. The desired speed was selected. The Viscometer motor switch was turned to the ON position. Allowed time for the indicated reading to stabilize. A minimum of 5 revolutions was recommended before taking any reading. To take a reading, the clutch lever was depressed and held it in the down position. With the lever still depressed, moved the motor switch to the "Pause" or "Off" position. The dial position was adjusted, to allow the red pointer to appear in the viscometer window. The readings were recorded, indicated by the red pointer on the dial: this number was known as % torque. % torque reading was converted to viscosity in centipoise (cP), multiplied the dial reading by the appropriate factor for the spindle and speed in use. Switched off the motor to change the spindle and samples. Removed the spindle before cleaning.

Dial reading * Factor = Viscosity in cP (mPa*s)

The factor was read from the factor table given by the manual of viscometer.

3.5.3 Whey separation (Drainage Method)

Materials

Electric balance (accuracy of 0.1mg)
A sieve (1 mm^2)
Time watch

Method

A cup of set or stirred yoghurt was taken out from the cold room. Approximately 30g of gel was cut in a single action by using a stainless steel ladle and the gel was weighed and drained on a 1 mm^2 pore size sieve for 15 min at room temperature. The way was weighed and the syneresis was expressed as the percent weight of the whey separated from the gel over the initial weight of the gel (Amatayakul *et al.*, 2006).

3.6 Sensory evaluation

Sensory analysis for sensory characteristics and overall acceptability of the yoghurt samples were carried out using a panel of judges selected from Lucky Lanka Dairies (pvt) Ltd using nine-point hedonic scale.

Materials

Yoghurt samples
Ballot papers
Pens
Spoons

Method

The yoghurt samples, which were incorporated chocolate in four percentage (both set and stirred) were subjected to sensory evaluation. The sensory evaluation was done by non-expertise 30 panelists with use of ballot papers. Each panelist was apart invisible to each other. The sensory evaluation was carried out by the panelists in the morning, in order to avoid intensity of influence of external sensory forces. Four yoghurt samples were presented in four identical containers to the panelist. The sample containers were coded with 3 digit random numbers.

The code numbers were:

174 = 20% chocolate + 80% yoghurt
910 = 15% chocolate + 85% yoghurt
526 = 10% chocolate + 90% yoghurt
839 = 05% chocolate + 95% yoghurt
100% yoghurt as a control for set yoghurt samples

186 = 20% chocolate + 80% yoghurt
955 = 15% chocolate + 85% yoghurt
221 = 10% chocolate + 90% yoghurt
642 = 05% chocolate + 95% yoghurt
100% yoghurt as a control for stirred yoghurt samples

Four yoghurt samples representing each treatment combination were placed before the panelist alone with a ballot paper. Level of preference for each sensory attribute (colour, smell, appearance, texture, taste and overall acceptability) in all four samples was recorded according to a 9-point hedonic scale. The obtained results were analyzed by Minitab statistical analysis package with the using of Friedman test at 5% significance level.

3.7 Shelf life evaluation of the yoghurt

The microbiological analysis of samples was carried out for yeast, mould count and coliform count by using standard methods.

3.7.1 Coliform analysis by direct plating

Materials

Autoclave	Distilled water
Bunsen burner	MacConkey agar
Water bath	Ringer solution
Electric balance (accuracy of 0.1mg)	
Incubator	
Petridish (90-100mm)	
Delivery pipettes (1ml& 10ml)	
Spatula	

3.7.1.1 Medium preparation for coliform test

43 g of MacConkey agar was dissolved in 1 liter of distilled water and heated in a water bath with frequent stirring until boiling. Then it was cooled to 45°C after completely dissolved the agar and was sterilized using an autoclave (121°C) for two hours. Then after the medium was cooled to 45°C in a water bath.

Method

Coliform was identified by direct plating method.1ml of yoghurt from each sample was transferred into McCarthy bottle (10^{-1} dilution), which contain 9 ml of sterilized ringer solution, and shaken well. 1ml of this mixture was blown out by a pipette on to a sterile petri dish and about 15 ml of MacConkey agar at 45°C was poured into each petri dish and the contents was mixed by applying three circular movements in clockwise manner as well as anti clock wise manner. Then agar was allowed for solidifying at room temperature for 2-3 minutes in a sterilized condition. The plates were then incubated in an inverted position, aerobically **36±1°C** for 24 hours. Then colonies were counted using colony counter.

This testing were done close to the bunson flame to achieve sterile air condition. This procedure was also done for blank and duplicate samples. (SLS 516 Part 1)

3.7.2 Yeast and mold counting method

Materials

Autoclave
Bunsen burner
Electric balance
Incubator
Petri dish
Pipettes (1ml)
Spatula
Water bath
Ringer solution
Distilled water
Yeast and Mould agar

3.7.2.1 Medium preparation for yeast and mould test

54g of Yeast and Mould Agar was dissolved in 1 liter of distilled water and heated in a water bath with frequent stirring until boiling. Then it was cooled to 45°C after completely dissolved the agar and was sterilized using an autoclave (121°C) for two hours. Then after the medium was cooled to 45°C in a water bath.

Method

Yeast and mould were identified by direct plating method. 1 ml of yoghurt from each sample was transferred into McCarthy bottle (10^{-1} dilution), which contain 9 ml of sterilized ringer solution and shaken well. 1ml of the diluted yoghurt sample and 15 ml of Yeast and Mould Agar at 45°C were transferred onto a sterile Petri dish and the contents were mixed by applying three circular movements in clockwise manner as well as anti clockwise manner. Then agar was allowed for settle at room temperature for 3-4 minutes in a sterilized condition. The plates were then incubated in an inverted position, aerobically in 25±1°C temperature for 4 days. Then colonies were counted using colony counter.

This testing were done close to the Bunsen flame to achieve sterile air condition. This procedure was also done for blank and duplicate samples. (SLS 516 Part 2)

CHAPTER 04

RESULTS AND DISCUSSION

4.1 Formulation of chocolate layered set yoghurt

After adding liquid chocolate to the milk, it was separated as a layer on the top of yoghurt gel and some amount of chocolate were incorporated in the yoghurt gel. Small amount of chocolate were deposited bottom of yoghurt cup. So there were three layers of chocolate since the density of chocolate compounds.

4.2 Formulation of chocolate incorporated stirred yoghurt

After adding liquid chocolate to the stirred gel of yoghurt, it was incorporated all over the yoghurt and given better appearance and taste too.

4.3 Chemical composition of both yoghurt types

The chemical composition of the chocolate layered set yoghurt samples is shown in Table 4.1.Total solid contents, fat and total soluble solid values increased with the addition of chocolate. Minimum values were achieved by the control samples that were not added the chocolate. Maximum values were achieved by the 20% of chocolate added yoghurt samples. There were not changeable of protein and ash values according to the incorporated chocolate percentage.

Table 4.1 Chemical composition of experimental set yoghurt samples.

Samples	Total solid	Fat (%)	Protein (%)	Brix value	Ash
Control	24.79	3.2	3.180	11.2	0.648
5%	25.68	4.8	3.414	14.8	0.874
10%	27.16	5.5	3.347	15.2	0.711
15%	32.35	6.2	3.342	18.0	0.671
20%	33.77	7.4	3.183	20.5	0.814

The chemical composition of the chocolate incorporated stirred yoghurt test sample is shown in Table 4.2. Total solid content, fat and total soluble solid values increased with the addition of liquid chocolate. Minimum values were achieved by the control samples that were not added the chocolate. Maximum values were achieved by the 20% of chocolate added yoghurt samples. There were not changeable of protein and ash values according to the incorporated chocolate percentage.

Table 4.2 Chemical composition of experimental stirred yoghurt samples.

Samples	Total solid	Fat (%)	Protein (%)	Brix value	Ash
Control	23.89	3.1	3.122	12.2	0.667
5%	25.71	4.7	3.333	14.0	0.614
10%	26.91	5.5	3.421	17.5	0.567
15%	32.11	6.1	3.212	18.5	0.663
20%	33.81	7.5	3.244	19.0	0.746

4.3.1 Variation of total solid with chocolate percentage

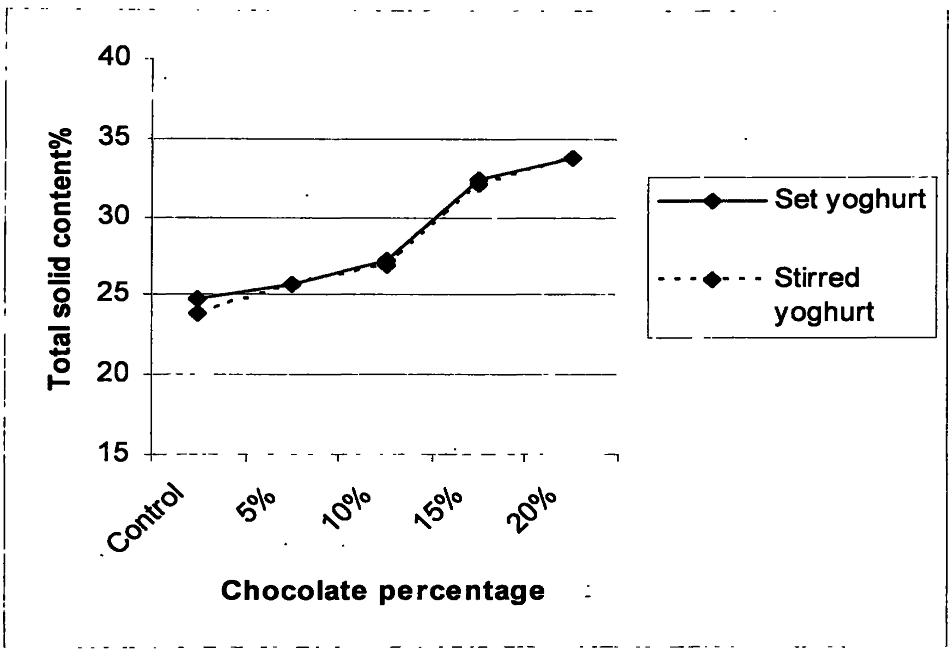

Figure 4.1 Changeability of total solid according to chocolate percentage

The figure 4.1 shows that the changeability of total solid according to chocolate percentage in both set and stirred yoghurt. The total solid increase with the increase of chocolate percentage in both set and stirred yoghurt. The data pattern is also very similar to each other in both products. Since the chocolate having the high amount of solid compounds cause to the increase of total solid level in both yoghurt products.

4.3.2 Variation of fat content with chocolate percentage

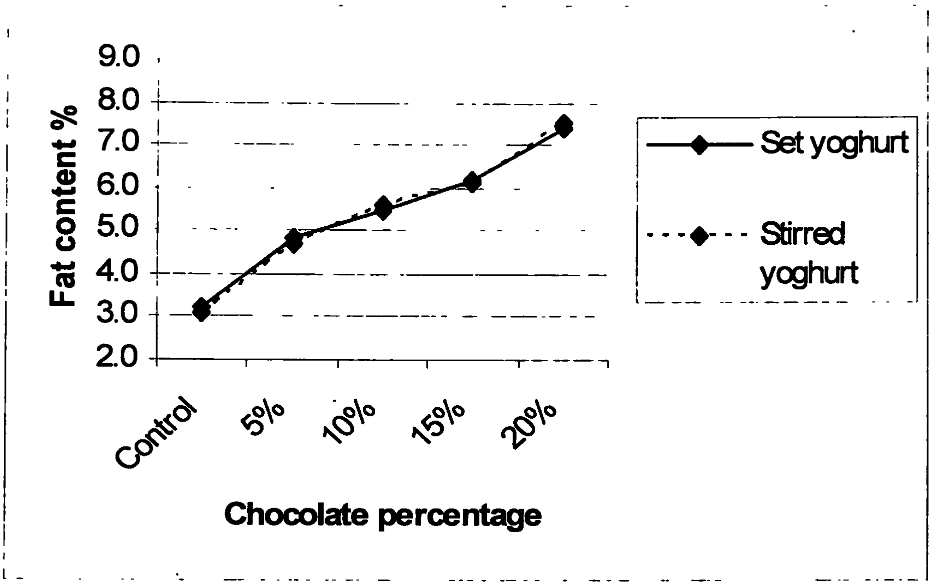

Figure 4.2 Changeability of fat content according to chocolate percentage

The figure 4.2 shows that the changeability of fat according to chocolate percentage in both set and stirred yoghurt. The fat level increase with the increase of chocolate percentage in both set and stirred yoghurt. The data pattern is also very similar to each other in both product types. Since the chocolate having the high amount of fat content cause to the increase of fat level in both yoghurt products with the increase of chocolate amount.

4.3.3 Variation of protein content with chocolate percentage

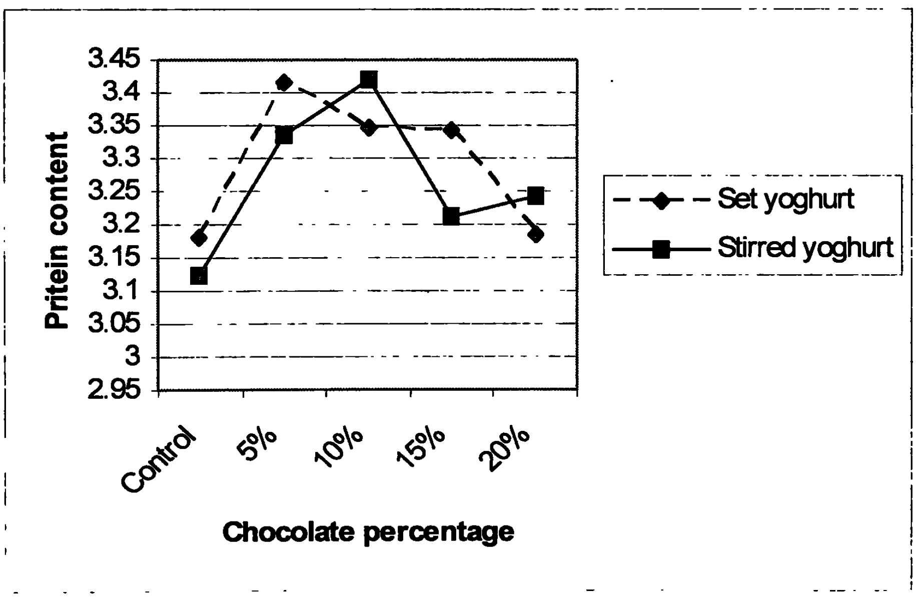

Figure 4.3 Changeability of protein content according to chocolate percentage

The Figure 4.3 shows that the changeability of protein, according to chocolate percentage in both set and stirred yoghurt. There is no increase or decrease of protein amount with the increase of chocolate percentage. The data patterns are irregular.

4.3.4 Variation of total soluble solid content with chocolate percentage

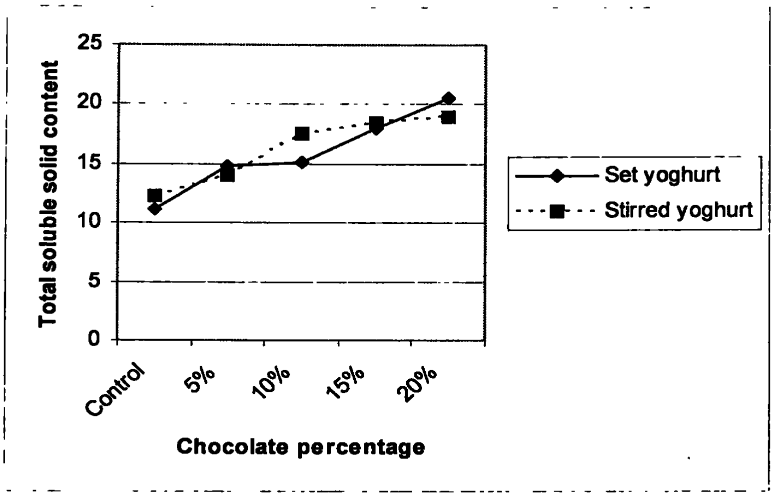

Figure 4.4 Changeability of total soluble solid according to chocolate percentage

The figure 4.4 shows that the changeability of total soluble solid according to chocolate percentage in both set and stirred yoghurt. The total soluble solid level increase with the increase of chocolate percentage in both set and stirred yoghurt. The data pattern is similar to each other in both product types. Since the chocolate having the high amount of sugars, minerals and other compounds cause to the increase of total soluble solid level in both yoghurt products with the increase of chocolate amount.

4.3.5 Variation of ash content with chocolate percentage

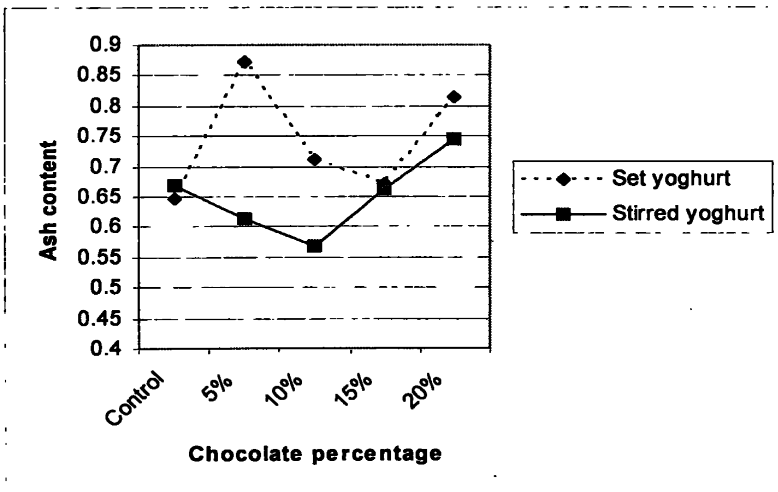

Figure 4.5 Changeability of ash content according to chocolate percentage

The Figure 4.5 shows that the changeability of ash, according to chocolate percentage in both set and stirred yoghurt. There is no increase or decrease of ash amount with the increase of chocolate percentage. The data patterns are irregular.

4.4 Physical and Chemical characteristics of yoghurts

4.4.1 pH value changes of the set yoghurt samples during the storage

The obtained results are summarized in Table 4.3

Table 4.3 pH changes during the storage of set yoghurt that was incorporated with different percentage of chocolate (measured by weekly).

Days	Samples				
	Control	5%	10%	15%	20%
1	4.38	4.51	4.63	4.84	4.88
7	4.17	4.43	4.48	4.51	4.54
14	4.13	4.36	4.46	4.47	4.49
21	4.10	4.38	4.43	4.47	4.47
28	4.11	4.31	4.42	4.34	4.56

4.4.2 Variation of pH in set yoghurt with time

Changes in pH with time at all chocolate percentage levels in storage are shown in below.

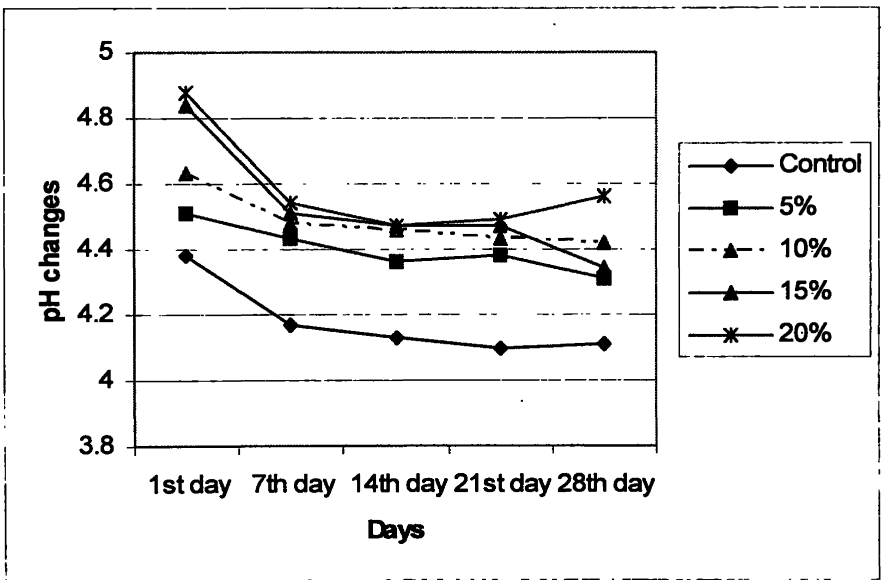

Figure 4.6 Changeability of pH with the time in set yoghurts

4.4.3 pH value changes of the stirred yoghurt samples during the storage

The obtained results are summarized in Table 4.4

Table 4.4 pH changes during the storage of stirred yoghurt that was incorporated different percentage of chocolate (measured by weekly).

Days	Samples				
	Control	5%	10%	15%	20%
1	4.32	4.76	4.88	5.02	5.07
7	4.15	4.71	4.79	4.91	4.93
14	4.10	4.69	4.77	4.88	4.84
21	4.12	4.58	4.68	4.79	4.79
28	4.10	4.69	4.66	4.79	4.81

4.4.4 Variation of pH in stirred yoghurt with time

Changes in pH with time at all chocolate percentage levels in storage are shown in below.

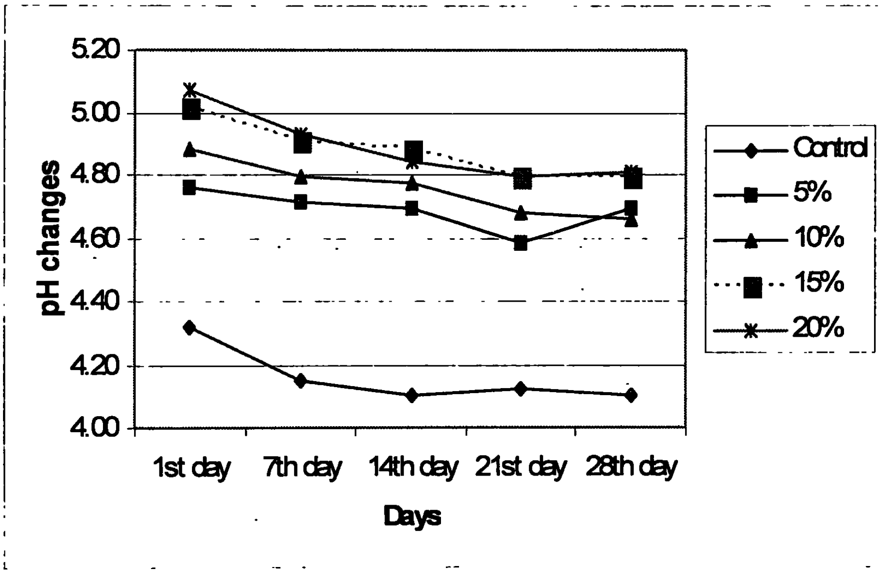

Figure 4.7 Changeability of pH with the time in stirred yoghurts

The pH values of set and stirred yoghurt at all chocolate percentage levels are summarized in Table 4.3 and Table 4.4. According to this data, pH increase with the increase of chocolate percentage that is incorporated to both yoghurt types. Minimum values are achieved by the control sample that was not added the chocolate. Maximum values are achieved by the 20% of chocolate added yoghurt samples. Chocolate compounds cause to the increase of pH values. pH values are some what high in stirred yoghurt than set yoghurt because chocolate are incorporated all over the yoghurt gel not as a layers. The activity of culture organisms and metabolic rate are slow down through the storage period. But there are some activity and make lactic acid, cause to the reduction of pH in all yoghurt types with the time being.

4.4.5 Variation of viscosity and whey separation in set and stirred yoghurt with chocolate percentage

Viscosity and whey separation of the chocolate layered set yoghurt and chocolate incorporated stirred yoghurt test samples are shown in Table 4.5 and Table 4.6.

Table 4.5 Viscosity and whey separation in set yoghurts

Samples	Viscosity	Whey separation
Control	2333	2.48
5%	2416	2.51
10%	2483	7.18
15%	3433	7.28
20%	3483	14.46

Table 4.6 Viscosity and whey separation in stirred yoghurts

Samples	Viscosity	Whey separation
Control	1633	3.44
5%	1683	6.41
10%	1916	7.45
15%	2383	7.22
20%	2800	6.11

4.4.5.1 Variation of viscosity in set and stirred yoghurts with chocolate percentage

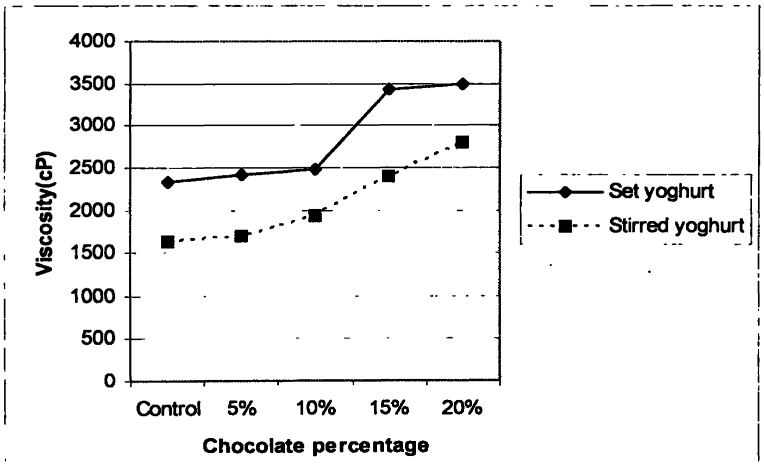

Figure 4.8 Changeability of viscosity according to chocolate percentage

This figure 4.8 shows that the viscosity increase with the increase of chocolate percentage in both set and stirred yoghurt. When increase the chocolate percentage cause to the increase of total solid content and effect for the increase of gel strength. The viscosity of set yoghurt is higher than the stirred yoghurt because stirred yoghurt process contains the gel braking/gel striking part. Liquid chocolate is a high viscosity fluid and cause to increase of viscosity of stirred yoghurt with the increase of chocolate percentage of the yoghurt.

4.4.5.2 Variation of whey in set and stirred yoghurt with chocolate percentage

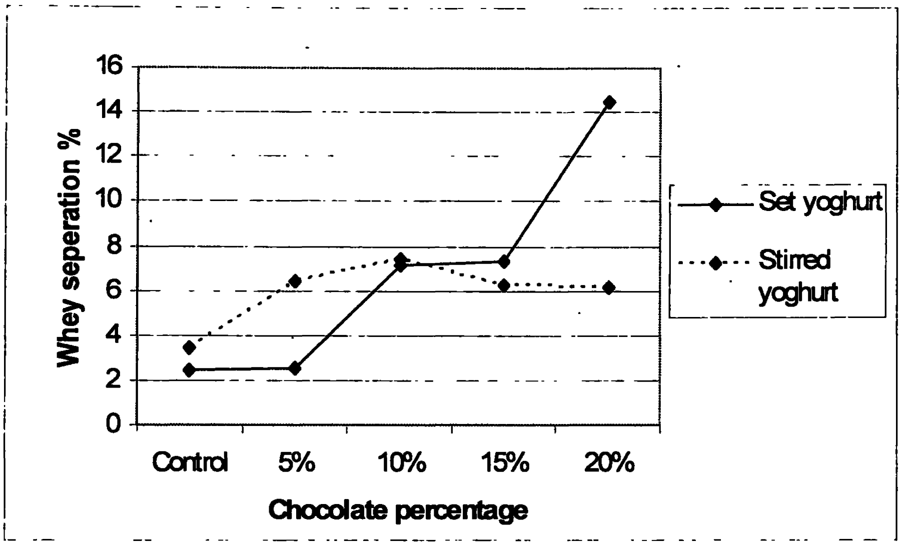

Figure 4.9 Changeability of whey separation according to chocolate percentage

This figure 4.9 shows that the changeability of whey separation of set and stirred yoghurt with the increase of chocolate percentage. Whey separation of set yoghurt, increase with the increase of chocolate percentage. But there is no clear pattern of whey separation data with the increase of incorporated chocolate percentage in stirred yoghurt.

4.5 Determination of microbiological quality of both set and stirred yoghurt with the different chocolate percentage.

4.5.1 Results of the coliform test of chocolate layered set yoghurt samples.

Coliform count data of the chocolate layered set yoghurt test samples are shown in Table 4.7.

Table 4.7- Coliform counts for set yoghurt samples with the increase of chocolate percentage.

Days	Samples				
	Control	5%	10%	15%	20%
1	2	1	2	5	4
7	0	2	3	1	2
14	0	0	0	2	0
21	0	1	0	0	0
28	0	0	2	1	0

4.5.2 Coliform growth with time in different chocolate added set yoghurt

Diagrammatic representation of changes in growth of coliform with time at all chocolate percentages in storage is shown in Figure 4.10.

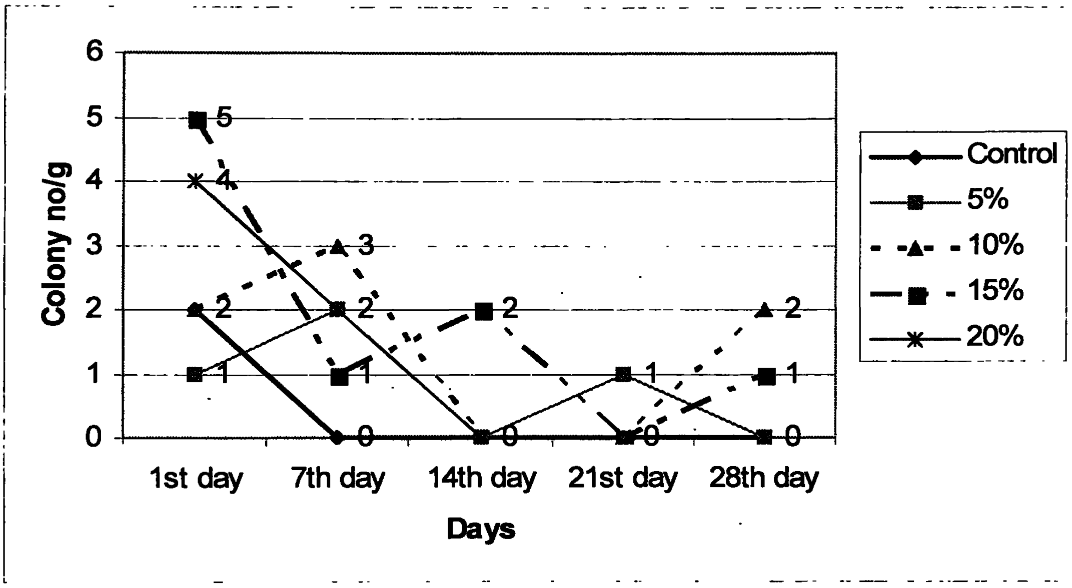

Figure 4.10 Coliform growths with the time in set yoghurt samples.

According to the above results, there was no increasing or decreasing of Coli form count with the increase of chocolate percentage. With the time being there was no increment of Coli form count. Preservatives and pH reduction affected to the growth of Coli forms and controlled the increment of Coliform colony counts.

4.5.3 Results of the coliform test of chocolate incorporated stirred yoghurt samples.

Coli form count data of the chocolate incorporated stirred yoghurt test samples are shown in Table 4.8.

Table 4.8 - Coliform counts for stirred yoghurt samples with the increase of chocolate percentage.

Days	Samples				
	Control	5%	10%	15%	20%
1	6	8	5	7	9
7	5	7	6	11	6
14	4	3	5	9	10
21	4	7	3	6	2
28	5	2	3	6	4

4.5.4 Coliform growth with time in different chocolate added stirred yoghurt

Diagrammatic representation of changes in growth of coliform with time at all chocolate percentages in storage is shown in Figure 4.11.

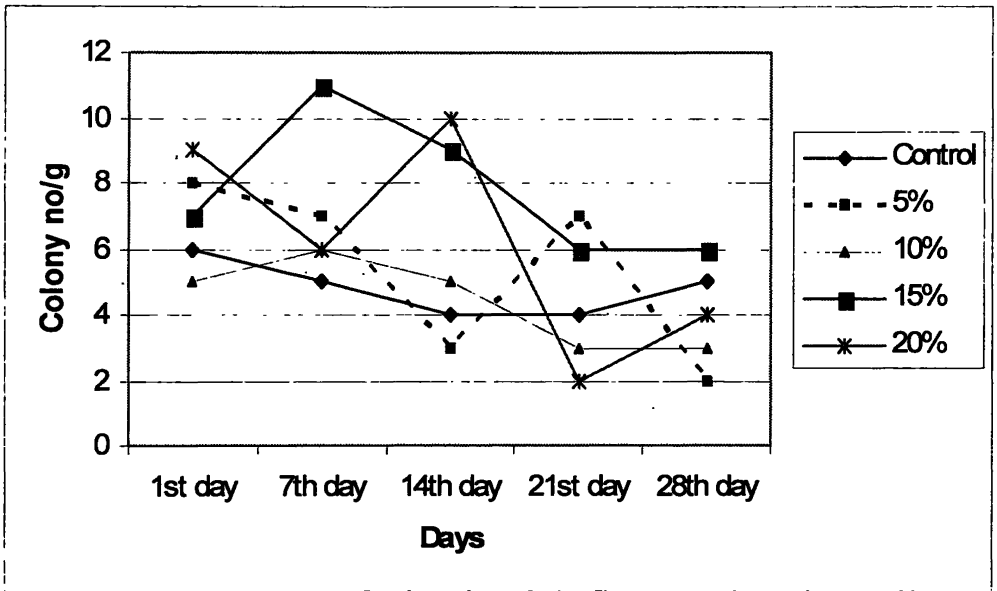

Figure 4.11 Coli form growth with time in stirred yoghurt samples.

According to the above results, there was no increasing or decreasing of Coli form count with the increase of chocolate percentage. With the time being there was no increment of Coli form count too. Preservatives and pH reduction affected to the growth of Coli forms and controlled the increment of Coliform colony counts.

Coliform counts were somewhat high in stirred yoghurt than set yoghurt. Cross contaminations specially in stirring process affected to give those results. But Coliform count was not show any relationship with the time and chocolate percentage in both set and stirred yoghurt samples.

4.5.5 Results of the yeast and mould test of set and stirred yoghurt samples

There were no yeast and mould count in both set and stirred yoghurt samples. Specially added preservatives (Sorbates) affected to the elimination of yeast and moulds in yoghurt samples.

4.6 Sensory analysis for the selection of best chocolate percentage for each characteristic in set yoghurt samples

The sensory evaluation was carried out using the same human subject serving for four samples at the same time and thus the data generated were dependent 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 those attribute, 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 summarize as appeared in the following table (table 4.9).

Table 4.9 Evaluation of sensory appeal to determine the best formulation in set yoghurt.

Sensory Attribute	P-Value	Average Ranks				Best Sample
		174	526	839	910	
Colour	0.295	74.5	77.5	66.5	81.5	....
Appearance	0.287	71.5	78.5	68.0	82.0	....
Smell	0.095	69.5	76.5	68.0	86.0	....
Texture	0.000	41.0	105.5	96.5	57.0	526
Taste	0.000	85.0	65.5	51.0	98.5	910
Overall Acceptability	0.000	45.0	99.0	93.0	63.0	526

When performing Friedman test for set yoghurt samples (174, 526, 839 and 910), the p-value (0.295) for colour indicates that there is sufficient evidence that all the treatment medians are equal when alpha is set at 0.05. This implies that there is no statistically significant difference in colour.

When performing Friedman test for set yoghurt samples (174, 526, 839 and 910), the p-value (0.287) for appearance indicates that there is sufficient evidence that all the treatment medians are equal when alpha is set at 0.05. This implies that there is no statistically significant difference in appearance.

When performing Friedman test for set yoghurt samples (174, 526, 839 and 910), the p-value (0.295) for smell indicates that there is sufficient evidence that all the treatment medians are equal when alpha is set at 0.05. This implies that there is no statistically significant difference in smell.

When performing Friedman test for set yoghurt samples (174, 526, 839 and 910), the p-value (0.000) for texture indicates that there is sufficient evidence that not all the treatment medians are equal when alpha is set at 0.05. This implies that there is a statistically significant difference in texture. Since sample code 526 shows the highest average rank (105.5) it could be considered as the best one out of these four. So 10% chocolate added set yoghurt was selected as the best one for texture.

When performing Friedman test for set yoghurt samples (174, 526, 839 and 910), the p-value (0.000) for taste indicates that there is sufficient evidence that not all the treatment medians are equal when alpha is set at 0.05. This implies that there is a statistically significant difference in taste. Since sample code 910 shows the highest average rank (98.5) it could be considered as the best one out of these four. So 15% chocolate added set yoghurt was selected as the best one for taste.

When performing Friedman test for set yoghurt samples (174, 526, 839 and 910), the p-value (0.000) for overall acceptability indicates that there is sufficient evidence that not all the treatment medians are equal when alpha is set at 0.05. This implies that there is a statistically significant difference in overall acceptability. Since sample code 526 shows the highest average rank (99) it could be considered as the best one out of these four. So 10% chocolate added yoghurt was selected as the best one for overall acceptability.

The P-values obtained clearly emphasize that there is a statistically significant difference between samples coded as 174, 526, 839 and 910 only in texture, taste and overall acceptability. While other three attributes did not show statistically significant difference. Based on corresponding average ranks, it is possible to say that **sample code 526 (10%) is the best sample in texture attribute, sample code 910 (15%) has the best taste and sample code 526 (10%) is the best sample in overall acceptability** because they bear the highest average rank.

4.7 Sensory analysis for the selection of best chocolate percentage for each characteristic in stirred yoghurt samples.

The sensory evaluation was carried out using the same human subject serving for four samples at the same time and thus the data generated were dependent 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 those attribute, 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 summarize as appeared in the following table (table 4.10).

Table 4.10 Evaluation of sensory appeal to determine the best formulation in stirred yoghurt.

Sensory Attribute	P-Value	Average Rank				Best Sample
		186	221	642	955	
Colour	0.159	83.0	75.5	78.0	63.5	....
Appearance	0.339	80.5	69.5	69.5	80.5	....
Smell	0.002	89.0	83.5	57.0	70.5	186
Texture	0.080	80.5	60.5	81.5	77.5	....
Taste	0.000	87.5	66.5	49.0	97.0	955
Overall Acceptability	0.000	84.5	69.5	52.0	94.0	955

When performing Friedman test for stirred yoghurt samples (186, 221, 642 and 955), the p-value (0.159) for colour indicates that there is sufficient evidence that all the treatment medians are equal when alpha is set at 0.05.This implies that there is no statistically significant difference in colour.

When performing Friedman test for stirred yoghurt samples (186, 221, 642 and 955), the p-value (0.339) for appearance indicates that there is sufficient evidence that all the treatment medians are equal when alpha is set at 0.05. This implies that there is no statistically significant difference in appearance.

When performing Friedman test for stirred yoghurt samples (186, 221, 642 and 955), the p-value (0.002) for smell indicates that there is sufficient evidence that not all the treatment medians are equal when alpha is set at 0.05. This implies that there is a statistically significant difference in smell. Since sample code 186 shows the highest average rank (89.0) it could be considered as the best one out of these four. So 20% chocolate added stirred yoghurt was selected as the best one for smell.

When performing Friedman test for stirred yoghurt samples (186, 221, 642 and 955), the p-value (0.080) for texture indicates that there is sufficient evidence that all the treatment medians are equal when alpha is set at 0.05. This implies that there is no statistically significant difference in texture.

When performing Friedman test for stirred yoghurt samples (186, 221, 642 and 955), the p-value (0.000) for taste indicates that there is sufficient evidence that not all the treatment medians are equal when alpha is set at 0.05. This implies that there is a statistically significant difference in taste. Since sample code 955 shows the highest average rank (89.0) it could be considered as the best one out of these four. So 15% chocolate added stirred yoghurt was selected as the best one for taste.

When performing Friedman test for stirred yoghurt samples (186, 221, 642 and 955), the p-value (0.000) for overall acceptability indicates that there is sufficient evidence that not all the treatment medians are equal when alpha is set at 0.05. This implies that there is a statistically significant difference in overall acceptability. Since sample code 955 shows the highest average rank (94.0) it could be considered as the best one out of these four. So 15% chocolate added stirred yoghurt was selected as the best one for overall acceptability.

The P-values obtained clearly emphasize that there is a statistically significant difference between samples coded as 186, 221, 642 and 955 only in smell, taste and overall acceptability. While other three attributes did not show statistically significant difference. Based on corresponding average ranks, it is possible to say that **sample code 186 (20%) is the best sample in smell attribute, sample code 955 (15%) has the best taste and sample code 955 (15%) is the best sample in overall acceptability** because they bear the highest average rank.

CHAPTER 05

CONCLUSION AND FURTHER STUDIES

5.1. Conclusion

Fat, total soluble solids, total solids, viscosity, whey separation were increased in both set and stirred yoghurt with the increase of chocolate percentage. pH was increased in both set and stirred yoghurt with the increase of chocolate percentage. But with the time being pH was decreased in both yoghurt types. Viscosity level was high in set yoghurt than stirred yoghurt. On the other hand, amount of whey separation was high in stirred yoghurt than set yoghurt. According to the sensory analysis data, Colour, Appearance and Smell sensory attributes was not show any statistically significant difference ($P>0.05$) and Texture, Taste and Overall acceptability showed the significant difference in set yoghurt samples($P<0.05$). The best sample for Texture, Taste and Overall acceptability was 10%, 15% and 10% chocolate added set yoghurts. Colour, Appearance and Texture sensory attributes was not show any statistically significant difference ($P>0.05$) and Smell, Taste and Overall acceptability showed the significant difference in stirred yoghurt samples ($P<0.05$). The best sample for Smell, Taste and Overall acceptability was 20%, 15% and 15% chocolate added stirred yoghurts.
The best sample for the set yoghurt was 10% chocolate added product and the best sample for the stirred yoghurt was 15% chocolate added product. Both yoghurt types were microbiologically safe. Coliform, Yeast and mould content were under SLSI standard level. But Coliform count was somewhat high in stirred yoghurt than set yoghurt.

5.2. Further Studies

In special comments obtained by sensory evaluation, it is recommended to increase the sweetening character in the chocolate layer by choosing an alternative chocolate source or some other means. A market research for the target group has to be conducted to analyze the true consumer requirements and their preference on the chocolate incorporated set yoghurt. There is a need to investigate the effect of incorporated chocolate on the viable count of culture bacteria in the course of their post acidification and survival during the shelf life. A chemical study on rancidity development in the final product has to be carried out.

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APPENDIX I

Sensory Evaluation Data

Name: ……………………………………………………………………………

Date: ……………………………………………………………………………..

Product Description: Chocolate incorporated set yoghurt

You are kindly requested to asses each food sample presented, with reference to under mentioned sensory attributes according to your preference considering the following scale.

9-Like Extremely
8-Like very much
7-Like moderately
6-Like Slightly
5-Neither Like nor dislike
4-Dislike Slightly
3-Dislike Moderately
2-Dislike Very Much
1-Dislike Extremely

Sample Code	Color	Appearance	Smell	Texture	Taste	Overall acceptability
174						
526						
839						
910						

Comments:……………………………………………………………………………
……………………………………………………………………………………
……………………………………………………………………………………
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……………………………………………………………………………………
……………………………………………………………………………………
……………………………………………………………………………………

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APPENDIX II

Sensory Evaluation Data

Name: ....

Date: ....

Product Description: Chocolate incorporated stirred yoghurt

You are kindly requested to asses each food sample presented, with reference to under mentioned sensory attributes according to your preference considering the following scale.

9-Like Extremely
8-Like very much
7-Like moderately
6-Like Slightly
5-Neither Like nor dislike
4-Dislike Slightly
3-Dislike Moderately
2-Dislike Very Much
1-Dislike Extremely

Sample Code	Color	Appearance	Smell	Texture	Taste	Overall acceptability
186						
221						
642						
955						

Comments:..
....
....
....
....
....
....

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Appendix III
Friedman Test Results of Sensory Analysis

For set yoghurt

(1) Preference versus Sample no. blocked by Assessor no. for Colour

Test hypothesis:

Ho: All the treatment medians are equal according to the colour.
H1: At least one treatment median is different from others.

```
S = 4.11   DF = 3   P = 0.250
S = 5.18   DF = 3   P = 0.159 (adjusted for ties)
```

Decision rule:
Reject Ho if p value < α
α=0.05
P value = 0.159
So P value > α

Not Reject Ho
So there is no significant difference in colour comparing to others.

Sample	N	Est Median	Sum of Ranks
186	30	7.6250	83.0
221	30	7.1250	75.5
642	30	6.8750	78.0
955	30	6.8750	63.5

Grand median = 7.1250

(2) Preference versus Sample no. blocked by Assessor no. for Appearance

Test hypothesis:

Ho: All the treatment medians are equal according to the appearance.
H1: At least one treatment median is different from others.

```
S = 2.42   DF = 3   P = 0.490
S = 3.36   DF = 3   P = 0.339 (adjusted for ties)
```

Decision rule:
Reject Ho if p value < α
α=0.05
P value = 0.339
So P value > α

Not Reject Ho
So there is no significant difference in appearance comparing to others.

Sample	N	Est Median	Sum of Ranks
186	30	7.5000	80.5
221	30	7.2500	69.5
642	30	7.5000	69.5
955	30	7.7500	80.5

Grand median = 7.5000

(3) Preference versus Sample no. blocked by Assessor no. for Smell

Test hypothesis:

Ho: All the treatment medians are equal according to the smell.
H1: At least one treatment median is different from others.

```
S = 12.25  DF = 3  P = 0.007
S = 14.94  DF = 3  P = 0.002 (adjusted for ties)
```

Decision rule:
Reject: Ho if p value < α
α=0.05
P value = 0.002
So p value < 0.05

Reject Ho
So there is a significant difference in Smell comparing to all the samples.

Sample	N	Est Median	Sum of Ranks
186	30	6.2500	89.0
221	30	5.8750	83.5
642	30	5.2500	57.0
955	30	5.6250	70.5

Grand median = 5.7500

(4) Preference versus Sample no. blocked by Assessor no. for Texture

Test hypothesis:

Ho: All the treatment medians are equal according to the texture.
H1: At least one treatment median is different from others.

```
S = 5.78  DF = 3  P = 0.123
S = 6.75  DF = 3  P = 0.080 (adjusted for ties)
```

Decision rule:
Reject Ho if p value < α
α=0.05
P value = 0.080
So P value > α
Not Reject Ho
So there is no significant difference in texture comparing to others.

Sample	N	Est Median	Sum of Ranks
186	30	7.3125	80.5
221	30	6.6875	60.5
642	30	7.4375	81.5
955	30	7.3125	77.5

Grand median = 7.1875

(5) Preference versus Sample no. blocked by Assessor no. for Taste

Test hypothesis:

Ho: All the treatment medians are equal according to the taste.
H1: At least one treatment median is different from others.

```
S = 27.77  DF = 3  P = 0.000
S = 29.33  DF = 3  P = 0.000 (adjusted for ties)
```

Decision rule:
Reject: Ho if p value < α
α=0.05
P value = 0.000
So p value < 0.05

Reject Ho
So there is a significant difference in taste comparing to all the samples.

Sample	N	Est Median	Sum of Ranks
186	30	7.000	87.5
221	30	6.000	66.5
642	30	5.250	49.0
955	30	7.750	97.0

Grand median = 6.500

(6) Preference versus Sample no. blocked by Assessor no. for Overall acceptability

Test hypothesis:

Ho: All the treatment medians are equal according to the overall acceptability.
H1: At least one treatment median is different from others.

```
S = 20.21  DF = 3  P = 0.000
S = 21.35  DF = 3  P = 0.000 (adjusted for ties)
```

Decision rule:
Reject: Ho if p value < α
α=0.05
P value = 0.000
So p value < 0.05

Reject Ho
So there is a significant difference in Overall acceptability comparing to all the samples.

Sample	N	Est Median	Sum of Ranks
186	30	7.000	84.5
221	30	6.000	69.5
642	30	5.250	52.0
955	30	7.750	94.0

Grand median = 6.500

For stirred yoghurt

(1) Preference versus sample no. blocked by Assessor no. for Colour

Test hypothesis:

Ho: All the treatment medians are equal according to the colour.
H1: At least one treatment median is different from others

```
S = 2.42  DF = 3  P = 0.490
S = 3.70  DF = 3  P = 0.295 (adjusted for ties)
```

Decision rule:
Reject Ho if p value < α
$\alpha=0.05$
P value = 0.295
So P value > α

Not Reject Ho
So there is no significant difference in colour comparing to others.

Sample2	N	Est Median	Sum of Ranks
174	30	7.5000	74.5
526	30	7.5000	77.5
839	30	7.5000	66.5
910	30	7.5000	81.5

Grand median = 7.5000

(2) Preference versus Sample no blocked by Assessor no. for Appearance

Test hypothesis:

Ho: All the treatment medians are equal according to the appearance.
H1: At least one treatment median is different from others.

```
S = 2.45  DF = 3  P = 0.484
S = 3.77  DF = 3  P = 0.287 (adjusted for ties)
```

Decision rule:
Reject Ho if p value < α
α=0.05
P value = 0.287
So P value > α

Not Reject Ho
So there is no significant difference in appearance comparing to others.

Sample2	N	Est Median	Sum of Ranks
174	30	7.5000	71.5
526	30	7.5000	78.5
839	30	7.5000	68.0
910	30	7.5000	82.0

Grand median = 7.5000

(3) Preference versus Sample no. blocked by Assessor no. for Smell

Test hypothesis:

Ho: All the treatment medians are equal according to the smell.
H1: At least one treatment median is different from others.

```
S = 4.05  DF = 3  P = 0.256
S = 6.36  DF = 3  P = 0.095 (adjusted for ties)
```

Decision rule:
Reject Ho if p value < α
α=0.05
P value = 0.095
So P value > α
Not Reject Ho
So there is no significant difference in smell comparing to others.

Sample2	N	Est Median	Sum of Ranks
174	30	7.5000	69.5
526	30	7.5000	76.5
839	30	7.5000	68.0
910	30	7.5000	86.0

Grand median = 7.5000

(4) Preference versus Sample no. blocked by Assessor no. for Texture

Test hypothesis:

Ho: All the treatment medians are equal according to the texture.
H1: At least one treatment median is different from others.

```
S = 57.45  DF = 3  P = 0.000
S = 60.47  DF = 3  P = 0.000 (adjusted for ties)
```

Decision rule:
Reject: Ho if p value < α
α=0.05

P value = 0.000
So p value < 0.05

Reject Ho
So there is a significant difference in texture comparing to all the samples.

Sample2	N	Est Median	Sum of Ranks
174	30	4.375	41.0
526	30	7.500	105.5
839	30	6.875	96.5
910	30	5.250	57.0

Grand median = 6.000

(5) Preference versus Sample no. blocked by Assessor no. for Taste

Test hypothesis:

Ho: All the treatment medians are equal according to the taste.
H1: At least one treatment median is different from others.

```
S = 26.37  DF = 3  P = 0.000
S = 28.56  DF = 3  P = 0.000 (adjusted for ties)
```

Decision rule:
Reject: Ho if p value < α
α=0.05
P value = 0.000
So p value < 0.05

Reject Ho
So there is a significant difference in Texture comparing to all the samples.

Sample2	N	Est Median	Sum of Ranks
174	30	6.719	85.0
526	30	5.844	65.5
839	30	4.969	51.0
910	30	7.344	98.5

Grand median = 6.219

(6) Preference versus Sample no blocked by Assessor no. for Overall acceptability

Test hypothesis:

Ho: All the treatment medians are equal according to the overall acceptability.
H1: At least one treatment median is different from others.

```
S = 38.88  DF = 3  P = 0.000
S = 41.36  DF = 3  P = 0.000 (adjusted for ties)
```

Decision rule:
Reject: Ho if p value < α

$\alpha=0.05$
P value = 0.000
So p value < 0.05

Reject Ho
So there is a significant difference in overall acceptability comparing to all the samples.

Sample2	N	Est Median	Sum of Ranks
174	30	4.875	45.0
526	30	7.250	99.0
839	30	7.000	93.0
910	30	5.375	63.0

Grand median = 6.125

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