SHELF LIFE EVALUATION OF NON-FAT YOGHURT AT ACCELERATED STORAGE CONDITIONS

BY

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DECLARATION

The work described in this thesis was carried out by me at Leading Dairy Company and Faculty of Applied Sciences under the supervision of Miss. I.Amarasekara and Mrs. P.S.Perera. A report of this has not submitted to any other university for another degree.

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AFFECTIONATELY DEDICATED

TO

MY BELOVED PARENTS AND TEACHERS

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ABSTRACT

Yoghurt is custard like cultured milk made by fermentation, slightly concentrated whole or skimmed milk with a mixed bacterial culture. It is a popular dairy product in the world as well as in Sri Lanka.

This study was carried out to detect the quality changes of non-fat yoghurt and the shelf life of the non-fat yoghurt at different storage conditions. Therefore a complete study on the physical, chemical and microbiological properties of non-fat yoghurt was carried out at the above conditions.

Firstly, the well-incubated yoghurt samples were kept at four different storage temperatures 3°C, 8°C, 20°C and 30°C for 28 days. The samples that were in different temperatures were tested using standard physical, chemical and biological methods at different time intervals. The chemical analysis conducted on samples showed that quick acidity development were shown and decrease in pH at higher temperature. Coliform test and yeast mould test were carried out to detect microbial deterioration. No coliform colonies were observed after one week of storage period at all temperatures due to the development of low pH condition. The results of yeast and mould test showed that colonies were uncountable on PDA media at 30°C and 20°C. As the growth rate of yeasts and mould was increased with decreasing pH. The samples stored at 3°C showed little microbial growth throughout the storage period.

The sensory evaluation was carried out to determine the organoleptic properties (colour, odour, appearance, texture and overall acceptability) of yoghurt. After analyzing the results obtained, statistically, it was found that the organoleptic characteristics were preferable at 3°C temperature conditions. It has a longer shelf life (25) than other storage conditions. However the organoleptic characteristics of yoghurt stored at 8°C also did not changed until 16 days of storage period. According to the results it can be concluded that the physical properties and the sensory characteristics of non-fat yoghurt at 3°C shows good eating quality and further it is microbiologically acceptable.

CONTENT

ABBREVIATIONS

C^{o} = Degree Centigrade

UHT = Ultra Heat Treatment

USA = United State America

HTST = High Temperature Short Time

SNF = Solid Not Fat

LAB = Lactic Acid Bacteria

/g = Per gram

g = Gram

NaOH = Sodium hydroxide

l = Liter

ml = Milliliter

N = Normality

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CHAPTER 01

INTRODUCTION

1.1 INTRODUCTION

Milk is the lacteal secretion, practically free from colostrums, obtained by the complete milking of one or more healthy cows. It is a natural liquid food containing a high percentage of water, and also it is a concentrated food designed to produce rapid growth in young mammals and contains more solid materials. However the high moisture content, abundant supply of nutrients together with the almost neutral acidity (p^H 6.7) and temperature of raw milk makes it practically prone to spoilage by microorganisms (Fox, 1995).

Therefore to extend the shelf life of raw milk much beyond a few days at ambient storage conditions, it must be converted to other forms. A range of ways are available to preserve milk by converting it into value added products such as yoghurt, butter, ice cream, cheese, etc.

Yoghurt is one of the cultured (fermented) milk products and the manufacturing process involves the controlled use of lactic acid producing microorganisms for prescribed time and temperature combinations to achieve a final product with the described characteristics of viscosity, texture, mouth feel, flavour and acidity. The most commonly used starter microorganisms are the members of the group of lactic acid bacteria. Two main types of bacteria commonly used in manufacturing of yoghurt are *Streptococcus thermophillus* and *Lactobacillus bulgaricus.* Yoghurt is sold as plain yoghurt, fruit yoghurt, drinking yoghurt, low fat yoghurt, non-fat yoghurt, etc.

Non-fat yoghurt is healthy food for adult people and for people who are suffering fromdiabetes, as it does not contain sugars and flavours and produced from non-fat milk powder or skimmed raw milk. Also this type of product is suitable for people with 'Hyperlipidaemia' and 'Cardiovascular Disease', and also which give more advantages to the man, as a treatment of patients who are suffering from 'Lactose Intolerance' (Fox, 1995).

But yoghurt has a relatively shorter shelf life compared to other cultured milk products. So this study is mainly focused on to detect microbiological, chemical and physical changes, which affect the final quality, by using sensory analysis and shelf life evaluation methods.

1.2 OBJECTIVES

1.2.1 Overall objective

- Monitoring keeping quality of non-fat yoghurt at accelerated storage conditions.

1.2.2 Specific objectives

- Determination of threshold levels and consumer acceptance in quality of non-fat yoghurt.
- Development of chemical analysis for standardized identification of sensory defects.
- Determines changes in selected quality characteristics over a period of time.
- Determination of best storage temperature, which influence long shelf life for non-fat yoghurt.

CHAPTER 02

LITERATURE REVIEW

2.1 CULTURED MILK PRODUCTS

Cultured milk is a collective term used for products such as yoghurt, kefir, koumiss, kishk, cultured buttermilk, etc. The word 'cultured' is used to describe the process of inoculation of milk with a combination of microorganisms, which ultimately convert lactose to lactic acid. Carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other substances that are formed via the metabolic pathways of these microorganisms and give resultant products of characteristic flavour, texture and aroma (Spainer *et al.*, 2001).

These products may be classified in a number of ways. But a system based on the type of starter microorganisms used.

- Lactic fermentation.
- Yeast lactic fermentation.
- Mould lactic fermentation.

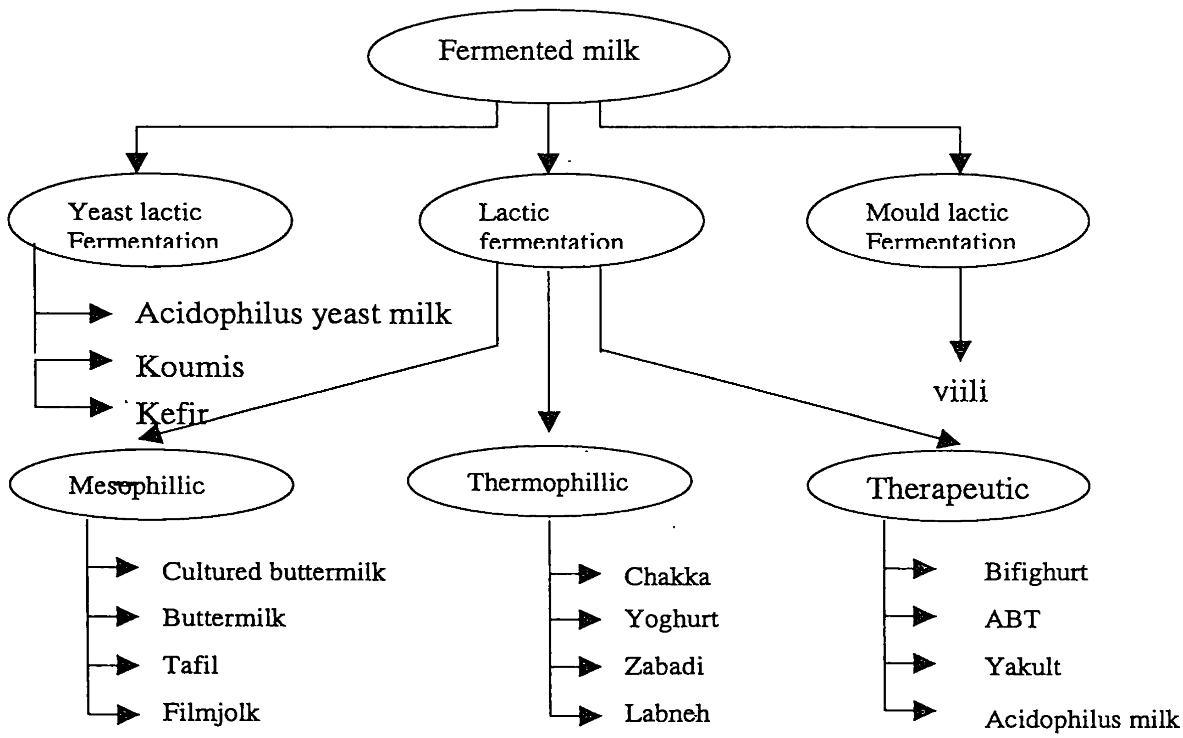

Figure 2.1- Scheme of classification of cultured milk product

2.2 OTHER CULTURED MILK PRODUCTS

2.2.1 Acidophilus milk

Acidophilus milk is traditional therapeutic milk fermented with *Lactobacillus acidophilus*. Skim or whole milk may be used, a heavy heat treatment of 95°C for one-hour, a tindallization process or UHT treatment being applied. Milk is inoculated at a level of 2-5% and incubated at 37°C until coagulated. Some acidophilus milk has acidity as high as 1% lactic acid, but for therapeutic purposes 0.6-0.7% is more common (Law, 1997).

2.2.2 Cultured buttermilk

For cultured buttermilk, buttermilk may be used, but skim or whole milk is now more common. A widely used process involves heating the milk to 80 °C, de-aerating, homogenizing, heating to 95 °C and cooling to 20-25 °C before addition of the starter. Starter is added at a level of 1-2 %, fermentation proceeding at 25 °C, to an acidity of 0.9% lactic acid. Separation may occur in the finished product and gelatin is some time added as stabilizer.

2.2.3 Cultured cream

The fat content of cultured cream is standardized between 12 and 30% depending on the required properties. Cream is heated to 75-80 °C before homogenization at high pressure and temperature to improve texture. The inoculation and fermentation conditions are similar to those for cultured buttermilk, but the fermentation is stopped at an acidity of 0.6% lactic acid. Post fermentation handling and inoculation adversely affect the consistency of cultured cream and fermentation may take place in the final retail pack (Reed, 1987).

2.2.4 Kefir

Kefir is a foamy effervescent drink, which is usually made from whole milk heat treated to 95 °C for 5 min. This process denatures whey proteins and improves product consistency, homogenization also in general use in modern practice. A portion of the processed milk is used to prepare the inoculums, being mixed kefir grains and incubated at 20-25 °C for 20 hours. At the end of this period the kefir grains are removed by sieving. The cultured milk is then used as starter at a level of 3 to 5%, the main fermentation also being at 20-25 °C for 20 hours. The kefir is then held for several hours during which time the coagulum stabilizes, the final product containing 0.9-1.1% lactic acid and 0.5-1.0 ethanol.

2.2.5 Koumiss

Koumiss is traditionally made from mares' milk, but similar products are made from whole or skimmed cow's milk containing added sucrose. *Lactobacillus acidophilus, Lactobacillus delbrueckii* and yeast are used as starters and added to cooled, heat-treated milk at a level of 10 –30 %. This gives and initial acidity of 0.45 % lactic acid, the inoculated milk being incubated at 26-28 °C until the desired levels of lactic acid and ethanol are present. After incubation the product is cooled and agitated to ensure the smooth consistency and to slightly aerate.

2.2.6 Scandinavian ropy milks

Scandinavian ropy milk has characteristic texture, which is both sticky and yet easily cut by a spoon. Milk is heated, deaerated, homogenized, reheated and cooling. Then the starter is added. After incubation stirring breaks coagulum and the product cooled and packed.

2.3 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 youghrt from the Turkish word "Jugurt" and these like products have been identified in many parts of the world.

Table 2.1- Selection of Yoghurt / Yoghurt like Product from Middle East and elsewhere

Countr	Traditional
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

The Western Europe, The USA and Australia yoghurt is mainly produce from cow's milk for manufacture of Greek yoghurt. Addition of salt to food is a traditional method of prevention and different types of concentrated yoghurt are made in Turkey by the addition of various quantities of salt. The yoghurt market has fragmented over the years with marketing strategies concentrating on calorific content, reduced or lower fat content additive free yoghurt microbiological content /health promotion and children's yoghurt.

2.4 COMMERCIAL STANDARD YOGHURT

Yoghurt is an acidified coagulated product obtained from milk by fermentation with lactic acid producing bacteria. The colour of plain yoghurt should be creamy white. If fruited it should represent the colour of the fruit without being too bright. The typical yoghurt flavour is slightly acidic, clean and fresh with the acetaldehyde.

Flavour, texture and aroma of yoghurt vary dependant upon country of origin, row materials, and formulation and manufacture process. In some areas yoghurt is produced in the form of highly viscous liquid, where as in other countries it takes the form of a softer gel. Yoghurt is also produced in drinking form and can be frozen or blended with other ingredients to create mousse type products, yoghurt ice cream or other form of dairy deserts.

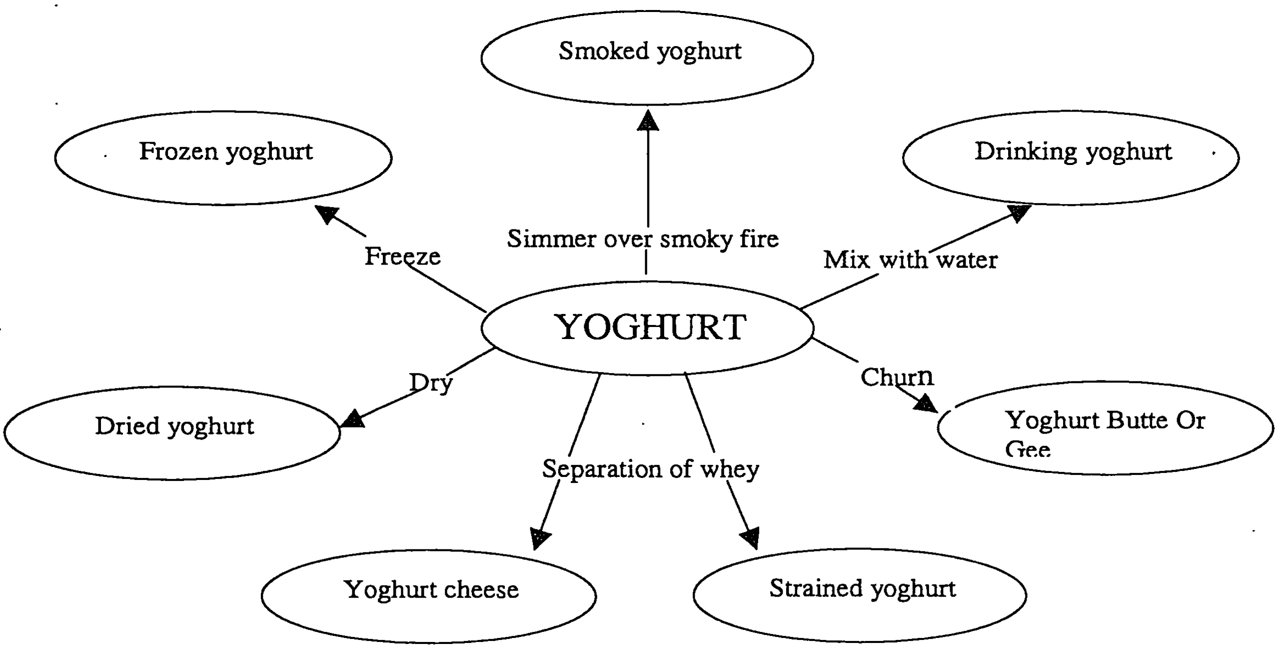

Figure 2.2- Different processes for the manufacture of yoghurt related food.

Yoghurt manufacturing methods vary considerable depending upon country row material used, size of operation, product formulation and type of product being manufactured. But there are number of common principles that determine the nature and quality of the final product these can be identified as;

1.Increasing the level of total solids in the milk.

2.Heat-treating the milk to a high temperature (≥80 °C) for a period of time consistent with denaturation of whey proteins.

3.Inoculating the cooled milk with a bacterial culture.

4.Incubating the inoculated milk in bulk or in retail containers under conditions, which promote the formation of a smooth viscous coagulum with the desired p^H, flavour and aroma.

5.Cooling and adding fruit and flavours as appropriate.

6.Packaging and chilled storage.

2.5 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 ofg8.5% by weight.

2.6 CLASSIFICATION OF YOGHURT

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- 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

How ever this products and in particular yoghurt are sub divided in to different grouping based on fat content, physical nature, flavours, post fermentation processing

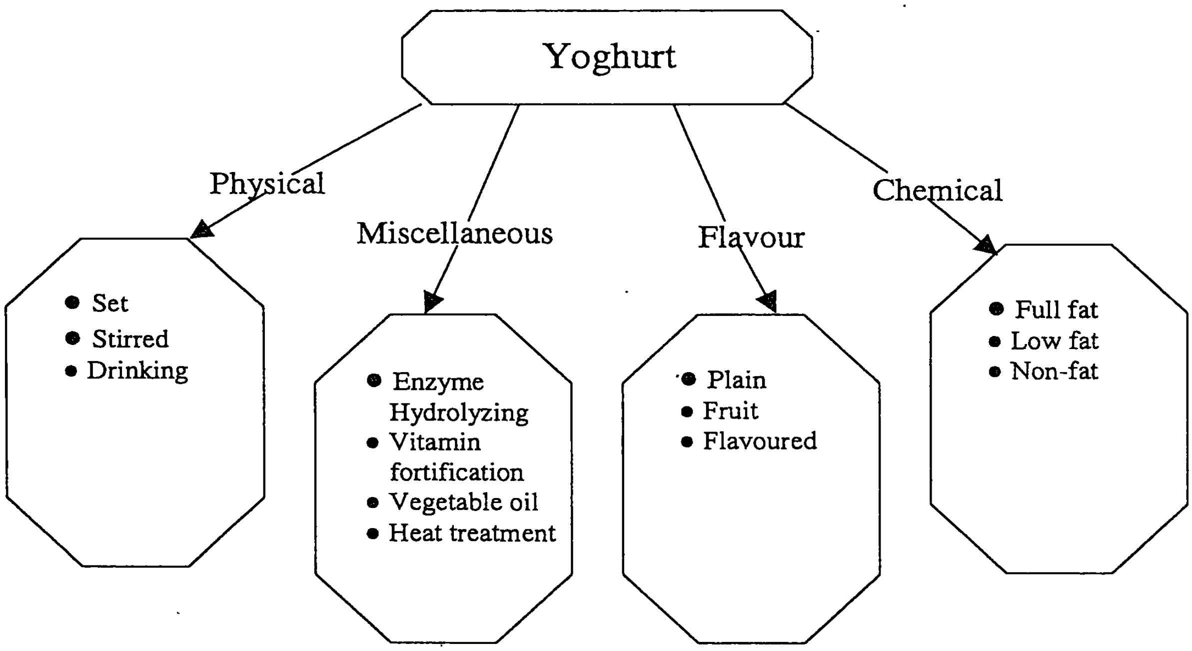

Figure 2.3- Scheme for the classification of yoghurt.

These different types of yoghurt are manufactured in either "set" or "stirred" form and each type has quite distinctive characteristics.

2.6.1 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 packed immediately after inoculation with bulk starter and is incubated in the packages.

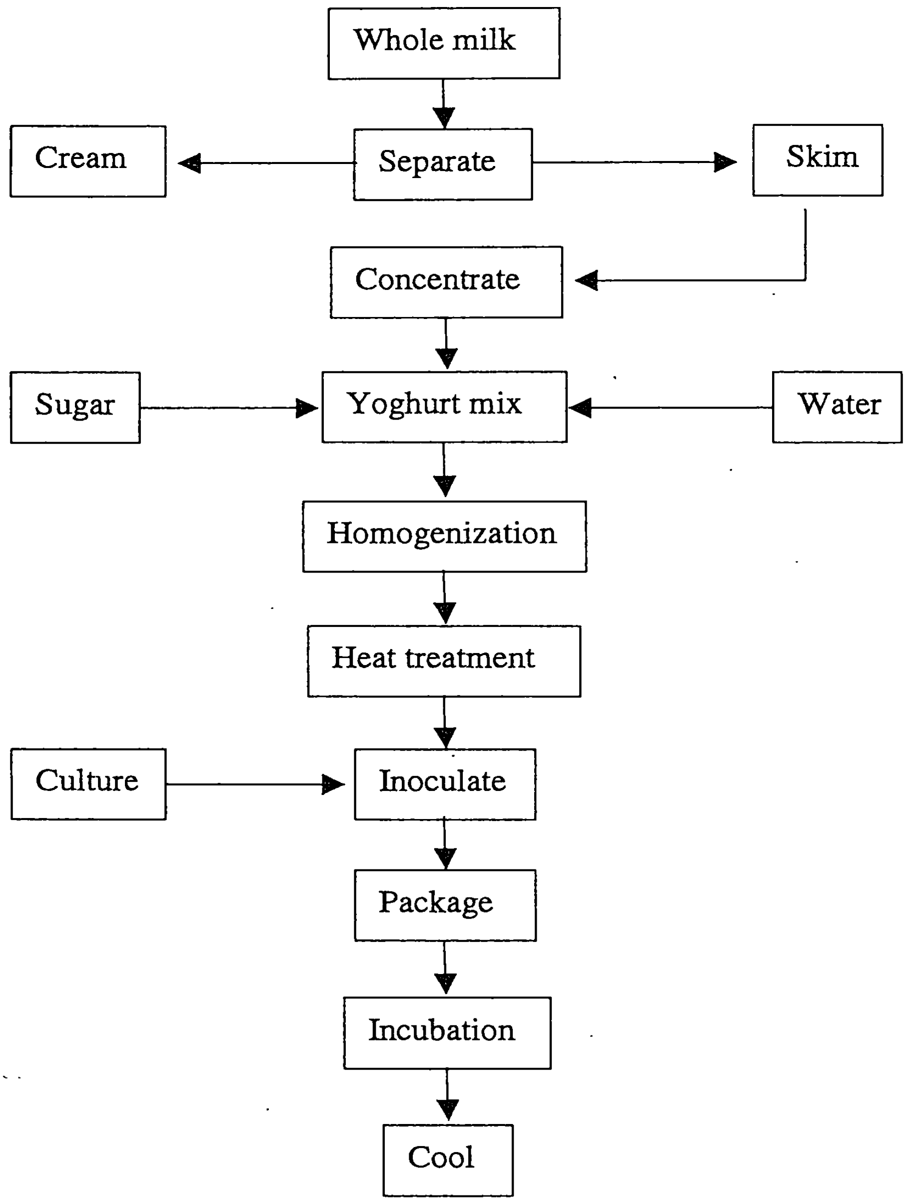

Figure 2.4- Set yoghurt technology.

2.6.2 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 tank. After incubation, the product is cooled before packed.

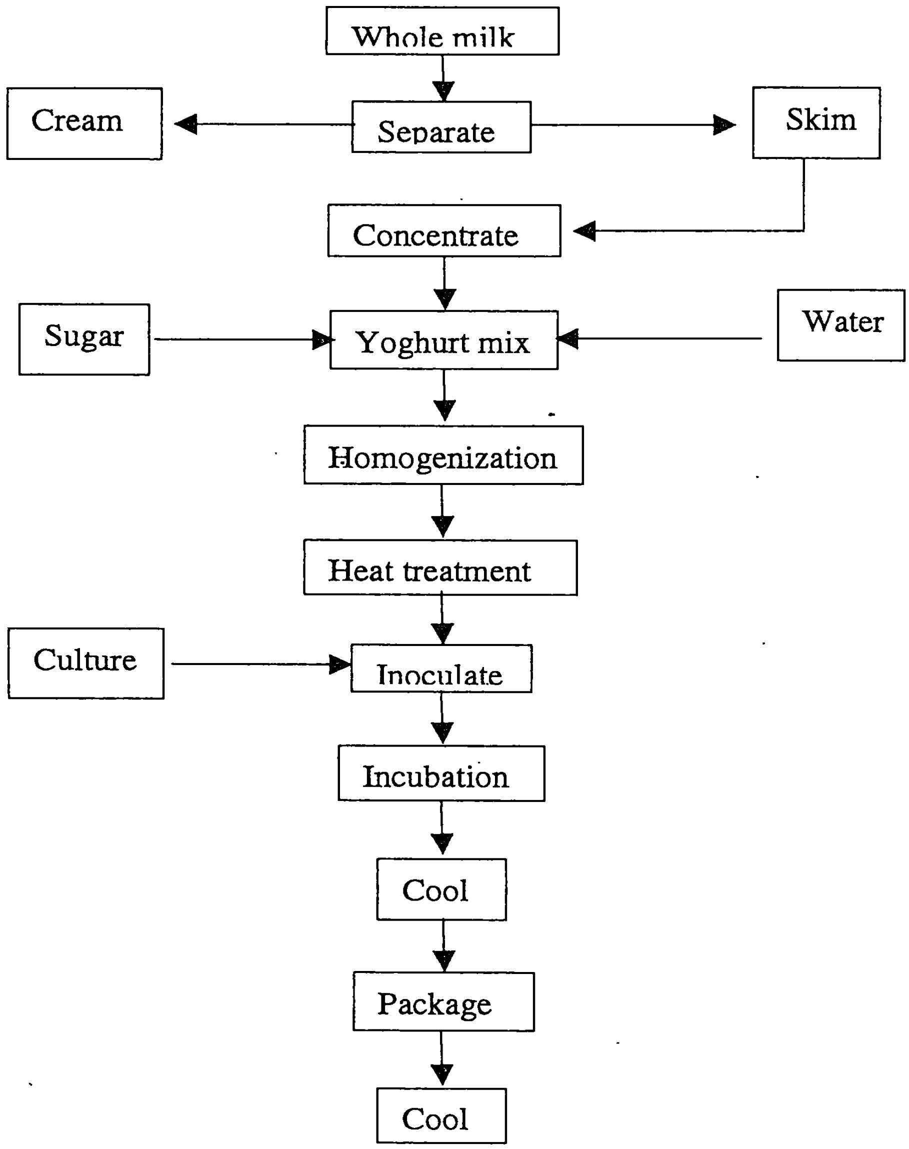

Figure 2.5- Stirred yoghurt technology.

2.7 TYPES OF YOGHURT

2.7.1 Frozen yoghurt

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).

2.7.2 Dried yoghurt

Traditionally dried yoghurt is produced by simply concentrating conventional yoghurt by boiling and sun drying, the dried product being reconstituted before consumption. But for large-scale manufacture for this purpose either freeze-drying or spray drying may be used.

2.7.3 Pasteurized yoghurt

The yoghurt may be heated either in a plate heat exchanger at 75-80 °C for 15 s or "heat shocked" in carton at 58°C for 5 min. 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.

2.7.4 Concentrated yoghurt

Concentrated or strained yoghurt is produced in a number of countries and may be known as "labneh". These yogurts are fermented by mesophilic starter cultures in conjunction, in some cases with yeast. Concentrated yoghurt may be considered as intermediate between conventional fermented milks and high moisture, unripened soft cheese and it is notable that suggested culinary uses as a spread or salad dip are more usually associated with soft cheese. The traditional process for preparation of concentrated yoghurt, straining in cloth bags, but is laborious and subject to microbiological contamination. The commercial process involves centrifugal separation of skimmed milk yoghurt to produce a concentrated base, which is the recombine with butter oil or cream to the desired fat content. More recently there has been much interest in the use of ultrafiltration either to concentrate into final product or to concentrate the milk prior to fermentation.

2.7.5 Drinking yoghurt

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. Two process are used, a HTST pasteurization coupled with aseptic packaging to give a life of several weeks at 2-4°C and a UHT process coupled with aseptic packaging to give a life of many weeks at room temperature.

2.7.6 Carbonated yoghurt

Carbonated yoghurt is extremely popular wide world. They can be manufactured either in a liquid or in a dry form. Additionally this type of yoghurt is less acidic and has P^H around 7.

2.7.7 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 is 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.

2.8 NUTRITIONAL VALUE OF YOUGHURT

Nutritional value of yoghurt depends upon it's composition, row materials used, ingradians added and the manufacturing process. This will have effects on carbohydrate, vitamins, proteins, fat and mineral matters.

Table 2.3- 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

2.8.1 Carbohydrate

2.8.1.1 "Available" carbohydrates

The expression "Available" carbohydrate is intended to cover all those carbon compounds that can be assimilated by the human body. In the case of natural yoghurt, a number of mono and disaccharides are present in trace amount, but lactose remains the dominant sugar, and is presenting in the finished product at similar levels to those founding milk. Yoghurt has been found to be acceptable in the diet of those who suffering from lactose intolerance.

2.8.1.2 "Unavailable" carbohydrates

Although natural yoghurt entirely depend on milk, stirred and fruit yoghurt are usually incorporated with stabilizers to reduce whey separation during the distribution. Some of the available carbohydrates (stabilizers) are reported to depress blood cholesterol level.

2.8.2 Protein

Yoghurt is an excellent source of protein. Proteins in yoghurt are totally digestible. A feature enhanced by the fact that some degree of initial proteolysis is caused by the starter organisms themselves. The other pertinent characteristic is that the milk proteins in yoghurt are already coagulated prior to ingestion, and the "soft clot" formed in the stomach may have other benefits.

2.8.3 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. Although there are yoghurts made with fat percentages approximating to that of whole milk 4%, the majority of yoghurt usually contains 0.5-1.2% fat and are described as 'low fat' or 'non fat'.

Normally fats are an integral part of "balanced" diet. Thus humans have double requirements for lipids in that these possess.

2.8.3.1 Storage fat

Storage fat composed of saturated fatty acids, and serving as a source of energy or as a protection for vial organisms

2.8.3.2 Structural fat

Structural fat with proteins forms many of the essential membranes in animal's cells particularly in areas like the brain.

2.8.4 Vitamins and Minerals

The relative availability of vitamins in yoghurt is much more difficult to assess. Because of unlike minerals many vitamins are sensitive to the condition of processing. The level of inorganic ions or unit weight is also going to be higher, but the position of calcium is, perhaps rather special. Thus not only can yoghurt act as a source of calcium for who suffers from lactose intolerance, but also calcium supplied by yoghurt may be better absorbed and utilized than calcium made available in other forms.

2.9 RAW MATERIALS

The raw materials most likely to be used for the manufacture of yoghurt are Milk, Milk concentrates, Milk powder, Cream, Cultures, Stabilizers, Flavoures, Colours, Sugar, etc.

2.9.1 Milk

Milk may be defined as the whole, fresh lacteal secretion, practically free from colostrums obtained by the complete milking of one or more healthy cow, which contains not less than 8¼ % of solids not fat (SNF) and not less than 3¼ % of milk fat.

The main ingredient of yoghurt is milk or ingredients derived from milk and the most commonly used milk based ingredients are,

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

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 microorganisms.

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 microorganisms.

2.9.1.1 Constituent of milk

Table 2.4- 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

2.9.1.1.1 Water

Milk has a high percentage of water (87%). Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. A small percentage of water in the milk us hydrate to the lactose and salts, and some is bound in the proteins.

2.9.1.1.2 Milk fat

Milk fat is the most variable of all milk components and any average value of 3.5 to 3.7% means little when applied to one breed one animal or one source of supply. The mixture of mixed trigliceride which makes up 98-99% of milk fat is peculiar to milk; though quite bland in taste, it imparts smoothness and palatability to yoghurt .The remaining 1-2% of milk fat is composed of phospholipids, sterols, carotenoids, the fat soluble vitamins (A, D, E and K) and some trace of free fatty acids (Fox, 1995).

The density of the milk fat globule is much lower than that of the milk serum and, as a result, milk fat globules rise to form a fat rich phase (Cream separating).

2.9.1.1.3 Lactose

This Lactose is the characteristic sugar of milk and for most purposes can be considered as the only carbohydrate present. Trace amounts of other carbohydrates also found in milk. Lactose is a disaccharide comprised of alpha –D-glucose linked to beta-D-galactose. The sugar may exist in alpha and beta forms and alpha form is less soluble than beta form.

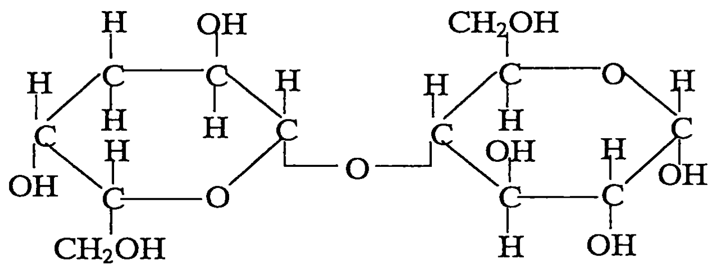

Figure 2.6- The structure of Lactose.

Lactose is a reducing sugar and, in some circumstances reacts freely with free amino groups in protein (Classical Maillard type reaction). Lactose is useful source of dietary energy and is thought to promote the adsorption of calcium from the diet.

2.9.1.1.4 Proteins

The proteins in milk fall into two distinct types, whey proteins and caseins. The casein content over 80 % of the total protein and this may subdivide into five main classes, the $alpha_{S1}$, $alpha_{S2}$, beta, gamma and kappa caseins. The major components of whey proteins are beta lactoglobulin and alpha lactalbumin. The relative proportion of whey protein to casein may vary over the season. In the first few days of lactation and in the late lactation, the whey protein content of milk may increase.

Casein is of modest size and does not posses an organized structure. Because of this property heating can't denature them. In contrast to the casein, the whey proteins are typical globular proteins, which may be denatured on heating at temperatures above 65°C.

The protein fraction of milk also includes the enzymes of milk, and perhaps some unidentified minor protein (Harding, 1995).

2.9.1.1.5 Ash

The ash content of milk is an analytical value indicating the amount of non-combustible matter in milk. In normal milk the value remains rather constant about 0.7%; a value much higher than this is indicative of abnormal conditions in the secreting glands.

2.9.1.1.6 Vitamins

Milk is good source of Vitamin A, Vitamin B, Vitamin B_2 and Thiamine. It provides small quantity of vitamin C or Ascorbic acid, vitamin D and Niacin. Other vitamins are presented in smaller quantities. Vitamin C and several vitamins of the B complex are water-soluble. Vitamin A, D, E and K are fat soluble, so they tend to be present in milk and products in proportion to the fat content (Harding, 1995).

2.9.2 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,

- Type of sweetening agent used.
- Consumer preference.
- Type of fruit used.
- Inhibitory effects on starter microorganism.
- Legal aspects.
- Economic consideration.

On average yoghurts may contain as high as 20g $100g^{-1}$ carbohydrates and these are derived from residual milk sugars (lactose, galactose and glucose), natural sugars present in the fruits and sugars added by the manufacturer.

2.9.3 Stabilizers and thickeners

The most common used stabilizer and thickeners are modified or natural starches, alginates, agar, carrageenan, edible gums, pectins and celluloses. There are recommendations with regard to addition levels of stabilizers. The food standard committee report suggests a total maximum addition of 0.5% by weight of final product except for the addition of gelatin, starches and pectin when the total maximum may be raised to 1.0% by weight of final product. Their main functions are to:

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

The addition of stabilizers and thickeners has been employed as a means of replacing milk solids and fat as apart of a cost reduction exercise (Imason, 1999).

2.9.4 Preservatives

A number of individual preservatives are used in yoghurt either directly into the milk it self 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.

2.9.5 Fruit preparations

Fruit and flavourings are usually added to yoghurt after cooling of the fermented yoghurt and prior to filling in retail container. Fruit can be supplied fresh, frozen or as a homogeneous mix including any colour, flavour, stabilizers, sweetener or preservative as require by individual customers.

2.9.6 Yoghurt culture

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 (Jay, 2000).

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.

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.

Generally in a mixed culture, initially the Streptococci utilize lactose and excrete galactose and the organism remains active until the pH falls to 4.6. Thereafter the Lactobacilli respond to increasing galactose levels.

Cultures are supplied in liquid, frozen or dried form. Liquid cultures will be supplied in sterilized, antibiotic free reconstituted skimmed milk powder at 10-12% SNF and can be kept at <8°C for 1-2 weeks. Deep frozen (-30 to –40°C) and ultra low frozen in liquid nitrogen (-196°C) can be kept for 3-6 months and dried forms (vacuum, spray, freeze dried or concentrated freeze dried) for over sixth month.

Freeze dried and liquid starters tend to be used for propagation purposes into mother culture and for sub culturing into liquid 'feeder system' in larger volumes. Concentrated frozen and concentrated freeze-dried products usually contain 10^9-10^{12}active cells per gram and are intended for direct inoculation into bulk starter vessels.

2.10 YOGHURT PROCESSING

Except the incubation stage, the essential steps in the manufacture of fresh stirred and set yoghurt are common to both yoghurt types.

2.10.1 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. 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. Sugar may be added as liquid but care must be taken not to add too much sugar to the pre-fermented base since this can result in dehydration of the starter organisms. Fortification of milk solids may be achieved in the addition of skimmed milk powder, dilution of evaporated milk and use of membrane technology to increase milk solids levels. Dry ingredients (milk powders, stabilizers, sugar) require sufficient time for rehydration and deaeration.

2.10.2 Homogenization / Emulsification

Homogenization means quite literally the provision of a homogeneous emulsion between two immiscible liquids, e.g. oil/fat and water. Yoghurt milk is atypical oil in water emulsion and as a result the fat has a tendency to separate upon standing. In order to prevent this, the milk base is subjected to high speed mixing or homogenization (Johnson and Alford, 1987).

Homogenization affects the fat phase of milk, which plays no direct role in formation of the yoghurt coagulum. The reduction of size and increase of number of fat globules resulting from homogenization does however modify the subsequent gel. In the first place the adsorption of 'small' fat globules into casein micelles increase viscosity and the effective total volume of suspended matter. Secondly syneries is decreased due to increase in hydrophilicity of casein micelles. Single stage or double stage homogenization can be used to emulsify the milk. The use of single stage or double stage homogenization is only critical in milk containing high levels of fat (e.g. cream).

2.10.3 Heat treatment

Heating of yoghurt mix has an important technological role in modifying the properties of proteins and contributing to the formation of stable coagulum. There is also a redistribution of calcium, magnesium, and phosphorus ions between the soluble and colloidal form, which tends to reduce coagulation time. The major direct consequence of heating on the proteins is the denaturation of the whey proteins and the interaction with k-casein. The objectives of heating 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 and to acids in the prevention of whey separation.
- Hydrate stabilizers, which require heat.

These objectives are usually achieved by the temperature of 85°C for 30 min. Functional properties of the whey proteins become more apparent after heating milk and whey begin to become denatured above 60°C. The effect of heat on protein is a two stage processes firstly the structured is altered, causing denaturation and secondly, aggregation takes place followed by coagulation (Beckett, 1995).

2.10.4 Pre Fermentation cooling

After heat treatment the milk is required to cool to a suitable temperature for inoculation. Inoculation temperature for short set method will approximate to 42°C. For short set incubation it is critical to achieve an accurate inoculation temperature since to high a temperature can inhibit and ultimately killed starter culture microorganisms and too low a temperature will result in unnecessary extension of fermentation time.

2.10.5 Starter addition

Normally a number of different forms of starter cultures are used to manufacture of yoghurt. A ratio of *Latobacillus* to *Strephtpcoccus* of is 1:1 is theoretically required in the in the yoghurt manufacture as the starter microorganisms. The reasons for selecting the combination of starter cultures to achieve the desired flavour characteristic of the product mainly lactate, aroma compounds (acetaldehyde) and provide the consumer with a good textured product. Some extends the method of addition is influence 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.

2.10.6 Fermentation

In yoghurt plant, stirred type and set type yoghurt are often produced in the manufacture of stirred type yoghurt bulk incubation is carried out in incubation tank. 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 use and the propose length of incubation. The incubation of retail containers in set type yoghurt takes place within a warm air incubation room (Shapton and Shapton, 1998).

2.10.7 Striking

This stage applies only to stirred and liquid type yoghurt and is essentially the operation of breaking the warm curd and reincorporation of the whey. It is usually sufficient 5 to 10 minutes to obtain homogeneous mix.

2.10.8 Post Fermentation cooling

. This step is used to control the metabolic activity of the starter culture and it's enzymes. Since the yoghurt organisms show limited growth activity around 10°C, the primary objective of cooling is to drop the temperature of the coagulum from 30-45°C to <10°C as quickly as possible so as to control the final acidity of the product. Cooling of the coagulum commences directly after the product reaches the desired acidity. The desired acidity will be dependent upon type of yoghurt, method of cooling, time taken to empty fermentation vessel and desired final acidity. Cooling will take place approximately pH 4.5-4.6.

2.10.9Chill storage

Yoghurt, which has not been subjected to any form of heat treatment in its final product form, be that via pasteurization, sterilization or UHT processes, needs to be kept cold until its reaches the customer. Which will have a shelf life of approximately 15-20 days.

2.11 SPOILAGE Of YOGHURT

Yoghurt, in general is of low pH value and high lactic acid concentration and are thus a highly selective environment favouring the growth of spoilage microorganisms.
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 and yoghurt containing added sugars are usually affected. 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.
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).

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.

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 2.5- Microbiologically requirements for Yoghurt

Test organism	Limit
Coliform	Not more than 10 /g
Yeast	Not more than 1000 /g
Mould	Not more than 10 /g

Mainly quick spoilage of yoghurt is due to,

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

2.11.1 Raw materials

The raw materials most likely to be used for the production of yoghurt introduce microorganisms and chemicals, which may affect the quality of the final product.
Microorganisms can gain access to the milk from the cow, personnel, or equipment used during milking or during transportation to and storage at the processing dairy. To keep the microbiological flora to a minimum it is essential to cool the milk to below 5°C directly after milking.
Culture management and the activity and viability of the organisms are vital to final product quality and to the shelf life of yoghurt. Culture preparation and handling demands the highest standards of hygiene combined with attention to detail in the manufacturing techniques and in the selection of plant. The risk of airborne contamination by yeasts, moulds, bacteria and bacteriophage must be eliminated.
For any other raw materials used, like sugar, Milk powder, stabilizers, etc. the source of the ingredient, approval of supplier, the identification of critical control points and hygiene standards are all important factors in the shelf life of yoghurt (Beckett, 1995).

2.11.2 Processing

The basic yoghurt mix is subjected to a relatively severe heat treatment. This is cause to destruction of any undesirable organisms including pathogens. After addition of the culture with optimum temperature and a medium with all the desired factors, but without any inhibitory substances culture organisms produce lactic acid to the desired pH level.
After completion of fermentation the yoghurt is cooled to below 20°C . These all steps are reduced the undesirable organisms to a desirable level. How ever, the lactic acid producing cultures required a lower incubation temperature, and could also be suitable for the growth of undesirable organisms.

2.11.3 Filling and packaging

The filling and packaging operation is the last direct contact that the manufacturer has with the product. The filling operation and choice of packaging materials are critical due to the incorrect choice of filling mechanism, poor hygiene practices, and inability of packing materials to protect the product can result in pre-mature shelf life failure.

2.11.4 Storage and distribution

Yoghurt may spoil during distribution due to;

- Growth of microorganisms.
- Bio chemical and chemical changes such as oxidation, rancidity development, and moisture migration.
- Attacks by vermin, birds and other pests.

The bio chemical and microbiological changes are greatly reduced by storing the yoghurt at low temperatures, such that yoghurt must be stored and distributed at temperatures of less than of 8°C.

2.11.5 Consumer storage

After selling, the shelf life of yoghurt mainly depends upon the maintaining 'cold chain' continuously at optimum Temperature. The way of handling the yoghurt by the consumer is mainly cause to shorter the shelf life of the product.

- The time that the product may be out of refrigeration, between being removed from the retailer's chill cabinet and being placed in the consumer's refrigeration.
- The number of days it will be stored in the consumer's refrigeration.
- The likely position in which it will be stored within the consumer's refrigeration.

CHAPTER 3.0

MATERIALS AND METHODALOGY

Sampling:

40 samples of yoghurt for each storage temperature were collected randomly with sterilized conditions and analyzed for physico-chemical, microbiological and organoleptic characteristics.

3.1 DETERMINATION OF THE SHELF LIFE OF NON FAT YOGHURT

As the first stage in a shelf life determination have to detect the microbiological safety and the changes of the physical attributes of the yoghurts, before attempting the shelf life evaluation.

3.1.1 Materials

- Incubated yoghurt samples (prior to post fermentation cooling).
- Incubators at four different temperatures (To create accelerated storage conditions)

3.1.2 Method

Yoghurt samples were stored in four different temperatures 3°, 8°, 20° and 30°C stored for 28 days. These samples were analyzed using standard physicochemical and microbiological analyzing methods through storage period with every two days. At fourteenth day of storage period, the sensory evaluation was carried out.

3.1.3 physicochemical analysis

3.1.3.1 Determination of pH

3.1.3.1.1 Materials

Digital pH meter (pH scan2, Singapore)

Beaker

Spoon

Distilled water

3.1.3.1.2 Method

The samples of the four storage conditions were taken into beakers separately and mixed thoroughly by a spoon, and standard for 3-5 minutes at 20°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.

3.1.3.2 Determination of acidity

3.1.3.2.1 Materials

Burette

Beaker

Dropper

Pipette

Spoon

Stand

White marble

Yoghurt (9 ml)

1.5% Phenolphthalein solution

0.1 NaOH

3.1.3.2.2 Method

Yoghurt samples were mixed thoroughly by a spoon until it becomes a liquid. The 1 g of the each sample was taken into a beaker by a pipette and 3 drops of 1.5% Phenolphthalein solution was added to it by using a dropper. Then the samples were titrated with 0.1 N Sodium Hydroxide until get a permanent pale pink colour. After that the burette readings were taken and the results were recorded. The results were expressed in terms of percentage. Titration was done on a white tile to detect an accurate colour change. The test was also done for duplicate samples and for blank test.

$$\text{Acidity present by mass} = \frac{nv}{m}$$

v = Volume in ml of 0.1 N NaOH required for titration

n = Normality of 0.1 N NaOH

m = Gram of the sample taken for the test

3.1.4 Microbiological analysis

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

3.1.4.1 Coliform analysis by direct plating

3.1.4.1.1 Materials

Autoclave	Ringer solution
Bunsen burner	Distilled water
Water bath	Violet red bile agar
Electric balance	Pipettes (1ml)
Incubator	Spatula
Petri dish	

3.1.4.1.2 Medium preparation for coliform test

39.5 g of Violet red bile agar was dissolved in 1 liter of distilled water and heated in a water bath with frequent stirring until boiling. The medium was cooled to 45°C after completely dissolving the medium.

3.1.4.1.3 Method

Coliform was 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 this mixture was blown out by a pipette on to a sterile petri dish and about 10 ml of violet red bile 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 30-45 minutes in a sterilized condition. The plates were then incubated in an inverted position, aerobically 30°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.

3.1.4.2 Yeast and mold counting method

3.1.4.2.1 Materials

Autoclave
Bunsen burner
Electric balance
Incubator
Petridish
Pipettes (1ml)
Spatula
Water bath
Ringer solution
Distilled water
Potato dextrose agar

3.1.4.2.2 Medium preparation for yeast and mould test

39 g of Potato Dextrose Agar was dissolved in 1 l 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.

3.1.4.2.3 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 10 ml of Potato Dextrose 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 30-45 minutes in a sterilized condition. The plates were then incubated in an inverted position, aerobically in room 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.

3.2 SENSORY EVALUATION

Sensory analysis for sensory characteristics and overall acceptability of the yoghurt samples were carried out using a panel of judges selected from Swiss Cheese Company (pvt) Ltd using five-point hedonic scale.

3.1.5 Materials

Yoghurt samples

Ballot papers

Pens

Spoons

3.1.6 Method

The yoghurt samples, which were in four temperatures, 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: 654 = Sample at 30°C

248 = Sample at 20°C

732 = Sample at 8°C

186 = Sample at 3°C

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, spoon-ability, appearance and overall acceptability) in all four samples was recorded according to a 5-point hedonic scale. The obtained results were analyzed by Minitab statistical analysis package with the using of Kruscal Wallis test at 5% significance level.

CHAPTER 04

RESULTS AND DISCUSSION

4.1 PHYSICAL CHANGES

4.1.1 Physical changes of Sample 01 (at 3°C)

No discoloration, undesirable odour formation or gas formation and syneries were observed until 25 days at 3°C.

4.1.2 Physical changes of Sample 02 (at 8°C)

No discoloration, undesirable odour formation or gas formation and syneries were observed until 16 days at 8 °C.

4.1.3 Physical changes of Sample 03 (at 20°C)

Discoloration, undesirable odour formation or gas formation and syneries were observed after keeping for 7 days at 20°C.

4.1.4 Physical changes of Sample 04 (at 30°C)

Discoloration, undesirable odour formation or gas formation and synerise were observed after keeping for 4 days at 30 °C.

Gas formation of the yoghurt is can be observed by doming of the lid. The presence of microbes (yeasts) may cause to release gaseous materials (odour). The samples at higher temperature levels and with long storage period cause to contaminate the yoghurt heavily. The higher doming of the lid may indicate higher contamination of yoghurt. In all treatments, syneresis increased during the storage period. Normally in the course of storage of the examined yoghurt, a significant increase was observed in their susceptibility to syneresis with the duration of storage, which was associated with the aging process of the curd. But at the highest sensitivity to syneresis was observed in the curd of the yoghurt at 30°C, after 3 days of storage. The sensitivity to syneresis of yoghurts at higher temperature levels was significantly higher than in yoghurts containing low temperature condition. There was less sensitivity to syneresis of samples at 3°C temperature level, as compared to other temperature level.

4.2 CHEMICAL CHANGES

4.2.1 Determination of post pH development of non fat yoghurt at different storage conditions

4.2.1.1 pH value changes of the samples during the storage

The obtained results are summarized in Table 4.1.

Table 4.1 - pH changes during the storage of non-fat yoghurt at different temperatures for 28 days

Days	3°C	8°C	20°C	30°C
1	4.34	4.34	4.34	4.34
2	4.34	4.25	4.00	3.92
3	4.29	4.20	3.84	3.74
5	4.24	4.21	3.82	3.70
6	4.24	4.18	3.76	3.68
7	4.15	4.16	3.73	3.66
8	4.13	4.12	3.72	3.67
10	4.12	4.12	3.73	3.66
12	4.12	4.11	3.71	3.67
14	4.10	4.09	3.69	3.62
16	4.11	4.10	3.70	3.62
18	4.12	4.09	3.72	3.64
20	4.12	4.06	3.69	3.61
22	4.12	4.08	3.71	3.60
24	4.10	4.07	3.68	3.60
26	4.11	4.07	3.69	3.58
28	4.11	4.08	3.68	3.59

The data was represented by the mean value of 3 replicates.

4.2.1.2 Variation of pH with time

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

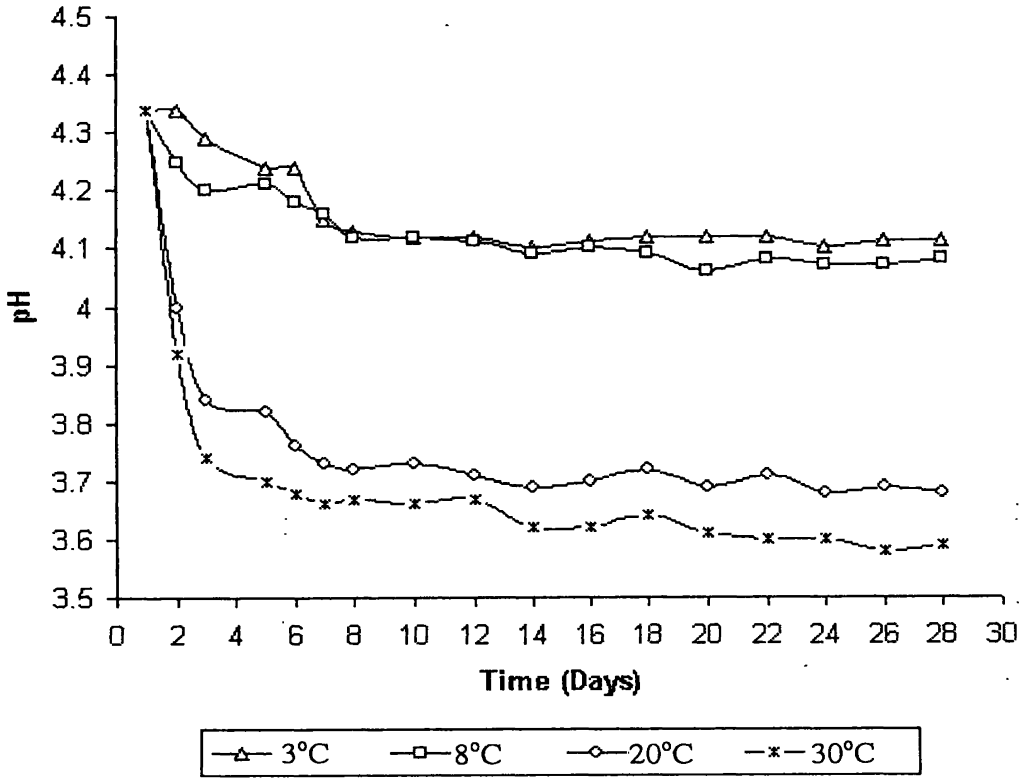

Figure 4.1- Variation of ph with time

The pH values of yoghurt at all temperature levels are summarized in Table 4.1.The post pH decreasing of non-fat yoghurt is mainly depending on storage temperature. In higher temperature levels the culture organisms are still viable. So their activity and metabolic rate are not slow down, through the storage period. So the acidity level can be increase with higher rate than lower temperature level. The results showed that the decrease in pH during storage slightly almost linearly on the highest temperature levels (20°C, 30°C). The temperature significantly influenced the decrease of pH and changes were more pronounced during storage for 1-4 days. The pH of the sample at 3°C was not showed much variation after 20 days at storage.

4.2.2 DETERMINATION OF ACIDITY CHANGES OF NON FAT YOGHURT AT DIFFERENT STORAGE CONDITONS

4.2.2.1 Acidity development of the samples during the storage

Table 4.2 -Acidity developments during the storage of non-fat yoghurt at different temperatures for 28 days

Days	3°C	8°C	20°C	30°C
1	0.90	0.90	0.90	0.90
2	0.88	0.95	1.07	1.22
3	1.00	1.02	1.35	1.41
5	0.93	1.3	1.35	1.68
6	1.03	1.18	1.78	1.85
7	1.13	1.18	1.69	1.98
8	1.22	1.23	1.70	1.99
10	1.28	1.30	1.79	2.02
12	1.31	1.38	1.88	2.04
14	1.16	1.23	1.85	2.20
16	1.21	1.29	1.88	2.18
18	1.33	1.37	1.92	2.22
20	1.33	1.33	1.95	2.19
22	1.31	1.35	1.89	2.24
24	1.30	1.22	1.80	2.15
26	1.29	1.23	1.85	2.18
28	1.30	1.21	1.83	2.17

The data was represented by the mean value of 3 replicates.

The total titratable acidities of all samples are shown in Table 4.2.

4.2.2.2 Variation of acidity with time

Diagrammatic representation of changes in acidity with time at all temperature levels in storage is shown in below.

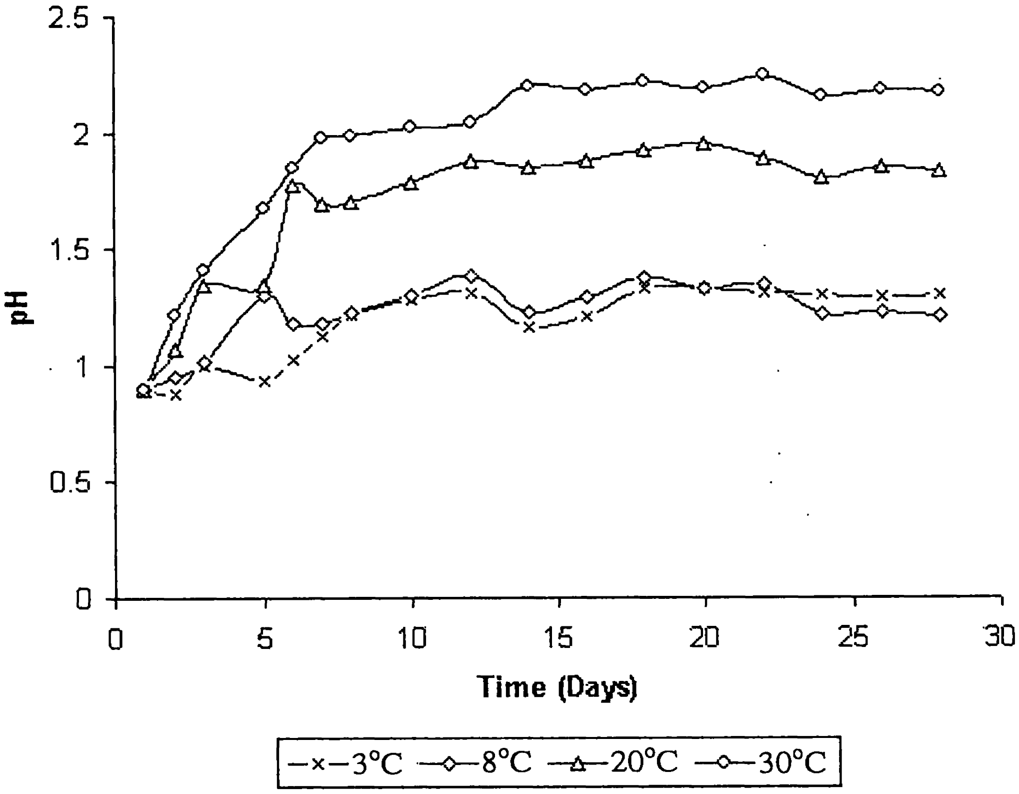

Figure 4.2- Variation of acidity with time

Titratable acidity is a reasonable indication of the performance of the starter culture. The post acidity development of nonfat yoghurt is mainly depending on storage temperature. According to the graph there was higher variation in acidity of lower and higher temperature levels. The results showed that the rate of increasing acidity is very high during storage at highest temperature levels (20°C, 30°C), because the activity and metabolic rate of culture organisms are get high at higher temperature levels. The high storage temperature is caused to increase in acidity.

4.3 DETERMINATION OF MICROBIOLOGICAL QUALITY OF NON FAT YOGHURT AT DIFFERENT STORAGE CONDITONS

4.3.1 Results of the coliform test of yoghurt samples

Table 4.3- Coliform counts for samples at all the temperature levels.

Days	3°C	8°C	20°C	30°C
1	3/1g	3/1g	3/1g	3/1g
2	0/1g	2/1g	1/1g	5/1g
3	1/1g	4/1g	0/1g	0/1g
5	5/1g	2/1g	0/1g	0/1g
6	2/1g	1/1g	0/1g	0/1g
7	0/1g	3/1g	0/1g	0/1g
8	0/1g	0/1g	0/1g	0/1g
10	0/1g	2/1g	0/1g	0/1g
12	0/1g	0/1g	0/1g	0/1g
14	0/1g	0/1g	0/1g	0/1g
16	0/1g	0/1g	0/1g	0/1g
18	0/1g	0/1g	0/1g	0/1g
20	0/1g	0/1g	0/1g	0/1g
22	0/1g	0/1g	0/1g	0/1g
24	0/1g	1/1g	0/1g	0/1g
26	0/1g	1/1g	0/1g	0/1g
28	0/1g	0/1g	0/1g	0/1g

The data was represented by the mean value of 3 replicates.

The coliform count of yoghurt at four temperature levels are shown in Table 4.3

4.3.1.1 Coliform growth with time

Diagrammatic representation of changes in growth of coliform with time at all temperature levels in storage is shown in below.

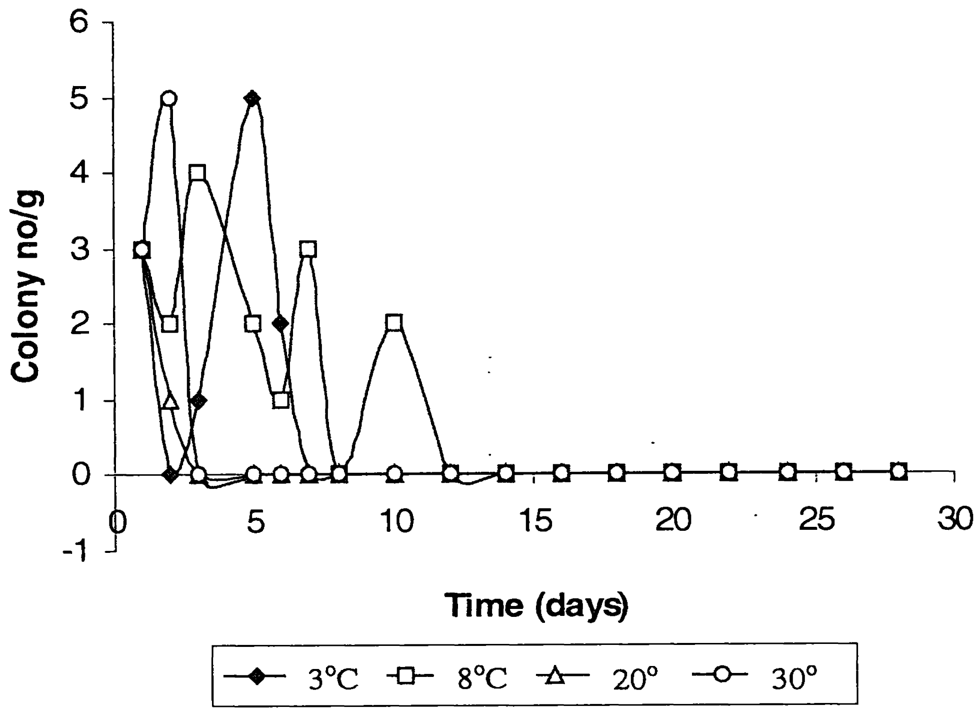

Figure 4.3- Variation of coliform growth with time

According to the above results, the growth of coliform bacteria was decreased with decreasing pH. No coliform colonies were observed after 3rd day at 30°C and 20°C due to the development of low pH condition. In highest pH condition growth rate of coliform bacteria is significantly reduced. Their initial presence in the yoghurt indicates contamination of the yoghurt before processed by, poor hygienic practices, raw materials, and contamination also can be done with packaging materials. But there is no unacceptable level of contamination with coliform of the sample. Because colony per gram value is less than unacceptable value recommended for yoghurt.

4.3.2 Results of the yeast and mould test of yoghurt samples

Table 4.4- Yeast and mould count for samples at all the temperature levels.

Days	3°C	8°C	20°C	30°C
1	5/1g	5/1g	5/1g	5/1g
2	13/1g	10/1g	5/1g	28/1g
3	2/1g	13/1g	16/1g	45/1g
5	1/1g	0/1g	20/1g	un
6	0/1g	0/1g	42/1g	un
7	0/1g	0/1g	35/1g	un
8	0/1g	0/1g	38/1g	un
10	5/1g	0/1g	un	un
12	0/1g	0/1g	un	un
14	0/1g	0/1g	un	un
16	0/1g	6/1g	un	un
18	3/1g	0/1g	un	un
20	0/1g	3/1g	un	un
22	0/1g	5/1g	un	un
24	2/1g	0/1g	un	un
26	0/1g	1/1g	un	un
28	0/1g	0/1g	un	un

▪un =Uncountable

The data was represented by the mean value of 3 replicates.

4.3.2.1 Yeast and mould growth with time

Diagrammatic representation of changes in growth of yeast and mould with time at all temperature levels in storage is shown in below.

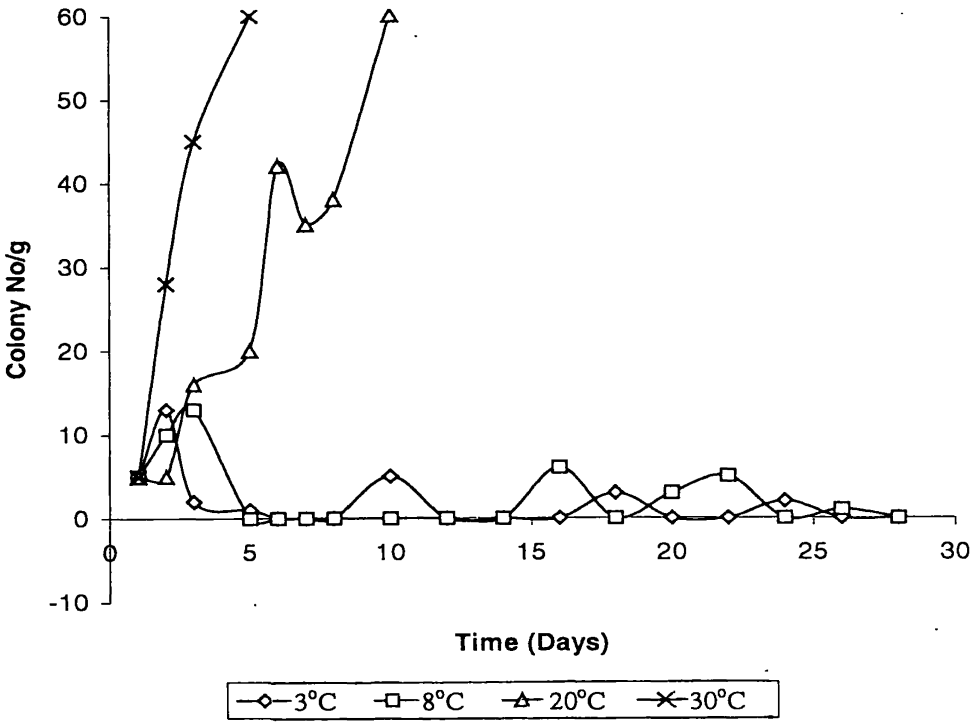

Figure 4.3- Variation of yeast and mould growth with time

The total viable count of yeasts and moulds of all the samples are shown in Table 4.4

The yeasts and moulds colonies are white in colour. After 10th day of storage period, yeasts and moulds colonies were uncountable on PDA media at 30°C and 20°C. Because these organisms gain higher rate of growth with high acidity conditions. According to the above results, the growth rate of yeasts and moulds were increased with decreasing pH. Growth rate of yeasts and mould is likely to be rapid in low pH condition.

4.4 SENSORY ANALYSIS FOR THE SELECTION OF BEST STORAGE TEMPERATURE FOR EACH CHARACTERISTIC.

The effect of the panel on the evaluation of quality parameters was not significant. So there was no any bias. Also the panelist to panelist's variation was not found in decision-making.

4.4.1 Kruskal Wallis non –parametric test for colour

According to the sensory evaluation, the best temperature for colour development of the yoghurt are given below.

Treatment	Ave Rank
186 (3°C)	13.8
248 (20°C)	39.1
356 (30°C)	45.9
732 (8°C)	23.3

H = 31.62 DF = 3 P = 0.000 P'= 0.05

According to the above results

P'< P

So, there are at least one treatment is significant with regard to colour. That means temperature is significantly affecting the changes of taste. The samples at 30°C have the highest average rank (45.9). That means this sample has highest changes of taste at storage period and the samples at 3°C have the lowest average rank (13.8). That means this sample has lowest changes of taste during the storage.

4.4.2 Kruskal Wallis non –parametric test for texture

According to the sensory evaluation test, temperature, which has best colour of the yoghurt at storage period, is selected bellow.

Treatment	Ave Rank
186 (3°C)	18.1
248 (20°C)	37.8
356 (30°C)	48.9
732 (8°C)	7.2

H = 35.70 DF = 3 P = 0.000 P'= 0.05

According to the above results

$$P' < P$$

So, there are at least one treatment is significant with regard to texture. That means temperature is significantly affecting the changes of texture. The samples at 30°C have the highest average rank (48.9). That means this sample has highest changes of taste at storage period and the samples at 8°C have the lowest average rank (7.2). That means this sample has lowest changes of texture during the storage.

4.4.3 Kruskal Wallis non –parametric test for appearance

Treatment	Ave Rank
186 (3°C)	10.2
248 (20°C)	37.1
356 (30°C)	50.8
732 (8°C)	23.9

H = 44.77 DF = 3 P = 0.000 P'= 0.05

According to the above results

$$P' < P$$

So, there are at least one treatment is significant with regard to appearance. That means temperature is significantly affecting the changes of appearance. The samples at 30°C have the highest average rank (50.8). That means this sample has highest changes of appearance at storage period and the samples at 3°C have the lowest average rank (10.2). That means this sample has lowest changes of appearance during the storage.

4.4.4 Kruskal Wallis non –parametric test for odour

Treatment	Ave Rank
186 (3°C)	16.0
248 (20°C)	43.7
356 (30°C)	45.1
732 (8°C)	17.2

H = 38.18   DF = 3   P = 0.000   P'= 0.05

According to the above results

$$P' < P$$

So, THERE are at least one treatment is significant with regard to odour. That means temperature is significantly affecting the changes of odour. The samples at 30°C have the highest average rank (45.7). That means this sample has highest changes of odour at storage period and the samples at 3°C have the lowest average rank (16.0). That means this sample has lowest changes of odour during the storage.

4.4.5 Kruskal Wallis non –parametric test for overall acceptability

Treatment	Ave Rank
186 (3°C)	14.1
248 (20°C)	37.4
356 (30°C)	48.0
732 (8°C)	22.6

H = 33.67 DF = 3 P = 0.000 P'= 0.05

According to the above results

P'< P

So, there are at least one treatment is significant with regard to overall acceptability. That means temperature is significantly affecting the changes of overall acceptability. The samples at 30°C have the highest average rank (48.0). That means this sample has highest changes of overall acceptability at storage period and the samples at 3°C have the lowest average rank (14.1). That means this sample has lowest changes of overall acceptability during the storage.

According to the sensory evaluation, there are significant changes in quality of non-fat yoghurt during storage at higher temperature levels. These changes were pronounced at 30°C in storage. According to the above results the sample stored at 3°C was of best quality yoghurt while samples stored on 30°C are sensory unsatisfactory to consume.

CHAPTER 05

CONCLUSIONS

- All examined samples were accompanied with increased acidity and lower pH. This was more pronounced with 30°C storage condition.
- The difference of temperature at the storage has significant effect on acidity development.
- Samples at 3°C and 8°C are microbiologically acceptable for consumption.
- It was identified that temperature of the storage condition, significantly affect the changes of organoleptic properties (colour, texture, odour, appearance and overall acceptability) of the non-fat yoghurts.
- According to the study the best storage temperature for non fat yoghurt is 3°C (cold room condition)
- Shelf life of the non fat yoghurt at 3°C is 25 days
- 8°C (domestic refrigerated condition) is suitable for storing non-fat yoghurt and the shelf life is more than two weeks (16 days).
- Non-fat yoghurt can be stored and distributed at temperatures of between 3°C and 8°C without changes in appearance, texture, odour and colour more than two weeks.

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APPENDIX I

RANKING TEST FOR NON FAT YOGHURT

Please indicate your assessment for these four samples for colour, appearance, texture, odour and overall acceptability, by filling the following table with the rating system given below.

- 1 for first best sample
- 2 for second best sample
- 3 for third best sample
- 1 for fourth best sample

Attribute	**356**	**248**	**732**	**186**
Colour				
Texture				
Odour				
Appearance				
Overall acceptability				

Comments:

....

....

APPENDIX II

Kruskal-Wallis Test: Rank versus Item

Kruskal-Wallis Test on Rank

Item	N	Median	Ave Rank	Z
186	15	1.000	13.8	-4.28
248	15	3.000	39.1	2.19
356	15	3.000	45.9	3.94
732	15	2.000	23.3	-1.85
Overall	60		30.5	

H = 31.62 DF = 3 P = 0.000
H = 33.66 DF = 3 P = 0.000 (adjusted for ties)

APPENDIX III

Kruskal-Wallis Test: Rank versus Item

Kruskal-Wallis Test on Rank

Item	N	Median	Ave Rank	Z
186	15	1.000	18.1	-3.18
248	15	3.000	37.8	1.88
356	15	4.000	48.9	4.72
732	15	1.000	17.2	-3.41
Overall	60		30.5	

H = 35.70 DF = 3 P = 0.000
H = 38.03 DF = 3 P = 0.000 (adjusted for ties)

APPENDIX IV

Kruskal-Wallis Test: Rank versus Item

Kruskal-Wallis Test on Rank

Item	N	Median	Ave Rank	Z
186	15	1.000	10.2	-5.20
248	15	3.000	37.1	1.68
356	15	4.000	50.8	5.20
732	15	2.000	23.9	-1.68
Overall	60		30.5	

H = 44.77 DF = 3 P = 0.000
H = 47.45 DF = 3 P = 0.000 (adjusted for ties)

APPENDIX V

Kruskal-Wallis Test: rank versus Item

Kruskal-Wallis Test on rank

Item	N	Median	Ave Rank	Z
186	15	1.000	16.0	-3.71
248	15	3.000	43.7	3.39
356	15	3.000	45.1	3.74
732	15	1.000	17.2	-3.41
Overall	60		30.5	

H = 38.18 DF = 3 P = 0.000
H = 40.57 DF = 3 P = 0.000 (adjusted for ties)

APPENDIX VI

Kruskal-Wallis Test: Rank versus Item

Kruskal-Wallis Test on Rank

Item	N	Median	Ave Rank	Z
186	15	1.000	14.1	-4.21
248	15	3.000	37.4	1.76
356	15	4.000	48.0	4.47
732	15	2.000	22.6	-2.02
Overall	60		30.5	

H = 33.67 DF = 3 P = 0.000
H = 36.41 DF = 3 P = 0.000 (adjusted for ties)

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