DEVELOPMENT OF MUNG BEEN ENRICHED STIRRED YOGURT

By

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DECLARATION

The work described in this thesis carried out by me at the Newdale Dairies (Pvt.)Ltd., 100, Delgoda Road, Biyagama under the supervision of Mr. N.S.Pathirana and Mis. W.M.Deepika Priyadharshini. A report on this has not been submitted to another university for another degree.

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Affectionately Dedicated

To

My loving parents

And

Teachers

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Abstract

Yogurt, one of the most popular fermented milk products, is known for its typical flavour, characteristic consistency and high nutritive and therapeutic value. The highest consumption of yogurt through out the country made an image to enrich the product with suitable pulse grains. Therefore studies were carried out to develop a green gram enriched yogurt with good sensory appeal.

Five samples were prepared by changing level of mung bean powder as 5%, 10%, 15%, 20%and 25%, while keeping other ingredients constant. Organoleptic qualities of sample were evaluated with the experienced tasting panel using seven-point hedonic scale. Acidity and pH of the samples were observed over the 29 days of storage at 4°C with 4 days interval. Final product was analyzed for total solids, pH, fat, acidity and Brix value. Microbiological evaluation was carried out for yeast, mould and coliform.

Significant difference existed between the samples in the attribute of the appearance, colour, flavour, mouth feel, and overall acceptability. Highest rating was obtained by the sample with 10% mung bean paste. Sample with 10% mung bean paste was further evaluated for its keeping quality. Time series analysis of pH forecasted the shelf life of product as 27 days when pH was 4.1 at 4°C. No significant differences were found in pH and acidity development throughout the storage. Microbiological data (yeast mould) showed no significant difference with the storage and no coliforms were detected throughout the storage.

The green gram enriched stirred yogurt with 26% total solids, 4.5%, 0.97 pH, acidity, 21° Brix and 3.5% fat showed 29 days of storage life at 4± °C without any deterioration of colour, texture and appearance.

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CHAPTER 1

1. Introduction

The concept of globalization has taken not only men but our women also towards the working environment. Busy mothers do not have enough time to prepare foods at their cuisine. Therefore with the diversification of employment and rapid urbanization, demand for processed ready to eat foods is being increased remarkably. The foods available in ready to eat form can be broadly classified on grain-based products, fruit and vegetable products milk products and fish and meat products. Among dairy products yogurt is the most demanded and popular milk product in the country. When buying processed foods for their diet they consider not only about the easiness, but also the nutrition value, as nowadays mothers are concer lot about the health of their family. Yogurt, non-alcoholic fermented product produced by two lactic acid bacteria as *Streptococcus thermophilus* and *Lactobacillus balgaricus* make nutritionally superior product by it. By its easy digestibility, superior protein quality, higher absorption of minerals, and it helps to recover wounds in intestinal track. On the other hand some investigators have recommended the use as fermented dairy product such as yogurt by lactose intolerance as and alternative mean of gaining the supervisor nutrition of milk without the accompanying discomfort.

Over the years the demand for yogurt has increased and product is now easily available at any part of the country. With the growing popularity yogurt is now available in a number of new forms such as pourable yogurt, low fat yogurt, stirred fruit yogurt etc. Besides, product diversification researches have tried to develop its nutritional quality by incorporating cereals. Enrichment of yogurt with cereals or pulse can satisfy the mothers,concern on nutritional requirement for their children. It is in this context therefore the enrichment of yogurt with pulse could be helped to reduce the incidence of malnutrition among children.

A method for the production of yogurt-cereal mixture has been discussed by Robinson and Cadena, 1978, 1979. Their studies revealed that the nutritional value of such mixture confirms the usefulness of it in addition to cereal based diets. According to Frankul, 1961, the similar product of cereal yogurt mixture named 'Kishk' is widely consumed in the rural area of Iraq, while a similar product is found in Turkey.

Mung bean (*Vigna radiata*) is a popular pulse in Sri Lanka and is an important source of protein in the national diet. It contains approximately 24% protein which is about two third the protein content in the Soya bean. Therefore, there are good reasons for proposing mung bean yogurt mixture with high protein content and with all other essential nutrients. Formulation of mung bean yogurt mixture provides the community with an alternative and nutritionally valuable ready to eat product at reasonable cost.

Therefore attempts are made to develop green gram enriched yogurt with the following objectives.

Objectives

1. Introduce a new nutritious processed ready to eat food product to the market.
2. Formulation of green gram enriched yogurt with good storage and sensory quality.

CHAPTER 2

2. Literature Review.

2.1. Cow's milk and its composition

2.1.1. Definition.

Fresh lacteal secretion (practically free from colostrums) obtained by the complete milking of one/more healthy cows or buffaloes without the addition of any substances or abstraction of fat or any other constituents.

2.1.2. Legal standards.

Milk is the most legally controlled of all commodities in many countries. The minimum standards for fat values ranges from 3.0% to 3.8% while it range from 11.2% to 12.25%for total solids. There are standards of composition and regulations against condition that would constitute adulteration for milk and all important milk products. In the case of fluid milk, Federal standards include a minimum of 3.25%fat and 8.25% milk solids non fat (Potter, 1973).

2.1.3. Composition.

When considering cow's milk there are quite large difference in composition depending on the breed and even in the same breed the composition of fresh milk varies from day-today depending on stage of lactation age of the animal health or infections of the herd, climatic conditions and season of the year, and even the intervals between milking (Varnam and Sutherland., 1994). Table 2.1 shows the average composition of cow's milk.

Table 2.1: The average composition of cow's milk.

Component	% Of liquid	% Of solid
Lactose	4.8	37.5
Fat	3.7	28.9
Protein	3.4	26.6
Non protein N	0.19	1.5
Ash	0.7	5.5

Source: Varnam and Sutherland (1994)

2.1.3.1. Milk protein.

The protein content of fluid milk 3% to 4%, depending on breed and a variety of environmental factors. A value of 3.5% protein is often considered average for milk. Milk proteins have been traditionally divided into two classes. Casein and whey proteins. The casein fraction contains a heterogeneous group of phosphoproteins that can be precipitated from raw skim milk by acidification to pH 4.6 at 20^0C. Proteins remain in solution after precipitation of casein are collectively know as whey or milk serum proteins. The casein fraction accounts for almost 80% of the total protein content of milk, with whey proteins making up the other 20% (Fennema, 1994) the major protein components in cow's milk are shown in table 2.2.

Table 2.2: Major protein components in cow's milk.

Components	Present of milk	Present of total proteins
Casein	276	78.0
B-lactoglobulin	0.43	12.0
α-lactalbulin	0.08	2.0
Immunoglobulins	0.07	2.0
Bovine serum albumin	0.03	1.0
Others	0.18	5.0

Source: Fennema (1994)

2.1.3.2. Milk fat.

The bulk of the fat in milk exists in the form of small globules, which average approximately 2 to 5μ in size. This is oil –in-water type emulsion. The surface of these fat globules is coated with an adsorbed larger of material commonly know as the fat globule membrane. The membrane contains phospholipids and proteins in the form of a complex.

Tri-acylglyoerols are dominant and contribute 93% of milk fat, together with small amount of di and mono acylglycerols and free-fatty acids. (Varna and Sutherland, 1994).

2.1.3.3 Lactose.

Lactose, the milk sugar, is a disaccharide and comprises α–D glucose and β-D galactose molecules. It generally rangers between 4.2% and 5.0% in cow's milk, being lowest in the late lactation milk, or in milk from animals suffering from udder disease.(Johnson,1982).

2.1.3.4 Minerals.

Minerals in milk marks principally with bicarbonates, Chlorides, Posphates, Citrates and bicarbonates of calcium, magnesium, Pottasium and Sodium.(Varnam and Sutherland,1994).

2.1.3.5 Minor components and micronutrients.

Among the large number of minor components in milk, urea, milk enzymes and vitamins play a major role in the composition of cow's milk. Further more, milk is a rich source of fat-soluble vitamins A, and water-soluble vitamins C, B1, B6, B12, pantothenic acid, nicotonic acid, biotin, choline and folic acid. (Jhonson, 1982)

2.1.4 Physical properties of milk.

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

Table 2.3:physical properties of cow milk

Physical properties	Representative value
Ph value (at 25 ^{0}C)	6.6
Specific gravity (at 20^{0}C)	1.032
Freezing point	-0.5400^{0}C
Boiling point	100.170 ^{0}C
Viscosity (at 20^{0}C)	1.6314 CPOISE
Surface tension (at 20^{0}C)	50dyneskm
Heat capacity	0.52cal/g^{0}C

Source: Nutting(1969)

a. Density

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

I. To estimate the solid content.

II. To convert volume to mass.

III. To calculate other physical properties.

b. Viscosity

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

I. The rate of creaming.

II. The flow condition of dairy processes.

III. Rate of mass and heat transfer.

c. Freezing point

This is mainly used in determine added water, it can be 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 concentrations 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 be used 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 6.6pH value. Many substances affecting the acidity of milk .Many components 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 is of the most variable properties of milk. It is varied by amount of water, fat non-fatty solids, adulterations etc.

f. Specific heat

In normal 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 than 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 its 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 flavour.

Milk has characteristic white opalescent colour, due to the scattering of light by the containing colloidal particles. The yellow colour of 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. (Johnson,1982)

2.2 Mung bean.

The mung bean (*Vigna radiate* (L.) wilezek) is a leguminous species, or pulse crop, grown principally for its protein – rich edible seeds.

Pulses are important world food crops because they provide and inexpensive source of vegetable dietary protein. In many densely populated area of the world, the economy does not support large – scale production and utilization of animal protein. In those areas, the protein in people's diets may be augmented by supplementation with the protein rich pulse grains. In addition to being less expensive than animal protein, pulse grains provide a source of rich protein for those people who prefer vegetable to animal protein in their diet for cultural or religious reasons.

Among the pulses, the mung bean is favored for children and the elderly due to its easy digestibility and low production flatulence. Protein in the seed averages around 24 percent. The protein is comparatively rich in Lycine, an amino acid deficient in cereal grains. Mung bean protein is deficient in Methionine, Cystine and Cysteins, Sulfur bearing amino acid found abundantly in cereal grains. A diet combining mung bean and cereal grains compensates for the deficiencies in protein quality found in either alone and provides a balanced amino acid diet. Sprouted mung bean seeds provide a succulent and nutrition vegetables, rich in proteins, minerals, and vitamins, available in all seasons of the year. (Poelman, 1991).

2.2.1 Mung bean production in Sri Lanka.

Mung bean is a popular pulse in Sri Lanka and is an important source of protein in the national diet. About 80 percent of cultivated area is grown as a rain fed crop.

Market demand was not being met by local production in the early seventies. So attention was given to increasing the domestic production and the reducing imports an effort that netted an as percent increase in production from 1974 to 1986. Production increases in both area planted and in yield. (Poelman, 1991).

2.2.2 Nutritional composition of mung bean.

The chemical composition of lot of different seed species will vary due to the production environment, genetic factors and the composition. Table 2.4 lists the proximate composition of mung bean. The mung bean seeds are not raw, they will be soaked, sprouted, cooked or milled before being consumed. Chemical and physical

changers will occur during processing that will affect the digestibility and nutritional value of the seeds.

The mung bean is good source of food energy, protein, carbohydrates, minerals and lipids.

Table 2.4:Proximate composition of mung bean.

Component	Amount in 100g
Water	9.05 g
Food energy	347 kcal
Proteins (n x 6.25)	23.86 g
Lipid (fat)	1.15 g
Carbohydrate (total)	62.2 g
Crude fibers	5.27 g
Ash	3.32 g

Source: Poelman(1991)

2.2.2.1. Protein.

The utility of the proteins in mung bean seeds as food is determined by its quantity and quality. Mung bean averages about 24 percent protein. Legume proteins contains roughly 70 percent globulins, is to 20 percent albumin and is to 20 percent glutelins. The globulins are storage proteins and the principal protein constituent in the seed, whereas the albumins and glutelins are non-storage proteins and are present in enzymes, membranes and structural units. The major storage globulin in mung bean is vicilin. Viciline is rich in the acidic amino acids and deficient in the sulphuramino acids legumin another storage globulin is present only is small amounts. Other proteins include hemagglutinins and trypsin inhibitors (Poelman, 1991).

2.2.2.2. Amino acids.

The nutritive value of mung been is affected by total protein content and balance among the nutritionally essential amino acids. In animal, the amino acids are generally present in proportion satisfy human nutritional needs, but in vegetable proteins one or more of the amino acids are deficient so that the protein is not balanced as required for the human diet. Amino acids are important human nutrition

that may be imbalanced in vegetable proteins are Lysine, Methionine, Cystine, Threonine and Trypotopal. A general rule is at the amino acids be supplied in the diet in a ratio of 4 parts Lysin: 2 parts Methionine: 2 parts threonine: 1 part tryptophan. When an amino acid is so low in the diet that the ratio is affected, it becomes the limiting factor in the nutritive value of the protein. Mungbean and other pulses are relatively rich in Lysine due to the high content of the Lysine in the protein and the high protein content in the seed. When cereal grains and mung bean are mixed in the diet. A better amino acid balance is possible than when either is consumed alone. A 70: 30 ratio of rice protein to mungbean protein was suggested as being optimum for human diets (Poelman, 1991).

2.2.2.3 Lipids.

Lipids are present in small amounts in seeds of pulse crops, generally in the range of one to three percent. The major fatty acid in mung bean and black gram is linolinic, with oleic and linoleic acids also present. Lipids influence development of flavors in legume seeds and are precursors of particular off – flavors. There is no cholesterol in mung bean of other pulse seeds (Poelman, 1991).

2.2.2.4 Carbohydrates.

Carbohydrate content of mung bean is similar to that of other pulse species. Carbohydrate, combined with proteins, makes mung bean a rich source of food energy. The carbohydrate contains water-soluble component, sugar and pectin and water insoluble components such as starch and cellulose. The major sugar is sucrose, but mung bean seeds also contain the oligosaccharids, raffinose and stachyose implicated in gastrointestinal flatulence. The traditional view that content of flatulence factors in mung bean is less than other pulses is confirmed by reports that raffinose combined with stachyose averages 2 percent in mung bean. The oligosaccharide content in raw seeds is reduced with seed germination and increased by cooking (Poelman, 1991).

2.2.2.5 Minerals.

Mungbean seeds are good source of minerals in diet, being particularly rich in calcium, iron and potassium. In this regard, mung bean seeds do not refer remarkably from seed of other pulse species in content of different minerals. The proximate content of mineral elements in mung bean is given in table 2.5.

Table 2.5: Mineral composition of mung bean

Minerals	Content in mg
Calcium	132
Iron	6.74
Magnesium	189
Phosphorus	367
Potassium	1.246
Sodium	15
Zinc	2.68
Copper	0.941
Manganese	1.035

Source: Poelman,(1991)

2.2.2.6 Vitamins.

The proximate vitamin content of mung bean is reported in table 2.6.Mungbean seeds are a good source of thiamine, niacine, pantathenic acid, folacin and vitamin A. Compared to soybean, mung bean is higher in niacin, pantothenic acid, folacin, and vitamin A and lower in ascorbic acid, thiamin and riboflavin. Vitamins are synthesized in the sprouting process, the sprout having higher vitamin content than raw seeds.

Table 2.6:Vitamin composition of mung bean amount in 100 grams Haytowitz and Matthiws, 1986.

Vitamin	Content
Ascorbic acid	4.8 mg
Thiamin	0.621 mg
Riboflavin	0.233 mg
Niacin	2.251 mg
Pantothenic acid	1.910 mg
B_6	0.382 mg
Folacin	624.9 µg
A	114 IU[b]

Source: Poelman(1991)

2.2.3 Digestibility, Biological value, and Nutritional value

Proteins quality may be evaluated with reference to its digestibility and biological value. Legume protein is considered to be difficult to digest due to anti nutritional components and flatus production that causes stomach and intestinal discomfort. Comparisons of " digestibility coefficients " for mung bean in table 2.7. Another measure of protein quality is " biological value " [amount of absorbed nitrogen retained in the body].

Table 2.7: Digestibility coefficient, biological value and nutritional value of mung bean

Mungbean	Digestibility Coefficient 79%	Biological value 72%	Nutritional value in relation to egg protein 32%

Source: Poelman(1991).

2.2.4 Flatulence Production.

The production of flatulence is a characteristic of most legume seeds consumed as food. The oligosaccharides- raffinose, stachyose and cerbascose – have been singled out as flatus producers. Intestinal enzymes do not digest these sugars so they accumulate in the large intestine where natural gut micro flora use the sugars and produce gas. Mungbean is regarded to be low in production of flatulence in comparison of other pulse species or soybeans. (Poelman, 1991).

2.2.5:antinutritional factors.

The nutritional value of legume proteins may be adversely affected by the presence of antiphysiological of toxic substances.(Poelman,1991).

2.2.5.1: Trypsin inhibitor.

Trypsin in a proteolytic enzyme of the pancreatic juice capable of converting proteins into peptone. Protease inhibitors have been isolated from plant and animal sources that inhibit the action of the trypsin enzyme and reduce the digestibility of protein in the digestive tract. The trypsin inhibitor is present in mung bean in all plant parts. The action of the trypsin inhibitors is diminished by heat. .(Poelman, (1991).

2.2.5.2 Tannins.

Tannins [polyphenols] present in food legumes reduces the digestibility of the dietary proteins.The tannin content [100 mg^{-1}] of mung bean is 612. The tannin content was reduced by soaking, germinating or cooking the seeds. .(Poelman, J. M.,(1991).

2.2.5.3 Hemagglutinins.

Hemagglutinins or lectins have the ability to agglutinate and break down red blood cells. They are present in dry bean and soybean but are not present in mung bean, chickpea or pigeonpea. .(Poelman, (1991).

2.3 Yoghurt

Yoghut is one of the most popular fermented milk products which is known for its typical flavour, characteristec consestency and high hutrityve and theraputic value.

2.3.1 Ingredients for yogurt.

2.3.2 Milk.

Milk provides the main fermentable substrate, i.e. Lactose, to the micro organisms and it is the main protein source. Lactose in the milk provides the energy source for the milk provides the energy source for the youhurt starter organisms, while protein plays an important fole in the formation of the coagulum.

2.3.1.2 Sugar.

Sugar is added to the yoghurt mix as a sweetening agent. The main objective If adding sweetning compounds to yoghurt mix is to lone down the acidity of the product and the level of imcorporation is depenent on;

1. the type of sweetning compound used.
2. Consumer preference.
3. The type of fruit used.
4. Possible inhibitory effects on the yoghurt storter organism.
5. Legal aspects
6. Econamic considaration [Tamime and Robinson, 1985]

2.3.1.3 Stabilizers/ Emulsifiers.

The primary aim of adding stabilizers to the basic mix is to enhance maintain the desirable characterristics in yoghurt, e.g. body and texture, viscosity / consistancy, appearance and mouthfeel. Their application in most countries is governed by legistative regulations.

2.3.1.4 Milk powder.

Skim milk powder and / or full cream milk powder is added in to the yoghurt mix to increase the total solid level in the yoghurt mix.

2.3.1.5 Starter Culture.

yoghrt is generally produced weth a yoghurt starter which is a mixed culture of *s. thermophilus* and *L.bulgaricus*, in a 1:1 ratio. Normally starter culture is added about 2%of the yoghurt mix in volume basis [Jay. 1992]

2.3.2 requirement for high quality yogurt.

Tamine and Robinson [1985] have reported that following factors directly influence the quality of yoghurt.

- Milk of good quality and adequate solid non-fat.
- Correct heat treatmint.
- An attractive, well balanced and contaminant- free starter culture.
- A clean and well maintained plant
- crrect incubation rate.

2.3.3 Yogurt production.

2.3.3.1 Basic principle in yogurt production.

The lactose in the yoghurt mix is converted into lactic acid by bacterial enzymes, increasing the acidity os the mix up to 0.6-0.7 % and that will curdle the milk protein at ordinary temperatures.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 \longrightarrow 4CH_3CHOHCOOH$$

Lactose Glucose Lactic acid

In addition, the coccus grows faster than rod and is primarily responsible for prodiction of lactic acid, while the ros adds flavours and aroma. The associative growth of these two organisms result in lactic acid production of rate greater than than produced by either when growing alone, and more acetaldehyde [the chief volotile flavour component of yoghurt] as well. There is a general agreement in the literatur than the aroma and flavour of yoghurts are basically due carbonyl compounds, and the wymbiotic actions of the S.*thermophilus*, and *L.bulunicus* are nicessary to obtain full disirable flavour and proper acid full desirable flavour and proper acid development in yoghurt. [Jay, 1992]

2.3.3.2 Yorgurt production process

The process of making yoghurt is a coplex and it combines both ' art ' and ' science ' together. The two main type of yohgurt are named as set and stirred where several other types exist as well.The specification for yogurt in Sri Lanka is given Appendix 2 The basic production technology of stirred yoghurt is summerized in the figure 2.1

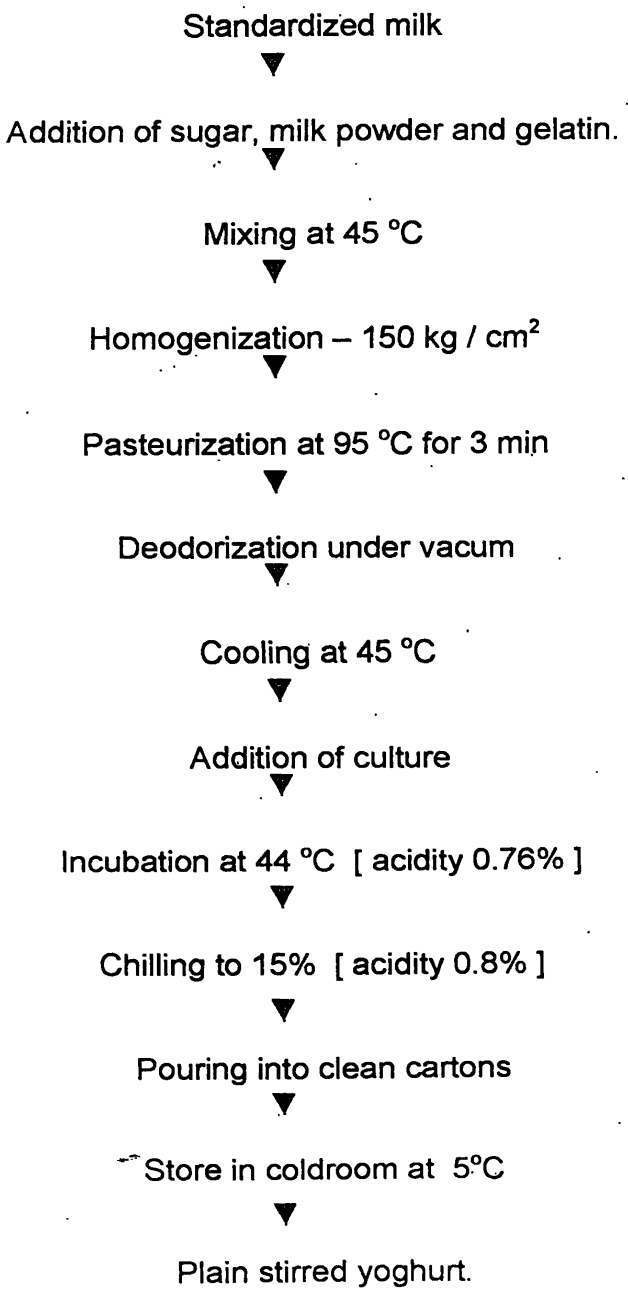


Figure:2.1 The basic production technology of stirred yoghurt.
Source: Tamine and Robinson, 1985

Some important operation in commercial yogurt are discribed in below.

Homoginisation.

When heat the milk, fat globules get together and come to the top to avoid this homogenisation es done before pasteurization. In order to prevent this and also to have a uniform consistency throughout. The yoghurt mix is homogenised to break the fat globules. The homoginisation will reduce the average diameter of the fat globules to less than 2 microns size preventing 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 homogenisar. Improvemet in consistency characteristics in yoghurt could be due to the change in the water holding capasity of the milk proteins which tend to reduce the syneresis characteristics and the increased amount of milk fat soluble membrane meterial in the skim phase of milk (Grant,1980)

Heat treatment.

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

The milk is heated before being inoculated 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 seperation 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 k-caein, there by hilping to give the yoghurt a stable 'body' (Grant,1980)

Incubation.

During the manufacture of yoghurt, the heat treated milk is cooled to the incubation temperature of the starter culture (*S.thermophillus* and *L. balgaricus*) and in general the milk is fermented at 40-45 °C i.e. the optimum growth condition for the mixed culture in the short incubation method.The incubation period can as short as 4 hours, assuming that the stater culture [3%] is an active one and the ratio between the rods and the cocci is well balanced. However, the larger incubation method, i.e. oveerhight, 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 withen the temperature range fo 32-47 °C . Temperature below 32 °C cause them to be inactivated. Anything little above 47 °C causes them to multiply too rapidly and become inactivated bécaule of aver crowding. At 49 °C and above they are killed by the heat. Maintianace of optimum incubation temperature 40-42°C is important to produce yogurt with good body. (Grant,1980)

Fermentation

Fermentation is an metabolic process in which organic meterial 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 ect. The variors type of products can be produced by the fermentation of the carbohydrate substrate by micro-orgamisms. Fermintation 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 micro-orgamisms. This process is called industrial fermentation. (Grant,1980)

Cooling.

Yoghurt production is a biological process, and cooling is one of the important process. After heat treatment the milk is required to be cooled to a suitable temperature for inoculation. Inoculation temperature for short set mithod will approximately 42 °C cooling of the coagulum commences directly after the product reaohes the desired acidity, e.g. around pH 4.6 or 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 refrigeration. This take about twelve hours. Yoghurt that is being chilled must hot to be allowed freeze. (Grant,1980)

2.3.4 Nutritional and therapeutic value of yogurt

2.3.4.1 Nutritional value

The protein content of yoghurt is similar to that of starting milk but the digistion of protein is twice that of raw milk due to partial breakdown of proteins. Starter micro-orgamisms alleviate the lactose malabsorbtion. Vitamins are initially metabolized and then synthesized by micro-organisms in yoghurt, so there isonly a little net effect, but the level of folic acid is increased by 100%. In yoghurt, which is a food suitable for use by lactose – intolerant individuals only a little change of energy value is ocurred due to the conversion of lactose into lactic acid or alcohol. The riboflavin content may be increased as a result of fermentation. However, vitamin C is completely destroyed practically during majufacture of yoghurt.

Some typica values of the major constituents of milk and yoghurt are shown in table 2.9 and some typical vitamin contents of milk and yoghurts are shown in table 2.10

Table 2.8 Some typical value of the major constituents of milk and tyghurt [units per 100 g]

Constituent	**Milk**		**Yoghurt**		
	Whole	Skimmed	Fullfat	Low fat	Fruit
Colories	67.5	36.0	72.0	64.0	98.0
Protein [g]	3.5	3.3	3.9	4.5	5.0
Fat [g]	4.25	0.13	3.4	1.6	1.25
Carbohydrates [g]	4.75	5.1	4.9	6.5	18.6
Ca [mg]	119.0	121.0	145.0	150.0	176.0
P [mg]	94.0	45.0	119.0	118.0	153.0
Na [mg]	50.0	52.0	47.0	51.0	--
K [mg]	152.0	145.0	186.0	192.0	254.0

From Tamine and Robinson, 1985

Table 2.9 Some typical vitamin contents of milk and yoghurt [units / 100 g]

Vitamin	Milk		Yoghurt	
	Whole	Skimmed	Full fat	Lwo fat
A [I .V.]	148	-	140	70
Thiamin(B_1) (μg)	37	40	30	42
Riboflavin[B_2] (μg)	160	180	190	200
Pyridoxine (B_6) (μg)	46	42	46	46
Cyanocobalamin(B_{12}) (μg)	0.39	0.4	-	0.23
C (mg)	1.5	1.0	-	0.7
D (I.U)	1.2	-	-	-
E (I.U)	0.13	-	-	Trace
Folic (μg)	480	-	-	125
Pantathonic acid (μg)	371	370	-	380
Biotin (μg)	3.4	1.6	1.2	2.6
Choline (mg)	12.1	4.8	-	0.6

Correct storage of retail product preferavly below 5 °C .

2.3.4.2 Theropeutic value of yoghurt.

Theropeutic yoghurt can be prepared using therapeutic starter cultures (*L. acidophillus* and *Bifidobacterium spp.)* alone or in the presence of normal starter bacteria. Therapeutic activity may enhance by the precence of normal starter cultures. Some of the therapeutic micro-organisms, properties they enhance and proposed michanisms are shown in the 2.10.

Table 2.10 Therapeutic micro-organisms properties they enhance and proposed michanisms.

Microorganism	**Property**	**Proposed mechanism**
Bifidobacterium spp.	Maintance of normal intestinal microflora	a. Production of inhibitors b. Stimulation of host immune system
Lactobacillus spp.	Alleiation of lactose	a. Reduce the lactose cotent of the product b. Auto digestion of lactose by starter derived β-lactosidase
Bifidobacterium spp. Various lactic acid bacteria	Anti carcanoganic activity	a. Removal of dietory procarcinogens b. Stimulation of host immusystem
Bifidum	Reduce the serum cholesterol level nutritinal enhancement Alleviation of effects of renal malfunction	Not known Synthesis of β-complex vitamins increase Calcium absorption Recuce Calcium absorption. Reduce the level of the toxic amines.

Source: Varnam and Sutherland, 1994

CHAPTER 3

3.0 Materials and Method

The research was conducted at the laboratory of the Newdale Daries (Pvt) Limited, 100, Delgoda road, Biyagama, Sri Lanka.

3.1 Experiment :Determination of appropriate incorporation form of mung bean.

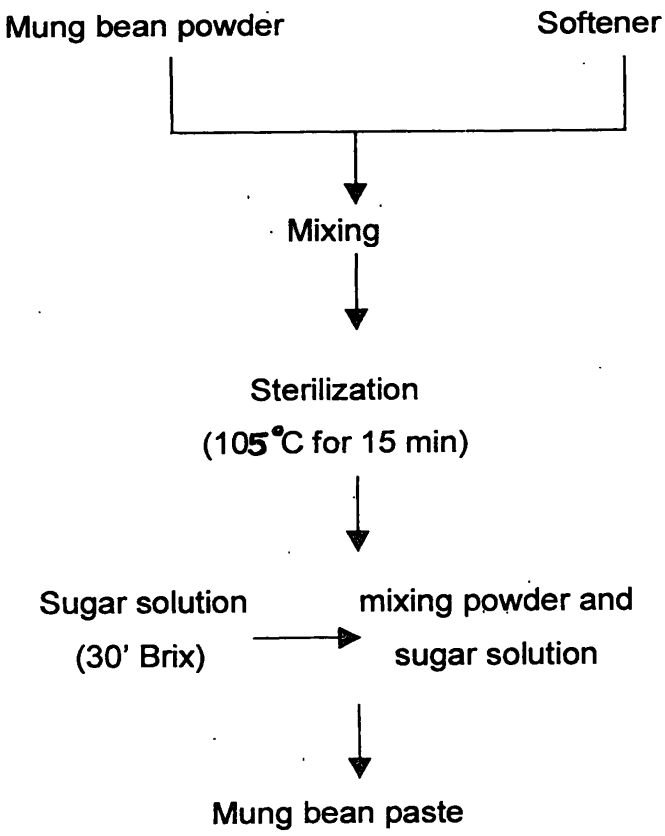
As a Preliminary test whole mung bean seed was incorporated to the stirred yogurt. Hard nature of abnormal seeds produced the product unpalatable. Therefore attempts were mode to incorporate mung bean powder.

3.1.1 Preparation of mung bean powder

Pest attacks free green gram was purchased form the local market. They were then screened and washed well. Washed grams were dried in a mechanical dryer and blended using kitchen blender to produce fine powder.

3.1.2 Preparation of mung bean paste

Mung bean powder and the softener (Mono – and diglycerides of fatty acids) were weighed by laboratory balance. All the ingredients were mixed thoroughly and sterilized for 15 minutes in IOSC. The sugar solution was prepared by using cane sugar portable water and pectin with the brix value as 30" brix. Sugar solution and mung bean powder were mixed and prepared mung bean paste. Sterilized powder containing mung bean powder and softener was mixed with sugar solution and mung bean paste was prepared. The flow chart represent the steps



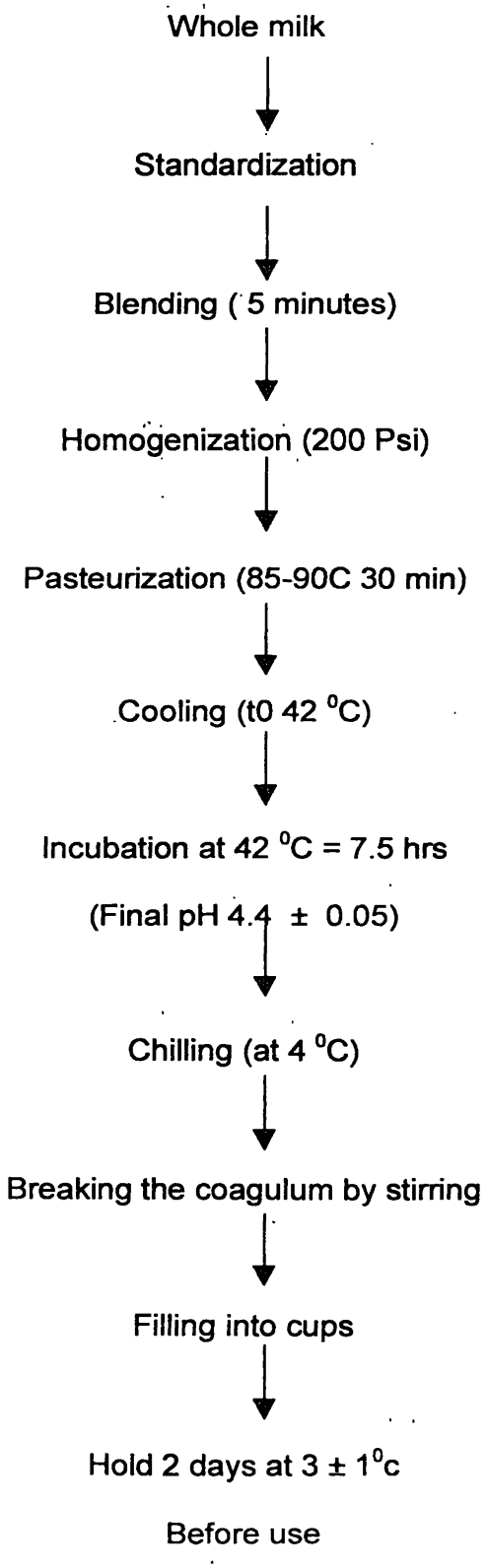
Preparation of sugar solution

Sugar, water and pectin weigh and put in to the blender after blending all ingredients (brix 30)

3.2 Experiment 2: Determination of acceptable percent level of mung bean.

3.2.1 Preparation of pulse based plain stirred yogurt.

The stirred yogurt was prepared according to the procedure described below. Fresh cow's milk standardized to 3.5% fat and 23.5% total solids, was used to prepare plain stirred yogurt. The procedure is given in the following flow diagram.


Whole milk
Standardization
Blending (5 minutes)
Homogenization (200 Psi)
Pasteurization (85-90C 30 min)
Cooling (t0 42 °C)
Incubation at 42 °C = 7.5 hrs
(Final pH 4.4 ± 0.05)
Chilling (at 4 °C)
Breaking the coagulum by stirring
Filling into cups
Hold 2 days at 3 ± 1°c
Before use

Yogurt mix was replaced with the mung bean paste at the levels of 5, 10,15 20 and 25 percents. After addition of mung bean paste it was stirred properly for even destruction within the stirred yogurt.Table 3.1.

Treatment levels and their codes are given in the table 3.1

Treatment	Treatment Code	Mung bean Paste %
1	497	5%
2	698	10%
3	327	15%
4	535	20%
5	705	25%

3.2.2 Assessment of sensory qualities

Sensory evaluation was carried out by three taste panels three months at the laboratory of New Daries (Pvt) Limited. The objective of conducting three taste panels using same panelists was to evaluate the sensory properties of three replicates of five treatments in order to reduce the bias. Appearance colour, flavour, spoon ability, mouth peel and overall acceptability were evaluated with 25 experienced panel members.

3.2.2.1 Testing Criteria

The seven point hedonic scale was used to evaluate the degree of liking for particular quality attribute. (Annexure1).

3.2.2.2 Serving of samples

The samples were coded with three digit random numbers drawn form a random number table samples were served in a random order and evaluated by one panelist at a time. The panelists were provided with distilled water and asked to rinse month before and after each basting.

3.2.2.3 Statistical analysis

A non parametric ranking procedure was used with Kruskall Wallis test for the evaluation of appearance, colour, flavour, spoon ability mouth feel and overall acceptability. Data were analyzed at the 0.05 level of significance using computer aided MINI TAB statically package.

3.2.2.4 Chemical Analysis

The final developed sample was analyzed for proximate composition of total solids, titrable acidity, pit and Brix value using standard methods.

3.3 Experiment 3: physico – chemical analysis of the product.

3.3.1 Proximate analysis

Sartorius AG GOTTINGEN MA-30-000X3 moisture analyzer was used to detect total solids in final product mettler Toledo NP 220 Pw meter was used to determined the pH. The Brix value was determined by using ATI Go referencetometer type IT.
All these test were done in replicates.

3.3.1.1 Determination of titrable acidity.

9 ml of the sample was taken into a 100 ml Erler mayer flask and 1 ml of 1 % phenolphthalein solution was added to it. Then the sample was titrated with O.I.M sodium hydroxide until a permanent very pale pink colour was observed.

$$\text{Titrable acidity \% (W/V)} = \frac{90 \times N.V. \times 100}{1000 \times 9}$$

Where N1 = Normality of sodium hydroxide

V1 = Burette reading

3.3.1.2 Determination of pH

To determine pH of yogurt was measure by the pH meter. Before reading the measurement g the part of measuring was dipped in distil water. Then the temperature was adjusted to the yogurt temperature pH of the yogurt was read, by dipping the measuring part in it.

3.3.2 Acidity and pH development during the storage

Acidity and pH of the samples were checked at 4 days intervals during time at $4\pm1^{0}C$ with two replicates for about 30 days.

Data were analyzed by using MINITAB. It was time series analysis.

3.3.3 Microbiological Analysis

Pulls based yogurt was analyzed for coliform and yeast and mold using standard method.

3.3.3.1 Coliform counting by direct plating

One gram of yogurt from each sample was transferred into sterile petri-dish. About 12 ml of violet red Blue agar at 4821 C was poured into each petri –dish followed by mixing the contents by rotating the closed petri-dishes. The agar was then allowed to solidify at room temperature. This procedure was done under sterilized laminar flow cabinet.

The plates were then incubator in an inverted position aerobically 30 I 1 C for 24 hours I 2 hours. The colonies were counted using a colony counter in subdued light and results were expressed as 'coliform', 'colony' forming units (C.F.U) per gram.

3.3.3.2 Yeast and mold counting method

Three replicates of 3.33 g of yogurt were transferred into 3 sterile petridish. About 15 ml of potato dextrose agar at 45 ± 1^{0}C was poured into each petriadish followed by mixing the contents by rotating the closed petrimdisheds. The agar was then allowed to solidify at room temperature. This procedure was done under sterilized laminar flow cabinet.

CHAPTER 4

4.0 Results and Discussion.

4.1 Results.

Preliminary investigation on form of mung bean application indicated that the whole seed were not acceptable. Therefore powdered form is selected for the development of the product and paste was prepared by mixing the mung bean powder with sugar solution.

The results of sensory evaluation conducted to determine most appropriate percentage of mung bean powder is described below.

4.1.1 The effect of the panel.

The effect of the panel on the evaluation of quality parameters was not significant. So there was no any bias effect on the decision made by the testing panel. The panelist to panelist variation was not found in decision making.

4.1.2 Appearance

The probability value (P= 0.000) of the test was less than the minimum probability value (P=0.05) that was required for the test to be significant. There are at least one treatment was significant with regard to appearance of yogurt. The sample number 2 (code No.698, with 10% of mung bean paste) gained the highest average rank (81.1) for appearance among five samples. Results of five samples were presented in the table 4.1.

Table 4.1: Average rank values for appearance.

Sample No.	Code No.	Average rank	Z value
1	497	79.5	2.55
2	698	81.1	2.80
3	327	63.2	0.02
4	535	52.1	-1.68
5	705	39.1	-3.69

4.1.3 Colour.

The probability value (P=0.001) of the test was less than the minimum probability value (P=0.05) that was required for the test to be significant. Therefore at least one treatment was significant with regard to colour of the yogurt. The sample No. 2 (code No. 698, with 10% of mung bean paste) gained the highest average rank of 79.5 for colour among five samples. Results of five samples were presented in the table 4.2.

Table 4.2: Average rank value for colour

Sample No.	Code No.	Average rank	Z value
1	497	78.1	2.33
2	698	79.5	2.54
3	327	61.5	-0.23
4	535	52.7	-1.59
5	705	43.2	-3.05

4.1.4 Flavour.

The probability value (0.000) of the test was less than minimum probability value (P=0.05) that was required for the test to be significant. Therefore at least one treatment was significant with regard to flavour of the pulse based yogurt. The sample No.2 (code No. 698, 10% of mung bean paste) gains the highest average rank of 79.1 for flavour among five samples. Results of five samples were presented in the table 4.3.

Table 4.5: Average rank value for mouth feel.

Sample No.	Code No.	Average rank	Z value
1	497	80.4	2.68
2	698	80.8	2.74
3	327	66.6	0.56
4	535	46.5	-2.55
5	705	40.7	-3.44

4.1.5 Spoon ability.

The probability value (0.126) of the test was more than minimum probability value (P=0.05) that was required for the test to be significant. Therefore no significant difference with regard to spoon ability of the pulse-based yogurt. Results of five samples were presented in the table 4.4.

Table 4.4: Average rank value for spoon ability.

Sample No.	Code No.	Average rank	Z value
1	497	76.0	2.00
2	698	70.2	1.11
3	327	60.0	-0.46
4	535	54.1	-1.37
5	705	54.7	-1.28

4.1.6 Mouth feel.

The probability value (0.000) of the test was less than minimum probability value (P=0.05) that was required for the test to be significant. Therefore at least one treatment was significant with regard to mouth feel of the pulse based yogurt. The sample No.2 (code No. 698, 10% of mung bean paste) gains the highest average rank of 80.8 for mouth feel among five samples. Results of five samples were presented in the table 4.5.

Table 4.5: Average rank value for mouth feel.

Sample No.	Code No.	Average rank	Z value
1	497	80.4	2.68
2	698	80.8	2.74
3	327	66.6	0.56
4	535	46.5	-2.55
5	705	40.7	-3.44

4.1.7 Overall acceptability.

The probability value (0.000) of the test was less than minimum probability value (P=0.05) that was required for the test to be significant. Therefore at least one treatment was significant with regard to overall acceptability of the pulse-based yogurt. The sample No.2 (code No. 698, 10% of mung bean paste) gains the highest average rank of 78.6 for overall acceptability among five samples. Results of five samples were presented in the table 4.6.

Table 4.6: Average rank value for overall acceptability.

Sample No.	Code No.	Average rank	Z value
1	497	76.5	2.09
2	698	78.6	2.41
3	327	66.8	0.58
4	535	52.5	-1.62
5	705	40.6	-3.45

According to results of the organoleptic test sample coded 698 selected as the best sample, which contained 10% of mung bean paste all the product developments. Chemical analysis, microbial analysis, shelf life evaluation was done to selected formulae. Physico –chemical and microbial quality assessment were done for the selected best sample.

4.2 Proximate composition.

Product with 10% mung bean powder shown the following composition as illustrated in the table 4.7.

Table 4.7: Proximate composition of the product.

Total solid	25.94%
Fat	3.5%
pH	4.5
Acidity	0.97
Brix	21^0

4.3 Changes during the storage.

4.3.1 Physical changes during the storage at 4^0C.

There was no gas formation, discolouration or mould growth observes in the yogurt even after 29 days storage at 4± 1^0C. A formation of small 'eyes' on the surface of the yogurt was observed after 32 days of storage.

4.4 Acidity and pH changes during the storage at 4^0C.

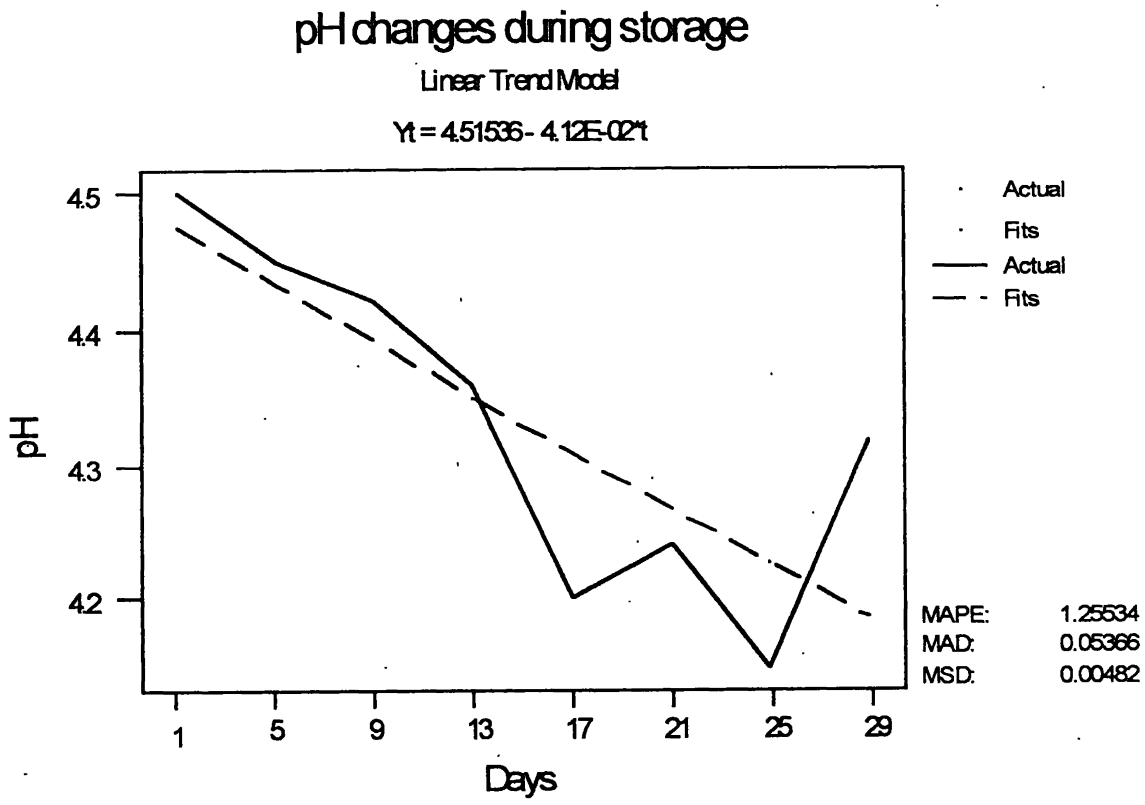


Figure 4.1:pH changes during storage.

According to the figure 4.1 it can be seen there was a downward trend. When we consider the measurements of accuracy of the trend line it can be seen that all the measurements (mean absolute percentage error, mean absolute deviation, mean squared deviation) are very small. Therefore the fitted trend line was good.

$Yt = 4.51536 - 4.12E - 02 * t$

According to this equation at pH 4.1, the shelf life of the product was 37 days.

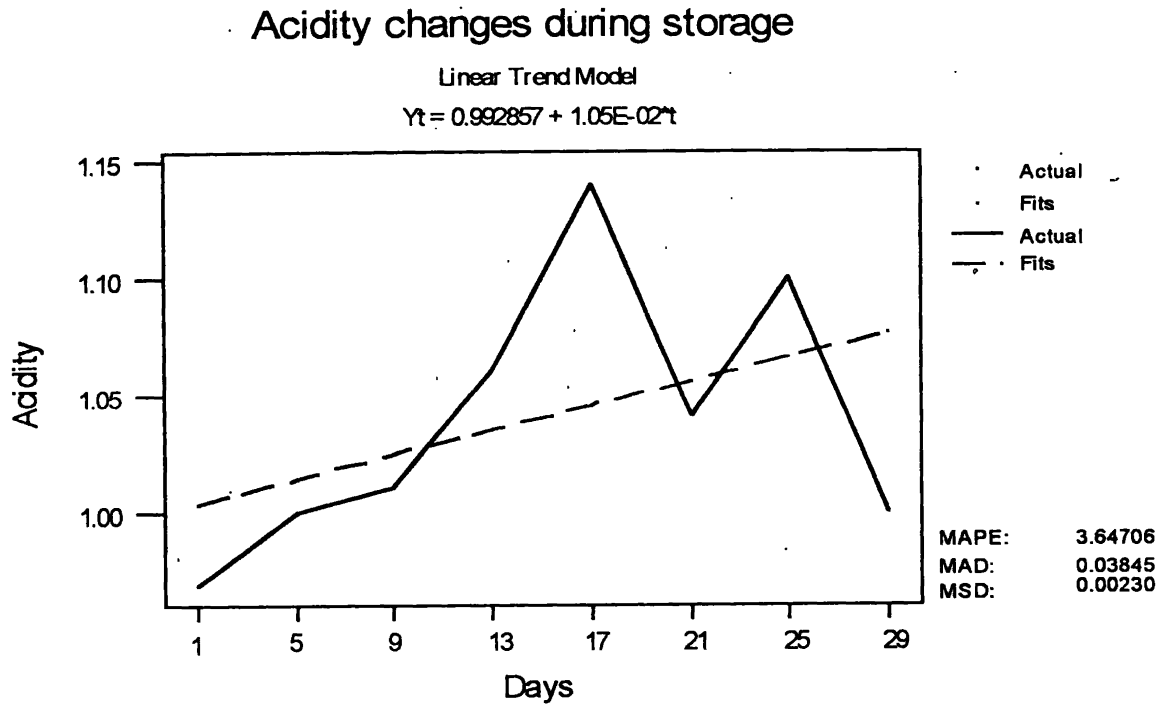


Figure 4.2: Acidity changes during storage.

According to the figure 4.2 it can be seen there was a upward trend. When we consider the measurements of accuracy of the trend line it can be seen that all the measurements (mean absolute percentage error, mean absolute deviation, mean squared deviation) are very small. Therefore the fitted trend line was good.

4.5 Microbial analysis.

4.5.1 Coliform, Yeast and mould test.

All the values of microbial count are within the standard limit for yogurt specified by SLS 824:1989. Microbial count is given in the table 4.8.

Table 4.8: coliform, yeast and mould counts.

Days	Yeast and Mould	Coliform
4	No count	No count
8	No count	No count
12	1/g	No count
16	1/g	No count
20	1/g	No count
24	1/g	No count
28	1/g	No count

4.6. Discussion.

There is a high demand for processed foods as mothers are busy nowadays hence they also contribute their energy to development of the country with the concept of globalization.

Busy mothers in urban community pay more attention to processed foods. Apart from the time saving, they are now very concern about the nutritions value of the food also. Therefore this mung bean based stirred yogurt is better solution as nutritions proceesed food for children as well as for elderly people.

Yogurt is a nutrition food that consumed by people all over the world since very ancient times. It very popular among all the young and old age groups, becaue of its delicious flavour. Mother give it to their children becaue of its easy digestability. On the other hand mothers health consionsness on procesed food made yogurt popular item because of its nutritional level.

Typical yogurt contain 3.9% of protein, 3.4% of fat and 4.9 of carbohydrate (Tamine and Robinson, 1984)

Apart from the plain natural yogurt there are flavoured yogurt as well as yogurt with a mix of fruit pieces (fruit yogurt), but yogurt with cereal or pulses is a new approach.Many effort have been done in incorperation of cereal to yogurt. In 1978, Robinson and cadena have developed dried cereal mixture incorperatng wheat grains. Apart from cereals addition of pulse can enhance availability of protein. Plant protein is the major food protein consumed by people in the densely populated regions of the of the world, because in those regions, animal protein are aften in hort supply or unavailable, their use may be prohibited by cost, or they may not be eaten meet due to dietary or cultural preferences.

Mung been is the major pulse crop consime in the country with high protein level (23.86%), hence it support to balance the protein intake of people. In corperation of mung been with yogurt, hence will increase the protein content of the yogurt.

Mung been also supplys vitamins and minerals hence it increses vitamin and minerals content of the yogurt. Therefore mung been was selected to enriched the yogurt. In corperation of mung been was tried with two major types of yogurt, stirred and set.But addition of mung been was not succeded in the set yogurt as it is deposit

in the bottom when it set. Therefore enrichment was carried out with the stirred type of yogurt.

More addition need to paid when selecting mung been for this type of experiments. Pest infestation damage free green coloured green gram were used for experiment.

Preliminary trial error test revealed that the incorperation of boild whole grains were not applicable due to presence of hard seeds. Those findings focused experiment on incorperation of green gram powder.

Thorough washing of mung been before blending reduce the risk of foreign contaminants and drying following washing reduce the moisture content in the powder. There by water activity is reduced other wise it could increase the microbial growth.

Demodan, company standared softner, containing mono and diglycerides of fatty acids was used as a softner to reduce the hardenes of the mung bean powder. Reaction of milk in yogurt with starch causes crystalization. Softner reduce the crystalization by coating the powder.

Inorder to increase the sweeteness of the powder it was mixed with sugar syrup. Syrup alo act as a preservation in the mixture.

Addition of stabilizer is an essential step in the yogurt production. It result in increase viscosity of the product by binding powder with yogurt mixture. Pectin, a popular stabilizer used in the food industry, was used as a stabilizing agent.

Analysis of data obtained by sensory evaluation revealed that the addition of 10 % mung bean paste, paste reffered to mixture of mung bean powder and sugar solution, was the acceptable level. Low scores were obtained for the samples with mung bean paste over 10 % due to undesirable colour, flavour, texture and appearance with the increased amount of mung bean paste. It was also found that, mung bean flavour was not much identified when amount is lower than 10 %.

The acidity of yogurt is due to partly to the natural acidity of the milk and, partly due to acidity develop by the bacterial flora (Robinson and Tamime, 1984). Once protein coagulation has been initiated, further production of lactic acid is controlled with a

view to palatability, consumer preference and shelf life of the product. Therefore acid development during production need to be carefully monitered. It was found that there was a fluctuation in pH after 17 days of storage at 4 ± 1^{0}C. This fluctuation in pH may be due to the packaging contamination. The consumer acceptable pH of yougurt ranges between 4.6-4.1. Time series analysis of the pH during storage indicated that product can be retained for 37 days at pH 4.1.

Besides the natural deterioration, yogurt can be spoiled after any contamination and growth by yeast and moulds. This deterioration is stronly dependent on the type of packaging material used. Aerobic yeast and mould grow very fast in yogurt packed in air pearmeable material or in packages with considerable head space. Though data revealed the 37 days of storage at pH 4.1 it could be argued that the storage life will vairy with the environment at retail shop. Temperature differences during storage and transportaion could lead the acid development there by reducing pH to an unacceptable level.

Microbiological analysis, direct plate counting of the product indicated that the product contained 1 / g of yeast and moulds up to the 16 days of storage There was no any Coliform count for the developed product.This was within the standard microbiological requirement for yogurt (Yeat not more than 1000 per g, mould not more than 1 per g, E-coli not more than 1 per g). Observations indicated that there was small eyes on the surface of the yogurt after 30 days of storage. This could probably be due to the growth of yeast, since highly selective environment flavourable for yeast is created, with low pH and lactic acid concentration.There was no difference occurred in texture, flavour and colour of the product through out the storage life. End product analysis indicated that the product containing 3.5 % fat and 21^0 Brix value.

It was interested to indicate that introduction of green gram enriched yogurt could able to divert the yogurt market, and mothers will have a choice to select nutritious ready to eat food product at the market with reasonable price. Finally it may some how be contribute to solve the protein malnutrition among children for some extent.

CHAPTER 5

5.1 Conclusion.

According to the results obtained it can be concluded that the most acceptable percentage of mung bean powder for incorporation on mung bean enriched yogurt is the 10% from the total weight of stirred yogurt.

The final product specification are within prescribed standards with a fair storage life of 29 days at 4± 1^0 C without any deterioration of quality parameters.

5.2 Recommendations for further studies.

1. Using another type of cereal can develop the product.
2. A market research should be carried out for consumer acceptability of the product.
3. Protein, crude fibre, carbohydrate content of this product should be analyzed.

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Appendix I

ORGANOLEPTIC TESTING OF GREEN GRAM BASED YOGURT

Date:.....

Name:.....

- This is green gram based yogurt.
- Please taste the five samples of yogurt and indicate your score against the sample code.
- The rating for such samles are given in numeric values ranging from 7 (Like extremely) to 1(Dislike extremely) as given below.

Like extremely.....7

Like very much.....6

Like slightly.....5

Neither Like nor dislike.....4

Dislike slightly.....3

Dislike very much.....2

Dislike extremely.....1

Panel score

Quality character	497	698	327	535	705
Appearance					
Colour					
Flavour					
Spoon ability					
Mouth feel					
Overall acceptability					

Comments:.....

.....

.....

.....

Thank you.

Appendix 2

Standonds for yoghurt.

The compositional standards for yoghurt very with different countries. Some examples of those fingures are shown in table 1 and table 2 the microbiological likits are shown in the table 3

Table 1 Examples of compositional [%] standards for yoghurt in different countries.

Character	**UK**	**USA**	**Australia**
Mean solid non-fat (MSNF)	8.5	8.25	8.5
Protein	3.0	N.S	N.S.
Acidity	N.S.	0.9	PH 4.5

N.S. = No standards.

Source: varnam and sutherland, 1994.

Table 2 Sri Lanka standards for yoghurt.

Character	**Yogurt**	**Low fat yogurt**	**Non fat yogurt**
Fat (%) w/w	3 min	0.5-3.0	<0.5
MSNF (%) w/w	8 min	8 min	8 min
Titrable acidity (%) w/w	0.8-1.25	0.8-1.25	0.8-1.25

min= minimum

source: SLS 824,1989

Table: 3 Microbiological limits for yogurt.

Micro-orgamism	Limits
E-coli	Not more than 01 per gram
Yeast	Not more than 1000 per gram
Moulds	Not more than 01 per gram

From SLS 824; 1989

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