

PREPARATION AND EVALUATION OF ALMOND MILK YOGHURT

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Technology degree in Biotechnology

by

DEEPAN T (37230012)

SANJITH S (37230076)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

**SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade “A” by NAAC
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600 119
APRIL– 2021**



SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with "A" grade by NAAC
Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai – 600 119
www.sathyabama.ac.in



DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Deepan T(37230012) who carried out the project entitled "Preparation and evaluation of almond milk yoghurt" under our supervision from January 2021 to March 2021

Internal Guide

Dr. SUDHA S

External Guide

Dr.ARUMUGAM P

Head of the Department

Dr. V RAMESH KUMAR

Submitted for Viva voce Examination held on _____

Internal Examiner

External Examiner

DECLARATION

I Deepan T (37230012) hereby declare that the Project Report entitled “**Preparation and evaluation of almond milk yoghurt**” done by me under the guidance of Dr.**SSudha** and Dr. **P Arumugam** at Armats biotek training and research institute is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE:

PLACE: Chennai

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr. V Ramesh Kumar, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr. S Sudha and Dr. P Arumugam for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work. I also thank them for taking me under their wing, for mentoring me and providing access for utilization of resources and facilities at the Armats biotek training and research institute despite their academic and professional commitments.

I express my sincere thanks to Ms. Stefi S M for providing feedbacks and suggestions that helped in troubleshooting of problems when needed.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

Yoghurt is a semisolid dairy product which is made from fermentation of milk by specific organisms which are viable, active and present in the product namely *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. Mainly yoghurts are made from cow milk, goat milk or sheep milk. But people with lactose intolerance and those who are allergen to animal resources couldn't consume. To sort out this problem, researches started to produce yoghurt from plant based milk. In this study yoghurt is made from almond milk and the microbial analysis is evaluated. Further the physico-chemical properties like fat content, sugar content are tested.

Key words: Yoghurt, almond milk, fermentation, physico-chemical properties

LIST OF CONTENTS

CHAPTER NO	TITLE	PAGE
	LIST OF FIGURES	vi
1		
	LIST OF TABLES	vi
	INTRODUCTION	1
	1.1 YOGHURT	
	1.2 NUTRITIONAL VALUE IN YOGHURT	2
	1.3 TYPES OF YOGHURTS	2
	1.3.1 Traditional yoghurt	2
	1.3.2 Greek yoghurt	2
	1.3.3 Probiotic yoghurt	3
	1.3.4 Drinkable yoghurt	3
	1.3.5 Organic yoghurt	3
	1.3.6 Non-Dairy yoghurt	3
	1.4 ANIMAL BASED YOGHURT	3
	1.4.1 Cow milk	3
	1.4.2 Goat milk	4
	1.4.3 Sheep milk	4
	1.5 PLANT BASED YOGHURT	4
	1.5.1 Almond milk	4
	1.6 STARTER CULTURE	5
	1.6.1 Lactic acid bacteria	5
	1.7 FLAVORS AND AROMA IN YOGHURT	6
	1.8 USES AND APPLICATIONS OF YOGHURT	6

2	LITERATURE SURVEY	8
3	AIM AND SCOPE	10
4	MATERIALS AND METHODS	11
	4.1 PREPARATION OF ALMOND MILK	11
	4.2 PREPARATION OF ALMOND MILK YOGHURT	11
	4.3 MICROBIAL ANALYSIS	11
	4.3.1 Total bacterial count	11
	4.3.1.1 Data interpretation	12
	4.3.1.2 Calculation	12
	4.3.2 Total yeast count	12
	4.3.2.1 Counting of plates	12
	4.3.2.2 Data interpretation	12
	4.4 ADDITION OF GRAPE FLAVOR	13
	4.5 PHYSICO-CHEMICAL PROPERTIES	13
	4.5.1 pH measurement	13
	4.5.2 Moisture content	13
	4.5.3 Ash content	13
	4.5.4 Protein content	14
	4.5.5 Fat content	14
	4.5.6 Carbohydrate content	14
	4.6 PERIODIC SHELF LIFE ANALYSIS	14
5	RESULTS AND DISCUSSION	15
	5.1 SAMPLE PREPARATION	15
	5.2 ADDITION OF STARTER CULTURE	16
	5.3 MICROBIAL ANALYSIS	18
	5.3.1 Total bacterial count	18
	5.3.2 Total yeast count	20
	5.4 ADDITION OF GRAPE FLAVOR	22

	5.5 PHYSICO-CHEMICAL PROPERTIES	23
	5.5.1 Analysis of cow milk culture	23
	5.5.2 Analysis of almond, cowmilk and curd	23
	5.5.3 Analysis of almond curd	24
	5.5.4 Analysis of almond culture	24
	5.6 PERIODIC SHELF-LIFE	25
6	SUMMARY AND CONCLUSION	26
	REFERENCES	27

LIST OF FIGURES

FIG 4.1	Almond	15
FIG 4.1.1	Soaked almond	15
FIG 4.1.2	Peeled off almond	15
FIG 4.1.3	Filtering of almond milk	16
FIG 4.1.4	Freshly prepared almond milk	16
FIG 4.2	Addition of culture and curd in almond milk	16
FIG 4.2.1	Addition of culture and curd in combination of cow and almond milk	17
FIG 4.4	Addition of grape flavour -Stirred type yoghurt	22
FIG 4.4.1	Set type yoghurt	22
FIG 4.4.2	Swiss type yoghurt	22
FIG 4.6	After 12 days	25

LIST OF TABLES

Table 4.3.1	Bacterial count for almond curd (OR YOUGURT)	18
Table 4.3.1.1	Bacterial count for almond added with cow milk	18
Table 4.3.1.2	Bacterial count for almond, cow milk and culture	19
Table 4.3.1.3	Bacterial count for almond culture	19
Table 4.3.2	Yeast count for almond curd	20
Table 4.3.2.1	Yeast count for almond added with cow milk	20
Table 4.3.2.2	Yeast count for almond, cow milk and culture	21
Table 4.3.2.3	Yeast count for almond culture	21
Table 4.5	Analysis of cow milk culture	23
Table 4.5.1	Analysis of almond, cowmilk and curd	23
Table 4.5.2	Analysis of almond curd	24
Table 4.5.3	Analysis of almond culture	24

PREPARATION AND EVALUATION OF ALMOND MILK YOGHURT

CHAPTER 1

INTRODUCTION

1.1 YOGHURT:

Nutrition is the most important source of physical and mental health of human. Milk and milk products plays a major role in nutritive and biological quality irrespective of animal and plant based foods. Milk industry is one of the most foremost sectors of agro-industrial complex. Sour milk products are mostly used in human diet because of its health benefits [Yogurt:US Food and Drug Administration,1April2016.] Production of sour milk products includes fermentation process and the quality of the products depends on the types of ferments used. Yoghurt is the most popular sour food which is consumed all over the world.

Yoghurt is a food which is produced by bacterial fermentation in milk. Bacteria which is used to make yoghurt is called yoghurt culture (*Lactobacillus delbrueckii*). The word is derived from Turkish *yoğurt* which means to thicken or coagulated. In milk the sugars are fermented by bacteria which produce lactic acid and act on milk protein to give the texture to yoghurt and characteristic flavor. Yoghurt is mainly made from cow milk but researchers started to make yoghurt from goat, buffalo, ewes, mares, camels, yaks and plant milks. The milk used may be homogenized and pasteurized. Yoghurt prepared from different types of sources gives different types of results. Yoghurt is consumed by peoples for several thousands of years. It is the earliest example of food processing which improve shelf life of food. Analysis of *L.delbrueckii* subsp. *bulgaricus* indicated that the bacteria may be originated from the surface of plants [Lee YK, et al,2012]. Milk have been spontaneously exposed to the plants or the bacteria have been transferred from the udder of milk producing animals. Origin of yoghurt is unknown but it is thought to have been developed in Mesopotamia around 5000 BC. The combination of yoghurt and honey is called “the food of the gods” in ancient Indian records. Yoghurt is prepared by heating milk to a particular temperature which denatures its proteins that are essential for making yoghurt [Eneobong, H.N.,2001]. Variety of plant milk yoghurts appeared from 2000s by using soy milk, rice milk and

nut milk like almond and coconut milk fermented with yoghurt cultures. These types of yoghurts are mainly suitable for vegans, people those are intolerance to dairy milk and those who prefer plant-based products. These types of yoghurts are environmentally friendly as they are produced with water and land resources.

1.2 NUTRITIONAL VALUE IN YOGHURT:

Yoghurt is taken into account as a nutrient dense food but the added ingredients and therefore the production methods are answerable for the ultimate nutritional content. As it is made from milk, yoghurt may be a good source of top quality protein and contain high amount of bioavailable sources. Full fat fruit yoghurt are sources of phosphorous, low fat and thick yoghurts are sources of riboflavin and other yoghurts except of thick and creamy are sources of iodine [Christian, A,2007]. Yoghurt from milk has 81% of water, 9% of protein, 5% of fat and 4% of carbohydrates which incorporates 4% of sugars. Yoghurt is a rich source of riboflavin, vitamin B₁₂, betaine, choline, phosphorous and selenium. The National Diet and Nutrition Survey concludes that riboflavin, vitamin D, magnesium, potassium and calcium are very less in diets of childrens, adolescents, womens and older adults.

1.3 TYPES OF YOGHURTS:

There are different types of yoghurt in different countries which are consumed by people.

1.3.1 TRADITIONAL YOGHURT:

This yoghurt has creamy texture and tart taste which are available in many flavours and textures. Varieties include whole milk, low fat and fat-free yoghurts. These types of yoghurts are less expensive and are sold by many supermarkets [Clark M,2017].

1.3.2 GREEK YOGHURT:

This type of yoghurt is created when the traditional yoghurt is strained for multiple times in order to remove the liquid. The result is thick as a tart product with more amount of protein than traditional yoghurt but with less amount of calcium. Greek yoghurt is more suitable for people who has lactose intolerance. It can withstand the heat better than the regular yoghurt and used in Mediterranean style of cooking. It is available in full fat, reduced or low fat and non-fat [YOGURT, Cambridge,2020].

1.3.3 PROBIOTIC YOGHURT:

Probiotics are live bacteria that are added to yoghurt for health benefits. These types of yoghurts are marketed as digestive aids for immune support. Probiotic yoghurt has nutrients similar to that of traditional yoghurt.

1.3.4 DRINKABLE YOGHURT:

These are made by adding water and additional flavours to traditional yoghurt and possess same nutrients of traditional yoghurts with high amount of sugar and calories.

1.3.5 ORGANIC YOGHURT:

This type of yoghurt is made from organic milk. If an item or ingredient is considered as organic it should meet certain standards, including limited or no use of most pesticides, hormones, chemicals, fertilizers or antibiotics at any stage of production. This types of yoghurts are very expensive but are increased in popularity with good health and environmental reasons [YOGURT, Cambridge, 2020].

1.3.6 NON-DAIRY YOUGHURT:

This yoghurts are alternative for people with milk allergies and those who has gastrointestinal problems. These are good for peoples who do not consume dairy products because of religious beliefs. Yoghurts made from soy milk and coconut milk can be found at local grocer. These yoghurts have similar proteins if they are made from calcium and vitamin D.

1.4 ANIMAL BASED YOGHURT:

Yoghurt was produced from animal milk mainly cow milk. Later researches produced it from other animals like goat, sheep, buffalo and camel.

1.4.1 COW MILK:

Cow milk is used for various purposes in food industry from a very early period. It is used in medicinal and spiritual purposes too. Cow milk and its dairy products were highly valued in ancient Egypt that only the wealthy people could afford to consume them. Cow milk is the most popular choice for culturing dairy products. Heating encourages the protein to coagulate which forms thick yoghurt [Sisay, M., T. Feyera, 2011].

1.4.2 GOAT MILK:

The milk has white-matte colour with the absence of β -carotene and has a pleasant freshly milked taste. The flavour of goat milk is due to the release of short chain fatty acids and has density ranging from 1.026 to 1.042. [Antonio Moniterio,Susana Matos,2019]. The goat milk is naturally alkaline and the milk has water, fat, protein, lactose and minerals. Water content is similar to cow milk and it contains higher amount of nitrogenous substances and few types of casein. Goat milk has a good coagulation capacity and contains higher amount of vitamins and minerals than cow milk. Hence this is also used in most of the dairy products [Marina Temerbayeva, Maksim Rebezov,2018].

1.4.3 SHEEP MILK:

Sheep milk has the white opacity and has an odour called suarda or sheepy. High amount of calcium, phosphorous and magnesium are present in sheep milk.

1.5 PLANT BASED YOGHURT:

In 2000s yoghurts was produced from plant milk like soy milk, rice milk and coconut milk. These types of yoghurts are more suitable for people who prefer plant based foods and those who are with lactose intolerance. Plant based products have different structures when compared to dairy milk. These types of yoghurts contain gum, stabilizers, high sweeteners and artificial colors [McClements, David Julian,2019]. Different bacterial strains are present in plant based yoghurts like *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Bifidobacterium bifidum*.

1.5.1 ALMOND MILK:

Demands for vegetable milk has experienced some problems of healthy life and protein allergenicity. Attention is given to almond milk because of its good nutritional value and functionality. The main reason for the popularity of almond milk is its high added value foodstuff. Almond milk is used as a substitute for cow's milk which is readily available and easy to get. The milk proves that it contains extra nutrients essential for a human body and act as a good option for those who do not consume any form of dairy products. The milk prepared from almond is rich in energy, proteins, lipids and fiber. Almond milk is good source of vitamins like calcium, iron, magnesium,

zinc, sodium and potassium. It also contains vitamins C, B-6, thiamine, riboflavin, folate and vitamin E [Ali Jahanban Esfahlan, Rashid Jamei,2009].

Almond milk has lot of health benefits which include improvement of vision, stronger bones, healthy heart and weight loss. The milk helps in building strong muscles, helps to maintain blood pressure and to maintain a healthy kidney. Almond milk act as a good alternative for nursing mother's milk.

1.6 STARTER CULTURE:

Starter culture are microorganisms which are used in the production of cultured dairy products like yoghurt and cheese. When certain heat treatments are given to milk the natural microflora is either inefficient, uncontrollable and unpredictable. Therefore the starter culture is used which can give controlled and predictable fermentation. These cultures when added in milk form lactic acid which provide flavour, proteolytic and lipolytic activities [Amatayakul, T.; Sherkat,2006].

1.6.1 LACTIC ACID BACTERIA:

These bacteria are found on plants but some species occurs in large number where there is milk. Some bacteria are found in intestine of animals. It includes both the group bacilli and cocci which forms chains of varying length but doesn't form spores. Lactic acid bacteria are mostly anaerobic and are killed by heating to 70°C. These bacteria prefer lactose as their source of carbon which ferment lactose to lactic acid. The fermentation process may be pure or impure ie., the end product can be lactic acid or may be other substances can also be produced. The bacteria forms between 0.5 and 1.5% but some species forms upto 3%. Bacteria need organic nitrogen compounds for growth. The most important types of lactic acid bacteria used in diary industry are *Streptococcus thermophilus*, *Lactococcus lactis*, *lactococcus cremoris*, *Lactobacillus bulgaricus*, *Bifidobacterium* etc. Starter cultures are divided on their preferred growth temperatures.

- Mesophilic bacteria- growth temperatures of 20 to 30 °C
- Thermophilic bacteria – optimal growth temperatures of 40 to 45 °C

1.7 FLAVOURS AND AROMA IN YOGHURT:

Flavour is a complex phenomenon and traditionally consist of odor, taste and somatosenses. The tastebuds located the mouth leads to combined complex sensation known as taste. These can be expressed as sweet, sour, bitter, umami and salty. Flavour is not only the characteristic property which controls the consumer acceptance but it is mainly associated with feeling of wellbeing. Yoghurt has its own peculiar and popular flavour. There are many factors that affect flavour formation, flavour release and perception. Some fruit and fruit flavours used in yoghurt are apple, blackberry, peach, pineapple, raspberry, mango and strawberry. Lactic acid is also suggested to a major compound which significantly contribute the flavour of yoghurt. Nearly 90 flavours has been identified so far [Getenesh Teshome Abdi Keba Zerihun,2017]. The aroma and taste of yoghurt are mainly due to the presence of non-volatile and volatile acids and carbonyl compounds. Some important aromatic compounds are acetone, acetaldehyde, butanoic and propanoic acids. Microbial factors, processing parameters, source of milk, chemicals and additives are several factors which affect the aromatic properties of yoghurt.

1.8 USES AND APPLICATIONS OF YOGHURT:

Yoghurt offer wide range of applications to humans in various ways. In some yoghurts probiotics are added which are healthy bacteria that benefit the gut. Probiotics in yoghurt decreases diarrhea, constipation, bloating, help with weight management and reduce the risk of cancer. A recent study revealed that some type of yoghurts containing probiotics may be healthier than others. Yoghurts are low in lactose content, so if a person finds lactose intolerance he/she could consume yoghurt. Such type of people lack calcium so consumption of yoghurt can be helpful in improving calcium content in them.

The most interesting fact about yoghurt is that it plays a major role in diet plan. This contain high amount of protein and it would be helpful for weight loss. Greek yoghurt is considered as the healthiest among all varieties because it has the perfect ratio of protein and carbohydrate and also act as a workout snack. Taking a bowl of Greek yoghurt within 60min it releases amino acids that repair muscles. People with obesity can consume yoghurt for as it helps in releasing the fat content in body [Dimitrellou, D.; Kandyliis,2019].

As yoghurt contain high amount of calcium it helps in preventing osteroporosis (weakening of bones). Research shows that individuals with osteoporosis will have low bone density and have high risk of bone fracture. To prevent this problem consuming three spoons of yoghurt would be helpful in fast recovery.

In different countries different types of yoghurt is made and consumed by people and studies shows that people who tend to eat yoghurt can eat more food comparatively than others. This is because of the nutrient content and low calorie content.

CHAPTER 2

LITERATURE SURVEY

Abdul Matin, Tuli Banik *et al.*, (2018) explained the microbiological quality of yoghurt sample collected from different places of Bangladesh. Total bacterial, coliform, yeast and mold count was tested for different samples. They indicated that the production of yoghurt needs good practice of manufacture and packaging. Adequate hygiene and quality should be considered to require a healthy yoghurt which may reduce the pathogenic.

Anand Mohan, Joshua Hadi *et al.*, (2019) described the effect of using antibacterial manuka honey on yoghurts. The test was done by spread plate method and stored for three weeks. The combination was developed as the synbiotic functional food by developing the sensory and physicochemical properties like texture, apparent viscosity and water holding capacity.

Zahra Hassanzadeh-Rostami, Seyed Mohammad Mazloomi *et al.*, (2014) evaluated the suitability of different mixtures of cow milk and soy milk as a probiotic food carriers. Mixture of soy milk and cow milk at different concentrations of was made by using single culture and the microbial studies and sensory evaluation was done. They stated that this combination would act as vessel for probiotics.

Getenesh Teshome, Abdi Kebaet *et al.*, (2017) demonstrated the microbial quality and sensory acceptance of different fruit flavored yoghurt and plain yoghurt. They showed that the addition of fruit flavored increased the titratable acidity level. The lactic acid bacteria was high in plain yoghurt whereas in fruit flavored the coliform, yeast and mold count was higher. From this they confirmed the addition of fruit flavored increased the sensory evaluation and physicochemical properties of the yoghurt.

Dimitra Dimitrellou, Nikoletta Solomakou *et al.*, (2020) indicated that the use of grape flavor like berries increased the phenolic content, antioxidant activity, viability of yoghurt starters acidity, color and texture. They also find out the use of fruit juices had no effect on the main physicochemical properties of the yoghurt. Use of fruit juices helps to optimize the benefits of probiotic products with higher level of phenolic compounds.

Ali Jahanban Esfahlan, Rashid Jamei *et al.*, (2009) explained almond has a good level of nutritional value. Different phenolic compound were identified from almond seed and the role of almond in prevention of degenerative diseases like cancer in increasing.

Alozie Yetunde E. and Udofia, Ukpong S. (2015) evaluated various tests for almond milk and concluded that it contains higher amount of milk yield and this can be preferred in terms of color, flavor, taste and overall acceptability. It also contains high amount of calcium, iron and potassium. It is a nutrient dense with lower calories when compared to other type of dairy milks.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The aim of the study is to prepare an imitation of yoghurt from almond milk with grape flavour and analysis its microbial and physiochemical properties.

3.2 SCOPE

- Preparation of almond milk
- Formation of yoghurt
- Microbial Analysis
- Addition of flavor
- Physicochemical properties
- Periodic shelf-life Analysis

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS:

Culture

PREPARATION OF ALMOND MILK:

Fresh almond (500g) was bought from market and it was soaked for overnight in water. The very next day almond peel was removed and 500ml of water was added. This was further grinded and the prepared milk was placed in a beaker separately.

4.2 PREPARATION OF YOGHURT:

The prepared almond milk was filtered to remove the foreign particles and pasteurized for 15 seconds at 72°C in order to kill the pathogenic microorganisms. The pasteurized milk was cooled to 40-45°C and it was divided into equal amount in to four beakers (A, B, C & D). As curd contains lactobacillus this was used which may help the almond milk to form yoghurt. In beaker A curd was added wherein beaker B starter culture was added to almond milk, in beaker C and D culture and curd are added to the combination of cowmilk and almond milk. This was kept in incubation at 42°C for 12 hours (overnight) until the pH of 4.3-4.5 was attained. The yoghurt was then placed in refrigerator at 6°C before analysis.

4.3 MICROBIAL ANALYSIS:

4.3.1. TOTAL BACTERIAL COUNT:

Bacterial count is most common microbial analysis which gives quantitative idea about the microorganisms present in the sample. This is done by two methods plate count and spectrophotometric method. Standard plate count is indirect measurement of cell density and gives result only for live bacteria whereas the spectrophotometric analysis measures all bacteria of dead and live indirectly.

In this study the bacterial count was done by plate count method by a standard procedure. 10 test tubes of each containing 9ml of diluents was taken and concentration was made upto 10^{-9} . 0.5ml of yoghurt sample was homogenized in 450ml diluents by making a suspension in beaker. Now from the original sample, 1ml

of sample is transferred to first test tube and it is mixed thoroughly. Transfer 1ml from first test tube to second test tube and this was continued till the last test tube. From the last test tube 1ml of sample was discarded. The plates containing 15ml of nutrient agar was added with 1ml of sample from the first test tube to last. The petri dish was rotated for 5-10 seconds where the sample can mix thoroughly with the media. The plates was then inverted and kept for incubation at 37°C for 24 hours [Yogurt—Enumeration of Characteristics Microorganisms,2003].

4.3.1.1 DATA INTERPRETATION:

After incubation period petri plates containing 30-300 colonies were selected because plates which has more than 300 colonies cannot be counted(TNTC) and plates with less than 30 colonies are very few to count(TFTC).

4.3.1.2 CALCULATION

Calculate number of bacteria per gram or millilitre of sample by dividing the number of colonies by the dilution factor multiplied by amount of specimen.

Number of colonies/dilution × amount plated = no of bacteria/ml.

4.3.2 TOTAL YEAST COUNT:

Plate count for yeast is a screening technique to evaluate whether the product is contaminated or not. The process was done by taking 0.5ml of sample and diluted with 10ml of peptone water. From this 1ml was diluted with the first test tube which has 9ml of water. The process was continued as mentioned in 4.3.1. the plates were placed for incubation for 5days at 37°C.

4.3.2.1 COUNTING OF PLATES:

The plates were counted after five days of incubation. If there is no growth keep it again for 48hrs. If the sample has yeast, plates with 150 colonies are mainly considered. Do not count the plates before incubation period because this may result in secondary growth from dislodged spores.

4.3.2.2 DATA INTERPRETATION:

Plates which has colonies of 10-150 are considered for counting. If the product or sample is contaminated in yeast count it should be further tested for better microbial protection. If there is no fungal contamination the sample can be taken to next level to test preservative efficacy, safety and stability.

4.4 ADDITION OF GRAPE FLAVOUR:

Flavour is added to yoghurt at different steps on the type of yoghurt. In set style yoghurt the fruit is added at the bottom of the bowl and the inoculated yoghurt is poured on top and it is fermented. In swiss style yoghurt the fruit is blended with the fermented yoghurt.

4.5 PHYSICO-CHEMICAL PROPERTIES:

The physico-chemical properties like fat content, sugar content, protein content and carbohydrates level were analysed.

4.5.1 pH MEASUREMENT:

pH of yoghurt was determined at room temperature using a digital pH meter. The pH meter was calibrated with standards of buffer at pH of 4 and 10 before use. 50ml of yoghurt was placed in beaker and the pH meter was inserted and the value was recorded. The measurement was observed on the opening of the yoghurt and the probe was rinsed thoroughly with distilled water before to used on the sample[Dr. William Horwitz, Dr. George 1995].

4.5.2 MOISTURE CONTENT:

The moisture content in yoghurt was identified by Association of Official Analytical Chemists Method[AOAC,Dr. William Horwitz, Dr. George1995]. 10g from each yoghurt was placed in oven at 105 C for 3hrs and reading was taken at a constant weight. The final moisture content was expressed as percentage of the dry weight of sample.

4.5.3 ASH CONTENT:

Ash content of yoghurt is expressed as the inorganic residue which is left as a percentage of total weight of yoghurt incinerated. This was determined at 550 C according to AOAC [Dr. William Horwitz, Dr. George 1995].

4.5.4 PROTEIN CONTENT:

The protein contents of the sample was determined by Association of Official Analytical Chemist [AOAC, 2005].

4.5.5 FAT CONTENT:

Fat content was determined by modified ether extraction method the extracted fat was dried to constant weight and it is expressed as percent fat per weight[Dr. William Horwitz, Dr. George 1995].

4.5.6 CARBOHYDRAE CONTENT:

The carbohydrate contents of the sample was determined by Association of Official Analytical Chemist [AOAC, 2005].

4.6 PERIODIC SHELF-LIFE ANALYSIS:

After the preparation of yoghurt this was placed in refrigerator with tightly closed container. The shelf life of yoghurt was checked after 7 days.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 SAMPLE PREPARATION:

Almond milk was prepared from grinding of soaked almond and it was filtered to collect the fresh milk.



Fig 4.1 Almond

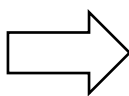


Fig 4.1.1 Soaked almond

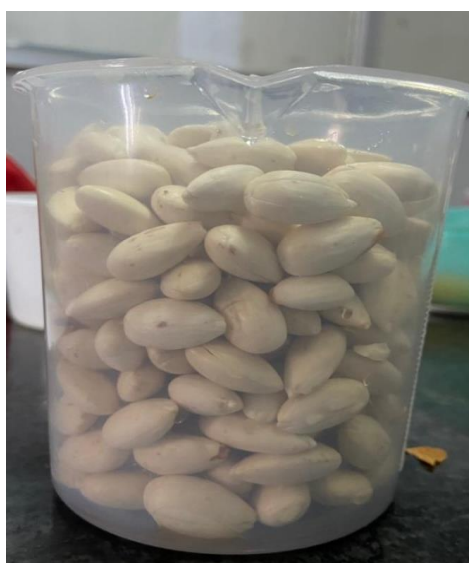


Fig 4.1.2 Peeled off almond

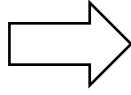


Fig 4.1.3 Filtering of almond milk

Fig 4.1.4 Freshly prepared almond milk

5.2 ADDITION OF STARTER CULTURE:

Starter culture of *Lactobacillus sporogenes*, *bifidobacterial* and curd was added to the freshly prepared milk in four combinations. The culture is added to ferment lactose to produce lactic acid. Increase in lactic acid decreases the pH and cause the milk to clot and form the soft gel of yoghurt.

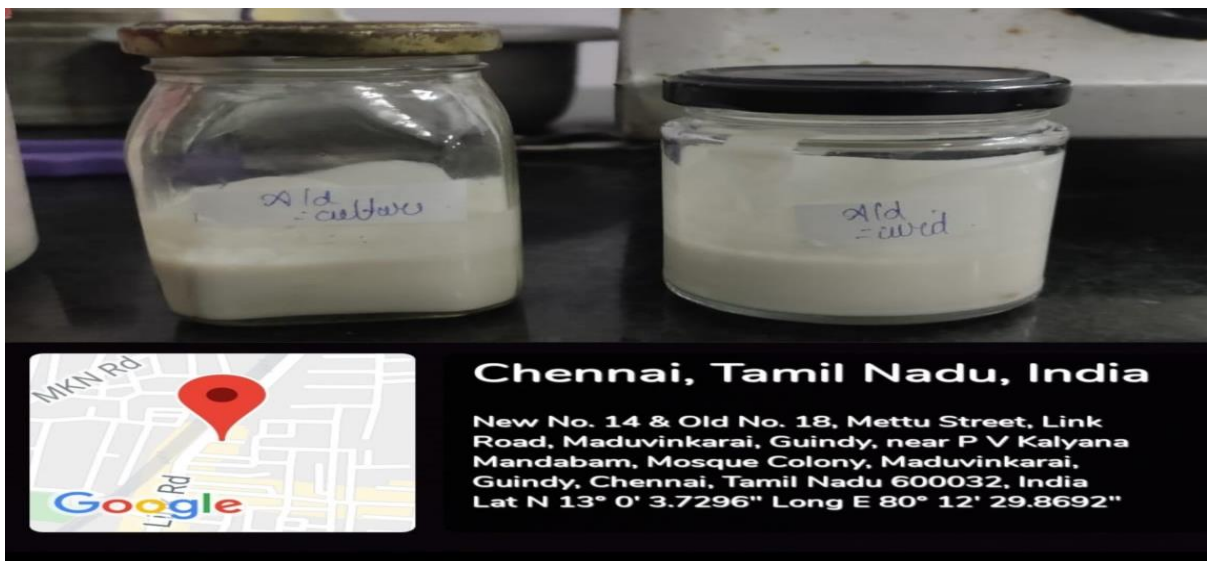


Fig 4.2 Addition of culture and curd in almond milk



Fig 4.2.1 Addition of culture and curd in combination of almond and cow milk

5.3 MICROBIAL ANALYSIS:

5.3.1 TOTAL BACTERIAL COUNT:

Bacterial count was done for all the four combinations to find out the best one. Plates with colonies of 30-300 were selected for counting.

Table 4.3.1 Bacterial count for almond curd

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond curd	10^{-1}	TNTC	Acceptable range
	10^{-2}	TNTC	
	10^{-3}	TNTC	
	10^{-4}	TNTC	
	10^{-5}	220	
	10^{-6}	156	
	10^{-7}	96	
	10^{-8}	50	
	10^{-9}	Nil	

Table 4.3.1.1 Bacterial count for almond added with cow milk

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond + cow curd	10^{-1}	TNTC	Acceptable range
	10^{-2}	TNTC	
	10^{-3}	TNTC	
	10^{-4}	TNTC	
	10^{-5}	204	
	10^{-6}	50	
	10^{-7}	21	
	10^{-8}	06	

	10^{-9}	Nil	
--	-----------	-----	--

Table 4.3.1.2 Bacterial count for almond, cow milk and culture

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond + cow +culture	10^{-1}	TNTC	Acceptable range
	10^{-2}	TNTC	
	10^{-3}	TNTC	
	10^{-4}	TNTC	
	10^{-5}	10	
	10^{-6}	0	
	10^{-7}	Nil	
	10^{-8}	Nil	
	10^{-9}	Nil	

Table 4.3.1.3 Bacterial count for almond and culture

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond + culture	10^{-1}	TNTC	Acceptable range
	10^{-2}	TNTC	
	10^{-3}	TNTC	
	10^{-4}	TNTC	
	10^{-5}	TNTC	
	10^{-6}	130	
	10^{-7}	86	
	10^{-8}	32	
	10^{-9}	09	

The combination of almond and curd, almond milk with cow milk and curd shows the good bacterial colonies and this could be the best from the four.

5.3.2 TOTAL YEAST COUNT:

The yeast count is performed for five days to find out the amount of yeast present in the sample. This was done by pour plate method four combinations and colonies with 10 to 150 were noted.

Table 4.3.2Yeast count for almond curd

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond curd	10^{-1}	88	Acceptable range
	10^{-2}	36	
	10^{-3}	02	
	10^{-4}	Nil	
	10^{-5}	Nil	
	10^{-6}	Nil	
	10^{-7}	Nil	

Table 4.3.2.1Yeast count for almond added with cow milk

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond + cow curd	10^{-1}	108	Acceptable range
	10^{-2}	77	
	10^{-3}	47	
	10^{-4}	Nil	
	10^{-5}	Nil	
	10^{-6}	Nil	
	10^{-7}	Nil	

Table 4.3.2.2 Yeast count for almond, cow milk and culture

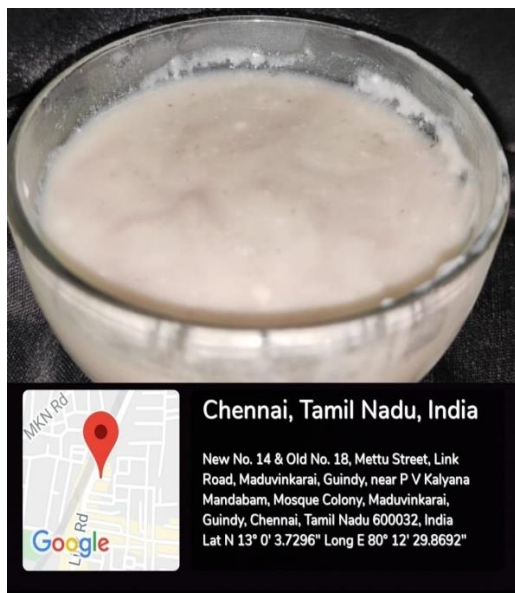
SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond + cow +culture	10^{-1}	232	Acceptable range
	10^{-2}	134	
	10^{-3}	94	
	10^{-4}	Nil	
	10^{-5}	Nil	
	10^{-6}	Nil	
	10^{-7}	Nil	

Table 4.3.2.3 Yeast count for almond and culture

SAMPLE NAME	DILUTIONS	RESULTS UNITS CFU/mL	RESULTS COMMENTS
Almond + culture	10^{-1}	75	Acceptable range
	10^{-2}	50	
	10^{-3}	04	
	10^{-4}	Nil	
	10^{-5}	Nil	
	10^{-6}	Nil	
	10^{-7}	Nil	

5.4 ADDITION OF GRAPE FLAVOR:

Grape fruit was brought from market and the fruit was squeezed to obtain the juice. 2-3ml of the juice was added to the yoghurt and this was mixed well and set apart which is referred as stirred type yoghurt. To another set of yoghurt the fruit was placed at the bottom and the yoghurt was added to the top which is referred as set-style of yoghurt.



4.4 Addition of grape flavour

4.4.1 Set style yoghurt

Stirred style yoghurt



4.4.2 Swiss style yoghurt

5.5 PHYSICO-CHEMICAL PROPERTIES

5.5.1 Analysis of cow milk culture:

The prepared cow milk culture was analyzed for protein, fat, moisture content and etc.. Here the protein content was 3.04, moisture content was 64.79, carbohydrate was 28.50, fat content was 2.55 for 100g. pH was at range of 4.3. Hence the total energy for this culture was found to be 149.11KCal.

Table 4.5 Analysis of cow milk culture

S.No	Parameters	Unit	Results
1	pH	-/100g	4.3
2	Moisture	g/100a	64.79
3	Ash	g/100a	1.12
4	Fat	g/100Q	2.55
5	Protein	g/100g	3.04
6	Carbohydrate	g/100g	28.50
7	Energy	KCal/100g	149.11

5.5.2 Analysis of almond, cowmilk and curd:

In this analysis the protein content was found to be higher as 5.97 due to the addition of almond milk. Fat content was low as 2.94 and carbohydrate content was 18.98 for 100g. the energy obtained here was 126.26Kcal.

Table 4.5.1 Analysis of almond, cowmilk and curd

S.No	Parameters	Unit	Results
1	pH	-/100g	4.4
2	Moisture	g/100s	71.09
3	Ash	g/100g	1.02
4	Fat	g/100s	2.94
5	Protein	g/100g	5.97
6	Carbohydrate	g/100s	18.98
7	Energy	KCal/100g	126.26

5.5.3 Analysis of almond curd:

In almond curd analysis also the protein was higher it proves that addition of almond in yoghurt gives higher quantity of protein to people who consume it. Similarly the fat content is also low as 2.79 for 100g.

Table 4.5.2 Analysis of almond curd

S.No	Parameters	Unit	Results
1	pH	-/100g	4.4
2	Moisture	g/100s	61.45
3	Ash	g/100g	1.15
4	Fat	g/100s	2.79
5	Protein	g/100g	4.54
6	Carbohydrate	g/100s	30.07
7	Energy	KCal/100g	163.55

5.5.4 Analysis of almond culture:

In almond culture the moisture content 59.45, protein was 4.59, fat content was 2.84, carbohydrate was 32.03 which shows the total energy is 172.04 for 100g.

Table 4.5.3 Analysis of almond culture

S.No	Parameters	Unit	Results
1	Ph	-/100g	4.4
2	Moisture	g/100s	59.45
3	Ash	g/100g	1.09
4	Fat	g/100s	2.84
5	Protein	g/100g	4.59
6	Carbohydrate	g/100s	32.03
7	Energy	KCal/100g	172.04

5.6 PERIODIC SHELF-LIFE ANALYSIS:

The yoghurt placed in refrigerator was checked for the shelf-life after 7days. The consistency was thick and there was no colour change due to the addition of flavour. The yoghurt remained the same for total of 12days.



4.6 After 12 days

CHAPTER 6

SUMMARY AND CONCLUSION

Fresh yoghurt was made from almond milk with the addition of grape flavor. The microbial analysis of bacterial and yeast count showed good results for almond with curd combination. Perfect range of pH was noted for the yoghurt for almond based of 4.4. Protein was higher in almond based analysis with addition of cow milk and curd with the range of 5.97 equal to 6. The fat content was low in all the samples therefore consumers can consume the yoghurt without fear. The total energy level was high and if someone lacks from protein content or any lactose intolerance can consume the yoghurt.

REFERENCES

1. "YOGURT" meaning in the Cambridge English Dictionary". dictionary.cambridge.org. 17 February 2020.
2. Yogurt: from Part 131 – Milk and Cream. Subpart B – Requirements for Specific Standardized Milk and Cream, Sec. 131.200". Code of Federal Regulations, Title 21, US Food and Drug Administration. 1 April 2016.
3. Lee YK, et al. "Probiotic Regulation in Asian Countries". In Lahtinen S, et al. (eds.). Lactic Acid Bacteria: Microbiological and Functional Aspects(Fourth ed.). Boca Raton: CRC Press. p. 712. ISBN 9780824753320.2012.
4. Chandan RC, Kilara A Dairy Ingredients for Food Processing. John Wiley & Sons. pp. 1–. ISBN 978-0-470-95912-1(22 December 2010).
5. Clark M. "Creamy Homemade Yogurt Recipe". NYT Cooking. 19 March 2017.
6. Eneobong, H.N., Eating Right: A Nutrition mechanisms and future research directions". Nutrition Guide. University of Calabar Press, pp: 87-89 2001
7. Gaman, P.M. and K.B. Sherrington. The Science Food. Jones and Barlett Publishers 2001.
8. Brain, F.A. and G.C. Alan, Food Science, Nutrition and Health. Edward Arnold, pp: 17-20. 1992.
9. Christian, A.,Fluted pumpkin (*Telfairia occidentalis* Hook F.) seed: a nutritional assessment. Electronic Journal of Environmental, Agricultural and Food Chem., 6(2): 1787-1793.2007.
10. Alozie Yetunde E. and Udofia, Ukpong S Nutritional and Sensory Properties of Almond (*Prunus amygdalu* Var. *Dulcis*) Seed Milk . World Journal of Dairy & Food Sciences 10 (2): 117-121, 2015 ISSN 1817-308X © IDOSI Publications, DOI: 10.5829/idosi.wjdfs.2015.10.2.9622, 2015.
11. Valerie Tournas, Michael E. Stack, Philip B. Mislivec, Herbert A. Koch and Ruth BandlerBAM Chapter 18: Yeasts, Molds and Mycotoxins April 2001.
12. King, A.D. Jr, J.I. Pitt, L.R. Beuchat, and J.E.L. Corry (eds.) Methods for the Mycological Examination of Food. Plenum Press, New York 1986
13. Beneke, E.S., and A.L. Rogers.. Medical Mycology Manual, 3rd ed. Burgess, Minneapolis 1971

14. Sisay, M., T. Feyera and O. Mohammed, 17. Berryman, C.E., A.G. Preston, W. Karmally, Microbiological Quality of Raw Cow's Milk from Four R.J. Deckdelbaum and P. M. Kris-Etherton, 2011.
15. Dairy Farms in Dire Dawa City, Eastern Ethiopia. Effects of almond consumption on the reduction World Journal of Dairy & Food Sciences, 10(1): 09-14 2015.
16. Goat and sheep milk as a raw material for yogurt Antonio Moniterio, Susana Matos, Paulo correia, March 9 2019.
17. Marina Temerbayeva, Maksim Rebezov Eleonora Okuskhanova, Oksana Zinina, Olga Gorelik, Oksana Vagapova, Tatiana Beginer, Svetlana Gritsenko, Ainur Serikova and Zhanibek Yessimbekov Development of Yoghurt from Combination of Goat and Cow Milk Annual Research & Review in Biology 23(6): 1-7, 2018; Article no.ARRB.38800 ISSN: 2347-565X, NLM ID: 101632869 February 8 2018
18. Barnes A "Choosing Dairy-Free In : Chobani Disrupting Yogurt Market With Plant-Based Product". Forbes. 30 March 2019.
19. McClements, David Julian; Newman, Emily; McClements, Isobelle Farrell. "Plant-based Milks: A Review of the Science Underpinning Their Design, Fabrication, and Performance". Comprehensive Reviews in Food Science and Food Safety. 18 (6): 2047–2067. doi:10.1111/1541-4337.12505 – via Wiley Online Library 12 February 2019.
20. Densie Webb. "The Scoop on Vegan Yogurts - Today's Dietitian Magazine". www.todaysdietitian.com. 2018
21. Amatayakul, T.; Sherkat, F.; Shah, N.P. Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. Int. J. Dairy Technol. 2006, 59, 216–221.
22. Liu, L.; Li, C.; Liu, J. Rheological and physical characteristics of non-fat set yogurt prepared with EPS-producing *Streptococcus thermophilus* and an H⁺-ATPase-defective mutant *Lactobacillus delbrueckii* subsp. *bulgaricus*. Int. J. Food Prop. 2017, 20, 745–753.
23. Microbiological quality analysis of yoghurt in some selected areas of Bangladesh Abdul Matin, Tuli Banik, Md. Rahim Badsha, Altaf Hossain,

- Mohammad Mozibul Haque, Monsur Ahmad International Journal of Natural and Social Sciences, 2018, 5(4): 82-86 August 2018
24. Naeem K and Rizvi A-u-R (1986). Studies on the physical and bacterial quality of dahi with special reference to public health. The Journal of Pakistan Medical Association, 36(4): 87-9. Olugbuyiro JA (2011).
 25. Physico-chemical and sensory evaluation of market yoghurt in Nigeria. Pakistan Journal of Nutrition, 10(10): 914-918. Oyeleke S (2009).
 26. Microbial assessment of some commercially prepared yoghurt retailed in Minna, Niger State. African Journal of Microbiology Research, 3(5): 245-248.
 27. Getenesh Teshome Abdi Keba Zerihun Assefa Bilatu Agza Firew Kassa Development of Fruit Flavored Yoghurt with Mango (*Mangifera indica* L.) and Papaya (*Carica papaya* L.) Fruits Juices. www.iiste.org ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online) Vol.67, 2017
 28. Dimitra Dimitrellou, Nikoletta Solomakou, Evangelos Kokkinomagoulos and Panagiotis Kandylisurts Supplemented with Juices from Grapes and Berries 21 August 2020
 29. Boycheva, S.; Dimitrov, T.; Naydenova, N.; Mihaylova, G. Quality characteristics of yogurt from goat's milk, supplemented with fruit juice. Czech J. Food Sci. 29, 24–30, 2011
 30. Calvo, M.M.; Diez, O.; Cobos, A. Use of rectified grape juice in yogurt edulcoration. J. Food Sci. 2002, 67, 3140–3143.
 31. Barat, A.; Ozcan, T. Growth of probiotic bacteria and characteristics of fermented milk containing fruit matrices. Int. J. Dairy Technol. 71, 120–129, 2018
 32. ISO. Yogurt—Enumeration of Characteristics Microorganisms—Colony Count Technique at 37 °C; ISO 7889:2003(E); IDF 117:2003(E); International Organization for Standardization: Geneva, Switzerland, 2003.
 33. Dimitrellou, D.; Kandylis, P.; Kourkoutas, Y. Assessment of freeze-dried immobilized *Lactobacillus casei* as probiotic adjunct culture in yogurts. Foods 8, 374, 2019
 34. Joseph A.O. Olugbuyiro and Joy E. Oseh Physico-chemical and Sensory Evaluation of Market Yoghurt in Nigeria Pakistan Journal of Nutrition 10 (10):

914-918, 2011 ISSN 1680-5194 © Asian Network for Scientific Information, 2011

35. Dr. William Horwitz, Dr. George W. Latimer, Jr., Assistant Editor Official Methods of Analysis of AOAC INTERNATIONAL 18th Edition, 2005
36. Obi, T.E., F.O. Henshaw and O.O. Atanda, 2010. Quality evaluation of plain-stirred probiotic yoghurt produced from skim and whole milk powder during refrigerated storage. EJEAFChe, 9: 1203-1213.
37. Karagul-Yuceer, Y., P.C. Coggins, J.C. Wilson and C.H. White, 1999. Carbonated yogurt-sensory properties and consumer acceptance. J. Dairy Sci., 82: 1394- 1398.
38. USDA, 2001. Specifications for Yogurt, Nonfat Yogurt and Lowfat Yogurt. Document 21CFR, Part 131.200-203