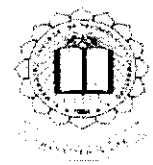

DEVELOPMENT OF FORTIFIED YOGHURT

A PROJECT REPORT

Submitted by

SIVASANKAR.G

In partial fulfillment for the award of the degree

of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE

ANNA UNIVERSITY: CHENNAI 600 025

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ANNA UNIVERSITY: CHENNAI 600 025

BONAFIDE CERTIFICATE

Certified that this project report **"DEVELOPMENT OF FORTIFIED YOGHURT"** is the bonafide work of **"SIVASANKAR.G"** who carried out the project work under my supervision.

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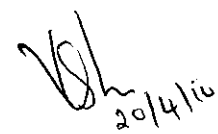

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The report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in Biotechnology of Anna University was confirmed to be the report of the work done by the above students and then evaluated.

DEDICATED TO OUR BELOVED PARENTS

&

RESPECTED GUIDE

INTRODUCTION

1. INTRODUCTION

FORTIFIED FOODS:

Fortified foods are items that have had vitamins and minerals that are not naturally occurring added. The purpose of fortification is to strengthen the foods that people eat. There are also foods on the market that are enriched. People often confuse these with fortified foods, but there is a major difference. Enrichment is performed because a lot of what people eat i not fresh or in its natural form. Processing foods often destroys vitamins and minerals. To repair the damage, food is sometimes enriched. This means that the vitamins and minerals that where destroyed are replaced after processing. The nutrients that are added to fortified foods are not generally to be found in foods that have been enriched, even if they had not been processed. Foods are fortified because the lack of proper nutrients can cause a number of health problems. Children who do not eat properly are often underweight and have stunted growth. Adults who do not eat properly tend to be more susceptible to illness and less productive than their peers who are properly nourished. Complications can also occur with pregnancies if the mother and unborn child are lacking certain nutrients.

We are highly thankful to all the non-teaching staff members of the Department of Biotechnology for their kind and patient help in all respects of this project work.

We wish to thank our friends for providing us with the moral support throughout our course of the project. Last but not the least, we express our gratitude to our parents for their motivation, unflinching support and encouragement which has enabled us to climb the ladder of success.

SIVASANKAR.G

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ACKNOWLEDGEMENTS

First and foremost, I thank the Almighty for giving me the courage and strength to carry out the project work successfully.

I wish to express our deep sense of gratitude and profound heartfelt thanks to **Dr. Stephen V. Rapheal**, Assistant Professor, for his valuable guidance, constant encouragement, constructive criticisms and enthusiastic suggestions in each and every step of this investigation.

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I wish to place on record our gratitude and heartfelt thanks to **Dr. S. Sadasivam**, Dean, for his constant motivation and support throughout the period of this investigation.

I wish to place on record our sincere thanks to **Dr. Ramalingam**, Assistant Professor and all our staff members for their constant support and valuable suggestions during the course of this study.

I wish to express our sincere gratitude to **Dr.S. Ramachandran**, Principal and the college management for their help and support during the period of this investigation.

ABSTRACT

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Yoghurt or yogurt is a dairy product produced by bacterial fermentation of milk. Fermentation of lactose produces lactic acid, which acts on milk protein to give yoghurt its texture and its characteristic tang. Sov voghurt, a non-dairy yoghurt alternative, is made from sov milk.

Today it is a common food item throughout the world. A nutritious food with unique health benefits, it is rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12.Yogurt is a fermented milk product with a custard-like consistency which differentiates it from other fermented milk products. The process of yoghurt production begins with milk with a fat level from 2.0-3.5%. The serum solids content of the milk is increased to 10.5-11.5% through a standardization process by adding condensed skim milk or nonfat dry milk. Milk is homogenized and heated to 185° to 195°F (85-90.5° C) for 30 to 60 minutes for pasteurization. The high heat treatment improves the body of the yogurt and limits whey expulsion. The milk is cooled to 104 to 106° F (40 to 41° C) and is inoculated with a lactic acid producing culture. Acid production is monitored and data (time/temperature/pH) is recorded.

The inoculated milk is incubated in a vat (stirred) or placed in consumer-sized sterile packages (set) to incubate in a temperature controlled environment. To attain yogurt with a pH of 4.0 the cooling process should begin when the fermenting milk reaches a pH of 4.3-4.4. To extend the shelf life of the product, yogurt can be heat treated after culturing is complete, destroying viable microorganisms. In the present study yoghurt preparation was standardized and fortified yoghurt was prepared. In addition flavor, color other nutrients was added and the properties of the yoghurt was studied.

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ENRICHED FOODS:

Enriched means that vitamins or minerals have been added to the food. The vitamins and minerals are added to replace the original vitamins and minerals that were lost during the refining process. For example, if the food originally had iron, but the iron was lost during the refining process, the food will be 'enriched' to add the iron back into the food.

Consumers often think enriched means 'added vitamins and minerals'. This assumption is incorrect. Enriched merely means to replace what was lost during the refining process.

CONTENTS:

Several ranges of food supplements are recognized:

- additives which repair a deficit to "normal" levels
- additives which appear to enhance a food
- supplements taken in addition to the normal diet

Many physicians today disagree with the premise that foodstuffs need supplementation, but accept that - for example - added calcium may provide benefit, or that adding folic acid may correct a nutritional deficiency specially in pregnant women on a more controversial level, but well founded in scientific basis, is the science of using foods and food supplements to achieve a defined health goal.

A common example of this use of food supplements is the extent to which body builders will use amino acid mixture vitamins and phytochemicals to enhance natural hormone production, increase muscle and reduce fat.

Moving on from this reasonably accepted usage, there is increasing evidence for the use of food supplements in established medical conditions. This nutritional supplementation using foods as medicine (nutraceuticals) has been effectively used in treating disorders affecting the immune system up to and including cancers. This goes beyond the definition of "food supplement", but should be included for the sake of completeness.

FOOD SUPPLEMENTS:

There are several main groups of food supplements which can be considered:

- Vitamins and co-vitamins
- Essential minerals
- Essential fatty acids
- Essential amino acids
- Glyconutrients
- Phytonutrients

EXAMPLES OF FORTIFIED FOODS:

Iodized salt has been used in the United States since before World War II. Folic acid is added to flour in many industrialized countries, and has prevented a significant number of neural tube defects in infants. It is, however, not uniform in its application, with more intake of folic acid through fortified flour among those who were already receiving high amounts through their diet.

Niacin has been added to bread in the USA since 1938 (when voluntary addition started), a programme which substantially reduced the incidence of pellagra. Vitamin D is added to a few foods (especially margarine). Fluoride salts are added to water and toothpastes to prevent tooth decay. Water fluoridation is a controversial topic in some segments of the general public, although less so amongst established scientific bodies.

Calcium is frequently added to fruit juices, carbonated beverages and rice."Golden rice" is a variety of rice which has been genetically modified to produce beta carotene.

A wide range of iron compounds, including ferrous sulfate, ferrous fumarate and even elemental iron powder are added to food (usually cereal flours, but also table salt, milk and condiments) in a number of countries to prevent iron deficiency anemia. Although iron intake is often sufficient in developing countries, the bioavailability of the dietary iron is low, due to such factors as polyphones and phytic acid binding the iron and preventing its absorption.

Major challenges in iron fortification are to avoid undesirable changes in the appearance and taste of the food, and to target the population segment that needs the fortification the most.

FORTIFICATION OF FOODS:

Nutrient supplementation of foods was mentioned for the first time in the year 400 B.C. by the Persian physician Melanpus, who suggested adding iron filings to wine to increase soldiers' "potency." In 1831 the French physician Boussingault urged adding iodine to salt to prevent goitre. However, it was between the First and Second World Wars (1924-1944) that supplementation was established as a measure either to correct or prevent nutritional deficiencies in populations or to restore nutrients lost during food processing. Thus, during this period the adding of iodine to salt, vitamins A and D to margarine, vitamin D to milk, and vitamins B1, B2, niacin, and iron to flours and bread was established.

Currently, food fortification encompasses a broader concept, and might be done for several reasons. The first is to restore nutrients lost during food processing, a process known as enrichment. In this case, the amount of nutrients added is approximately equal to the natural content in the food before processing. A second reason is to add nutrients that may not be present naturally in food, a process known as fortification.

In this case, the amount of nutrient added may be higher than that present before processing. Fortification also standardizes the contents of nutrients that show variable concentrations. A typical example is the addition of vitamin C to orange juice to standardize vitamin C concentration and compensate for changes due to seasonal and processing variations. Finally, for technological purposes, a preservative or colouring agents are added to processed foods.

Therefore, depending on the reasons for adding nutrients, the objectives may be: to maintain the nutritional quality of foods, keeping nutrient levels adequate to correct or prevent specific nutritional deficiencies in the population at large or in groups at risk of certain deficiencies (i.e., the elderly, vegetarians, pregnant women, etc.); to increase the added nutritional value of a product (commercial view); and to provide certain technological functions in food processing.

According to these principles, currently in several countries nutrients are added to a wide variety of food carriers, such as cereals, flours, bread, milk, margarine, infant formulas, soy milk, orange juice, salt, sugar, monosodium glutamate, tea, dietetic beverages, and even parenteral and enteral solutions (table 1). Most fortifying agents are vitamins and minerals, and in some cases essential amino acids and proteins. These additions have helped to solve public health problems, such as salt iodization to prevent goitre .

1.2 YOGHURT:

Yoghurt or yogurt is a dairy product produced by bacterial fermentation of milk. Fermentation of lactose produces lactic acid, which acts on milk protein to give yoghurt its texture and its characteristic tang. Soy yoghurt, a non-dairy yoghurt alternative, is made from milk. Dairy yoghurt is produced using a culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* bacteria. Milk is heated and cooled for an hour. While it is heated, the bacteria are added for fermentation. People have been making and eating yogurt for at least 5,400 years. Today it is a common food item throughout the world. A nutritious food with unique health benefits, it is rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12.

CONTENTS:

HISTORY:

There is evidence of cultured milk products being produced as food for at least 4,500 years. The earliest yoghurts were probably spontaneously fermented by wild bacteria Lactobacillus delbrueckii subsp. bulgaricus native to and named after Bulgaria.

The oldest writings mentioning yogurt are attributed to Pliny the Elder, who remarked that certain nomadic tribes, including the Bulgars, knew how "to thicken the milk into a substance with an agreeable acidity". The use of yoghurt by medieval Turks is recorded in the books Diwan Lughat al-Turk by Mahmud Kashgari and Kutadgu Bilig by Yusuf Has Hajib written in the 11th century.

Both texts mention the word "yoghurt" in different sections and describe its use by nomadic Turks. An early account of a European encounter with yoghurt occurs in French clinical history: Francis I suffered from a severe diarrhoea which no French doctor could cure. His ally Suleiman the Magnificent sent a doctor, who allegedly cured the patient with yoghurt. Being grateful, the French king spread around the information about the food which had cured him Raita is a condiment made with yoghurt and popular in India.

Until the 1900s, yoghurt was a staple in diets of people in the Russian Empire (and especially Central Asia and the Caucasus), Western Asia, South Eastern Europe/Balkans, Central Europe, and India. Stamen Grigorov (1878–1945), a Bulgarian student of medicine in Geneva, first examined the micro flora of the Bulgarian yoghurt. In 1905 he described it as consisting of a spherical and a rod-like lactic acid bacteria. In 1907 the rod-like bacteria was called Lactobacillus bulgaricus (now Lactobacillus delbrueckii subsp. bulgaricus).

The Russian Nobel laureate biologist Ilya Ilyich Mechnikov, from the Institute Pasteur in Paris, was influenced by Grigorov's work and hypothesised that regular consumption of yoghurt was responsible for the unusually long lifespans of Bulgarian peasants. Believing Lactobacillus to be essential for good health, Mechnikov worked to popularise yoghurt as a foodstuff throughout Europe.

A Sephardic Jewish entrepreneur named Isaac Carasso industrialized the production of yoghurt. In 1919, Carasso, who was from Ottoman Salonika, started a small yoghurt business in Barcelona and named the business danone ("little Daniel") after his son. The brand later expanded to the United States under an Americanized version of the name:
Dannon. Tarator is a cold soup made of yoghurt popular in Bulgaria.

Yoghurt with added fruit jam was patented in 1933 by the Radlická Mlékárna dairy in Prague. It was introduced to the United States in 1947, by Dannon.

Yoghurt was first introduced to the United States by Armenian immigrants Sarkis and Rose Colombosian, who started "Colombo and Sons Creamery" in Andover, Massachusetts in 1929.

Colombo Yogurt was originally delivered around New England in a horse-drawn wagon inscribed with the Armenian word "mad zoon" which was later changed to "yogurt", the Turkish name of the product, as Turkish was the lingua franca between immigrants of the various Near Eastern ethnicities who were the main consumers at that time. Yoghurt's popularity in the United States was enhanced in the 1950s and 1960s, when it was presented as a health food. By the late 20th century yoghurt had become a common American food item and Colombo Yogurt was sold to General Mills in 1993.

NUTRITIONAL VALUE AND HEALTH BENEFITS:

Tzatziki is an appetizer made with yoghurt, popular in Greece and Bulgaria, where it is called Dry Tarator.

Yoghurt is nutritionally rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12.It has nutritional benefits beyond those of milk. People who are moderately lactose-intolerant can enjoy yoghurt without ill effects, because much of the lactose in the milk precursor is converted to lactic acid by the bacterial culture.

Yoghurt also has medical uses, in particular for a variety of gastrointestinal conditions and in preventing antibiotic-associated diarrhea. One study suggests that eating yoghurt containing L. acidophilus helps prevent vulvovaginal candidiasis, though the evidence is not conclusive.

Yoghurt is believed to promote good gum health, possibly because of the probiotic effect of lactic acids present in yoghurt.

A study published in the International Journal of Obesity (11 January 2005) also found that the consumption of low-fat yoghurt can promote weight loss. In the trial, obese individuals who ate 3 servings of low-fat yoghurt a day as part of a low-calorie diet lost 22% more weight than the control group who only cut back on calories and did not have extra calcium. They also lost 81% more abdominal fat.

VARIETIES AND PRESENTATION:

Dadiah sold in Bukittinggi Market Dadiah, or Dadih, is a traditional West Sumatran yoghurt made from water buffalo milk. It is fermented in bamboo tubes.

Yoghurt is popular in Nepal, where it is served both as an appetizer or dessert. Locally called dahi , it is a part of the Nepali culture, used in local festivals, marriage ceremonies, parties, religious occasions, family gatherings, and so on. The most famous type of Nepalese yoghurt is called juju dahu, originating from the city of Bhaktapur.

Tarator and Cacık are popular cold soups made from yoghurt, popular during summertime in Albania, Bulgaria, Republic of Macedonia, and Turkey. They are made with ayran, cucumbers, dill, salt, olive oil, and optionally garlic and ground walnuts.

Rahmjoghurt, a creamy yoghurt with much higher fat content (10%) than most yoghurts offered in English-speaking countries (Rahm is German for "cream"), is available in Germany and other countries.

Cream-top yoghurt is yoghurt made with un homogenized milk. A layer of cream rises to the top, forming a rich yoghurt cream. Cream-top yoghurt was first made commercially popular in the United States by Brown Cow of Newfield, New York, bucking the trend toward low- and non-fat yoghurts.

Jammed is yoghurt which is salted and dried to preserve it. It is popular in Jordan. Zabady is the yoghurt made in Egypt. It is particularly associated with Ramadan fasting, as it is thought to prevent thirst during all-day fasting.

Raita is a yoghurt-based South Asian/Indian condiment, used as a side dish. The yoghurt is seasoned with cilantro (coriander), cumin, mint, cayenne pepper, and other herbs and spices. Vegetables such as cucumber and onions are mixed in, and the mixture is served chilled. Raita has a cooling effect on the palate which makes it a good foil for spicy Indian dishes.

Dudh is a Sindhi-curd, popular in India. People drink dudh along with food at intervals, to help digestion and make food more delicious. In some places dudh is also served with plain rice.

Dahi is a yoghurt of the Indian subcontinent, known for its characteristic taste and consistency. The word dahi seems to be derived from the Sanskrit word dadhi, one of the five elixirs, or panchamrita, often used in Hindu ritual. Dahi also holds cultural symbolism in many homes in the Mithilanchal region of Bihar. It is found in different flavours, two of which are famous: sour yoghurt (tauk doi) and sweet yoghurt (meesti or podi doi).

In India, it is often used in cosmetics mixed with turmeric and honey. Sour yoghurt is also used as a hair conditioner by women in many parts of India. Dahi is also known as Thayiru (Malayalam), doi (Assamese, Bengali), dohi (Oriya), perugu (Telugu), Mosaru (Kannada), Thayir (Tamil), or Qəzana a pəəner (Pashto).

Srikhand, a popular dessert in India, is made from drained yoghurt, saffron, cardamom, nutmeg and sugar and sometimes fruits such as mango or pineapple.

SWEETENED AND FLAVORED YOGURT:

To offset its natural sourness, yoghurt can be sold sweetened, flavored, or in containers with fruit or fruit jam on the bottom. If the fruit has been stirred into the yoghurt before purchase, it is commonly referred to as Swiss-style. Most yoghurt in North America have added pectin, found naturally in fruit, and/or gelatin to artificially create thickness and creaminess at lower cost.

This type of adulterated product is also marketed under the name Swiss-style, although it is unrelated to the way yoghurt is eaten in Switzerland. Some specialty yoghurts, often called "cream line", have a layer of fermented fat at the top. Fruit jam is used instead of raw fruit pieces in fruit yoghurts to allow storage for weeks.

Sweeteners such as cane sugar are often present in large amounts in commercial yoghurt.

In the USA, sweetened, flavored yoghurt is the most popular type, typically sold in single-serving plastic cups. Typical flavors are vanilla, honey, or fruit such as strawberry, blueberry, blackberry, or peach.

STRAINED YOGHURTS AND YOGHURT CHEESE:

Main article: strained yoghurt

Strained yoghurts are types of yoghurt which are strained through a paper or cloth filter, traditionally made of muslin, to remove the whey, giving a much thicker consistency and a distinctive, slightly tangy taste.

Labneh is a strained yoghurt used for sandwiches popular in Arab countries. Olive oil, cucumber slices, olives, and various green herbs may be added. It can be thickened further and rolled into balls, preserved in olive oil, and fermented for a few more weeks. It is sometimes used with onions, meat, and nuts as a stuffing for a variety of pies or kebbeh balls.

Shankleesh is a type of cheese made from cured dried labneh, featured in the gastronomy of Lebanon and surrounding areas. The labneh is salted, dried and rolled into balls. It comes in different varieties ranging from the fresh variant in olive oil and thyme to the "aged" balls covered with spices.

Some types of strained yoghurts are boiled in open vats first, so that the liquid content is reduced. The popular East Indian dessert, a variation of traditional dahi called mishit dahi, offers a thicker, more custard-like consistency, and is usually sweeter than western yoghurts. Strained yoghurt is also enjoyed in Greece and is the main component of tzadziki, a well-known accompaniment to gyros and souvlaki pita sandwiches.

BEVERAGES:

Ayran or dhalla is a yoghurt-based, salty drink popular in Albania, Bulgaria, Turkey, Azerbaijan, Iran, Republic of Macedonia, Kazakhstan and Kyrgyzstan. It is made by mixing yoghurt with water and (sometimes) salt. The same drink is known as doogh in Iran; tan in Armenia; laban ayran in Syria and Lebanon; shenina in Iraq and Jordan; laban arbil in Iraq; majjiga (Telugu), majjige (Kannada), and moru (Tamil and Malayalam) in South India; lassi in Punjab and all over India.

A similar drink, doogh, is popular in the Middle East between Lebanon, Iran and Afghanistan; it differs from ayran by the addition of herbs, usually mint, and is carbonated, commonly with seltzer water.

Lassi is a yoghurt-based beverage originally from the Indian subcontinent that is usually slightly salty or sweet. Lassi is a staple of Punjab. In some parts of the subcontinent, the sweet version may be commercially flavored with rosewater, mango or other fruit juice to create a very different drink.

Salty lassi is usually flavored with ground, roasted cumin and red chillies; this salty variation may also use buttermilk, and is interchangeably called ghol (Bengal), mattha (North India), tak (Maharashtra), or chaas (Gujarat). Lassi is also very widely drunk in Pakistan.

YOGHURT-LIKE PRODUCTS:

Kefir is a slightly alcoholic (up to 0.88%) fermented yogurt-like product originating in the North Caucasus and has been a main food staple in Russia and the other republics of the former Russian Empire. For many years some American dairies have offered a drink called "kefir" with fruit flavours but without carbonation or alcohol.

Matsoni is a yoghurt-like dairy product, popular in Georgia and Armenia. It is started with Lactococcus lactose subsp. cremoris and Acetobacter oriental is species and has a viscous, honey-like texture.

It is milder in taste than other varieties of yoghurts since it is less sour and has less alcohol than kefir.Matsoni is called "Caspian Sea Yogurt" in Japan, where it is believed to have been introduced in 1986 by researchers returning from a trip to the Caucasus region of Georgia and Armenia. Ideally, Caspian Sea yoghurt is made at home because it requires neither special equipment nor unobtainable culture.

It can be made at room temperature (20–30°C) in 10 to 15 hours. In Japan, freeze-dried starter cultures are sold in department stores and online, although many people obtain starter cultures from friends.
A Central Asian Turco-Mongolian drink made from mare's milk is called kumis, or airag in Mongolia.

Sweetened yoghurt drinks are the usual form in Europe (including the UK) and the US, containing fruit and added sweeteners. These are typically called "drinking / drinkable yoghurt", such as Yop and BioBest Smoothie. Also available are "yoghurt smoothies", which contain a higher proportion of fruit and are more like smoothies.

In Ecuador, yoghurt smoothies flavored with native fruit are served with pan de yuca as a common type of fast food.

TOTAL YOGHURT FAT:

Nutritional value per 100 g (3.5 oz), Energy 257 kJ (61 kcal) ,Carbohydrates 4.7 g, Sugars 4.7 g ,(*) Fat 3.3 g, saturated 2.1 g monounsaturated 0.9 g, Protein 3.5 g, Vitamin A equiv. 27 µg (3%) Riboflavin (Vit. B2) 0.14 mg (9%) Calcium 121 mg (12%).

FORTIFIED YOGHURT PRODUCTION:

Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44^0c.

This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42^{0}c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops.

The growth of lactic acid culture, which is thermophilic. In the present study yoghurt preparation will be standardized and fortified yoghurt will be prepared. Amla powder and Pomegranate seed powder And Tulsi seeds powder were added to the yogurt and it was found there was no microbial growth.

Tests will be carried out for these samples to find the composition of the nutrients like Vitamin C, Iron, Calcium. In addition flavor, colour other nutrients will be added and the properties of the yoghurt will be studied.

OBJECTIVES

2. OBJECTIVES:

1. Formulation of fortified yoghurt with enhanced mineral and vitamin content.

2. Cost analysis of production.

3. Analysis of scale up considerations.

LITERATURE REVIEW

LITERATURE REVIEW

3.LITERATURE REVIEW

3.1 PLAIN YOGHURT PRODUCTION:

Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44^0c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42^0c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic.

3.2 FORTIFIED YOGHURT:

Milk is heated upto 85 for 15 minutes. Adding 25ml of plain yoghurt. The three tablets Vitamin c, calcium, zincovit are added to the above experiment. While plain yoghurt +100mg calcium, plain yoghurt +200mg zincovit and plain yoghurt +300mg are added and kept inside incubator after four hours micro organisms get developed.

While adding 50mg calcium and 100mg zincovit the micro organism is not developed. For every half an hour ph level is noted. Instead of adding tablets we can add easy nutrients available in market like amla powder, pomegranate seeds powder and tulsi seeds powder are added. While adding 300mg amla powder, 200mg pomegranate seeds powder and 50mg tulsi seeds powder the growth of micro organisms is nill.ph level is noted.

0.1g of kesari colour and lemon yellow is added to 5ml of water. From that 2ml is added to plain yoghurt. 2ml of Chocolate flavor and strawberry flavor is added to the plain yoghurt and ph level is noted. In plain yoghurt amla 300mg and 2ml of lemon yellow ,kesari colour is added and sugar is not added. In plain yoghurt amla 300mg and 2ml of lemon yellow ,kesari colour is added and 10g of sugar is added.ph level is noted.

In plain yoghurt tulsi 50mg and 2ml of lemon yellow ,kesari colour is added and sugar is not added. In plain yoghurt tulsi 50mg and 2ml of lemon yellow ,kesari colour is added and 10g of sugar is added.ph level is noted.

In plain yoghurt pomegranate 200mg and 2ml of lemon yellow ,kesari colour is added and sugar is not added. In plain yoghurt pomegranate 200mg And 2ml of lemon yellow ,kesari colour is added and 10mg of sugar is Added. Ph level is noted.

Amla powder, tulsi seeds powder and pomegranate seeds powder are Added.ph level is noted for every half an hour and for every one hour gram Staining is done. In plain yoghurt amla seeds powder, tulsi seeds powder , colours and flavours are added and protein estimation is done every one hour. In plain yoghurt amla seeds powder , tulsi seeds powder , colours and Flavours are added . these are refrigerated for four hours and then protein Estimation is carried out.

3.3 PH TITRATION:

A typical titration begins with a beaker or Erlenmeyer flask containing a precise volume of the reactant and a small amount of indicator, placed underneath a burette containing the reagent. By controlling the amount of reagent added to the reactant, it is possible to detect the point at which the indicator changes color.

As long as the indicator has been chosen correctly, this should also be the point where the reactant and reagent neutralize each other, and, by reading the scale on the burette, the volume of reagent can be measured.

As the concentration of the reagent is known, the number of moles of reagent can be calculated (since Morality = moles / volume (L)). Then, from the chemical equation involving the two substances, the number of moles present in the reactant can be found. Finally, by dividing the number of moles of reactant by its volume, the concentration is calculated.

A typical titration curve of a diprotic acid, oxalic acid, titrated with a strong base, sodium hydroxide. Each of the two equivalence points is visible.

A titration curve is a curve in the plane whose x-coordinate is the volume of titrant added since the beginning of the titration, and whose y-coordinate is the concentration of the analyte at the corresponding stage of the titration (in an acid-base titration, the y-coordinate is usually the pH of the solution at the corresponding stage).

Often it is the case that the titration curve of a titration reflects the nature of the titration quite well; for instance, it reflects the nature of all solutions involved in the titration.

In the case of acid-base titrations, titration curves reflect the strength of the corresponding acid and base. For instance, in a strong acid and strong base titration, the titration curve will be relatively smooth, although very steep for points near the equivalence point of the titration. Since in this case, small changes in the volume of the titrant result in large changes of the pH near the equivalence point, an extensive range of indicators would be appropriate (for instance litmus, phenolphthalein or bromothymol blue).

On the other hand, if one of the constituents of an acid-base titration is either a weak acid or a weak base, and the other is either a strong acid or a strong base, the titration curve is fairly irregular near the equivalence point (and the pH does not change as much due to the addition of small volumes of titrant). For instance, the titration curve for the titration between oxalic acid (a weak acid) and sodium hydroxide (a strong base) is depicted in the image above.

Here, the equivalence point occurs at a pH of about 8-10, and thus the analyte is basic at the equivalence point (more precisely, the sodium salt produced by the reaction hydrolyses in water to produce hydroxide ions). An indicator such as phenolphthalein would be appropriate for this particular titration.

The titration curve corresponding to a weak base and strong acid ion is similarly behaved. In this case, indicators such as methyl orange mothymol blue are regularly used.

On the other hand, titration curves corresponding to acid-base titrations in which the constituents are a weak acid and weak base are quite irregular in nature. Due to the nature of such titrations, no definite indicator may be appropriate, and thus pH meters are often used.

3.4 GRAM STAINING

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The Gram stain is almost always the first step in the identification of a bacterial organism.

While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram variable and Gram in determinant groups as well.

This method is named after its inventor, the Danish scientist Hans Christian Gram (1853–1938), who developed the technique in 1882 and published it in 1884 to discriminate between two types of bacteria with similar clinical symptoms: Streptococcus pneumoniae (also known as the pneumococcus) and Klebsiella pneumoniae bacteria.

The word Gram is always spelled with a capital, referring to the name inventor of the Gram staining.

EXAMPLES:

GRAM-NEGATIVE BACTERIA:

Main article: Gram-negative bacteria

The proteobacteria are a major group of Gram-negative bacteria. Other notable groups of Gram-negative bacteria include the cyan bacteria, spirochetes, green sulfur and green non-sulfur bacteria.
These also include many medically relevant Gram-negative cocci, bacilli and many bacteria associated with nosocomial infections.

GRAM-POSITIVE BACTERIA:

Main article: Gram-positive bacteria

In the original bacterial phyla, the Gram-positive forms made up the phylum Formicates, a name now used for the largest group. It includes many well-known genera such as Bacillus, Listeria, Staphylococcus, Streptococcus, Enterococcus, and Clostridium. It has also been expanded to include the Mollicutes; bacteria like Mycoplasma that lack cell walls and so cannot be stained by Gram, but are derived from such forms.

3.5 TOTAL PROTEIN ESTIMATION:

1. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube as given in the table. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05 to 1 mg/ ml.

2. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well.

3. This solution is incubated at room temperature for 10 mins.

4. Then add 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) to each tube and incubate for30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660nm.

5. Plot the absorbance against protein concentration to get a standard calibration curve.

6. Check the absorbance of unknown sample and determine the concentration of the unknown sample using the standard curve plotted above.

A Water Sample conc. Sample vol Alk. CuSO4 Lowry reagent O.D.

Write short notes on the following points in the report:

1. Beer-Lamberts law

2. Compare Folin-Lowry method with other methods of protein estimation.

3.6 NUTRITIONAL VALUE AND HEALTH BENEFITS:

Yoghurt is nutritionally rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12.It has nutritional benefits beyond those of milk. People who are moderately lactose-intolerant can enjoy yoghurt without ill effects, because much of the lactose in the milk precursor is converted to lactic acid by the bacterial culture.

Yoghurt also has medical uses, in particular for a variety of gastrointestinal conditions and in preventing antibiotic-associated diarrhea.One study suggests that eating yoghurt containing L. acidophilus helps prevent vulvovaginal candidiasis, though the evidence is not conclusive.

Yoghurt is believed to promote good gum health, possibly because of probiotic effect of lactic acids present in yoghurt.

3.7 SWEETENED AND FLAVORED YOGHURT:

To offset its natural sourness, yoghurt can be sold sweetened, flavored, or in containers with fruit or fruit jam on the bottom. If the fruit has been stirred into the yoghurt before purchase, it is commonly referred to as Swiss-style. Most yoghurts in North America have added pectin, found naturally in fruit, and/or gelatin to artificially create thickness and creaminess at lower cost.

This type of adulterated product is also marketed under the name Swiss-style, although it is unrelated to the way yoghurt is eaten in Switzerland. Some specialty yoghurts, often called "cream line", have a layer of fermented fat at the top. Fruit jam is used instead of raw fruit pieces in fruit yoghurts to allow storage for weeks.

Sweeteners such as cane sugar are often present in large amounts in commercial yoghurt.

In the USA, sweetened, flavored yoghurt is the most popular type, typically sold in single-serving plastic cups. Typical flavors are vanilla, honey, or fruit such as strawberry, blueberry, blackberry, or peach.

3.8 FORTIFIED YOGHURT PRODUCTION:

Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44^0c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42^0c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic. In the present study yoghurt preparation will be standardized and fortified yoghurt will be prepared. Amla powder and Pomegranate seed powder And Tulsi seeds powder were added to the yogurt and it was found there was no microbial growth. Tests will be carried out for these samples to find the composition of the nutrients like Vitamin C, Iron, Calcium. In addition flavor, colour other nutrients will be added and the properties of the yoghurt will be studied.

The plain yoghurt which is mixed with milk was prepared and taken in a vessel. The mixture is cooled down to 42-44°C using cold water bath. Inorder to fortify tablets are added that are rich in vitaminC, calcium and zincovit. The proportional weights of each components is as follows: Vitamins of weight 100mg, Calcium of weight 100mg, Zincovit of weight 100mg, 200mg, 300mg. Then the milk is kept inside the incubator maintained at 42°C for one hour. Once the milk kept inside the incubator the milk turns to yoghurt. After one hour the yoghurt is taken for measuring the pH value. Now a check for the growth of micro-organisms is carried out. During this check it is seen that organisms has grown in the yoghurt containing 100 mg vitaminC, 200mg zincovit and 300mg zincovit. Thus to prevent the growth of organisms we are taking yoghurt containing 50 mg calcium. It is seen that no organisms have grown in this sample.

Thus fresh fortified yoghurt has been prepared. Since addition of tablets increase the cost of the product so in order to reduce the cost we are utilizing commonly available fruits and herbal plants that are rich in nutrients.

In order to add nutrients to the plain yoghurt amla powder, pomegranate seed power, tulsi seed powder are added. Each 300 mg of each is taken and added to the plain yoghurt and tested. No growth of micro-organisms were observed. Thus the plain yoghurt has been converted to fortified yoghurt.

MATERIALS AND METHODS

MATERIALS AND METHODS:

MATERIALS:

1. Vessel
2. Heat source
3. Incubator
4. Thermometer
5. Milk
6. Plain yoghurt from stores
7. Amla powder
8. Pomegranate seeds powder
9. Tulsi seeds powder
10. Colours
11. Flavours
12. Crystal violet
13. Gram iodine
14. Gram's decolourizer
15. Safranin
16. Alkaline copper
17. Folin's reagent
18. Sodium carbonate
19. Copper sulphate
20. Sodium potassium tartarate
21. PH Meter

METHODS:

PLAIN YOGHURT PRODUCTION:

Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44^0c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42^0c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic.

FORTIFIED YOGHURT:

Heat 500ml of milk in a vessel slowly at 85°c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44°c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42°c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic. In the present study yoghurt preparation will be standardized and fortified yoghurt will be prepared. Amla powder and Pomegranate seed powder And Tulsi seeds powder were added to the yogurt and it was found there was no microbial growth. Tests will be carried out for these samples to find the composition of the nutrients like Vitamin C, Iron, Calcium. In addition flavor, colour other nutrients will be added and the properties of the yoghurt will be studied.

pH TITRATION:

Titration is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of a known reactant. Because volume measurements play a key role in titration, it is also known as volumetric analysis.

A reagent, called the titrate or titration a known concentration (a standard solution) and volume is used to react with a solution of the analyte or titrand whose concentration is not known. Using a calibrated burette to add the titrant, it is possible to determine the exact amount that has been consumed when the endpoint is reached. The endpoint is the point at which the titration is complete, as determined by an indicator (see below).

This is ideally the same volume as the equivalence point—the volume of added titrant at which the number of moles of titrant is equal to the number of moles of analyte, or some multiple thereof (as in polypro tic acids). In the classic strong acid-strong base titration, the endpoint of a titration is the point at which the pH of the reactant is just about equal to 7, and often when the solution takes on a persisting solid color as in the pink of phenolphthalein indicator. There are however many different types of titrations (see below).

Many methods can be used to indicate the endpoint of a reaction; titrations often use visual indicators (the reactant mixture changes color). In simple acid-base titrations a pH indicator may be used, such as phenolphthalein, which becomes pink when a certain pH (about 8.2) is reached or exceeded. Another example is methyl orange, which is red in acids and yellow in alkali solutions.

Not every titration requires an indicator. In some cases, either the reactants or the products are strongly colored and can serve as the "indicator". For example, a redox titration using potassium permanganate (pink/purple) as the titrant does not require an indicator. When the titrant is reduced, it turns colorless. After the equivalence point, there is excess titrant present. The equivalence point is identified from the first faint persisting pink color (due to an excess of permanganate) in the solution being titrated.

Due to the logarithmic nature of the pH curve, the transitions are, in general, extremely sharp; and, thus, a single drop of titrant just before the endpoint can change the pH significantly—leading to an immediate colour change in the indicator. There is a slight difference between the change in indicator color and the actual equivalence point of the titration. This error is referred to as an indicator error, and it is indeterminate.

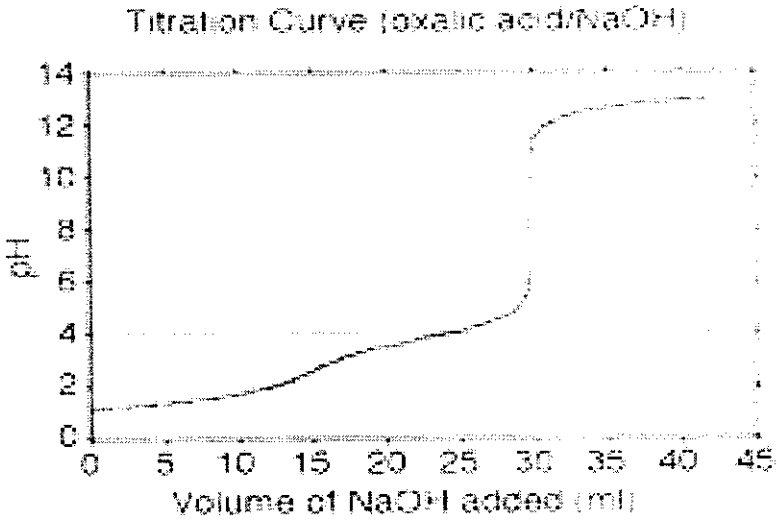
PROCEDURE:

A typical titration begins with a beaker or Erlenmeyer flask containing a precise volume of the reactant and a small amount of indicator, placed underneath a burette containing the reagent. By controlling the amount of reagent added to the reactant, it is possible to detect the point at which the indicator changes color. As long as the indicator has been chosen correctly, this should also be the point where the reactant and reagent neutralize each other, and, by reading the scale on the burette, the volume of reagent can be measured.

As the concentration of the reagent is known, the number of moles of reagent can be calculated (since Morality = moles / volume (L)). Then, from the chemical equation involving the two substances, the number of moles present in the reactant can be found. Finally, by dividing the number of moles of reactant by its volume, the concentration is calculated.

TITRATION CURVES:

MAIN ARTICLE: TITRATION CURVE



A typical titration curve of a diprotic acid, oxalic acid, titrated with a strong base, sodium hydroxide. Each of the two equivalence points is visible.

A titration curve is a curve in the plane whose x-coordinate is the volume of titrant added since the beginning of the titration, and whose y-coordinate is the concentration of the analyte at the corresponding stage of the titration (in an acid-base titration, the y-coordinate is usually the pH of the solution at the corresponding stage).

Often it is the case that the titration curve of a titration reflects the nature of the titration quite well; for instance, it reflects the nature of all solutions involved in the titration.

In the case of acid-base titrations, titration curves reflect the strength of the corresponding acid and base. For instance, in a strong acid and strong base titration, the titration curve will be relatively smooth, although very steep for points near the equivalence point of the titration. Since in this case, small changes in the volume of the titrant result in large changes of the pH near the equivalence point, an extensive range of indicators would be appropriate (for instance litmus, phenolphthalein or bromothymol blue).

On the other hand, if one of the constituents of an acid-base titration is either a weak acid or a weak base, and the other is either a strong acid or a strong base, the titration curve is fairly irregular near the equivalence point (and the pH does not change as much due to the addition of small volumes of titrant). For instance, the titration curve for the titration between oxalic acid (a weak acid) and sodium hydroxide (a strong base) is depicted in the image above.

Here, the equivalence point occurs at a pH of about 8-10, and thus the analyte is basic at the equivalence point (more precisely, the sodium salt produced by the reaction hydrolyses in water to produce hydroxide ions). An indicator such as phenolphthalein would be appropriate for this particular titration.

The titration curve corresponding to a weak base and strong acid titration is similarly behaved. In this case, indicators such as methyl orange or bromothymol blue are regularly used.

On the other hand, titration curves corresponding to acid-base titrations in which the constituents are a weak acid and weak base are quite irregular in nature. Due to the nature of such titrations, no definite indicator may be appropriate, and thus pH meters are often used.

HISTORY AND ETYMOLOGY:

The word "titration" comes from the Latin word titulus, meaning inscription or title. The French word titre, also from this origin, means rank. Titration, by definition, is the determination of rank or concentration of a solution with respect to water with a pH of 7 (which is the pH of pure H2O under standard conditions).

The origins of volumetric analysis are in late-18th-century French chemistry. Francois Antoine Henri Descroizilles developed the first burette (which looked more like a graduated cylinder) in 1791. Joseph Louis Gay-Lussac developed an improved version of the burette that included a side arm, and coined the terms "pipette" and "burette" in an 1824 paper on the standardization of indigo solutions.

A major breakthrough in the methodology and popularization of volumetric analysis was due to Karl Friedrich Mohr, who redesigned the burette by placing a clamp and a tip at the bottom, and wrote the first textbook on the topic, Lehrbuch der chemisch-analytischen Titrirmethode (Textbook of analytical-chemical titration methods), published in 1855.

PREPARING A SAMPLE FOR TITRATION:

In a titration, both titrant and analyte are required to be in a liquid (solution) form. If the sample is not a liquid or solution, the samples must be dissolved. If the analyte is very concentrated in the sample, it might be useful to dilute the sample.

Although the vast majority of titrations are carried out in aqueous solution, other solvents such as glacial acetic acid or ethanol (in petrochemistry) are used for special purposes.

A measured amount of the sample can be given in the flask and then be dissolved or diluted. The mathematical result of the titration can be calculated directly with the measured amount. Sometimes the sample is dissolved or diluted beforehand, and a measured amount of the solution is used for titration. In this case the dissolving or diluting must be done accurately with a known coefficient because the mathematical result of the titration must be multiplied with this factor.

Many titrations require buffering to maintain a certain pH for the reaction. Therefore, buffer solutions are added to the reactant solution in the flask to maintain the pH of the solution.

Some titrations require "masking" of a certain ion. This can be necessary when two reactants in the sample would react with the titrant and only one of them must be analysed, or when the reaction would be disturbed or inhibited by this ion. In this case another solution is added to the sample, which "masks" the unwanted ion (for instance by a weak binding with it or even forming a solid insoluble substance with it).

Some redox reactions may require heating the solution with the sample and titration while the solution is still hot, in order to increase the reaction rate. For instance, the oxidation of certain oxalate solutions requires heating the solution to approximately 60 degrees in order to maintain a reasonable rate of reaction.

Milk is heated upto 85 for 15 minutes. Adding 25ml of plain yoghurt . The three tablets Vitamin c, calcium , zincovit are added to the above experiment. While plain yoghurt +100mg calcium, plain yoghurt +200mg zincovit and plain yoghurt +300mg are added and kept inside incubator. After four hours micro organisms get developed. While adding 50mg calcium and 100mg zincovit the micro organism is not developed. For every half an hour ph level is noted.

Instead of adding tablets we can add easy nutrients available in market like amla powder, pomegranate seeds powder and tulsi seeds powder are added.

While adding 300mg amla powder, 200mg pomegranate seeds powder and 50mg tulsi seeds powder the growth of micro organisms is nill.

pH level is noted. 0.1g of kesari colour and lemon yellow is added to 5ml of water. From that 2ml is added to plain yoghurt. 2ml of Chocolate flavor and strawberry flavor is added to the plain yoghurt and ph level is noted. In plain yoghurt amla 300mg and 2ml of lemon yellow, kesari colour is added and sugar is not added. In plain yoghurt amla 300mg and 2ml of lemon yellow, kesari colour is added and 10g of sugar is added.ph level is noted. In plain yoghurt tulsi 50mg and 2ml of lemon yellow, kesari colour is added and sugar is not added. In plain yoghurt tulsi 50mg and 2ml of lemon yellow, kesari colour is added and 10g of sugar is added ph level is noted. In plain yoghurt pomegranate 200mg and 2ml of lemon yellow, kesari Colour is added and sugar is not added. In plain yoghurt pomegranate 200mg and 2ml of lemon yellow, kesari colour is added and 10mg of sugar is added.ph level is noted. Amla powder, tulsi seeds powder and pomegranate seeds powder are added. PH level is noted for every half an hour and for every one hour gram staining is done.

GRAM STAINING:

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The Gram stain is almost always the first step in the identification of a bacterial organism.

While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram variable and Gram in determinant groups as well.

This method is named after its inventor, the Danish scientist Hans Christian Gram (1853–1938), who developed the technique in 1882 and published it in 1884 to discriminate between two types of bacteria with similar clinical symptoms: Streptococcus pneumoniae (also known as the pneumococcus) and Klebsiella pneumoniae bacteria.

The word Gram is always spelled with a capital, referring to the name of the inventor of the Gram staining.

CONTENTS:

USES:

Gram staining is used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls. Gram staining is not used to classify archaea, since these microorganisms yield widely varying responses that do not follow their phylogenetic groups.

RESEARCH:

Gram staining is a bacteriological laboratory technique. The technique is used as a tool for the differentiation of Gram-positive and Gram-negative bacteria, as a first step to determine the identity of a particular bacterial sample.

The Gram stain is not an infallible tool for diagnosis, identification, or phylogeny, however. It is of extremely limited use in environmental microbiology, and has been largely superseded by molecular techniques even in the medical microbiology lab. Some organisms are Gram-variable (that means, they may stain either negative or positive);

Some organisms are not susceptible to either stain used by the Gram technique. In a modern environmental or molecular microbiology lab, most identification is done using genetic sequences and other molecular techniques, which are far more specific and information-rich than differential staining.

MEDICAL:

Gram stains are performed on body fluid or biopsy when infection is suspected. It yields results much more quickly than culture, and is especially important when infection would make an important difference in the patient's treatment and prognosis; examples are cerebrospinal fluid for meningitis and synovial fluid for septic arthritis.

Being able to identify bacteria by stain result/ photo, or negative stain and history is typical Medical Boards question. Special Stains and Growth Media, such as India Ink for Cryptococcus and actual appearance of hemolytic streps on growth plate are seen on test questions.

STAINING MECHANISM:

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink.

Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolonization with alcohol or acetone, and counterstaining with safranin.

Basic fuchsin is sometimes substituted for safranin since it will more intensely stain anaerobic bacteria but it is much less commonly employed as a counter stain. Crystal violet (CV) dissociates in aqueous solutions into CV+ and chloride (Cl –) ions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV+ ion interacts with negatively charged components of bacterial cells and stains the cells purple.

Iodine (I – or I3 –) interacts with CV+ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color the cell.
When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane.

A gram-negative cell will lose its outer membrane and the lipo polysaccharide layer is left exposed. The CV–I complexes are washed from the gram-negative cell along with the outer membrane.

In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolonization step is critical and must be timed correctly; the crystal violet stain will be removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolonization, the gram-positive cell remains purple and the gram-negative cell loses its purple color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized gram-negative bacteria a pink or red color.
Some bacteria, after staining with the Gram stain, yield a Gram-variable pattern: a mix of pink and purple cells are seen.

The genera Actinomyces, Arthobacter, Corynebacterium, Mycobacterium, and Propionibacterium have cell walls particularly sensitive to breakage during cell division, resulting in Gram-negative staining of these Gram-positive cells.

In cultures of Bacillus, Butyrivibrio, and Clostridium a decrease in peptidoglycan thickness during growth coincides with an increase in the number of cells that stain Gram-negative. In addition, in all bacteria stained using the Gram stain, the age of the culture may influence the results of the stain.

EXAMPLES:

GRAM-NEGATIVE BACTERIA:

Main article: Gram-negative bacteria

The proteobacteria are a major group of Gram-negative bacteria. Other notable groups of Gram-negative bacteria include the cyan bacteria, spirochetes, green sulfur and green non-sulfur bacteria.
These also include many medically relevant Gram-negative cocci, bacilli and many bacteria associated with nosocomial infections.

GRAM-POSITIVE BACTERIA:

Main article: Gram-positive bacteria

In the original bacterial phyla, the Gram-positive forms made up the phylum Formicates, a name now used for the largest group. It includes many well-known genera such as Bacillus, Listeria, Staphylococcus, Streptococcus, Enterococcus, and Clostridium.

It has also been expanded to include the Mollicutes; bacteria like Mycoplasma that lack cell walls and so cannot be stained by Gram, but are derived from such forms.

TOTAL PROTEIN ESTIMATION: (FOLIN-LOWRY'S METHOD)

OBJECTIVE:

To determine the concentration of proteins by Lowry's method.

REAGENTS REQUIRED:

1. BSA stock solution (1mg/ml),

2. Analytical reagents:

(a) 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solutions (0.4 gm in 100 ml Distilled water.)

(b) 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium potassium tartarate

Solution. Prepare analytical reagents by mixing 2 ml of (b) with 100 ml of (a)

3. Folin - Ciocalteau reagent solution (1N) Dilute commercial reagent (2N) with an equal volume of
Water on the day of use (2 ml of commercial reagent + 2 ml distilled water)

PRINCIPLE:

The phenolic group of tyrosine and tryptophan residues (amino acid) in a protein will produce a blue purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteau Reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Most proteins estimation techniques use Bovine Serum Albumin (BSA) universally as a standard protein, because of its low cost, high purity and ready availability. The method is sensitive down to about 10 µg/ml and is probably the most widely used protein assay despite its being only a relative method, subject to interference from Trisbuffer, EDTA, nonionic and cationic detergents, carbohydrate, lipids and some salts. The incubation time is very critical for a reproducible assay. The reaction is also dependent on pH and a working range of pH 9 to10.5 is essential.BL 301-1.

PROCEDURE:

1.Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube as given in the table. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05 to 1 mg/ ml.

2.From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well.

3.This solution is incubated at room temperature for 10 mins.

4.Then add 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) to each tube and incubate for30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660nm.

5.Plot the absorbance against protein concentration to get a standard calibration curve.

6.Check the absorbance of unknown sample and determine the concentration of the unknown sample using the standard curve plotted above.

7.BSA Water Sample conc. Sample vol Alk. CuSO4 Lowry reagent O.D.

Write short notes on the following points in the report:

1. Beer-Lamberts law

2. Compare Folin-Lowry method with other methods of protein estimation.

In plain yoghurt amla seeds powder, tulsi seeds powder, colours and flavours are added and protein estimation is done every one hour.

In plain yoghurt amla seeds powder, tulsi seeds powder, colours and flavours are added. These are refrigerated for four hours and then protein estimation is carried out.

SWEETENED YOGHURT PRODUCTION:

To offset its natural sourness, yoghurt can be sold sweetened, flavored, or in containers with fruit or fruit jam on the bottom. If the fruit has been stirred into the yoghurt before purchase, it is commonly referred to as Swiss-style. Most yoghurt in North America have added pectin, found naturally in fruit, and/or gelatin to artificially create thickness and creaminess at lower cost.

Sweeteners such as cane sugar are often present in large amounts in commercial yoghurt.

FLAVORED YOGURT PRODUCTION:

This type of adulterated product is also marketed under the name Swiss-style, although it is unrelated to the way yoghurt is eaten in Switzerland. Some specialty yoghurts, often called "cream line", have a layer of fermented fat at the top. Fruit jam is used instead of raw fruit pieces in fruit yoghurts to allow storage for weeks.

In the USA, sweetened, flavored yoghurt is the most popular type, typically sold in single-serving plastic cups. Typical flavors are vanilla, honey, or fruit such as strawberry, blueberry, blackberry, or peach.

FORTIFIED YOGHURT PRODUCTION:

Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44^0c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42^0c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic. In the present study yoghurt preparation will be standardized and fortified yoghurt will be prepared. Amla powder and Pomegranate seed powder And Tulsi seeds powder were added to the yogurt and it was found there was no microbial growth. Tests will be carried out for these samples to find the composition of the nutrients like Vitamin C, Iron, Calcium. In addition flavor, colour other nutrients will be added and the properties of the yoghurt will be studied.

The plain yoghurt which is mixed with milk was prepared and taken in a vessel. The mixture is cooled down to 42-44°C using cold water bath. Inorder to fortify tablets are added that are rich in vitaminC, calcium and zincovit. The proportional weights of each components is as follows: Vitamins of weight 100mg, Calcium of weight 100mg, Zincovit of weight 100mg, 200mg, 300mg. Then the milk is kept inside the incubator maintained at 42°C for one hour. Once the milk kept inside the incubator the milk turns to yoghurt. After one hour the yoghurt is taken for measuring the pH value. Now a check for the growth of micro-organisms is carried out. During this check it is seen that organisms has grown in the yoghurt containing 100 mg vitaminC, 200mg zincovit and 300mg zincovit. Thus to prevent the growth of organisms we are taking yoghurt containing 50 mg calcium. It is seen that no organisms have grown in this sample. Thus fresh fortified yoghurt has been prepared.

Since addition of tablets increase the cost of the product so inorder to reduce the cost we are utilizing commonly available fruits and herbal plants that are rich in nutrients.

Inorder to add nutrients to the plain yoghurt amla powder, pomegranate seed power, tulsi seed powder are added. Each 300 mg of each is taken and added to the plain yoghurt and tested. No growth of micro-organisms were observed. Thus the plain yoghurt has been converted to fortified yoghurt.

In the above fortified yoghurt food colour is added and then subjected for pH testing. In the same yoghurt flavours are added including chocolate and strawberry. Again the pH is noted. Once the pH is noted the youghurt is subjected to gram staining. After coducting gram staining the pH level is noted.

From the fortified yoghurt we are taking 0.1 mL is taken and protein estimation is carried out. From the values the optical density is determined.

The time taken from the heating of the milk to its cooling and additin of plain yoghurt the time taken is 45 minutes.

RESULTS AND DISCUSSION

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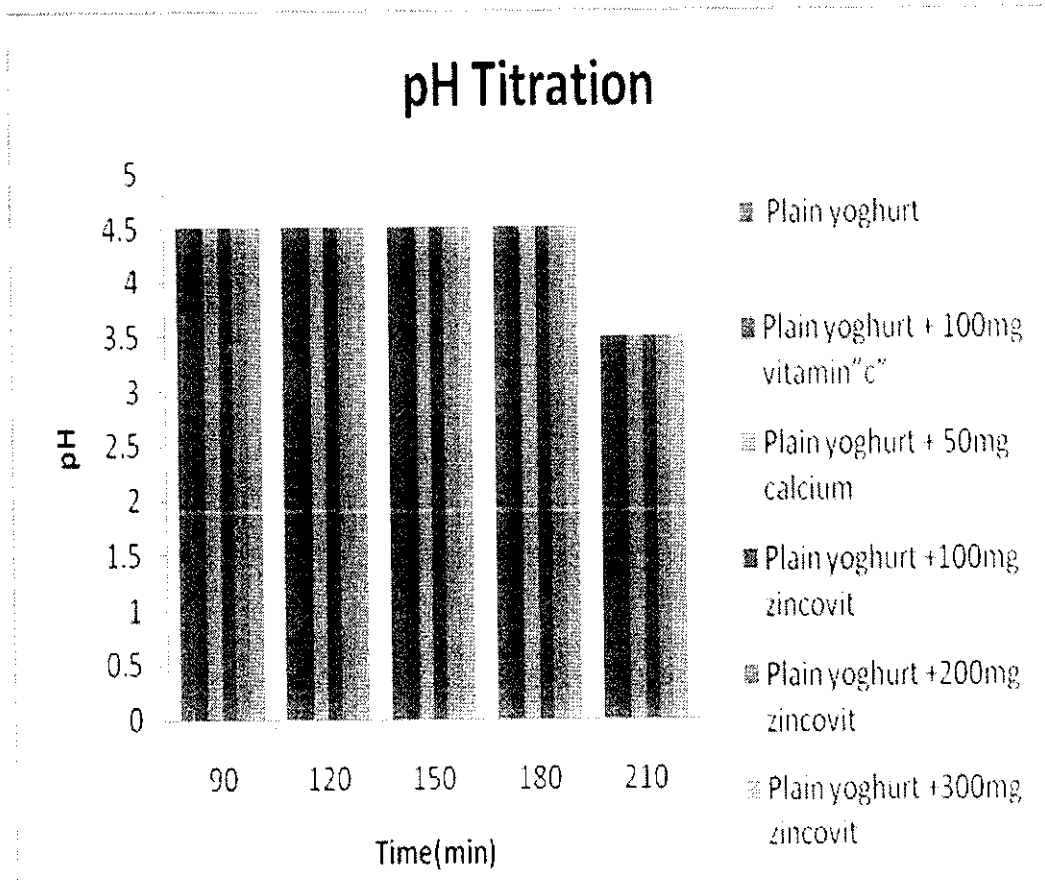
TABLE: 0.1 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt		4.5	4.5	4.5	4.5	4.5
2	Plain yoghurt + 100mg vitamin"c"		4.5	4.5	4.5	4.0	3.5
3	Plain yoghurt + 100mg calcium	PH measurment	4.5	4.5	4.5	4.0	3.5
4	Plain yoghurt + 100mg zincovit		4.5	4.5	4.5	4.0	3.5
5	Plain yoghurt + 200mg zincovit		4.5	4.5	4.5	4.0	3.5
6	Plain yoghurt + 300mg zincovit		4.5	4.5	4.5	4.0	3.5

Milk is heated upto 85 for 15 minutes. Adding 25ml of plain yoghurt. The three tablets Vitamin c, calcium, zincovit are added to the above experiment. While plain yoghurt +100mg calcium, plain yoghurt +200mg zincovit and plain yoghurt +300mg are added and kept inside incubator after four hours micro organisms get developed.

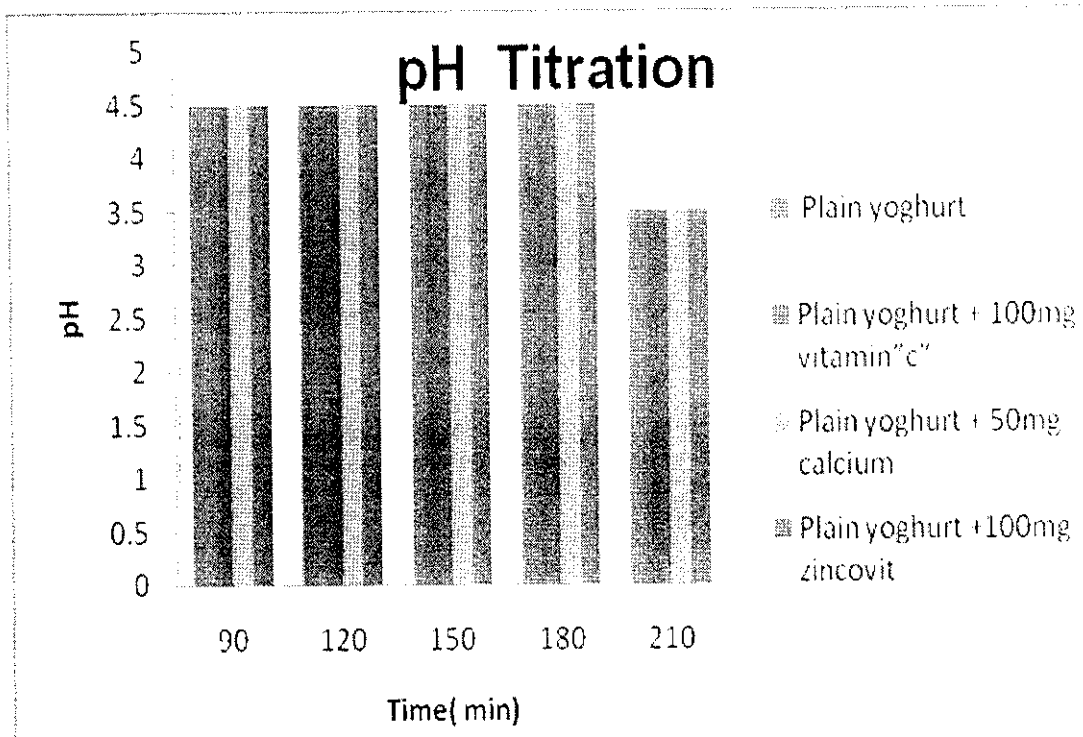


GRAPH: 0.1 pH TITRATION:

While adding 50mg calcium and 100mg zincovit the micro organism is not developed. For every half an hour ph level is noted.

TABLE: 0.2 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt		4.5	4.5	4.0	3.5	3.5
2	Plain yoghurt + 100mg vitamin"c"		4.5	4.5	4.0	3.5	3.5
3	Plain yoghurt + 50mg calcium	PH measurment	4.5	4.5	4.0	3.5	3.5
4	Plain yoghurt + 100mg zincovit		4.5	4.5	4.0	3.5	3.5

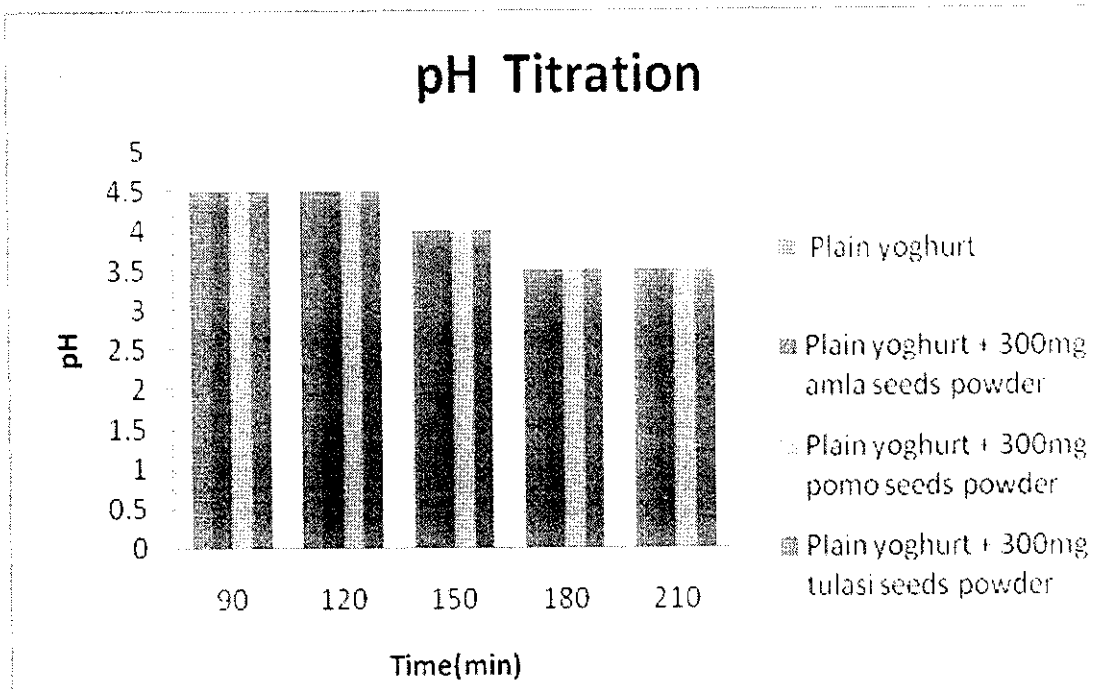


GRAPH:0.2 ,H TITRATION:

Instead of adding tablets we can add easy nutrients available in market like amla powder, pomegranate seeds powder and tulsi seeds powder are added. While adding 300mg amla powder, 200mg pomegranate seeds powder and 50mg tulsi seeds powder the growth of micro organisms is nill.ph level is noted.

TABLE:0.3 ;H TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt		4.5	4.5	4.5	4.0	3.5
2	Plain yoghurt + 300mg amla seeds powder	PH measurment	4.5	4.5	4.5	4.0	3.5
3	Plain yoghurt + 300mg pomogranate seeds powder		4.5	4.5	4.5	4.0	3.5
4	Plain yoghurt + 300mg tulasi seeds powder		4.5	4.5	4.5	4.0	3.5

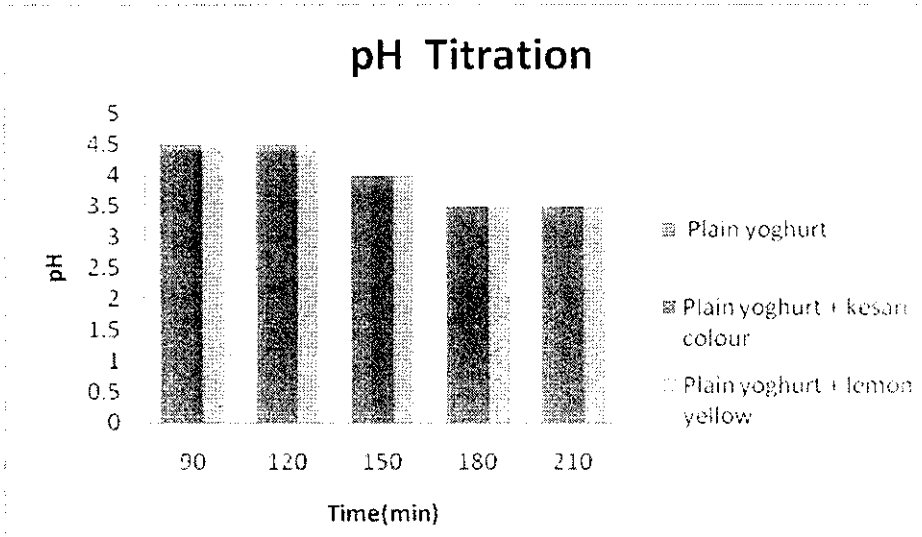


GRAPH:0.3 PH TITRATION

0.1g of kesari colour and lemon yellow is added to 5ml of water. From that 2ml is added to plain yoghurt.

TABLE:0.4 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt		4.0	4.0	3.5	3.5	3.0
2	Plain yoghurt + kesari colour	PH measurment	4.0	4.0	3.5	3.5	3.0
3	Plain yoghurt + lemon yellow		4.0	4.0	3.5	3.5	3.0

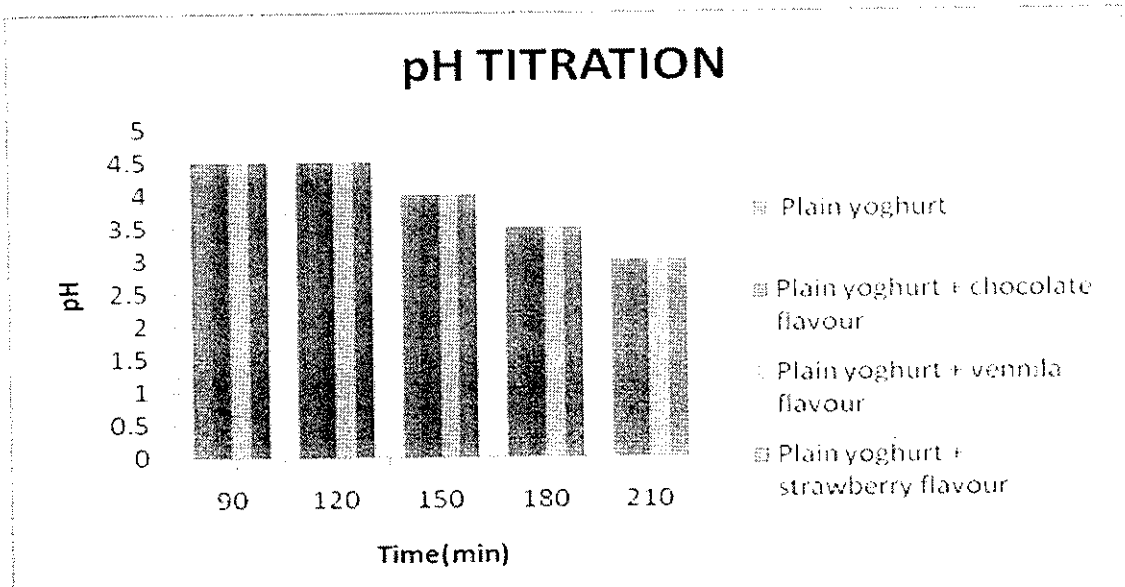


GRAPH:0.4 pH TITRATION

2ml of Chocolate flavor and strawberry flavor is added to the plain yoghurt and ph level is noted.

TABLE:0.5 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt		4.5	4.5	4.0	3.5	3.0
2	Plain yoghurt + chocolate flavour	PH measurement	4.5	4.5	4.0	3.5	3.0
3	Plain yoghurt + vennila flavour		4.5	4.5	4.0	3.5	3.0
4	Plain yoghurt + strawberry flavour		4.5	4.5	4.0	3.5	3.0

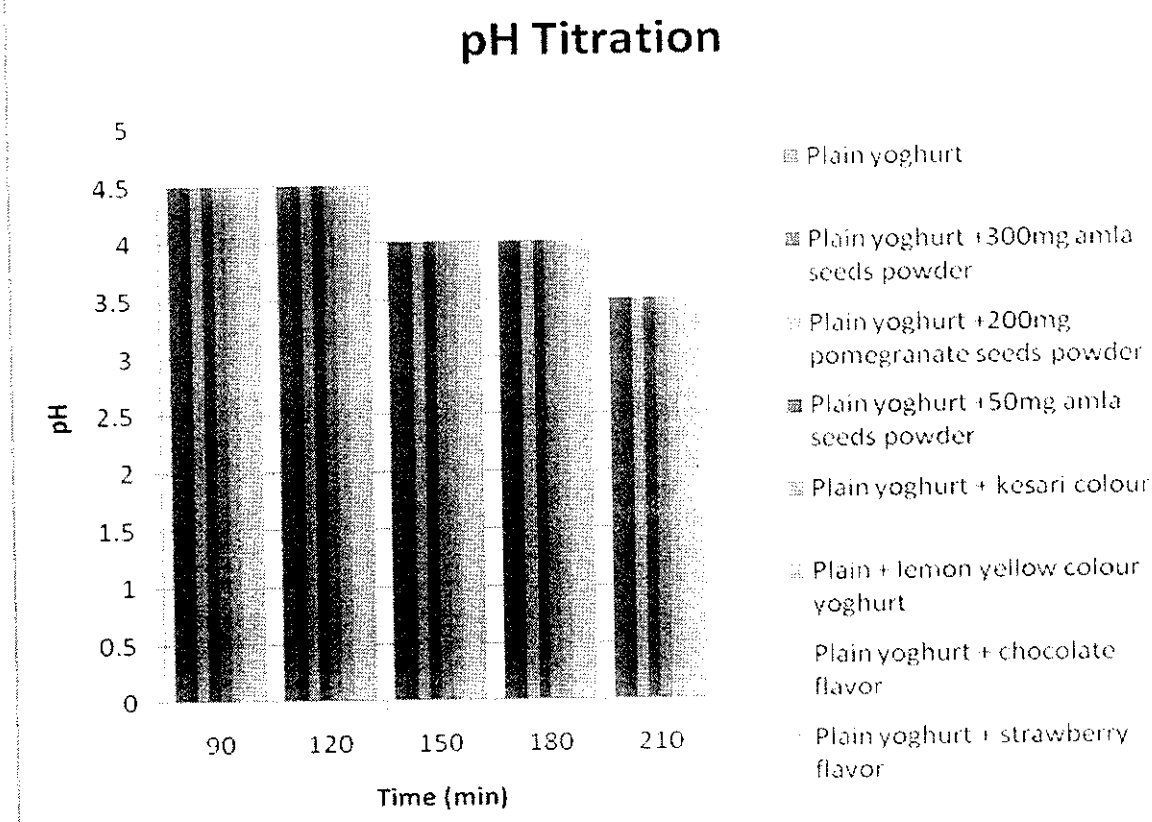


GRAPH:0. H TITRATION:

In plain yoghurt amla 300mg and 2ml of lemon yellow ,kesari colour is added and sugar is not added. In plain yoghurt amla 300mg and 2ml of lemon yellow ,kesari colour is added and 10g of sugar is added.ph level is noted.

TABLE:0.6 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt		4.5	4.5	4.0	4.0	3.5
2	Plain yoghurt +300mg amla seeds powder		4.5	4.5	4.0	4.0	3.5
3	Plain yoghurt +200mg pomegranate seeds powder		4.5	4.5	4.0	4.0	3.5
4	Plain yoghurt +50mg amla seeds powder	PH measurement	4.5	4.5	4.0	4.0	3.5
5	Plain yoghurt + kesari colour		4.5	4.5	4.0	4.0	3.5
6	Plain + lemon yellow colour yoghurt		4.5	4.5	4.0	4.0	3.5
7	Plain yoghurt + chocolate flavor		4.5	4.5	4.0	4.0	3.5
8	Plain yoghurt + strawberry flavor		4.5	4.5	4.0	4.0	3.5

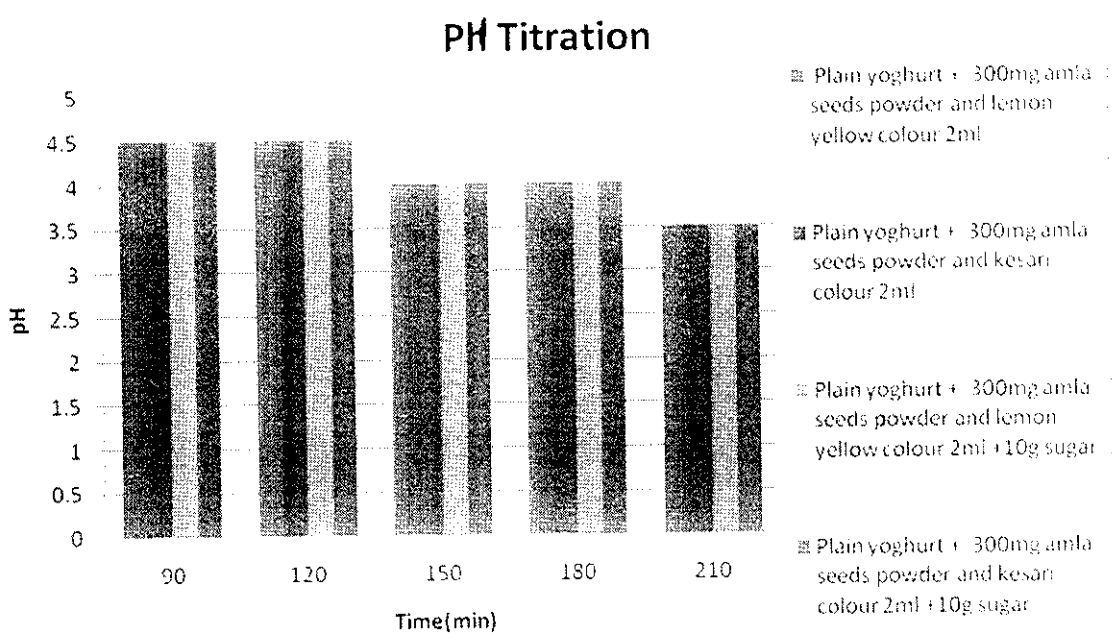


GRAPH:0.6 PH TITRATION:

In plain yoghurt tulsi 50mg and 2ml of lemon yellow ,kesari colour is added and sugar is not added. In plain yoghurt tulsi 50mg and 2ml of lemon yellow ,kesari colour is added and 10g of sugar is added.ph level is noted.

TABLE:0.7 pH TITRATION:

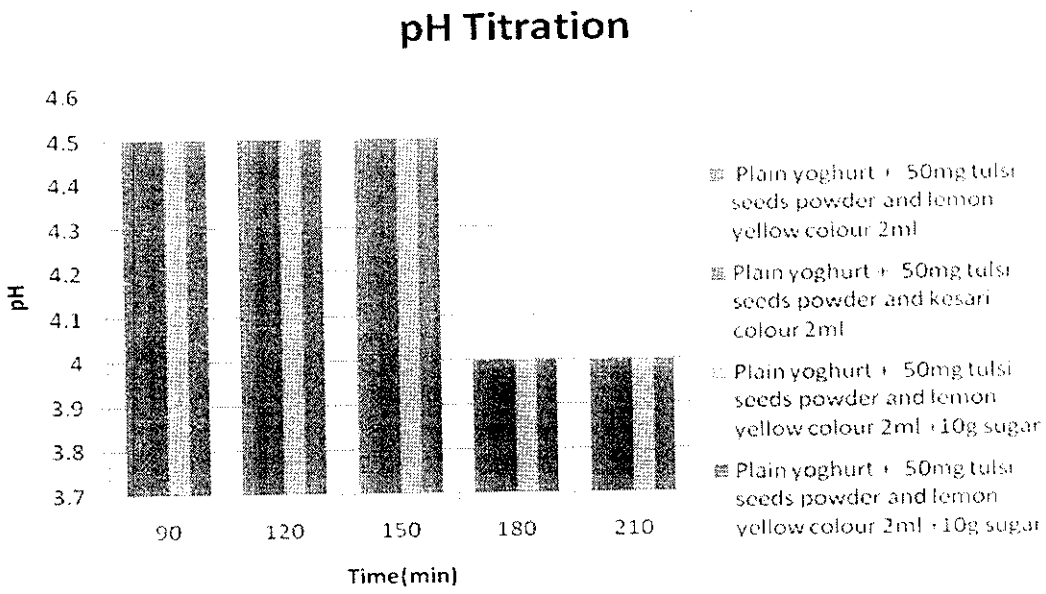
			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt + 300mg amla seeds powder and lemon yellow colour 2ml		4.5	4.5	4.0	4.0	3.5
2	Plain yoghurt + 300mg amla seeds powder and kesari colour 2ml	PH measurement	4.5	4.5	4.0	4.0	3.5
3	Plain yoghurt + 300mg amla seeds powder and lemon yellow colour 2ml +10g sugar		4.5	4.5	4.0	4.0	3.5
4	Plain yoghurt + 300mg amla seeds powder and kesari colour 2ml +10g sugar		4.5	4.5	4.0	4.0	3.5



GRAPH:0.7 pH TITRATION

TABLE:0.8 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt + 50mg tulsi seeds powder and lemon yellow colour 2ml		4.5	4.5	4.5	4.0	4.0
2	Plain yoghurt + 50mg tulsi seeds powder and kesari colour 2ml		4.5	4.5	4.5	4.0	4.0
3	Plain yoghurt + 50mg tulsi seeds powder and lemon yellow colour 2ml +10g sugar	PH measurment	4.5	4.5	4.5	4.0	4.0
4	Plain yoghurt + 50mg tulsi seeds powder and kesari colour 2ml +10g sugar		4.5	4.5	4.5	4.0	4.0

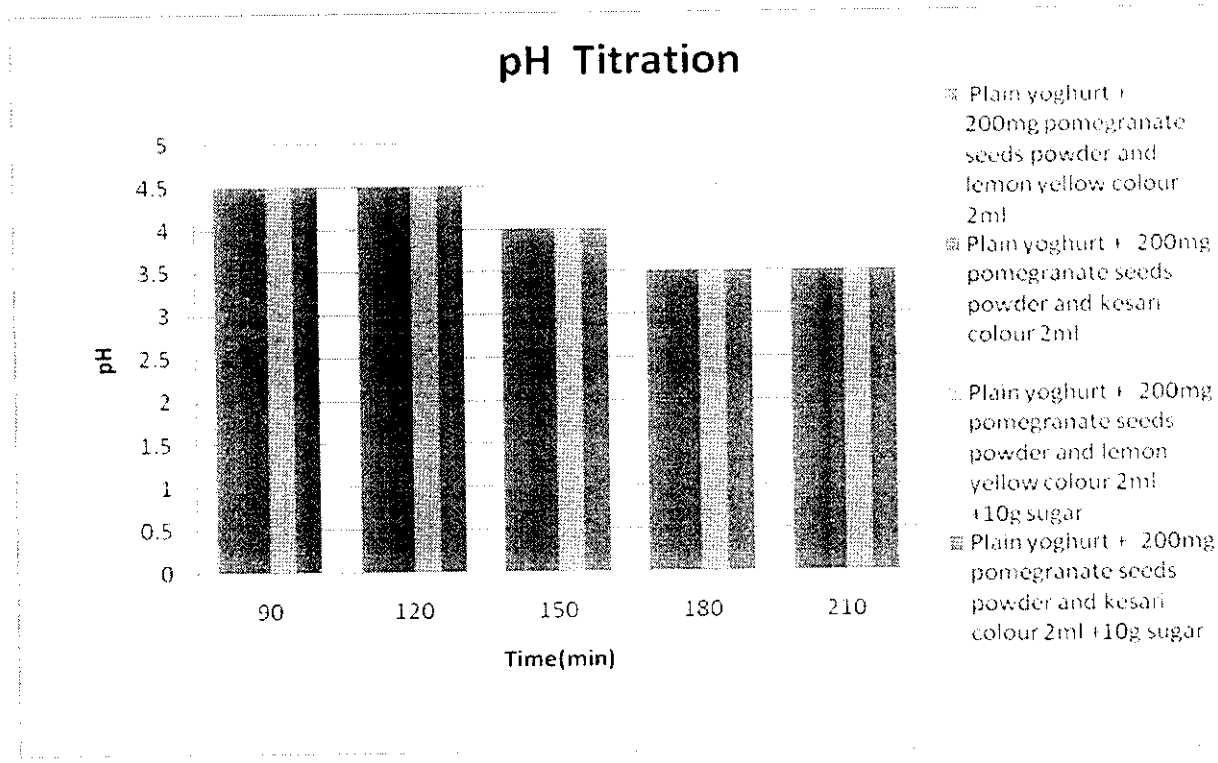


GRAPH:0.8 pH TITRATION:

TABLE:0.9 pH TITRATION:

			Time				
s.no	Reagents		90	120	150	180	210
1	Plain yoghurt + 200mg pomegranate seeds powder and lemon yellow colour 2ml	PH measurement	4.5	4.5	4.0	3.5	3.5
2	Plain yoghurt + 200mg pomegranate seeds powder and kesari colour 2ml		4.5	4.5	4.0	3.5	3.5
3	Plain yoghurt + 200mg pomegranate seeds powder and lemon yellow colour 2ml +10g sugar		4.5	4.5	4.0	3.5	3.5
4	Plain yoghurt + 200mg pomegranate seeds powder and kesari colour 2ml +10g sugar		4.5	4.5	4.0	3.5	3.5

Amla powder, tulsi seeds powder and pomegranate seeds powder are Added.ph level is noted for every half an hour and for every one hour gram Staining is done.



GRAPH:0.9 pH TITRATION:

TABLE: 1.0 PROTEIN ESTIMATION:

S.No	REAGENTS	B	S1	S2	S3	S4	S5	T1	T2
1	Volume of working solution (ml)	–	0.2	0.4	0.6	0.8	1.0	–	–
2	Conc. Of working std. solution	–	20	40	60	80	100	–	–
3	Volume of unknown solution(ml)	–	–	–	–	–	–	0.4	0.4
4	Volume of distilled water(ml)	1.0	0.8	0.6	0.4	0.2	–	0.6	0.6
5	Volume of alkalin copper reagent (ml)	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
	Keep at room temperature for 10 minutes								
6	Volume of folin's reagent(ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Keep at room temperature for 20 minutes								
7	Optical density	0.01	0.01	0.02	0.03	0.04	0.05	0.06	0.06

In plain yoghurt amla seeds powder, tulsi seeds powder , colours and flavours are added and protein estimation is done every one hour. In plain yoghurt amla seeds powder , tulsi seeds powder , colours and Flavours are added . these are refrigerated for four hours and then protein Estimation is carried out.

TABLE: 1.1 PROTEIN ESTIMATION :

B	S1	S2	S3	S4	S5	A1+ (LY)	A2+ (K)	A3+ (LY)	A4+ (K)	T1+ (LY)	T2+ (K)	T3+ (LY)	T4+ (K)
–	0.080	0.125	0.047	0.113	0.107	1.18	1.27	1.35	1.47	1.48	1.49	1.55	1.58
–	0.080	0.125	0.047	0.113	0.107	1.16	1.28	1.37	1.39	1.44	1.52	1.63	1.67
–	0.080	0.125	0.047	0.113	0.107	1.19	1.22	1.33	1.37	1.40	1.44	1.49	1.52
–	0.080	0.125	0.047	0.113	0.107	1.21	1.27	1.29	1.33	1.38	1.40	1.42	1.44

TABLE: 1.2 PROTEIN ESTIMATION :

B	S1	S2	S3	S4	S5	A1+ (LY)	A2+ (K)	A3+ (LY)	A4+ (K)	T1+ (LY)	T2+ (K)	T3+ (LY)	T4+ (K)
–	0.052	0.069	0.077	0.082	0.089	1.08	1.23	1.27	1.34	1.40	1.45	1.49	1.53
–	0.052	0.069	0.077	0.082	0.089	1.05	1.09	1.25	1.28	1.35	1.44	1.59	1.67
–	0.052	0.069	0.077	0.082	0.089	1.04	1.22	1.27	1.30	1.34	1.39	1.40	1.48
–	0.052	0.069	0.077	0.082	0.089	1.09	1.24	1.28	1.31	1.35	1.39	1.45	1.53

CONCLUSION

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Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-44^0c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 42^0c for 8hrs.The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic.

In the present study yoghurt preparation will be standardized and fortified yoghurt will be prepared. Amla powder and Pomegranate seed powder were added to the yogurt and it was found there was no microbial growth. Tests will be carried out for these samples to find the composition of the nutrients like Vitamin C, Iron, and Calcium. Tulsi seed powder, Alovera gel and Spinach powder will be added to the Yogurt and the composition of the nutrients in this Yogurt will be determined. In addition flavor, colour other nutrients will be added and the properties of the yoghurt will be studied.

The plain yoghurt which is mixed with milk was prepared and taken in a vessel. The mixture is cooled down to 42-44°C using cold water bath. Inorder to fortify tablets are added that are rich in vitaminC, calcium and zincovit. The proportional weights of each components is as follows: Vitamins of weight 100mg, Calcium of weight 100mg, Zincovit of weight 100mg, 200mg, 300mg. Then the milk is kept inside the incubator maintained at 42°C for one hour. Once the milk kept inside the incubator the milk turns to yoghurt. After one hour the yoghurt is taken for measuring the pH value. Now a check for the growth of micro-organisms is carried out. During this check it is seen that organisms has grown in the yoghurt containing 100 mg vitaminC, 200mg zincovit and 300mg zincovit. Thus to prevent the growth of organisms we are taking yoghurt containing 50 mg calcium. It is seen that no organisms have grown in this sample. Thus fresh fortified yoghurt has been prepared. Since addition of tablets increase the cost of the product so inorder to reduce the cost we are utilizing commonly available fruits and herbal plants that are rich in nutrients.

In order to add nutrients to the plain yoghurt amla powder, pomegranate seed power, tulsi seed powder are added. Each 300 mg of each is taken and added to the plain yoghurt and tested. No growth of micro-organisms were observed. Thus the plain yoghurt has been converted to fortified yoghurt.

In the above fortified yoghurt food colour is added and then subjected for pH testing. In the same yoghurt flavours are added including chocolate and strawberry. Again the pH is noted. Once the pH is noted the youghurt is subjected to gram staining. After coducting gram staining the pH level is noted.

From the fortified yoghurt we are taking 0.1 mL is taken and protein estimation is carried out. From the values the optical density is determined.

The time taken from the heating of the milk to its cooling and additin of plain yoghurt the time taken is 45 minutes.

FUTURE PERSPECTIVES

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In the above fortified yoghurt food colour is added and then subjected for pH testing. In the same yoghurt flavours are added including chocolate and strawberry. Again the pH is noted. Once the pH is noted the youghurt is subjected to gram staining. After coducting gram staining the pH level is noted.

From the fortified yoghurt we are taking 0.1 mL is taken and protein estimation is carried out. From the values the optical density is determined.

The time taken from the heating of the milk to its cooling and additin of plain yoghurt the time taken is 45 minutes.

Thus we can also include more herbal plants and fruits that are rich in other nutrients we can classify based on the age groups. This project can be done on large scale and then marketing can be done.

APPENDICES

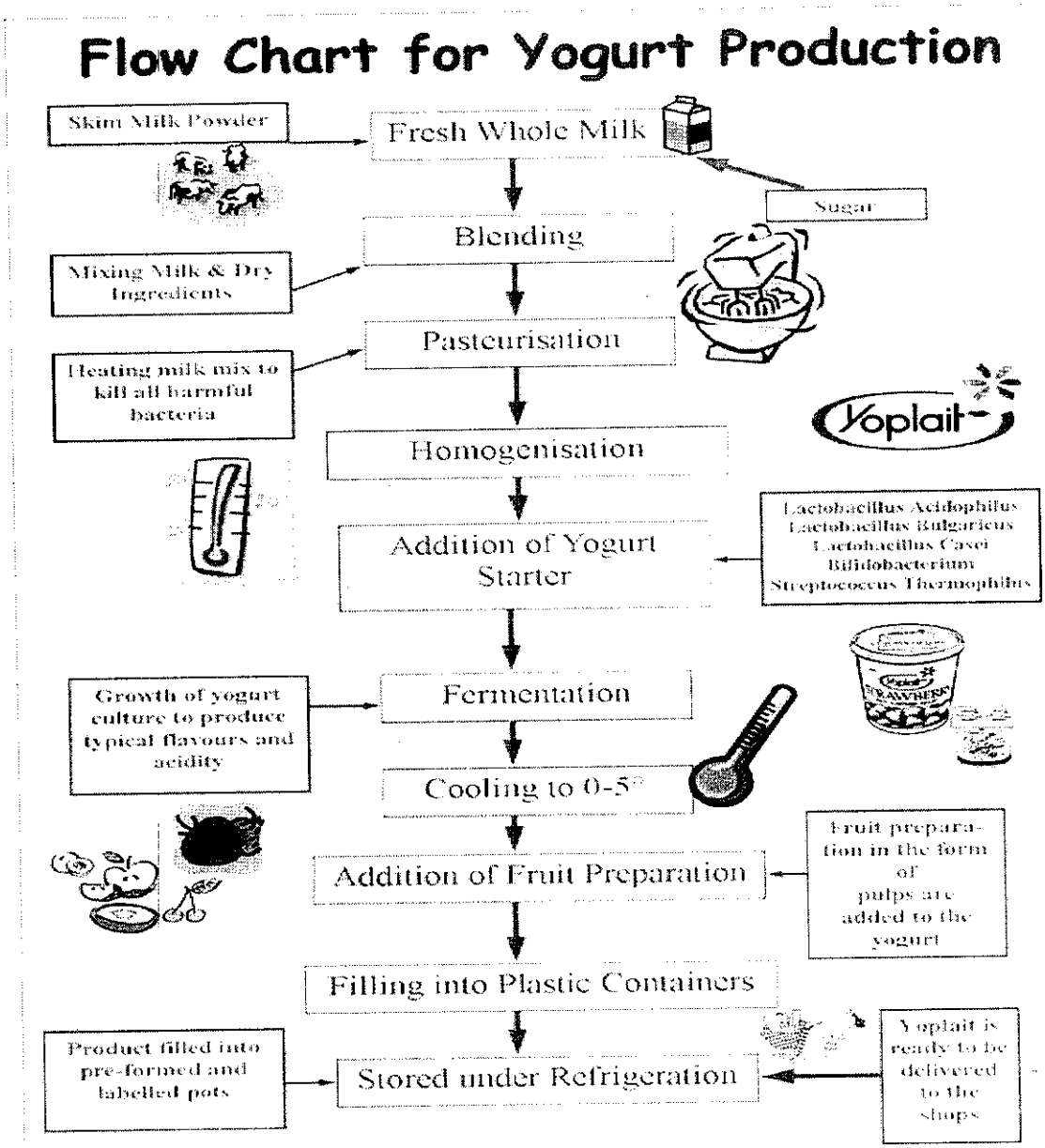

FIGURE:1.1: YOGHURT PRODUCTION:

PLAIN YOGHURT PRODUCTION:

Heat 500ml of milk in a vessel slowly at 85^0c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation.Cool milk in a cold water bath to 42-44^0c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod.Cover the container to minimize the possibility of contamination incubate at 42^0c for 8hrs.

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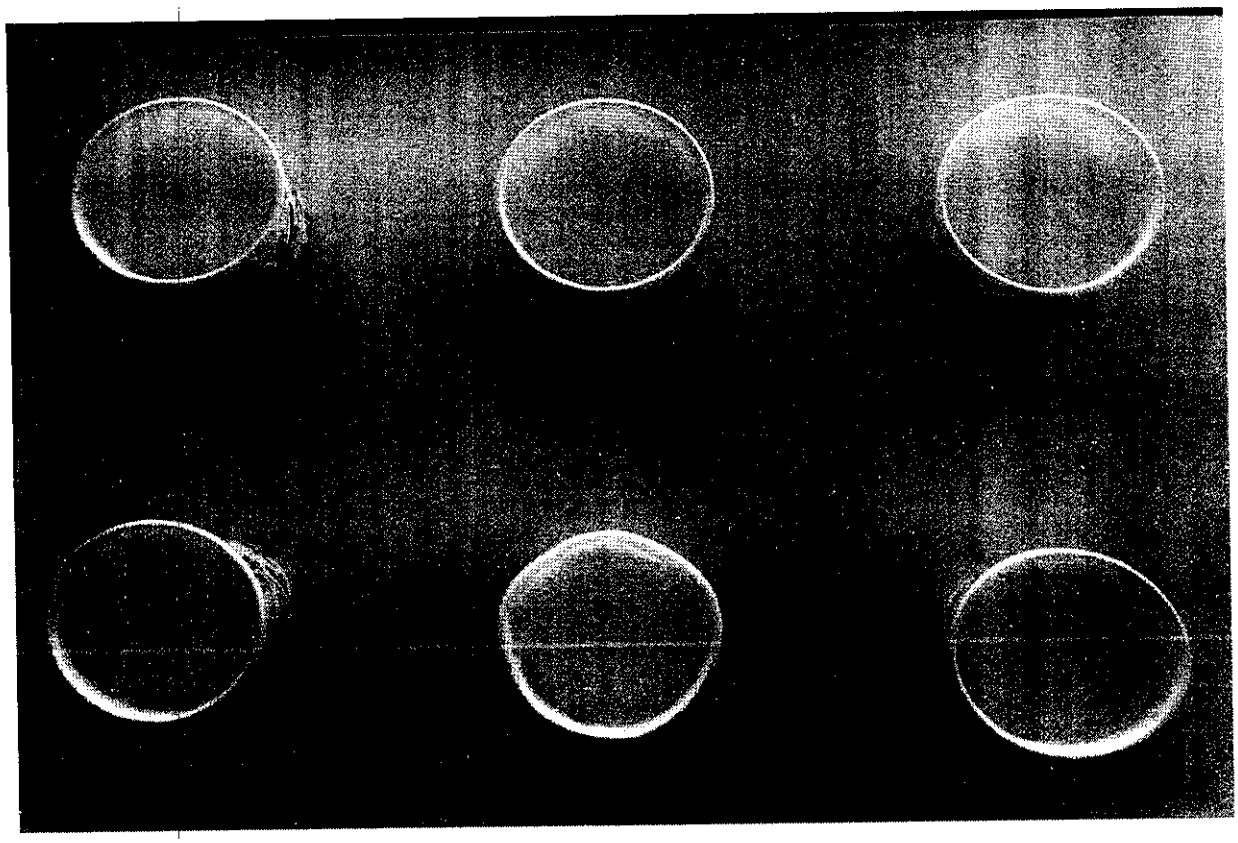
FIGURE:1.2:

PLAIN YOGHURT:



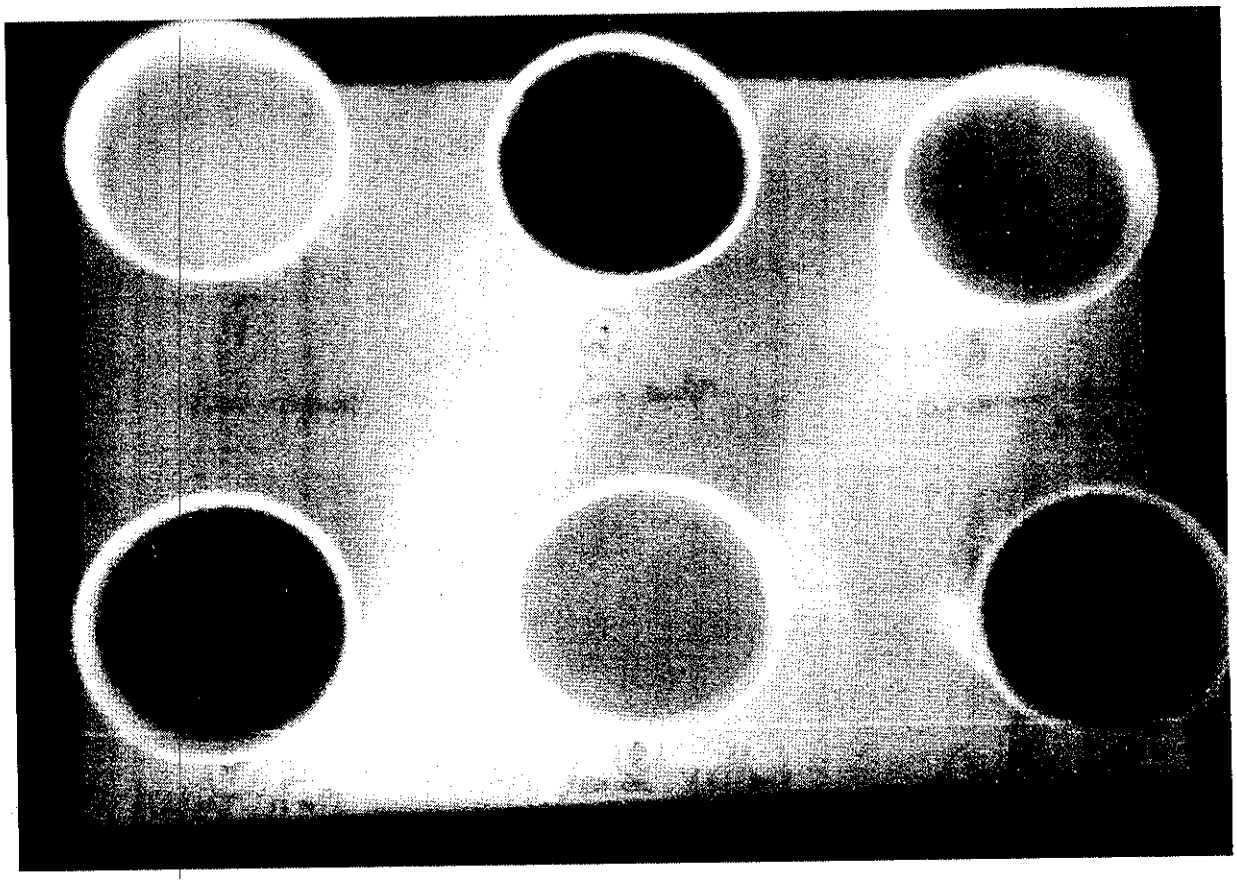
FORTIFIED YOGHURT:

Heat 500ml of milk in a vessel slowly at 85°c and maintain at the temperature for 2min.This step kills undesirable contaminant microorganisms. It also denature inhibitory enzyme that retard the subsequent yoghurt fermentation. Cool milk in a cold water bath to 42-440c. This cooling process should take about 15min.Add 25ml of plain yoghurt from stores to the cooled milk and mix a glass rod. Cover the container to minimize the possibility of contamination incubate at 420c for 8hrs.

The fresh made yoghurt is ready for consumption when it is set, however you may want to refrigerate it first if you are not accustomed to warm yoghurt refrigeration also stops. The growth of lactic acid culture, which is thermophilic. In the present study yoghurt preparation will be standardized and fortified yoghurt will be prepared. Amla powder and Pomegranate seed powder And Tulsi seeds powder were added to the yogurt and it was found there was no microbial growth. Tests will be carried out for these samples to find the composition of the nutrients like Vitamin C, Iron, Calcium. In addition flavor, colour other nutrients will be added and the properties of the yoghurt will be studied.

FIGURE:1.3:

FORTIFIED YOGHURT:



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