DEVLOPMENT OF LOW CALORIE YOGHURT

by

D.C.S. HALLALA

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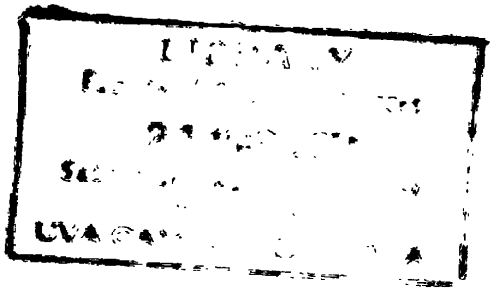
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka,
Buttala,
Sri Lanka.



DECLARATION

The work described in this thesis was carried out by me at the Gaya Cream Yoghurt, 172/4 Gothatowa, Angoda and Faculty of Applied Sciences under supervision of Dr. K.K.D.S. Ranaweera and Mrs. J.A.N. Jayakodi. A report on this has not been submitted to any other University for another degree.

D.C.S.Hallala

Date: _____

Certified by,

Mrs. J.A.Nalani Jayakodi
External Supervisor,
Manager,
Gaya Cream Yoghurt,
172/4 Gothatowa,
Angoda

Date: _____

Dr K K D S Ranaweera
Internal Supervisor,
Dean,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala,

Sri Lanka

Date: 15/12-20

Mr M A Jagath Wansapala,
Course coordinator,
Degree program of Food Science and Technology,
Department of Natural Resources,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala.

Sri Lanka

Date 15/12/20

AFFECTIONATEY DEDICATED
TO MY
LOVING PARENTS, TEACHERS
AND
MY FRIENDS

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ABSTRACT

Milk is a high nutritive balanced food. It consists of valuable material such as proteins, fat, milk sugar(lactose), vitamins and etc. A range of ways are available to preserve milk by converting it in to value added product, such as preparation of butter, cream, curd, cheese and yoghurt etc. Among them there is a big demand for yoghurt due to its flavor, taste, appearance, and nutritive value. It also has a relatively longer shelf life and easy to process at small to large scales. Yoghurt is produced from milk by lactic acid fermentation *Streptococcus thermophilus* and *Lactobaccilus bulgaricus* are used in this process. Yoghurt can be produced in 2 types; set and stirred. Major categories are full fat, low fat and non fat

Low calorie yoghurt is healthy food in which sugar is replaced by an artificial sweetener Low calorie yoghurt is produced from skim milk or raw milk (by removing fat) with use of artificial low calorie sweeteners. These type of products are suitable for people who are suffering from Hyperlipidaemia, diabetics and cardiovascular disease. The main low calorie sweeteners are aspartame, cyclamate, saccharin and etc. The main ingredients are raw milk, sweetener, gelatin, colouring, flavor and starter culture. Some operations are involved in preparation of low calorie yoghurt such as homogenization, heat treatment, fermentation, incubation and cooling A study was carried out to find out a sweeteners suitable for a low calorie yoghurt formula.

According to sensory evaluation, sorbitol is a appropriate sweetener in production of low calorie yoghurt. It is mostly resemble to the sugar and not remain sweeten aftertaste and also it has minimal effect of carcinogenic and other defects.

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CHAPTER 01

1. Introduction

Milk is a balance food that very essential to human beings and other animals. Nutritionally milk is the most nearly perfect and natural food to us. Milk contains mainly carbohydrates, fats, vitamins and minerals and has pH of about 6.6. It provides and excellent growth medium for micro organisms. There are so many food varieties which are made from milk by fermentation.

Yoghurt is an acidified coagulated semi solid product obtain from milk by fermentation with lactic acid producing bacteria. Yogurt is originated from Balkans and Eastern Mediterranean countries and traditionally have been made with whole milk obtain from cows, goats and sheep. Health foods such as low calorie, non-sugar and non-fat foods have big potential in consumer demand worldwide. Low calorie yoghurt is also healthy food in which preparation is same way as the normal yoghurt preparation but reduce the fat level below to 1.5% and sugar is replaced by an artificial sweetener.

Manufacturing of yoghurt is depending upon country raw material used, product formulation, size of operation and type of product. The popularity of the product will depend to a large extent on the ability to product which means adoption of right technological parameters like , optimum heat and homogenisation treatment and culturing in addition to maintain high standards on composition. The product should have a uniformly smooth body, texture, characteristic and pleasing flavour with minimum whey separation.

Low calorie Yoghurt is produced from skim milk which give more advantages to the man as a,

Value added product.

Treatment of patient who are suffering from lactose intolerance.

Yoghurt stimulates the stomach movements and enhance acid digestion.

Reduction of microbial population in gastro - intestinal tract and prevent the same disturbances of the intestinal tract.

Yoghurt bacteria has effect on break down the cholesterol.

Anti microbial properties are comprised in the yoghurt.

In low calorie yoghurt milk fat level is below 1.5 %, Titrable acidity as lactic acid range from 0.8 to 1.25 %, Milk Solid- Not - Fat (M.S.N.F) about 10%, Total Soluble Solid is about 12.5% and The final pH is around 4.6 to 4.7

- Investigation of suitable sweeteners for low calorie yoghurt with view to provide the product for a specific consumer group

CHAPTER .2

2. Literature review

2.1 Milk composition, chemical constituent and physical properties

Milk is a dynamically balanced mixture of proteins, fat, carbohydrates, salts and water co-existing as emulsions, colloidal suspensions and true solutions. Milk and diary product gain demand because of its nutritional and organoleptic properties, Although there is no perfect natural food. Milk is considered as the nearly perfect natural food of human beings. It is defined as the normal whitish liquid secretion of the mammary glands of lactating mammals (milk which take from the first few days of mammals after the birth of carves contain a large number of blood cells and hence unpalatable, therefore it should be avoided.). The cow is, of course, the most important of all these animals as a supplier of food for man, but buffalo or goat milk is more important in some parts of the world..

2.1.2. Composition of milk

Milk consist of complex mixture of lipids, carbohydrates, proteins, and many other organic compounds and inorganic salts dissolved or dispersed in water, some of these compounds such as the carbohydrate, lactose, and most of the salts and vitamins are soluble in water Other such as lipids, Proteins and di calcium phosphate are dispersed through the water in the colloidal or near colloidal state.

The major determinants of the milk composition from individual cows are breed of cow, age and stage of lactation. There are some factors affect the composition in raw milk, both as regards fat and solids-non-fat content. These factors are,

- Breed of cow
- Feeding
- Seasonal variation
- Health of the animal
- Age of the animal
- Efficiency of the milker
- Intervals between milking
- Period of lactation
- Individuality of the cow

Table 2.1.1: The average composition of cow milk,

Water	87.2%
Fat	3.7%
Protein	3.5%
Lactose	4.9%
Minerals	0.7%

Source : Dairy Processing, Food Cycle Technology Source

2.1.3. Milk fat

Milk fat is present in fine globules and in the form it is easily digested. It contains fat soluble vitamins such as vitamin A, D, E and K and essential fatty acids such as linoleic and linolenic acids. Milk fat provides a good source of energy.

The small amount of cholesterol in milk comes directly from blood, plasma. The density of the milk fat is lesser than serum and therefore fat globules rise to top of the whole milk.

The milk fat globules membrane is a weak structure and it can be easily broken by physical or thermal shock. Damage of fat globules react with lypolytic enzyme and resulting Rancid flavours and odours. (Srivastava, S.B. 1993)

The cream separator concentrates the milk fat into a small portion of the milk. The product is known as " cream". Milk fat exists in milk in the form of minute globules in a true emulsion of the oil-in-water type, the fat globules being in the dispersed phase. Milk with high fat content is creamy and smooth and yields more butter and cheese.

(Eckels, A.L. Combs, W.B Macy, H. 1993)

2.1.4. Milk proteins

Proteins may be defined as substances composed principally of amino acids chemically combined. Milk proteins can be classified in to 2 groups , they are casein and whey proteins. Both casein and whey protein consist of essential amino acids. The relative proportion of whey protein to casein vary over the season, typical mid season values are shown below,

(Eckels, A L. Combs, W.B Macy, H. 1993)

Table 2.1.2 : protein distribution in skim milk

Protein type	Percentage
Casein	82.2
Whey protein	
Beta lactoglobulin	9.6
Alpha lactoalbumin	3.8
Bovine serum albumin	1.4
Minor components	3.0

Source : Data from Davies and Law (1980)

a) Caseins

The caseins of the milk can be subdivided in to five main classes, α_{s1}, α_{s2}, β, γ, κ caseins. α_{s1} caseins are sensitive to calcium due to the presence of phosphate groups and the precipitate in the presence of calcium iron at a pH value of 7. Casein is a yellowish-white granular substances. In its pure state it is snow-white, odourless, and tasteless. It contributes to the whiteness of milk. When milk is separated of the centrifugal separator, a large proportion of the casein goes with the skim milk. The serum of the cream contains the same proportion as the serum of the original milk. Casein may be precipitated from milk by dilute acids, rennin and alcohol. The casein precipitated with weak acids is free of calcium. The casein precipitated with alcohol is calcium caseinate. When precipitated with rennin, paracasein is formed.

Casein is present in the form of micelles. Micelles are pores and highly solvated. Hydrophobic forces play a great role in micelle organization. calcium is necessary to micelle stability and removal result in disruption into sub units. (Varnam, A.h. and Sutherland J.p., 1996)

b) Whey protein

Minor amounts of Lactalbumins and lactoglobulin which are essential for the disease resistance in the young animals. The major whey protein consist on sulfur amino acid, on heating may participate sulphydryl exchange reactions leading to inter and intra-molecular cross linkage (Varnam, A h and Sutherland J p , 1996)

2.1.5 Milk sugar

Lactose is a main sugar, present in raw milk. Lactose is a disaccharide comprised with alpha-D- glucose linked to beta-D-galactose and is made up of by combining two simple sugars, glucose an galactose. Lactose contain C,H,O as energy source, Not only it provides source of energy but also it provides valuable source of galactose needed for the development of cerebroside. (Eckels, A.L. Combs, W.B Macy, H. 1993)

Lactose in milk help to enhancing the absorption of Calcium in intestinal track and important in the production of fermented milk products like youghurt and cheese. However, some persons can't digest this lactose in appreciable quantities. This incident is called as "lactose intolerant" and such people can use fermented milk products help to overcome this problem. Because, fermented milk products such as youghurt, cheese, have low amount of lactose, due to the conversion of some lactose in to lactic acid by microbial activities. (Byron.H.Webb, Arnold H. Johnson, John A. Alford, 1987)

2.1.6. Mineral in milk

All of the minerals are distributed between a soluble phase and a cooloidel phase.

Table 2.1.3: Mineral percentage in milk

	Percentage	
	Soluble phase	**Collodial phase**
Calcium total	33	67
Calcium (ionized)	100	0
Chloride	100	0
Citrate	94	6
Magnesium	67	33
Phosphorus (total)	45	55
Phosphorus (inorganic)	54	46
Potasium	93	7
Sodium	94	6

Milk contain most of essential minerals required by men, women and children of all ages. Calcium is essential in building the body skeleton as well as muscles. Calcium is necessary for the formation of micelles. (Srivastava, S.B. 1993)

2.1.7. Enzymes in milk

A number of enzymes are present in milk naturally, while some enter as a result of microbial contamination. Enzymes present in milk can be divided in to following groups:

(I) Oxidizing enzymes (peroxidase) which liberates oxygen from H_2O_2 in fresh milk.

(II) Amylase (diastase) which hydrolyses starch

(III) Lipolytic enzymes which hydrolyze fat

(IV) Catalase which decomposes H_2O_2

(V) Phosphatase which decomposes phosphorous esters

(VI) Lactases which hydrolyze lactose

(VII) Reductase which reduce certain organic materials in milk.

(VIII) Proteolytic enzymes which hydrolyse proteins.

They are denatured and inactivated by high temperatures, they possess a pH of optimum activity, and they exhibit specificity for certain substrates. (Srilakshmi, B. 1997)

2.1.8. The vitamins in milk

Milk is a useful source of some vitamins. There are two types of vitamins present in milk. They are :

- Fat soluble vitamins A, D, E and K
- Water soluble vitamins :

Vitamin B_1 (Thiamin or Aneurin)

Vitamin B_2 group - Riboflavin, Nicotinic acid, Pyridoxine, Pantothenic acid, Biotin, Vitamin B_{12}, Folic acid

Vitamin C (Ascorbic acid)

But, particularly milk is a good source of vitamin A and B_2 group (especially riboflavin) and it is also a good source of Thiamin. Other vitamins contain in smaller quantities in the milk. The feed is known to be a factor influencing the amount of vitamins in milk (Srilakshmi, B. 1997)

2.1.9. Non-protein nitrogenous substances

Milk contains a group of nitrogenous substances in addition to the proteins. These substances do not occur in large quantities but they are known to be present. The non-protein nitrogenous substances of milk may be listed as follows: urea, nitrogen , amino nitrogen, creatinine, creatine, uric acid adenine, and guanine. The substances are measured in parts per million, ranging from 1.5to10 in milk. (Eckels, A.L. Combs, W.B Macy, H. 1993)

2.1.10. Flavouring substances

In milk slightly sweet and salty taste result from the balance between lactose and milk minerals. Compounds involved in determining flavour include alkanals, lactones, esters sulphur compounds, aliphatic and aromatic hydrocarbons. (Eckels, A.L. Combs, W.B Macy, H. 1993)

2.1.11. Physical properties of milk

Milk is an aqueous solution of lactose, salts and few other minor compounds, which is emulsified with fat.

Table 2.1.4: physical properties of cow milk

	Representative value
pH value (at 25°C)	6.6
Specific gravity (at 200C)	1.032
Freezing point	-0.5400C
Boiling point	100.170C
Viscosity (at 200C)	1.6314cpoise
Surface tension (at 200C)	50 dynes/cm
Heat capacity	0.52 cal/g 0C

* *Data from B H Webb, A.H. Johnson, J.A Alford, Fundermentals of dairy chemistry second Edition*

a) Density

The density of milk and milk products is used for determining the following factors

- To estimate the solids content
- To convert volume to mass
- To calculate other physical properties

b) Viscosity

Milk is more viscous than water due to fat emulsion and colloidal particles. Therefore, variation of that compounds alters the viscosity of milk.
viscosity of the milk is necessary to determining

- The rate of creaming
- The flow condition of dairy processes
- Rate of mass and heat transfer

c) Freezing point

This is mainly used in determine added water, it can also used determine lactose content in milk, estimate whey powder contents in skim milk powder and determine water activity. A pure solvent freezes at a higher temperature than the solution. The depression of the freezing point depends on the concentration of dissolved substances. Also that point can decrease with the function of osmotic pressure of the solution.. Freezing point of milk is a constant value. So, it can use to detect the adulterations by the depressing of that point.

d) Acid – Base equilibria

pH is used to measure the milk acidity. The milk has 6.6 pH value. Many substances affecting the acidity of milk. Many component of milk provide buffering action and the major buffering groups are casein and phosphate

e) Specific gravity

The average specific gravity of milk is 1.0032 at 15.5 ^{0}C , but it is one of the most variable properties of milk. It is varied by amount of water, fat, non-fatty solids, adulterations etc.,

f) Specific heat

In normally milk has a specific heat of 0 93. This value is approximately same to warm milk in which fat is in liquid condition But, that value is less then the cold milk (below 19^{0} C), because some of the heat supplied to the milk at a temperature near the melting point of the fat is used to supply the fat with it's latent heat of fusion

g) Boiling point

A pure solvent boils at a lower temperature than a solution, due to the absent of dissolved substances. The boiling point of milk is constant to it and shows value of 100.45^0 C.The adulterations of milk increase to that point.

h) Colour and flavor

Milk has characteristic white opalescent colour, due to the scattering of light by the containing colloidal particles. The yellow colour of carotene is in fat, and so becomes prominent in cream and butter.

The sweet taste of milk sugar (lactose) is balanced by the salty tastes of minerals specially chlorides and both are damped down by proteins.

(Early, R., 1998)

2.2. FERMENTED DIARY PRODUCTS.

Fermented dairy products are produced by the modification of raw material by the activities of microorganisms. The organisms were cultured in the laboratory and pure cultures added to raw materials to give fermentation that were more reliable and give a consistent product.

The manufacture of fermented dairy products involves the addition of lactic acid bacteria as starter cultures to milk, which is normally pasteurized before the addition of starter. Historically fermentation process were based upon spontaneous souring of milk caused by inherent microflora. Modern processes effect milk fermentation under predictable, controllable and exacting condition to yield cultured diary products of high nutritional and sanitary conditions.

The bacteria are employed in cultured diary foods are ; for acid production, *Lactobacillus acidophilus*, *Streptococcus lactis*, *Streptococcus thermophillus* and *Streptococcus cremoris* for acid production and flavour development *Lactobacillus bulgaricus* and *Streptococcus lactis* are used for flavour production, *Leuconostoc cremoris*. (Garbutt, J 1997)

2.2.1. Cultured butter milk

Cultured butter milk is a by product of the butter making process. Cultured butter milk is obtained from pasteurised skim milk cultured with lactic and aroma producing organisms They are *Streptococcus lactis*, *streptococcus cremoris* and *Leuconostoc cremoris* However, cultured butter milk is a viscous, containing a characteristic pleasing aroma and flavour

.(Garbutt, J 1997)

2.2.2. Butter

Butter can be defined as agglomerated mass of a mixture of milk fat, butter milk and water, usually add colouring and salt. Butter differs from other dairy products in that is a very highly concentrated fatty food with small amount of milk constitution. (Early, R., 1998)

2.2.3. Acidophilus milk

Acidophilus milk is produced by fermentation of milk with *Lactobacillus acidophilus.* Skim milk or whole milk may be used, Tyndallization process or Ultra High Temperature treatment may be applied to reduce the microbiological load and favour the slow growing *Lactobacillus acidophilus.* For moderating the sour taste, acidophilus milk may be mixed with cultured buttermilk. (Early, R., 1998)

2.2.4. Cheese

Cheese is fermented milk product obtained by coagulation of proteins by using selected bacteria and acid or enzymes or a mixture of them and separating the coagulant or curd from the whey. The texture taste etc., of the cheese produced varies with the types of coagulating media is used. Long curing or ripening of cheese is requried to in order to develop the suitable characteristics of body, texture, flavour and aroma. (Early, R., 1998)

2.2.5. Curd

Curd is obtain from coagulation of cow or buffalo milk by the growth organism of *Streptococcus lactis, Streptococcus diacetilactis , Sterptococcus cremoris* singly or in combination with leuconostoc species. Cured contain not less than 5% of fat by weight and 8 5% of milk solid non fat by weight and pH 4.5 maximum. The digestibility is better when compared to ordinary milk . (Early, R., 1998)

2.2.6. Cream

Cream is a part of a milk, rich in milk fat ranging from 18- 30% whipping cream is used as topping and in certain other preparations such as manufacture of butter, cheese etc (Early, R , 1998)

2.2.7. Yoghurt

Yoghurt is a fermented milk product obtain from coagulation of milk by the growth of organisms of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* It is semisolid, slightly acidic, flavoured or non flavoured and minimum whey separation (Early, R , 1998)

2.3. Classification of yoghurt , Nutritive value of yoghurt and yoghurt production

2.3.1. Classification of yoghurt

Yoghurt is broadly classified in to two types according to the method of poduction: they are set Yoghurt and stirred Yoghurt. The deference between set Yoghurt and stirred Yoghurt is that set Yoghurt after addition of culture coagulates in the package itself and stirred Yoghurt after coagulating, is stirred and packed.

Major categories of set Yoghurt available at the market

- Full fat
- Low fat
- Non fat

The above categories can be further dived as follows,

- Plain Yoghurt
- Sweetened and flavoured Yoghurt
- Fruit Yoghurt
- Salad Yoghurt

Yoghurt which gives all the nourishment, like milk and curd is considered as healthy food. Low fat Yoghurts are produce to special consumer group such as diabetic patients, heart disease persons etc. (Vamam, A.h. and Sutherland J.p., 1996)

2.3.2. Nutritive value of low calorie yoghurt

2.3.2.1. Milk fat in the diet of the man

The determining of the palatability of food the presence of fat is an important factor. Milk fat consist of small molecular size, short chain fatty acid etc. These milk fat also provide energy value

The unhealthy image associated with dairy products from three factors: the high fat content of whole milk and whole milk products, the cholesterol content and the saturated nature of the fatty acids A dietary fat intake is associate with obesity and with hyperlipidaemia (excessively high levels of blood lipoproteins) Hyperlipidaemia is recognized as a risk factor predisposing to heart disease For this reason most of the countries reduction in fat consumption In some cases dairy industry introduction the products such as skim milk and low fat yoghurt, etc

Both dietary cholesterol and saturated have been associated with serum cholesterol levels and regarded as risk factor for cardiovascular disease. (Early, R., 1998)

2.3.2.2. Benefits of using low calorie yoghurt

- Improvement in nutrient quality

Increase the vitamin B content , antinutritional factors such as lectins, phytates and glucosinolates may be removed by the fermentation process and also increase the availability of the minerals.

- Preservation

In Raw milk has a very shorter shelf life and is highly perishable.

- Health benefits

Low calorie yoghurt can reduce the serum cholesterol level and help avoid Cancers. These types of yoghurts are restorative effect on the gut micro flora, assisting recovery of a normal balanced flora after oral antibiotic therapy.

- Improve digestibility

Most of the fermented foods are more digestible than the original raw material. Some people cannot digest lactose completely who can consume some type of dairy products such as yoghurt. In take of dairy product release unabsorbed lactose in the gut which is fermented by the normal gut flora and which cause abdominal pain, flatulence and diarrhoea. During the fermentation these lactose is converted to more easily digested lactate.

- Detoxification of raw material

The fermentation process may remove toxic compounds such as antibiotics.

(Garbutt, J.1997)

2.3.3. The manufacture of low calorie yoghurt

The manufacturing process of yoghurt is uniform. compositional values of yoghurt is vary as do yoghurt types, whish there by affect the principle of manufacture. Number of factors must be carefully control during the manufacture process in order to produce a high quality yoghurt with the required taste , aroma, appearance, consistency, viscosity and shelf life. In law calorie yoghurt fat content should be less than1.5 %

Reduced fat level leads to

- Reduced resistance to syneresis
- Reduced smoothness
- Reduced creaminess
- Inferior mouth feel
- Reduced viscosity

These effects can be avoided by

- Increasing the protein content
- Adding stabilisers

Table 2.3.1: Yoghurt with reduced fat content

	Full fat	Low fat	Non fat
Fat %	3.5	1.5	-
MSNF %	8.0	10.0	12.0
Stabiliser system %	0.8	1.0	1.2
Total solids %	12.3	12.5	13.2
Kcal/100g	67.0	58.0	53.0
% reduction	---	13.0	21.0

Source : Internet

These include

- Choice of milk base
- Ingridient added
- Heat treatment
- Emulsification
- Culture preparation

2.3.4. Ingredients using in yoghurt production

Most of the ingredients are common in yoghurt industry, but formula can be different. Chemical composition of yoghurt is very essential specially in Fat and Milk Solid Not Fat (MSNF) % level.

- Milk
- Water
- Sweeteners
- Stabilisers
- Preservatives

2.3.4.1. Milk ingredients

The main ingredient of yohgurt is milk or it's derivatives and most commonly used ingredients are

- Whole milk
- Skim milk
- Concentrated skim milk
- Cream
- Skimmed milk powder

In low fat formulation, fresh skimmed milk or concentrated skim milk are the major ingredients. SNF content can be adjust y the addition respectively, of skimmed milk powder or water. The minimum milk SNF in yoghurt should be 8.2%(FAO/WHO, 1976)

(Early, R., 1998)

2.3.4.2. Sweeteners

Sweetening compound are added to the yohgurt via fruit concentrate or milk base. The level of incorporation of sweetening agent will depend upon factors such as

- The type, level and acidity of fruit used
- The type of sweetening compound used
- Consumer preference
- Legal requirements
- Inhibitory effect on starter organisms
- Be non carcinogenic

Generally sucrose, glucose, sugar, fructose like sweeteners are added Non-calorific sweeteners are used in law calorie formulations, for e g aspartame cyclamate and

saccharine. To satisfy dieters, among whom diabetics are an important category, sweeteners should be used. (Jones, 1998)

Table 2.3.2: Sweetness of artificial sweeteners

Sweetener	Sweetness Relative to sucrose (Sucrose =1)
Acesulfame K	200
Alitame	2000
Aspartame	180
Cyclamate	30
Saccharin	300
Sucrolase	600
Sorbitol	0.5

Source : Adapted from Munro (1989).

a) Cyclamate

It's sweetness coupled with a less bitter aftertaste than saccharin made it very viable as an artificial sweetener. It was stable under condition of high heat, therefore it can be used wide variety of food products. Cyclamate is initial approval in the USA. But the National Academy of Science (NAS) reported that reasonable quantity of cyclamate posed no hazard to humans. In 1985 the NAS reviewed all existing data and concluded that Cyclamate and it's incriminated metabolite, cyclohexamine, were not them selves carcinogens but could be cancer promoters (NAS/NRC, 1985). The structure of the Cyclamate is shown below,

(Jones, 1998)

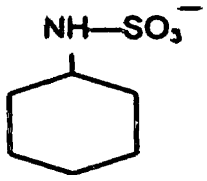


Figure 1: structure of cyclamate

b) Saccharin

Saccharin is 1,2 –benzisothiazolin-3-one-1,1-dioxide It was originally used as an antibacterial agent and food preservative Many products could be sweeten with saccharin because it was stable over a wide range of temperature and condition The only disadvantage to it's use was a slighter bitter aftertaste. this is due to the presence of impurities, additives such as glucono-delta-lactone are used to make this aftertaste

Impurities of saccharin is responsible for variations in the results obtained in toxicity test, but it did not have any mutagenic effect.

It also recommends careful of any adverse effect of saccharin and warns that young children and pregnant women should carefully consider the use of saccharin (AMA, 1985,1986).

Legally , saccharin is now classified as a carcinogen (tumor promoter) with very low potency. Extrapolations suggest that saccharin at 30-300 mg/day dose not increase human cancer risk (Byard, 1984). The structure of the Saccharin is shown below,

(Jones, 1998) (Linden & loriet ,1994)

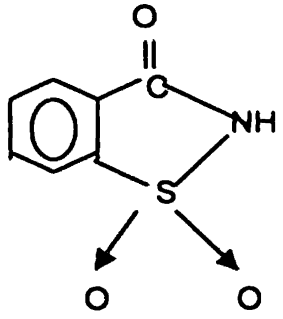


Figure 2: structure of saccharin

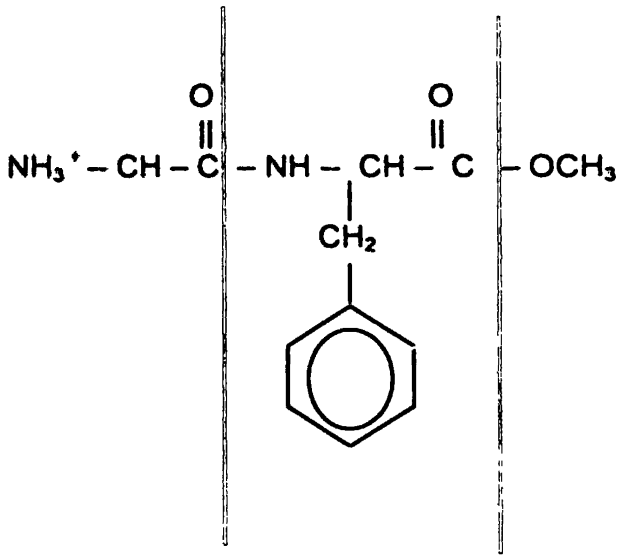
c) Aspartame

Aspartame is a dipeptide of L-aspartic acid and L-phenylalanine in the form of it's methyl ester. Aspartame is metabolized same way as the amino acid. It's solubility depend on pH and temperature. Aspartame is now permitted numerous countries i.e. recommended daily allowance is between 40-50 mg/kg. The stability of aspartame is depend on the pH moisture and length of storage. However aspartame is not stable to high heat, so it use In certain type of food is not feasible (Homler, 1984). During digestion of aspartame some methanol is produced because it contains a methyl ester. However, blood level of methanol were far below those considered to have any adverse effect even when aspartame was fed at 6 times the estimated daily intake.(Stegnick and Filer, 1984). Methanol toxicity result from it's metabolism to formate and the build up of formates.

Phenylalanine can cause neurotoxic potential of high plasma level Investigation showed that dose of aspartame similar to 24 liters of diet soda or 600 sweeter tablets doubled the plasma phenylalanine level. Increase phenylalanine may cause toxic symptoms of all groups including infants, pregnant women and people with phenylketonuria (PKU)

With respect to the aspartic acid component, the concerns focused on possible focal brain lesions or endocrine disorders from aspartic acid along or in combination with glutamate from the diet The structure of the aspartame is shown below

(Jones 1998) (Linden & loriet 1994)



L-Aspartic acid L-Phenylalanine Methanol

Figure 3: structure of aspartame

d) Acesulfame – K

Acesulfame – K is derivative of acetoacetic acid (Wells 1989). It has some structure similar to saccharin (Newberne and Conner, 1986). Acesulfame – K has several advantages over aspartame, when aspartame and Acesulfame – K used together, the sweetness quality is better than with either sweetener along. (Jones, 1998)

e) Sugar alcohols

Sorbitol, mannitol, maltitol and xylitol are all sugar alcohols that occur in naturally in small amounts in fruit and vegetables. Theoretically, these sugar alcohol yield the same number of calories as sugar, but ion actually they are incompletely metabolized, so the net energy yield is lower (Wursch and Anantharaman, 1989). They may have an effect on appetite If diet containing sorbitol or xylitol were less likely to consume excess food and over weight than when diet containing sucrose They absorbed more slowly and help reduce to formation toxic ketone bodies One study showed that the population of the decay producing bacteria *Streptococcus mutans* increase with the consumption of the sorbitol (Hirsch, 1985)

(Jones, 1998)

2.3.4.3 Stablisers

Hydrophilic colloids can bind water. The main functions are

- Increase viscosity during processing & to improve of viscosity of final products.
- Affect structure and the texture.
- Help to prevent whey separation during storage and transporting.
- Assist suspension of fruit particles.
- Influence creaminess and mouth feel.

Commonly use stabilizers are gelatin, pectin, agar, starch. The solubility, dissolution, solidification and temperature stability characteristic require to be taken in to account when using stabilizers in order to achieve optimum performance (Tamine and Robinson, 1985). Stabilizers usually added before homogenization and heat treatment and this has the obvious advantage of destroying vegetative microbial pathogens and potential spoilage of organisms. Stabilizers are added after pasteurization with a carrier such as flavoring or colouring. (Early, 1998)

2.3.4.4.Colouring matter

The addition of colour to fruit and flavoured yoghurts is aimed at making the products more attractive, and the active agents may be of natural origin or synthetic organic dyes.

(Srivastava, S.B. 1993)

2.3.4.5. Starter culture

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

The starter is added to the product and allowed to grow there under controlled conditions. The fall in pH which ensues when the bacteria ferment lactose to lactic acid has a preservative effect on the product, while at the same time its nutritional value and digestibility are improved.

(Early, 1998)

2.3.4.6. Preservatives

A number of preservatives are used either directly in to the milk prior to fermentation The most common preservatives are potassium sorbate, sodium benzoate and sulphur dioxide The main function of the preservatives are prevent the growth of yeast and moulds (Early 1998)

2.3.5. Important operations in yoghurt production

2.3.5.1. Homogenisation

When heat the milk, fat globules get together and come to the top to avoid this homogenisation is done before pasteurisation. In order to prevent this and also to have a uniform consistency throughout, the yoghurt mix is homogenised to break the fat globules. The homogenisation will reduce the average diameter of the fat globules to less than 2 microns size prevent cluster formation and tendency for the fat to the surface. Homogenisation depends on the correct level of fat content in the mix and correct temperature and pressure of homogenisation.

Improvement in consistency characteristics in yoghurt could be due to the change in the water holding capacity of the milk proteins which tend to reduce the syneresis characteristics and the increased amount of milk fat soluble membrane materials in the skim phase of milk (Dannenberg and dessler, 1986).

2.3.5.2. Heat treatment

The heating of milk to proper time temperature combination is of utmost importance to prepare good quality yoghurt. This, apart from reducing the undesirable micro-organisms, will result in improvement in the physico-chemical properties yoghurt.

The milk is heated before being inoculated with starter in order to

- Improve the properties of the milk as a substrate for the bacteria culture.
- Ensure that the coagulum of the finished yoghurt will be firm.
- Reduce the risk of the whey separation on the finished products.

Optimum results are achieved by heat treatment at 90-95 °C and a holding time is about 5 minutes that temperature time combination denatures about 70-80% of the whey proteins. In particular the β-lactoglobulin, which is the principal whey protein interact with the κ-casein , there by helping to give the yoghurt as a stable 'body'

(Dannenberg and dessler, 1986)

2.3.5.3. Incubation

During the manufacture of yoghurt, the heat treated milk is cooled to the incubation temperature of the starter culture (*S.thermophillus* and *L.bulgaricus).* and in general the milk is fermented at 40-45°C i.e. the optimum growth condition for the mixed culture the short incubation method. In some cases the incubation period can be as short as 4 hours, assuming that the starter culture (3%) is an active one and the ratio between the rods and the cocci is well balanced. However, the longer incubation method, i.e. Overnight, can be used, and the incubation conditions are 30°C for about 18 hours.

Most of the micro organisms which form yoghurt sourish are activated within the temperature range of 32°-47°C. Temperatures below 32°C cause them to be inactivated. Anything little above 47°C causes them to multiply too rapidly and become inactivated because of over crowding. At 49°C and above they are killed by the heat. Incubation to get the inoculated milk to be activated is the most critical stage in yoghurt making. It is important that optimum incubation temperature is maintained, around 40°C-42°C a range which is believed to produce yoghurt with good body. (Dannenberg and dessler, 1986).

2.3.5.4. fermentation

Fermentation is an metabolic process in which organic material are broken down to release energy without involvement of oxygen. In glycolysis glucose is converted to 2 pyruvic acid and 2 ATP. If the absence of oxygen these pyruvic acid is converted to lactic acid, acitic acid and etc. The various type of products can be produced by the fermentation of the carbohydrate substrate by microorganisms.

Fermentation can be applied for industrial process and that produces materials which are useful to human and this process is depend on the activity of one or more microorganisms. This process is called industrial fermentation (Garbutt, 1997)

2.3.5.5. Cooling

Yoghurt production is a biological process, and cooling is one of the popular method After heat treatment the milk is required to be cooled to a suitable temperature for inoculation Inoculation temperature for short set method will approximately 42°C

Cooling of the coagulum commences directly after the product reaches the desired acidity, e g around pH 4 6 or 0 9% lactic acid depending on the type of yoghurt produced, the method of cooling used and/or the efficiency of heat transfer

The yoghurt continues to thicken in the refrigerator This take about twelve hours

Yoghurt that is being chilled must not be allowed to freeze (E R Vedamuthu)

CHAPTER 03

3 MATERIAL AND METHODS

3.1 MATERIALS USED

- Raw Milk or non fat milk
- Gelatin
- starter culture
- Egg yellow
- Vanilla
- Artificial sweetness (Aspartame, Maltitol, Sorbitol, Honey)
- Refractometer (Erma hand refractometer, Tokyo, Japan) (0-30).
- Thermometer (0-100) ^{0}C

3.2 METHOD

The fat was removed from the Raw milk by using cream separator. Then skim milk was dissolved with sorbitol and filtered. Gelatin was dissolved in warm water (at 60 ^{0}C) and it was added while stirring. The mixture was heated at 93 ^{0}C for 5 minutes , while stirring. The mixture was cooled to about 42 ^{0}C and well mixed fresh culture, colouring and flavors were added and uniformly mixed. Then the mixture was poured into the clean sterile yoghurt cups. The yogurt was incubated at 42^{0}C for 3-4 hours in incubator. After set, it was transferred to refrigerator and stored in refrigerator.

METHOD

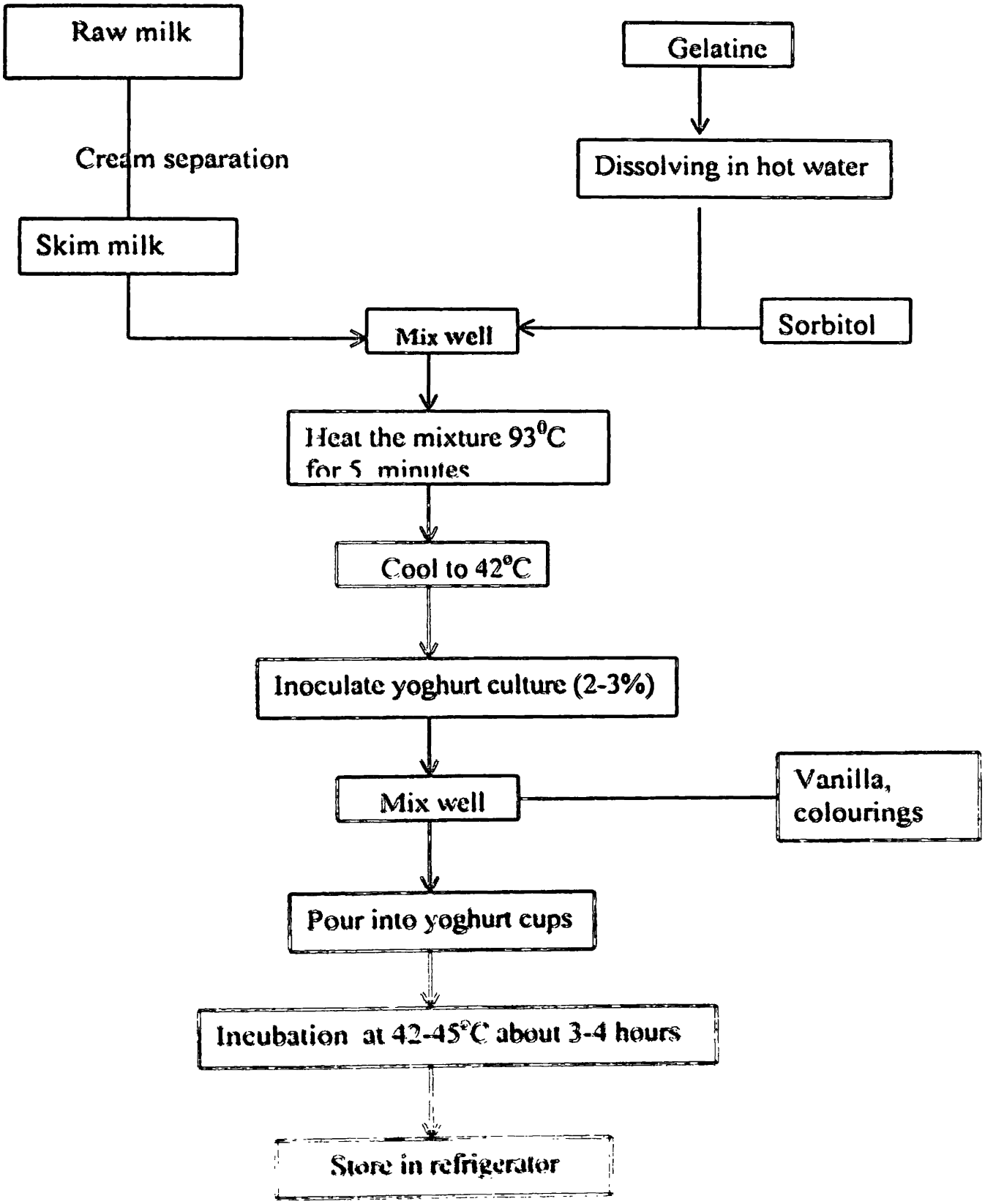


Figure 4 : Flow diagram -Yoghurt making

3.2.1.1. Preparation of samples

Samples were prepared according to the following formula,

Ingredients	Sample number			
	212	336	471	649
Raw milk	1750 ml	1750 ml	1750 ml	1750 ml
Aspartame	1.2 g	--	--	--
Sorbitol	--	400 g	--	--
Maltitol	--	--	180 g	--
Saccharin	--	--	--	0.9 g
Gelatin	14g	14g	14g	14g
Culture (cup)	1	1	1	1
Egg yellow (drops)	4	4	4	4
Colourings (drops)	4	4	4	4

3.2.1.2. Method of sensory evaluation

In all, using ranking test under four parameters ranked 4 types of samples.

3.2.1.2.1. Ranking

The panelist receives three or more coded samples and he asked to rank them for the intensity of some specific characteristics. The result of a ranking test can be checked for significant differences by using tables prepared by Kramer et al. (1974).

3.2.1.2.2. Method .

Eight judges ranked the 4 types of sweeteners using the score sheet in the Appendix on page 30.

3.2.2.1. Preparation of samples.

Another one sample was prepared by using sorbitol and other ingredients are same as the above formula.

3.2.2.2. Method of sensory evaluation.

Triangle test was used to compare with duplicate sample with odd sample.

3.2.2.2.1.Triangle test

The panelist receives three coded samples. He is told that two of the samples are the same and one of deferent and he is asked to identify the odd sample. This method is very useful and quality control work to ensure that samples from different production lots are the same, it is also used to determine if ingredient substitution or some other changes in the manufacturing result in the detectable deference in the product.

The result of triangle test indicate whether or not there is detectable deference between two samples higher levels of significance do not indicate that the deference is greater.

3.2.2.2.2. Method.

Ten judges were done the triangle test using the score sheet in chart is in the Appendix on page 31.

3.2.3. Methods for proximate analysis.

- Total soluble solids (TSS).

For each sample, TSS was tested by Erma hand type Refractometer (Brix® meter)

- Determination of fat

The fat percentage is determine by Gerber test method.

CHAPTER .4

4.1. Result and Discussion.

4.1.1. Results.

4.1.1.1. Results of ranking.

The ranks given the samples by the judges are shown below

Judges	Samples			
	212	336	471	649
1	3	1	2	4
2	4	1	3	2
3	4	2	1	3
4	3	2	1	4
5	4	1	3	2
6	3	2	4	1
7	4	2	1	3
8	4	1	3	2
9	4	3	1	2
10	3	1	2	4
Total	36	16	21	27

The rank totals were compared with the values in chart 1 of the Appendix on page 32 (Kramer et al,1974). When there were 4 samples and 10 judges, the tabular entries were 17-33: the lowest insignificant rank total was 17 and the highest insignificant rank total was 33. If one or more rank sums were lower than the upper left value in the block (17) or higher than the upper right value in the block (33), statistical significance at the 5% level of significance was indicated.

All of the rank sums were now compared to the lower pair of values in the block to determine which samples were significantly low (better) high (less good), sample B which received rank sum of 16 is significantly better than sample received a rank sum of 36.

4.1.1.2 Results of Triangle test

The odd sample was correctly identified by eight judges, according to the statistical chart – in the Appendix on page 33, eight correct judgement out of 10 in a triangle test indicate a significant deference at the 1% level. The degree of deference indicated by the eight judges who correctly identified by the odd sample was

Nomber of judges	value	
Slight	3	*(1) = 3
Moderate	2	*(2) = 4
Much	2	*(3) = 6
Extreme	1	*(4) = 4
Total		17
Mean		2.1

The average deference was 2.1 (moderate)

4.1.1.3 Results of proximate analysis.

Total Soluble solids 22.5%

Fat percentage 1.45 %

4.1.2 Discussion

According to the ranking test results sorbitol is better than other sweeteners. When using sorbitol some problem arises, sorbitol is less sweeter than sucrose (about ½) and it should be added in bulk. Therefore total soluble solids higher than normal range, it is above 22%

Proper amount of sorbitol effects to yoghurt. Because of more sobitol inhibiting the activity of *Steptococcus thermophillus*. They are responsible for the producing acidity When it inhibits yoghurt will not set.

Aspartame is also low calorie sweetener, at typical usage levels in concentrates, solubility is not a factor, however aspartame does dissolve faster under acidic conditions and at a temperature approaching 70°F. Nutra sweet aspartame is a methyl ester of a dipeptide composed of a two amino acids, phenylalinine and aspartic acid Aspartame is easily metabolized by the body In recently discovered aspartame is neurotoxic and cause ,phenylketonurea it mouth feel also unacceptable It gives sweeten to the yoghurt but after eating better taste remain in the mouth Aspartame is band in certain countries such as America

Saccharin is also low calorie sweetener it 400 time sweeter than sucrose & it requires very small amounts But it may cause bone decaying & it may affect the blood formation Saccharin is band in some countries like Canada

According to the triangle test results, odd sample is moderately deferent from duplicates sample. In this experiment low calorie yoghurt was prepared from manually and under laboratory conditions, therefore lot of errors may be arises such as milk is not properly homogenized, gelatin is not pure, incorrect heating temperature etc.

Proper amount of fresh starter culture is good for quality yoghurt. bulk amount of starter culture and prolonged incubating period can lead to increase the acidity. If the milk consist of more water percentage it can affect the coagulation of yoghurt. There fore less water content the more the coagulation rate and the quicker the formation of yoghurt. Among additives used in yoghurt making, gelatin, vanilla, egg yellow and other essence are significant. These additives should be added in proper quantities and at optimal temperature, because it can affect the quality of final product.

In order to minimize the contamination connected with uncleanness of utensils, it can be recommended to sterilized the vessels used properly.

Some troubleshooting arise preparation of low calorie yoghurt they are,

- Low viscosity and syneresis.

Causes : low fat content & Solid Non Fat content, incorrect heat treatment, insufficient inoculation of culture, fermentation temperature incorrect, stirring the coagulum at pH greater than 4.6, fast cooling of the yoghurt from 25-5 ^{0}C, air incorporating during processing and filling.

- Lumpy or grainy structure

Causes : SNF content is too high, fermentation is too fast , the coagulum has not been stirred up sufficiently

- Sandy texture

Causes : excessive addition of milk powder, uneven stirring of the coagulum, shaking the yoghurt during incubation

- Gummy/ gluey/ mealy texture

Causes : use of unsuitable stabiliser, excessive quantities of stabilisers added level of protein too high

CHAPTER 05

5. Conclusion and Suggestions

5.1 Conclusion

- According to the result Sorbitol is better sweetener than others because it is less carcinogenic or cancer promoter, not bitter taste remain aftertaste and less expensive.
- Compare the percentage of substances between final product and market available yoghurt.

Substances	Market available yoghurt	Final product
Total Soluble solids	12.5%	22.5%
Fat percentage	3.5%	1.45%
pH	4.6	4.6
Acidity	1.2%	0.9%
Milk Solid- Not - Fat	8.0%	10%

5.2 Suggestions

- Sorbitol and aspartame mixture can be used for the preparation of the yoghurt. By using both, we can maintain the Total Solubility Solid of the product.

- It is better to determine the calorie value in low calorie yoghurt.

- Honey is also low calorie sweetener, but high cost and some problems arises during setting of the yoghurt. It can be avoided by properly homogenization before incubation.

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Appendix 1

QUESTIONNAIRE FOR RANKING

Name ..... Date .....

.....

Rank these samples for sweetness. The sweetest sample is ranked first, the second sweetest sample is ranked second, the third sweetest sample is ranked third, and the least sweet sample is ranked fourth. Place the code numbers on the appropriate lines. Test the sample on following order: 212, 336, 471, 649

_____	_____	_____	_____
1	2	3	4

comments :

Appendix 2

QUESTIONNAIRE FOR TRIANGLE TEST

NAME ..... DATE .....

PRODUCT .....

Two of these samples are identical, the third is different

1. Taste the sample in the order indicate and identify the odd sample

Code	Check odd sample
314	.....
628	.....
542	.....

2 indicate the degree of difference between the duplicates sample and the odd sample.

Slight	.....
Moderate	.....
Much	.....
Extreme	.....

3. Acceptability

Odd sample more acceptable

Duplicates more acceptable

4 comments

STATISTICAL CHART 5

Rank totals

Rank totals required for significance at the 5% level ($P<0.05$). The four figure blocks represent lowest insignificant rank sum any treatment–highest insignificant rank sum, any treatment. Lowest insignificant rank sum, predetermined treatment–highest insignificant rank sum predetermined treatment

No. of reps.	Number of treatments, or samples ranked																			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2				3-9	3-11	3-13	4-14	4-16	4-18	5-19	5-21	5-23	5-25	6-26	6-28	6-30	7-31	7-33	3-39 7-35
3		6-8	6-11	4-14 5-13	4-17 6-15	4-20 6-18	4-23 7-20	5-25 8-22	5-28 8-25	5-31 9-27	5-34 10-29	5-37 10-32	5-40 11-34	6-42 12-36	6-45 12-39	6-48 13-41	6-51 14-43	6-54 14-46	7-56 15-48
4		5-11 5-11	5-15 6-14	6-18 7-17	6-22 8-20	7-25 9-23	7-29 10-26	8-32 11-29	8-36 13-31	8-40 14-34	9-43 15-37	9-47 16-40	10-50 17-43	10-54 18-46	10-58 19-49	11-61 20-52	11-65 21-55	12-68 22-58	12-72 23-61
5	6-9	6-14 7-13	7-18 8-17	8-22 10-20	9-26 11-24	9-31 13-27	10-35 14-31	11-39 15-35	12-43 17-38	12-48 18-42	13-52 20-45	14-56 21-49	14-61 23-52	15-65 24-56	16-69 25-60	16-74 27-63	17-78 28-67	18-82 30-70	18-87 31-74
6	7-11 7-11	8-16 9-15	9-21 11-19	10-26 12-24	11-31 14-28	12-36 16-32	13-41 18-36	14-46 20-40	15-51 21-45	17-55 23-49	18-60 26-53	19-65 27-57	19-71 29-61	20-76 31-65	21-81 32-70	22-86 34-74	23-91 36-78	24-96 38-82	25-101 40-86
7	8-13 8-13	10-18 10-18	11-24 13-22	12-30 15-27	14-35 17-32	15-41 19-37	17-46 22-41	18-52 24-46	19-58 26-51	21-63 28-56	22-69 30-61	23-76 33-65	25-80 35-70	26-86 37-75	27-92 39-80	29-97 42-84	30-103 44-89	31-109 46-94	32-115 48-99
8	9-15 10-14	11-21 12-20	13-27 15-25	15-33 17-31	17-39 20-36	18-46 23-41	20-52 25-47	22-58 28-52	24-64 31-57	25-71 33-63	27-77 36-68	29-83 39-73	30-90 41-79	32-96 44-84	33-103 47-89	35-109 49-95	37-115 52-100	38-122 54-106	40-128 57-111
9	11-16 11-16	13-23 14-22	15-30 17-28	17-37 20-34	19-44 23-40	22-50 26-46	24-57 29-52	26-64 32-58	28-71 35-64	30-78 38-70	32-85 41-76	34-92 45-81	36-99 48-87	38-106 51-93	40-113 54-99	42-120 57-105	44-127 60-111	45-135 63-117	47-142 66-123
10	12-18 12-18	15-25 15-24	17-33 19-31	20-40 23-37	22-48 26-44	25-55 30-50	27-63 33-57	30-70 37-63	32-78 40-70	34-86 44-76	37-93 47-83	39-101 51-89	41-109 54-96	44-116 57-103	46-124 61-109	48-132 64-116	51-139 68-122	53-147 71-129	55-155 75-135
11	13-20 14-19	16-29 18-26	19-35 21-34	22-44 25-41	25-52 29-48	28-60 33-55	31-68 37-62	34-76 41-69	36-85 45-76	39-93 48-83	42-101 53-90	45-109 57-97	47-118 60-105	50-126 64-112	53-134 68-119	55-143 72-126	58-151 76-133	60-160 80-140	63-168 84-147
12	15-21 15-21	18-30 19-29	21-39 24-36	25-47 28-44	28-56 32-52	31-65 37-60	34-74 41-67	38-82 45-75	41-91 50-82	44-100 54-90	47-109 58-98	50-118 63-105	53-127 67-113	56-136 71-121	59-145 76-128	62-154 80-136	65-163 84-144	68-172 89-151	71-181 93-159
13	16-22 17-22	20-32 21-31	24-41 26-39	27-51 31-47	31-60 35-56	35-69 40-64	38-79 45-72	42-88 50-80	45-98 54-89	49-107 58-97	52-117 64-105	56-126 69-113	59-136 74-121	62-146 78-130	66-155 83-138	69-165 88-146	73-174 93-154	76-184 97-163	79-194 102-171
14	17-25 18-24	23-34 23-33	26-44 29-42	30-54 33-51	34-64 38-60	38-74 44-68	42-84 49-77	46-94 54-86	50-104 59-95	54-114 65-103	57-125 70-112	61-135 75-121	65-145 80-130	69-155 85-139	73-165 91-147	76-176 96-156	80-186 101-165	84-196 106-174	88-206 111-183
15	19-26 19-26	23-37 25-35	28-47 30-45	32-58 36-54	37-68 42-63	41-79 47-73	46-89 53-82	50-100 59-91	54-111 64-101	58-122 70-110	63-132 75-120	67-143 81-129	71-154 87-138	75-165 92-148	79-176 98-157	84-186 104-166	88-197 109-176	92-208 115-185	96-219 121-194
16	20-28 21-27	25-39 27-37	30-50 33-47	35-61 39-57	40-72 46-67	45-83 51-77	49-95 57-87	54-106 63-97	59-117 69-107	63-129 75-117	68-140 81-127	73-151 87-137	77-163 93-147	82-174 100-156	86-186 106-166	91-197 112-176	95-209 118-186	100-220 124-196	104-232 130-206
17	22-29 23-29	27-41 28-40	32-53 35-50	38-64 41-61	43-76 48-71	48-88 54-82	53-100 61-92	58-112 67-103	63-124 74-113	68-136 81-123	73-148 87-134	78-160 94-144	83-172 100-155	88-184 107-165	93-196 113-176	98-208 120-186	103-220 126-197	108-232 133-207	113-244 139-218
18	23-31 24-30	29-43 30-42	34-56 37-53	40-68 44-64	46-80 51-75	51-93 58-86	57-105 65-97	62-118 72-108	68-130 79-119	73-143 86-130	79-155 93-141	84-168 100-152	90-180 107-163	95-193 114-174	100-206 121-185	106-218 128-196	111-231 135-207	116-244 142-218	122-257 149-229
19	24-33 25-32	30-46 32-44	37-59 39-56	43-72 47-67	49-84 54-79	55-97 62-90	61-110 69-102	67-123 76-114	73-135 84-125	78-150 91-137	84-163 99-148	90-176 106-160	96-189 114-171	102-202 121-183	107-216 128-195	113-229 136-206	119-242 143-216	124-256 151-229	130-269 158-241

Triangle test, difference analysis

Number of tasters	Number of correct answers necessary to establish level of significance			Number of tasters	Number of correct answers necessary to establish level of significance		
	•	••	•••		•	••	•••
	5%	1%	0.1%		5%	1%	0.1%
7	5	6	7	57	27	29	31
8	6	7	8	58	27	29	32
9	6	7	8	59	27	30	32
10	7	8	9	60	28	30	33
11	7	8	9	61	28	30	33
12	8	9	10	62	28	31	33
13	8	9	10	63	29	31	34
14	9	10	11	64	29	32	34
15	9	10	12	65	30	32	35
16	10	11	12	66	30	32	35
17	10	11	13	67	30	33	36
18	10	12	13	68	31	33	36
19	11	12	14	69	31	34	36
20	11	13	14	70	32	34	37
21	12	13	15	71	32	34	37
22	12	14	15	72	32	35	38
23	13	14	16	73	33	35	38
24	13	14	16	74	33	36	39
25	13	15	17	75	34	36	39
26	14	15	17	76	34	36	39
27	14	16	18	77	34	37	40
28	15	16	18	78	35	37	40
29	15	17	19	79	35	38	41
30	16	17	19	80	35	38	41
31	16	18	19	81	36	38	41
32	16	18	20	82	36	39	42
33	17	19	20	83	37	39	42
34	17	19	21	84	37	40	43
35	18	19	21	85	37	40	43
36	18	20	22	86	38	40	44
37	18	20	22	87	38	41	44
38	19	21	23	88	39	41	44
39	19	21	23	89	39	42	45
40	20	22	24	90	39	42	45
41	20	22	24	91	40	42	46
42	21	22	25	92	40	43	46
43	21	23	25	93	40	43	46
44	21	23	25	94	41	44	47
45	22	24	26	95	41	44	47
46	22	24	26	96	42	44	48
47	23	25	27	97	42	45	48
48	23	25	27	98	42	45	49
49	23	25	28	99	43	46	49
50	24	26	28	100	43	46	49
51	24	26	29	200	80	84	89
52	25	27	29	300	117	122	127
53	25	27	29	400	152	158	165
54	25	27	30	500	188	194	202
55	26	28	30	1000	363	372	383
56	26	28	31	2000	709	722	737

National Digitization Project

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