EFFECT OF LEMONGRASS DISTILLATE ON PHYSICO-CHEMICAL PROPERTIES OF YOGHURT

By

Miss. GAWADE BHARATI CHANDRAKANT
(Reg. No. 015/143)

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree

Of

MASTER OF SCIENCE (AGRICULTURE)

In

DAIRY SCIENCE

DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRY SCIENCE
POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
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2017

CANDIDATE'S DECLARATION

I hereby declare that this thesis or a part thereof has not been submitted by me or any other person to any other University or Institute for Degree or Diploma.

Place: M.P.K.V., Rahuri **(B. C. Gawade)**

Date : / / 2017

Dr. R. J. Desale
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Maharashtra, India.

C E R T I F I C A T E

This is to certify that the thesis entitled, "**EFFECT OF LEMONGRASS DISTILLATE ON PHYSICO-CHEMICAL PROPERTIES OF YOGHURT",** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, M.S. for the award of the degree of **MASTER OF SCIENCE** (AGRICULTURE) in **DAIRY SCIENCE**, embodies the results of a *bona fide* research carried out by **Miss. GAWADE BHARATI CHANDRAKANT**, under my guidance and supervision and that no part of the thesis has been submitted for any other Degree or Diploma.

Place: M.P.K.V., Rahuri **(R. J. DESALE)**
Dated: / /2017 Research Guide

Dr. J.V. Patil
Associate Dean,
Post Graduate Institute,
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Place: M.P.K.V., Rahuri **(J. V. Patil)**

Dated: / /2017

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Many have not been mentioned but none is forgotten.

Place: M.P.K.V., Rahuri.

Date: / / 2017 **(B. C. Gawade)**

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LIST OF ABBREVIATIONS

%	: Per cent
@	: at the rate
^0C	: Degree Centigrade
Amt	: Amount
Anon.	: Anonymous
Ca	: Calcium
CC	: Coliform Count
CD	: Critical Difference
c.f.u	: Colony forming Unit
Cm	: Centimeter
EO	: Essential Oil
et al.,	: And other (et alli)
G/gm (s)	: Gram (s)
GOI	: Government of India
H_2SO_4	: Sulphuric Acid
$CuSO_4$	: Copper Sulphate
Hrs. (s)	: Hour (s)
i.e.	: that is
IS	: Indian Standards
K_2SO_4	: Potassium Sulphate
L/lit.	: Liter
LA	: Lactic Acid
LAB	: Lactic Acid Bacteria
mg	: milligram
mg/L	: milligram per liter
ml	: milliliter
mm	: millimeter
MT	: Million Tonnes

N	: Normality
Na	: Sodium
No.	: Number (s)
NS	: Non-Significant
Qty.	: Quantity
S. E.	: Standard Error
Sig.	: Significant
SNF	: Solid Not Fat
SPC	: Standard Plate Count
TS	: Total Solids
Viz.	: Namely
Vol	: Volume
VRBA	: Violet Red Bile Agar
YMC	: Yeast and mould count

ABSTRACT

EFFECT OF LEMONGRASS DISTILLATE ON PHYSICO-CHEMICAL PROPERTIES OF YOGHURT

By

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A candidate for the degree of

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2017

Research Guide	: Dr. R. J. Desale
Discipline	: Animal Husbandry and Dairy Science
Major Field	: Dairy Science

The present study entitled "Effect of lemongrass distillate on physico-chemical properties of yoghurt" was carried out in Department of Animal Husbandry and Dairy Science laboratory, Post Graduate Institute, M.P.K.V., Rahuri.

The main objective of this research work was to optimize the levels of lemongrass distillate in yoghurt, to investigate the physico-chemical and sensory changes of yoghurt added by extract additives and to analyze microbiological properties of yoghurt.

The yoghurt samples were prepared by using cow milk procured from Research cum Development Project (RCDP) on Cattle, M.P.K.V., Rahuri, Dist. Ahmednagar.

The chemical attributes determined were fat, protein, lactose, ash, total solids, acidity, pH and moisture of yoghurt. The sensory evaluation was carried out in respect of flavour, body and texture, colour and appearance, taste and overall acceptability.

The level of lemongrass distillate for final experimental trials were finalized in pre-experimental trials on the basis of organoleptic evaluation.

The levels were selected as 0.01, 0.02 and 0.03 per cent for further study. The results obtained from chemical, microbiological and organoleptic evaluation were analyzed by Completely Randomized Design (CRD).

The treatment T_2 (Plain yoghurt + 0.02% of lemongrass distillate) was rated best among yoghurt samples and was comparable to control yoghurt. The chemical composition of treatment T_2 was 2.10, 2.86, 5.33, 0.58, 17.97, 0.63, 4.22 and 82.03 per cent of fat, protein, lactose, ash, total solids, titrable acidity, pH and moisture, respectively. The treatment T_0 was microbiologically superior over other treatments.

The addition of lemongrass distillate in yoghurt affected flavor, colour and appearance, body and texture and flavour significantly and overall acceptability differed significantly.

It may be concluded that treatment T_2 having addition of 0.02 per cent lemongrass distillate in yoghurt was most acceptable among other treatment in respect of chemical, organoleptic and microbiological qualities.

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CHAPTER I

INTRODUCTION

1.1 Background information

The importance of milk and milk products in India is realized since Vedic period. Milk has always vital status in human nutrition because of its palatability and digestibility. India ranks first amongst the milk producing countries in the world. Total annual milk production is 155.5 million tones which is sufficient to provide 337 gm per capita availability per day (Annual Report 2014-15, GOI). Recommended consumption of milk and milk products by Nutrition Advisory Committee is 279 gm per head per day (Anonymous, 2012). Out of total milk production 43 per cent milk production is contributed by cow milk, 56 by buffalo milk and 3 by sheep and goat milk. This has been largely achieved through combined efforts of constructive policies and institutional network, which has supported to the millions of rural milk producers, through small scale dairy farming.

Milk utilization pattern in India is given below-

Sr.no.	Product	% Utilization
1)	Liquid milk	46
2)	i) Ghee/Makkhan	33
	ii) Dahi (Curd/ Chakka)	7
	iii) Khoa	7
	iv) Channa & Paneer	3
3)	Western Type Products	4

(Anonymous, 2014)

Dairy industry has emerged as a fast growing and large scale producer of novel and nutritionally enriched milk based products, with proficient, which is a very good achievement in the world market. "However, the dairy market is highly fragmented with the top ten players occupying less than 60% of the market share."

Fermentation is a slow decomposition reaction of organic substances accelerated by microorganisms or enzymes that essentially convert complex substances into simpler ones as carbohydrates to alcohols or organic acids (FAO, 1998). Fermented milk products have better keeping quality and are easily digestible because of breakdown of proteins into peptides and free amino acids as a result of microbial action. The substances like lactic acid, alcohol, non-protein substances, volatile acids, calcium and phosphorous appear in more assimilable level in yogurt as compared to milk (Laxminarayan and Shankar, 1980).

Probiotics are defined as living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora. In addition to improving gut health, probiotics may play a beneficial role in several medical conditions including lactose intolerance, cancer, allergies, hepatic disease, *Helicobacter pylori* infections, urinary tract infections, hyperlipidemia and assimilation of cholesterol (Ejtahed *et al.,* 2011). Probiotic microorganisms that are known to be beneficial to human health can be ingested through fermented dairy products, enrichment of various foods with these bacteria and consumption of pharmaceutical products that are obtained by using viable cells (lyophilized preparations and tablets). Probiotics are defined as viable microorganisms which can be consumed separately or with foods, which assist dietary and microbial balance by regulating the mucosal and systemical immunity and beneficially affect the consumer's health. A great variety of dairy products contain probiotic bacteria (Ziemer & Gibson, 1998; Moayednia *et al.,* 1999; Kanmani *et al.,* 2013).

Yoghurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* (Bourlioux and Pochart, 1988) and is a popular product throughout the world. Yoghurt should contain at least 3.25% of milk fat and 8.25% of milk solid not fat(MSNF)with a titrable acidity of not less than 0.9%.Yoghurt has smooth and a mildy sour and pleasant flavor. It is obtained from pasteurized or boiled milk soured by naturally occurring, or lactic acid fermenting bacteria i.e. *Lactobacillus bulgaricus and Streptococcus thermophillus* .The sensory characteristics of yoghurt are due to its content of carbonyl, mainly acetaldehyde, acetone, diacetyl and ethanol produced by yoghurt bacteria. The highest consumption of yoghurt is in Mediterranean, Asian countries, central Europe (Sahana *et al.,* 2008).Yoghurt has been known for its nutraceutical, therapeutic and probiotic effects such as digestion enhancement, Immune system boosting, anticarcinogenic activity and reduction of serum cholesterol (Penna *et al.*, 2007; Najafi *et al.,* 2008).

Cymbopogon flexuosus (Lemongrass) is an economically important plant that has been used for centuries, as a medicine because of its wide-ranging therapeutic properties included relief of rheumatic and other pain and healing effect on ulcers (Fenwick *et al.,* 1990). Flavonoids extracted from Lemongrass are of considerable interest as natural plant components with antioxidant and antifungal activity (Pratt and Hudson, 1991; Nieto *et al.,* 1993; and Abu-Seif, *et al.,* 2009). Of the flavonoids present in Lemongrass, licochacone A and licochacone B which have equal antioxidant activity of vitamin E, and glabrene which is 3 times as active when compared with vitamin E (Okuda *et al.,* 1989). The use of Lemongrass herb in this study was due that Lemongrass is naturally occurring material, widely cultivated, cheap, had a medical functions and safe. These properties and the antifungal activity, if possible, make lemongrass oil may be potential multi-functional food additives.

1.2 Importance of study

The report says that the yoghurt market in India grew at a CAGR of 28.9% between 2011 and 2015. The country's yoghurt market is projected to grow at an even faster pace over next five years and touch $1bn by 2021.Easy availability of product and rising emphasis on low calorie and low fat content of food products is driving yoghurt consumption in India. Yoghurt are gradually eating away the share of traditional dairy product in urban and semi urban cities, due to increasing health awareness, better quality of packaged yoghurt and increasing yoghurt flavors. Due to all those factors; it is necessary to investigate potential of yoghurt and scope for value addition in it.

1.3 Future promises of yogurt

Yogurt has the potential to be the vital player in the spectrum of food products that provide a wide range of health benefits to individuals through specific influence on their intestinal microbiota. To reach this potential, however, important strides in both scientific understanding and regulatory oversight must be made. The scientific understanding of the intestinal microbiota is still being assembled. For yogurt, how much of the intestinal microbiota and its influence on whole-body health are alterable by diet. For regulatory oversight, the scientific, industrial, and regulatory communities must agree on quantifiable measures of those microbiota dependent health properties. Within such a context, companies can then show with these metrics that these health properties have been significantly improved by their dietary interventions. Industry must invest in the development of yogurt's potential. Industrial processes and products will need to become more transparent and their expectations for claimable health benefits more clearly defined. Industry will also need to participate in the development, validation, and implementation of technologies that accurately measure yogurt products and their quality, safety, and efficacy; it would be most efficient if the science and its regulatory applications were pursued in parallel.

1.4 Objectives

The present investigation on “Effect of lemongrass distillate on physico-chemical properties of yoghurt” was carried out with following objectives-

1. To optimize level of lemongrass distillate

2. To investigate the Physico-chemical and sensory changes of yoghurt added by extract additives
3. To analyze microbiological properties of yoghurt

CHAPTER II

REVIEW OF LITERATURE

Yoghurt is a very popular fermented milk product in the different parts of world and now getting popularity in India. The popularity of fermented milk products have gained for its therapeutic and nutritive values.

The research on the yoghurt has been taken by few institutes. An attempt has been made in this investigation to prepare yoghurt enriched with different plant extract additives.

In this chapter research carried out by past workers in context to use of herbal extract for preparation of yoghurt has been reviewed and presented in different sub-parts -

2.1 Yoghurt

Yoghurt is custard like curd of smooth and firm texture with a pleasant sour taste. It is prepared from cow, buffalo or goat milk (Banerjee, 1960).

Yoghurt is snow-white custard like cultured milk made by fermenting a slightly concentrated whole milk with a mixed bacterial culture, probably consisting of L. *bulgaricus and S. thermophilus (*Nelson and Trout, 1964).

Nambudripad (1969) described yoghurt as an acidic dahi, with a thick consistency and pleasant acid taste and aroma.

Yoghurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from pasteurized or concentrated milk with or without optional additions (milk powder, skim milk powder, whey powder, etc.) (FAO, 1976).

Sinha (1984) defined yoghurt as a coagulated milk product formed by the action of *Streptococcus thermophilus and Lactobacillus bulgaricus* and containing no stabilizers.

Yoghurt is snow white custard like cultured milk made by fermenting a slightly concentrated whole milk with mixed bacterial culture consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Rangnatham and Gupta, 1987).

Yadav *et al.* (1993) stated that frozen yoghurt can be prepared in a conventional manner but subsequently deep frozen to -20 °C. It required higher level of sugar and stabilizer for maintaining the consistency of the coagulum during freezing.

2.2 Origin of yoghurt

Yoghurt is derived from the Turkish word “Jugurt” used for any fermented food with acidic taste (Younus *et al.*, 2002). It is likely that the origin of yoghurt was from Middle East after domestication of milk producing animals began around 9000 B.C. It is also reported that the Russian biologist IlyaIlyich Mechnikov, co-winner of 1908 Nobel Prize in physiology, had an unproven hypothesis that regular consumption of sour milk could provide protection against enteric infections and their possible role to help attain a physiological old age and normal death (Schmalstieg and Goldman, 2008). Believing *Lactobacillus* to be essential for good health, Mechnikov worked to popularize yoghurt as a foodstuff throughout Europe.

Traditionally, different bacteria have been involved in the fermentation of milk but according to the Codex Alimentarius definition (FAO, 1992); the coagulated, fermented milk product can only be called "yoghurt" if the bacteria synergically grown in the milk are *Streptococcus thermophilus* (new nomenclature: *Streptococcus salivarius* ssp. *thermophilus*) and *Lactobacillus bulgaricus* (new nomenclature: *Lactobacillus delbrueckii* sp. *bulgaricus*

2.3 Varieties and types of yoghurt

2.3.1 Varieties-

A) On basis of chemical composition-

Parameter	Regular	Low fat	Non-fat
Fat	>3.25	0.5-2	<0.5
SNF	>8.25	>8.25	>8.25
Titrable acidity	>0.9	>0.9	>0.9
PH	<4.5	<4.5	<4.5

- Adopted from FDA

B) On basis of physical nature – Solid, Semisolid or fluid

C) On basis of flavor – Plain, Flavored

D) Yoghurt related product- Pasteurized and UHT, Frozen, Dried

2.3.2 Types of yoghurt-

Balken style, Greek style, European style, French and Fruit style, respectively.

2.4 Yoghurt production

Modern yoghurt production is a well-controlled process that utilizes milk, milk powder, sugar, fruit, inoculum, colouring agent, emulsifiers, stabilizers, and specific cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (in a ratio of 1:1) for the fermentation (Robinson and Tamime, 1975).

Though the term yoghurt is usually associated with acidification of cow milk, other raw materials have been successfully employed in the production of yoghurts. The use of goat milk, sheep milk, soybean milk, coconut milk, tiger nut milk, and combinations of some of these milk sources and types are reported by several researchers (Imele, 2001; Maria *et al.*, 2002; Farinde *et al.*, 2008; Sanful, 2009a; Sanful 2009b). Yoghurt is usually prepared from normal whole milk although skim milk, full cream milk with added skim milk powder, or partially evaporated milk are also used. When whole cows' milk is used, its water content is usually reduced by about a quarter in a vacuum pan or by adding about 5% milk solids followed by water reduction. Whole milk is also sometimes fortified with dairy ingredients such as skim milk powder to increase the total solids and the concentration of protein. The milk is homogenized and pasteurized by heating at about 80-90˚C for 15 to 60 minutes, with higher temperatures requiring less time. This treatment kills vegetative bacteria and expels most of the oxygen and produces reducing substances which help to initiate and maintain anaerobic conditions in the milk suitable for the growth of the inoculums. After pasteurization the milk is cooled to 45-48˚C, care being taken to prevent the uptake of oxygen, and the inoculums is added aseptically at around 2%, by volume, with gentle mixing. Incubation is done in small bottles or cartons at about 42-45˚C for about 3 to 5 hours till coagulation occurs, or until a pH of 4.5 is attained. At the end of the incubation period the fermented product is rapidly cooled to about 5˚C to stop lactic acid production (Jay, 2000).

Apart from this general method for producing plain yoghurt, some additives such as fruit pieces, nectars, jams and honey could be added after the fermentation process to obtain different varieties of the product (Lutchmedial *et al.*, 2004). Manufacturing process of set and stirred yoghurt by Lee and Lucey (2010) is given below -

Standardization of milk

↓

Homogenization (55-65˚c and 15-20/mpa)

↓

Pasteurization (90-95˚c for 5 min.)

↓

Cooling to incubation temperature (43-45˚c)

↓

Inoculation of Standard culture (2%v/v)

Set yoghurt	Stirred yoghurt
↓	↓
Packing into individual container	Incubation (42-45˚)
↓	↓
Incubation (42-45˚)	Cooling
↓	↓
Cooling and cold storage (<4˚c)	Stirring
↓	↓
Set yoghurt	Packaging and cold storage (<4˚c)
	↓
	Stirred yoghurt

2.5 Methods of preparation

2.5.1 Type of milk

Abrahamsen and Holman (1981) reported that, yoghurt was originally made from sheep, cow and buffalo milk and sometimes from goat milk. However, goat milk may be used as a replacement for cow milk in diet in some cases with advantage for those who suffering from allergy to cow milk, there is a demand for yoghurt made from goat milk.

Gupta and Patel (1984) reported that, soy milk could be effectively used for the preparation of Dahi and Yoghurt with addition of glucose, lactose and sometimes sucrose, because the carbohydrates of soy milk namely stachyose and raffinose are in general poorly utilized by lactic acid bacteria.

Geetha *et al.* (1994) observed good quality results for yoghurt prepared from skim milk having fat<0.2 per cent fortified by 4 per cent (w/v) skim milk powder and 3 per cent (w/v) cane sugar.

Mallikarjun *et al.* (2013) prepared yoghurt from toned mixed milk (TS 11.5%) vacuum concentrated to 23% TS, using yoghurt culture mixed culture of *S. thermophilus* and *L. bulgaricus*) and its quality was evaluated by acidity, pH, penetrometer – hardness, wheying – off and sensory characteristics. The concentrated milk diluted to toned milk solids level, with and without added sugar yielded good quality yoghurt whose physico-chemical quality characteristics were comparable to those of toned milk yoghurt.

Ehirim *et al.* (2013) analyzed the proximate composition, microbiological and sensory properties of yoghurt produced from cow milk and goat milk blend. All Samples showed no significant difference ($p>0.05$) for mouth feel and colour of yoghurt samples. Generally, goat milk yoghurt samples (100%), (75%), (50%) were mostly significantly preferred to 25% goat milk yoghurt sample at ($p>0.05$).

2.5.2 Standardization

Kulkarni and Vishweshwairaih (1983) suggested that the composition of milk is adjusted either by concentration or by incorporating skim milk powder, whole milk powder and concentrated milk etc.

Dave (1984) stated that, milk for yoghurt making should be standardized in order to ensure consistency of the end product and to exercise control over flavour, viscosity, and nutritive value. It has been suggested that the minimum milk solids not fat should be 8.5 per cent and fat 3 per cent.

According to Sinha (1984) milk should be standardized and should be of fixed compositional quality in order to ensure consistency of the end product.

Shukla *et al.* (1986) stated that, the fat per cent in milk for yoghurt preparation was adjusted using skim milk and cream, and solid not fat content adjusted with the addition of skim milk powder.

Tamime and Robinson (1988) reported that methods employed for the standardization of fat in the milk by removal of fat from full cream milk, mixing full cream with skim milk, addition of cream to skim milk and the use of standardizing centrifuge for yoghurt making.

Chawla and Balchandran (1993) reported that high fat content in milk helped to reduce yoghurt setting time.

Chawla and Balchandran (1994) reported that skim milk powder addition is widely used in the industry to increase the SNF level in the liquid milk for producing thick consistency yoghurt. However, excessive addition may adversely affect yoghurt quality.

2.5.3 Heat treatment

Iyenger *et al.* (1967) studied the effect of heat treatment on buffalo and cow milk in the manufacturing of yoghurt. The consistency and body was superior with buffalo milk whereas the flavour score was better for cow milk.

Campbell and Marshall (1975) reported that, high heat treatment of milk used in manufacturing of yoghurt was essential to denature whey proteins, this increasing the capacity of protein to bind water and liberate amino acids that promote growth of *Lactobacillus bulgaricus.*

Nelson (1975) studied the duration of heat treatment of milk in yoghurt manufacture using milk with 8.9 to 10.2 per cent SNF. The milk was heated at 75 to 95°C for periods of 15 to 20 minutes. The best results were obtained when the milk was heated at 90 °C for 5 to 10 minutes.

Dave (1984) reported that milk containing 9.5 to 12.00 percent total solids results good quality yoghurt when milk is heated at 90 ^{0}C for 5 minute.

Labropoulous *et al.* (1984) suggested three methods for heat treatments of raw whole milk which is used for yoghurt preparation.i.e.Ultra heat treatment (UHT) at 149°C for 3.3 seconds, Minimum vat pasteurization at 63°C for 30 minutes, Conventional Vat system at 82°C for 30 minutes.

Sarkar (1995) revealed that indirect heating of milk is preferred than direct heating by steam injection as the later treatment resulted in a yoghurt with a more fragile structure. In commercial application, milk is generally heated to either 85°C for 30 minute or 90 to 95°C for 5 minutes during yoghurt manufacture.

2.5.4 Starter culture used

The important role played by starter culture organisms in successful production, which could determine the success or failure in quality of yoghurt. It is an indisputable fact that the enormously increasing popularity of yoghurt is primarily based on the cultural and biochemical performance of lactic cultures namely, *S. therrnophilus and L. bulgaricus.*

Nikolvin (1960) observed that, good quality yoghurt having typical flavour could be obtained when the mixed culture contained equal proposition of S. *thermophilus and L. bulgaricus.*

Madanlal *et.al* (1978) reported that, successful preparation of yoghurt depends upon proper symbiotic growth between S. *thermophilus and L. bulgaricus* in proportion of 1:1 produced better product than the other combination.

Khanna and Singh (1979) while working on the type of milk used for yoghurt making, comparison of cultures for flavour production observed that *L. bulgaricus* showed greater proteolytic activity than S. *thermophilus.*

Deeth (1984) reported that, *L. bulgaricus and S. thermophilus* produced antibacterial substance in milk products.

Mittal (1984) reported that, soy milk is satisfactory medium for the growth of lactic cultures. Lactic cultures could be successfully used for manufacturing of fermented product of soy milk.

Gupta *et al.* (1997) prepared acidophilus yoghurt by L. *acidophilus* 301 and *L. acidophilus* 1899 individually or in combination. They showed that acid production by combination of (1:1) was greater than produced by either of strain individually.

Kongphoothorn *et al.* (2005) to develop soya milk yoghurt lactic acid bacteria isolated from soya milk, *Enterococcus faecium* KUA29, *Pediococcus pentosaceus* KUB3, *P. pentosaceus* KUC1 and *Lactobacillus plantarum* KUC7 supplemented with 2% isolated soya protein and incubated at 37 and 42 ^{0}C for 12 hours. Changes in the content of some components including pH, titratable acidity and viable counts of lactic acid bacteria during fermentation were examined. Results revealed that *P. pentosaceus* KUC1 and *L. plantarum* KUC7 incubated at 37 degrees C was better than 42 ^{0}c, pH was reduced from 6.53 to 4.05, titratable acidity of lactic acid was increased from 0.14 to 0.63%, viable cell counts were 9.81 Log CFU/ml after fermentation. There was no coliform bacteria, yeast and mould. Its viscosity was 1176 centipoise, 4.10% protein. Sensory evaluation for texture, colour, flavour and sourness were acceptable. Results show that soymilk yoghurt with lactic acid bacteria could be a novel type of health food.

Ersan and Kurdal (2014) studied the yoghurt and bio-yoghurt using different commercial probiotic combinations of

S. thermophilus, L. bulgaricus, L. acidophilus, Bifidobacterium ssp., *L. lactis* and *L. casei*. The samples were analyzed for microbiological, physico-chemical and sensorial properties at a 5-day interval during storage. Culture combinations and storage time significantly influenced some properties of the samples. While titratable acidity and lactic acid (%) increased syneresis, pH, lactose and acetaldehyde decreased during storage. Changes in fat, ash, protein contents of yoghurt samples during storage period were not remarkable. Viable probiotic bacterial counts in all bio-yogurts were above 10^7 c.f.u g-1 at the end of storage.

2.6 Biochemistry of yoghurt production

In yoghurt manufacture, the high heat treatment of milk prior to fermentation leads to the interaction between whey protein and casein. Exposure of previously buried hydrophobic groups in the unfolded whey proteins promotes hydrophobic interaction which will later be crucial to gel formation during the fermentation process (Smits and Van Brouwershaven, 1980).

Fermentation begins with *Streptococcus thermophilus* which grows faster than *Lactobacillus bulgaricus,* increasing the acidity of the milk and producing anaerobic conditions so that the milk becomes more suitable for the rapid growth of the latter. The *S. thermophilus* is responsible for initial acidification of the milk and together the two lactic acid bacteria (LAB) can produce more acid than when either is used alone. Once the *Lactobacilli* have started growing the acidity increases further and substances are produced which are beneficial for the continued growth of the streptococci.

These LAB ferment about 35% of the lactose in milk through hydrolysis to glucose and galactose. Only the glucose is changed into lactic acid, while the galactose moiety is released mainly by the coccus into the extracellular environment (Goodenough and Klein, 1976).

The lactic acid produced acts on milk protein to give yoghurt its texture. During acidification, the unfolded whey proteins (caused by heat treatment), which are either associated with casein micelles or free in the serum, interact with each other when the pH is close to their iso-electric point (pH 5.2–5.3), causing gel formation dominated by protein-protein interactions. This is an important stage of yoghurt formation, which when not properly executed may cause a deformation in the gelation, and an eventual poor mouth feel of the final product. Robinson (1981) reported that slow acidification of milk to form yoghurt causes development of grains in yoghurt. As fermentation progresses and pH continues to reduce, there is rearrangement of the gel network due to aggregation of casein particles as they reach their iso-electric point (pH 4.6). This eventually leads to casein-casein interactions dominating the gel network (Lucey *et al.*, 1997; Lucey *et al.*, 1998; Lucey and Singh, 1998).

2.7 Additives used

Shabaan *et al.* (2011) conducted study to investigate the antifungal and food additive potential of medicinal plants. Herbal decoction and essential oil (EO) extracts of *Cymbopogon flexuosus* (lemongrass) leaves and stems were tested for their inhibitory action against spoilage organisms and mycotoxins formation in two separated experiments. The results indicate that the addition of the appropriate EO concentration (0.1%, w/v) improved the physicochemical properties as well as sensory characteristics of yoghurt, could be used for decontamination of dairy products such as yoghurt from mycotoxigenic fungi and mycotoxins formation, beside its beneficial properties as a functional food.

Dari (2013) studied with main objective of the research is to assess the proximate composition of the fruit and also develop a new product (butternut squash yoghurt) using butternut squash. Butternut squash was acquired from Kukobila in the Savelugu district of Northern region, Ghana and samples prepared for proximate analyses and the development of the new product; butternut squash yoghurt. Butternut squash yoghurt was prepared following the process for preparing Greek yoghurt in the proportion of 2 kg of butternut squash to 360 g of skimmed milk. Finished butternut squash yoghurt was analyzed for proximate composition, and consumer acceptance using a taste panel of sample size thirty.

Leder and Thomson (1973) used gelatin at 0.1 to 1.0 per cent concentration in the manufacture of plain and fruit yoghurt from skim milk with 0.5 to 3.5 per cent fat and whole milk. For reducing whey separation in yoghurt, addition of 0.3 - 0.6 per cent high bloom gelatin gave good results. The gelatin suppressed mealiness in yoghurt and moderated flavour.

Shukla *et al.* (1986) reported that the wheying off is major defect in yoghurt. Therefore, stabilizers and additives are used to check wheying off in yoghurt. Gelatin, carboxyl methyl cellulose (CMC), gum acacia and sodium alginate were used at 0.1 per cent.

Rossi *et al.* (1990) studied the concentration of stabilizers, such as gelatin, guar gum and xanthan gum for improvement of quality of soya whey yoghurt. Optimum stabilizers combination (g/ litre) was for gelatin 1.27, Guar gum 2.32 and xanthan gum 0.19. The combination gave a better texture than that obtained with gelatin alone.

Jawalekar *et al.* (1993) showed that gelatin was the most suitable stabilizer for improving the body and texture of yoghurt, significantly with reduced whey separation. Rheological properties like viscosity were significantly improved by using gelatin.

Gupta and Prasad (2000) studied the effect of stabilizer on body and texture. Incorporation of 0.3 - 0.6 per cent gelatin could reduce the whey separation satisfactory without addition of skim milk powder in yoghurt. But more than 0.7 percent gelatin causes typical jelly like structure.

Lee *et al.* (2011) revealed that the concentrations (1 and 3%, w/v) of yam powder could be used to produce yam powder-added yogurt without significant adverse effects on physicochemical, microbial and sensory properties, and enhance functional components from the supplementation.

Mbaeyi *et al.* (2014) evaluated flavoured yoghurt enriched with soursop (Annona muricata). The most acceptable flavoured yoghurt contained 60% yoghurt and 40% soursop pulp and had a general acceptability of 7.15. Soursop could be used to produce acceptable beverage.

Sinham *et al.* (2014) performed quality evaluation of set yoghurt supplemented with turmeric powder and concluded that the effect of turmeric powder was significant on the physico-chemical properties of the set yoghurt. Furthermore, the addition of turmeric powder significantly affected the microbiological quality and organoleptic characteristics of the set yoghurt.

Jaylalita *et al.* (2015) formulated value enriched yoghurt with soymilk and mango pulp. There were significant changes in protein and total solids content in control yoghurt and value enriched yoghurt. Highest values for protein and SNF of yoghurt with 30% soy milk and 15% mango pulp were 7.12 and 14.31% respectively.

Kavas *et al.* (2016) worked on Probiotic Yoghurt (PY) by adding skimmed milk powder (5% w/v) and probiotic culture (7% w/v) (*Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium* ssp.) to heat-treated camels (*Camelus dromedarius*) milk with strawberry guava fortification. In all PFY samples, the relationships between fat content, sahlep ratio and fruit fortification with the viability of the microorganisms were significant ($P < 0.05$).

2.8 Chemical composition of yoghurt

Yoghurt is similar to milk in its overall composition from which it made, but certain compositional differences are likely to takes place owing to higher total solids, use of additives and fermentation brought about by starter culture.

Grosser (1978) reported the following composition of yoghurt;

Sr. No.	Composition	Percentage
1	Milk fat	2
2	Milk SNF	10
3	Sucrose	9
4	Corn syrup solids	9
5	Stabilizer	0.3
6	Emulsifier	0.2
7	Total solids	30.5

Tomar (1988) reported the composition of typical full fat yoghurt as follows:

Sr. No.	Composition	Percentage
1	Carbohydrates	4.9
2	Proteins	3.9
3	Fat	3.4
4	Calcium	0.14
5	Phosphorous	0.11
6	Sodium	0.04
7	Potassium	0.18

2.9 Nutritive and therapeutic value of yoghurt

Campbell and Marshall (1975) stated that, yoghurt contained more quantities of proteins, lactose and B – vitamins with good digestibility.

Bhandari (1983) stated that yoghurt restored helpful bacteria. Yoghurt has been successfully used in treating gastrointestinal disturbances like hepatic diseases, loss of appetite and intestinal intoxication. Like milk, yoghurt is a good source of both protein and minerals and was tolerated by those who cannot normally digest cow or buffalo milk.

Balsubramanya *et al.* (1984) reported that yoghurt should have higher levels of available calcium and phosphorus than milk from which it was made. The available calcium and phosphorus from yoghurt were high about 7 per cent and 11 per cent respectively than milk.

Tomar and Prasad (1989) reported that inclusion of yoghurt in diet had a hypocholesteromic effect on human subjects.

Malik (1993) stated that there was a second generation of bacteria, which could be used in yoghurt. These were known as *Bifidobacetrium spp.* and *L. acidophilus.* Both existed naturally in a healthy gut, where they aid digestion, help to keep restriction on harmful bacteria and guard against intestinal upset. *Bifidobacetrium Spp.* has pushed into the spotlight recently for its potential benefits in intestinal disorders. It may also correct large bowel flora imbalances caused by antibiotics or conditions such as cancer, liver or kidney disease and impaired gastrointestinal function in the elderly.

Patel and Schauen (1997) reported that yoghurt and other fermented products are reported to be effective in the treatment of a variety of disorders including colitis, constipation, and diarrhea, recolonization of the intestine with pathogens after antibiotics treatment, flatulence, gastric acidity, gastroenteritis, gingivitis, hypercholesterolemia and hepatic encephalopathy.

Sarkar and Mishra (2003) revealed that the dietetic culture could be stored for 7 days at 8 ± 1°C with all desirable characteristics. The viable counts of probiotic culture in the products were within the range required for successful seeding in the intestine.

2.10 Sensory evaluation

Kumar *et al.* (2004) reported that the effect of stabilizer addition on physicochemical, sensory, textural properties and starter culture counts of mango soymilk fortified yoghurt (MSFY) were studied. Three stabilizers, namely gelatin, pectin and sodium alginate, were used. The addition rate of stabilizer was 0.2%, 0.4% and 0.6%, w/w. Gelatin gave better effect on appearance and colour, body and texture, flavour and overall acceptability in comparison with other stabilizers at 0.4% addition rate.

Yang *et.al* (2010) prepared soyyoghurt and concluded that, with appropriate germination, the physicochemical and textural properties as well as sensory characteristics of soy-yoghurt can be improved significantly.

Ndife *et al.* (2014) investigated the potential of producing acceptable symbiotic yoghurt enriched with coconut-cake. Yoghurt samples A (Control), B, C and D were produced at 0%, 10%, 20%, and 30% of milk substitutions with coconut-cake. The sensory evaluation result showed significant differences ($0.05<p$) in all the organoleptic attributes analyzed. Sample D

with 30% coconut inclusion had the highest overall acceptability score.

Ukwo (2015) Studied sensory attribute of plain yoghurt blended with cowmilk and soymilk. The result of sensory evaluation revealed that substituting cowmilk with soymilk upto the level of 50% had no significant ($p>0.05$) effect on the appearance, taste, aroma and overall acceptability of the produced yoghurt.

Amal *et al.* (2016) prepared fruit flavoured yoghurt and suggested that yoghurt containing papaya pulp had the highest overall acceptability score as compared to other fruit yoghurt sample and also plain yoghurt.

2.11 Lemongrass distillate

The scientific name of lemongrass is *Cymbopogon citratus*. The *Cymbopogon* word derives from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the arrangement of the spike of the flower. The word *citratus* derives from the old Latin, meaning lemon-scented leaves (Shah *et al.*, 2011). This grass is native to India (Parikh & Desai, 2011). Because of its pleasant flavor, in Mexico is consumed as infusion in water or milk just because the herbs intake is a custom in the Mexican population (Juárez-Rosete *et al.*, 2013). Lemongrass (*Cymbopogon citratus*) is common in drinks, a sweet ingredient in exotic foods and recognized as a medicinal herb. The volatile compounds in lemongrass that provide its desirable flavor are neral, geranial, limonene, citronellal, caryophyllene, 6-methyl hept-5-en-3-one, linalool and beta-myrcene (Ashurst, 1999; Kasali *et al.*, 2001; Schaneberg, 2002). The essential oil of *Cymbopogon citratus* has shown to have anti-inflammatory, analgesic and antipyretic properties, (Gbenou *et al.*, 2013) besides having antimicrobial effects (Hammer *et.al* 1999; Pranoto *et.al* 2005; Adukwu *et.al* 2012). Tzortzakis & Economakis (2007) reported that the essential oil of lemongrass inhibited the growth of *Botrytis cinerea*.

Gagan *et al.* (2011) identified compounds in *Cymbopogon citratus* are mainly terpenes, alcohols, ketones, aldehyde and esters. Some of the reported phytoconstituents are essential oils that contain citral α, citral β, neral, geraniol, citronellal, terpinolene, geranyl acetate, myrecene and terpinol methylheptenone. The plant also contains reported phytoconstituents such as flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apiginin. Studies indicate that *Cymbopogon citratus* possesses various pharmacological activities such as antiamoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties. Various other effects like antimalarial, antimutagenic, antimycobacterial, antioxidants, hypoglycemic and neurobehavioral have also been studied. These results are very encouraging and indicate that this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects.

Tover *et al.* (2011) performed distillation of lemongrass using short path distillation process. High-quality essential oil was confirmed because of citral concentration increases from 19.816 mgcitral·mL–1 (initial sample) to 40.963 mgcitral·mL–1 (at 120 °C and 4.5 mL·min–1), reaching a concentration of the bioactive compound (citral) in the distillate stream of 2.1 times the concentration in the original sample. The density, dynamic viscosity, and free fatty acids were 0.901 g·cm–3, 2.069 mPa·s–1, and 1.26 wt. % (oleic acid), respectively, satisfying the oil quality criterion and avoiding thermal degradation.

Mirghani *et al.* (2012) investigated bioactivity of lemongrass (*Cymbopogan citratus*) essential oil. GCMS analysis revealed the major constituents of the lemongrass essential oil which compromise 67.769% and 67.328% of the total oil respectively. In this study, with the total of geranial (44.29%), neral (31.36), geraniol (10.01%), limonene (6.09%) and β-myrcene (3.56%), comprising 95.31% of the total oil.

Tajidin *et al.* (2012) analyzed chemical composition and citral content in lemongrass essential oil at different maturity stages and he found that (β-myrcene, 3-undecyne, neral, geranial, neral, Geranyl acetate and juniper camphor) had a concentration of greater than 1%. The citral content at 6.5 months after planting was higher by 11.4% than at 5.5 months after planting. The citral content decreased by 5.4% when lemongrass was harvested at 6.5 compared to at 7.5 months after planting. Citral content peaked at 6.7 ± 0.3 months after planting. Thus, maturity stage at harvest influenced essential oil and citral contents of lemongrass. Therefore, lemongrass should be harvested at the appropriate level of maturity in order to achieve high quality essential oil and lower production cost.

Mohammed *et al.* (2014) suggested Lemongrass essential oil (LGEO) as a potentially valuable antifungal and anti-inflammatory agent for the prevention and treatment of acute inflammatory skin conditions. Furthermore, there is growing evidence that LGEO in vapor phase is an effective antifungal system that has several advantages over the liquid phase, such as greater potency so that lower doses are required for the same effect.

Olorunnisola *et al.* (2014) given review on 'Biological properties of lemongrass' and concluded that Lemongrass tea contains several biocompounds in its decoction, infusion and essential oil extracts. Anti-oxidant, anti-inflammatory, anti-bacterial, anti-obesity, antinociceptive, anxiolytic and antihypertensive evidences of lemongrass tea were clearly elucidated to support initial pharmacological claims. Lemongrass tea was non-toxic, non-mutagenic and receives wide acceptance among alternative medicine practitioners in several developing countries. Traditionally, tea made from lemongrass leaves is popular among countries of South America, Asia and West Africa having been widely utilized as antiseptic, antifever, antidyspeptic, carminative and anti-inflammatory effects. Others are febrifuge, analgesic, spasmolytic, antipyretic, diuretic, tranquilizer and stomachic agent (Sawyerr, 1982, Viana *et al.*, 2000, Negrelle and Gomes, 2007; Adejuwon and Esther, 2007; Tatiana *et al.*, 2011).

Component	Activity	References
Myrcene	Antibacterial	Grace *et al.*(1984)
Citral	Antibacterial	Grace *et al.(1984)*
Geranial	Antibacterial	Viana *et al.(2006)*
Neral	Not available	Dharmendra *et al.(2001)*

Sirinath *et al.* (2014) used Lemongrass or pandan leaf extracts for improving flavor of soy ice-cream. In which

Lemongrass or pandan leaf extracts with water in concentrations of 10:100, 15:100, and 20:100 w/w were examined using sensory evaluation for the best flavor acceptance. The best concentration of fresh lemongrass or pandan leaf extracts for improving the sensorial flavor of soy ice cream was 10:100 (w/w). The beany flavor in soy protein isolate solution and soy ice cream was mainly composed of hexanal, pentanal, benzaldehyde, 2-pentyl-furan and 1-octen-3-ol. The flavor compounds in pandan leaf extract were 2-acetyl-1-pyroline and 3-methyl-2(5H)-furanone, while those of lemongrass extract were β-myrcene, α-pinene, 3-carene, neral, geranial and geraniol. This indicated that lemongrass and pandan leaf extracts could mask the beany flavor and improve the sensorial quality of soy ice cream.

Maria *et al.* (2015) showed following chemical compound content in lemongrass distillate;

Chemical compound	%	Chemical compound	%
3-Methyl-2-butenal	0.16	Geranial 22.63	22.63
Neral	1	Methyl acetate	2.56
Limonene	0.46	Oxiranecarboxaldehyde,3methyl-3-(4-methyl-3-pentenyl)	25.29
Citronellal	0.21	Cis-pulegone oxide	3.25
2-cyclohexen-1-one	0.33	Neric acid	9.19
Cis-linalool oxide	0.4	Carotol	0.88
Linalool	0.86	7-methyl-tetradecen-1-ol-acetate	0.25

2.12 Microbiological assessment

Ghalem *et al.* (2013) assessed microbiological properties of yoghurt enriched with *Rosmarinus officinalis* oil. The total and fecal coliform count, *Staphylococcus aureus* count, *Salmonella* count, yeast and mold counts were determined in yoghurt samples at two, seven and 21 days of storage period. One notes complete absence of the total and fecal coliforms, *Staphylococcus aureus*, *Salmonellas* yeast and mould in the two categories of yoghurt.

Erturk *et al.* (2014) investigated microbiological properties of herbal yoghurt and found that only C samples containing *C.sinensis* extract showed a weak antibacterial activity against the *Staphylococcus aureus,* the other samples did not show any antibacterial activity against the *S. aureus.* The samples of dioca extract added sample (B) and C showed the highest antibacterial activity against the *Pseudomonas aeruginosa* and *Escherichia coli.* The antibacterial activity against the Gram-positive bacteria was pronounced more than the Gram-negative.

Kirti and Sangita (2014) analyzed herbal yoghurt fortified with cinnamon and revealed that total viable count of control and herbal yogurt and found that treatment Ci3 (100.2) for *L.bulgaricus* and (44.6) recorded highest viability for *S.thermophilus.*

CHAPTER III
MATERIAL AND METHODS

The present investigation entitled "Effect of lemongrass distillate on physico-chemical properties of yoghurt" was carried out at the Department of Animal Husbandry and Dairy science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.1 Materials

Following materials were used in laboratory for analytical purpose during the entire research work.

3.1.1 Chemicals

All the chemicals used in study for the analytical purpose were of analytical reagent (AR) or guaranteed reagent (GR) grade Merk, India Ltd /Glaxo India Ltd. throughout study.

3.1.2 Glassware

The borosil glassware's were used to analyze milk and yoghurt etc.

3.1.3 Electronic balance

The electronic precision balance (BT 2245, Sartorius ISO 9001) was used for weighing samples and chemicals during research work.

3.1.4 Media Ingredients

The chemicals and ingredients of Qualigens, Glaxo India Ltd. were used for preparation of different media required for microbiological examination of yoghurt.

3.1.5 Hot Air Oven

The hot air oven manufactured by York Scientific Private Limited was used for sterilizing the glassware's throughout the study period.

3.1.6 Incubator

The digital temperature controlled B.O.D. incubator manufactured by M/S. Neutronic, Bombay was used for incubation of *Lb. acidophilus*, coliform and yeast and moulds counts from the prepared products.

3.1.7 Autoclave

To sterilize/autoclave the microbiological media and buffer solutions, Equitron autoclave manufactured by M/S Medical Instrument Company, Bombay was used throughout the study.

3.1.8 Colony Counter

A colony counter with magnifying lens and hand operated tally operator was used for counting the colonies, developed by microorganisms.

3.1.9 Laminar Air Flow

An instrument manufactured by Kirloskar Electrodyne Ltd. was used for microbiological work.

3.1.10 Digital pH meter

Digital pH meter manufactured by Systronics (India) Limited, Ahmedabad was used.

3.1.11 Thermometer

Thermometer made by M/S Type thermometer was used.

3.1.12 Muffle furnace

Tempo make (India) muffle furnace was used for determination of ash content in the samples.

3.1.13 Milk

The fresh, clean, composite samples of crossbred cow milk were procured from Research Cum Development project on Cattle, Department of Animal Husbandry and Dairy Science, Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.1.14 Lemongrass distillate

Lemongrass distillate was obtained from All India Co-ordinated Research Project on Medicinal and Aromatic Plant (Dhanvantari medicinal garden, MPKV, Rahuri).

3.2 Starter culture, its maintenance and propagation

The freeze dried mix culture of *Lactobacillus bulgaricus* and *streptococcus thermophillus* (strain- NCDC 145) were procured from the National Collection of Dairy Cultures (NCDC), Division of Dairy Microbiology, National Dairy Research Institute, Karnal, Haryana (India). The cultures were maintained in sterilized skim milk test tubes.

The sterilized skim milk test tubes were separately inoculated with these cultures and incubated at 42 ^{0}C for 8 hrs and there after stored at refrigerated temperature. In order to keep these cultures active, they were propagated at frequent intervals.

3.3 Preparation of yoghurt added with lemongrass distillate (*Cymbopogon flexuosus*)

The various technological and analytical methods are outlined as under.

3.3.1 Phase I: Preliminary trials

Preliminary trials were conducted to finalize the levels of lemongrass distillate in yoghurt. The samples of product were subjected to sensory evaluation. On the basis of the results of sensory evaluation, treatments were finalized for experimental trials.

3.3.1.1 Optimization of lemongrass distillate

Acceptability of extent of lemongrass distillate level (1,0.8,0.6,0.3,0.1,0.05,0.04,0.03,0.02,0.01%) in the finished product by the judges was determined by preparing product samples having variable levels of lemongrass distillate. On the basis of results of sensory evaluation, most acceptable lemongrass distillate levels i.e. 0.01, 0.02, and 0.03% were selected for final experimental trials.

3.3.1.2 Preparation of yoghurt

The yoghurt was prepared as per the method given by Gupta and Prasad, (2000) with slight modifications.

Flow chart for preparation of yoghurt

Milk

↓

Filtration

↓

Heat treatment (93-95^{o}C for 15 min.)

↓

Cooling at 42 ^{o}C

↓

Inoculation with yoghurt culture @ 2% (v/v) (*S. thermiphillus* and *L. bulgaricus*

↓

Filling of container

↓

Incubation (42 ^{o}C for 6 to 8 hrs)

↓

Storage (6±2 ^{o}C)

3.3.2 Phase II: Experimental trials

Yoghurt was prepared by using standardized procedure as finalized in preliminary trials.

Treatments

Three levels of lemongrass distillate viz., 0.01, 0.02 and 0.03 per cent selected in the preliminary trials were included for preparation of yoghurt in this part of experiment. Thus, yoghurt prepared in all four treatment combinations as given below were studied in detailed.

Treatment combinations

Treatment

T_0: plain yoghurt (without addition of lemongrass distillate)

T_1: plain yoghurt + 0.01 per cent lemongrass distillate

T_2: plain yoghurt + 0.02 per cent lemongrass distillate

T_3: plain yoghurt + 0.03 per cent lemongrass distillate

The prepared fresh and refrigerated stored samples of yoghurt were studied for sensory and microbiological qualities.

Preparation of lemongrass added yoghurt

Receiving milk

↓

Filtration/Clarification

↓

Pasteurization of milk 93^{0}C for 15min

↓

Cooling milk (42^{0}C)

↓

Addition of lemongrass distillate

↓

Inoculation with starter culture @ 2%

↓

Incubation at 42^0 C (8 hours)

↓

Cooling and storage (5^{0}C)

3.4 Chemical analysis of milk

3.4.1 Fat

The fat content of yoghurt was determined as per Gerber method described in IS: 1224, Part I, 1977.

3.4.2 Protein

The protein content was determined by Micro-Kjeldahl method as per the procedure recommended in IS: 1479 (part-II) (1961).

3.4.3 Total Solids

The total solids content was determined as per the gravimetric method given in IS: 1479 Part-II (1961).

3.4.4 Moisture

The moisture content was determined as per the method given in IS: 1479 Part-II (1961).

3.4.5 Titratable acidity (% lactic acid)

The titratable acidity was determined as per the method given in IS: 1479, Part –I (1960).

3.4.6 Lactose

The lactose content was determined as per IS: 1479 (Part-II) 1961, By Lane and Eynon method.

3.4.7 Ash

The ash content was determined by as per IS: 1981.

3.4.8 pH

The pH of the health was determined by using Systronic digital pH meter.

3.5 Sensory Quality of Yoghurt

The samples of yoghurt were evaluated for sensory qualities by using 9 point hedonic scale as per IS: 6273, (Part-II), 1971 described by Gupta (1976) for various sensory attributes. The flavour, colour and appearance, body and texture and overall acceptability were the characteristics considered for the purpose of scoring the products (Appendix-I).

3.6 Chemical Analysis of Yoghurt

3.6.1 Fat

The fat of yoghurt samples were determined by Gerber method as described in IS: 1224, Part-I (1977). Exactly 10gm of well mixed sample of yoghurt was weighed in a glass beaker. Three to four ml hot distilled water was added to make the paste. The 10 ml Gerber sulphuric acid (90:10, acid: Distilled water) was then transferred into the same beaker. The content of the beaker was quantitatively transferred into butyrometer followed by washing the beaker with 1 ml Isoamyl alcohol and 2 to 3 ml hot distilled water and then transferred into butyrometer. The butyrometer was properly stopper and the content was vigorously shaken to digest nonfat substances. Liquid level in the butyrometer was brought to calibration by addition of required amount of distilled water. It was then centrifuged in Gerber centrifuge machine for 5 min. Fat column was read after tempering butyrometer for 5 min. in water bath maintained at 65 ^{0}C and then recorded the fat percent of fermented drink sample.

3.6.2 Protein

The protein was determined by estimating the per cent nitrogen by Micro-Kjeldahl method as recommended in IS: 1479 Part-II (1961). The per cent nitrogen was multiplied by 6.25 to find out protein percentage.

Three gram of sample of yoghurt was taken in Kjeldhal's flask. It was added with 10 g of catalyzed mixture (K2SO4: CuSO4-=1:10). The 25 ml of pure nitrogen free H_2SO_4 and 5ml of H_2O_2 was added. The flask was heated gently in an inclined position when the frothing was continued strongly, taking care that the liquid boiled moderately the flasks was rotated occasionally to wash down any carbonaceous matter remaining on the side of the flask, when the content became clear, it was allowed to cool. This was diluted with water and content were transferred into a distillation flask with 100 ml of ammonia free water. A 25 ml of 40 per cent NaOH was added to the flask, so as to form a distinct layer at bottom. A 20 ml of 4% Boric acid was taken in beaker; to this few drops of methyl red indicator was added. This was then placed below the condenser tube at the receiving end of the distillation apparatus. The steam containing ammonia vapors was condensed by the condenser and was collected in Boric acid solution in a beaker, when about 200 ml of distillate were collected the tube was disconnected and the end of the condensing tube was washed with distilled water into the beaker containing the distillate. The distillate was titrated with 0.01 N HCl solution till methyl red indicator changed from red to yellow when end point reached. A blank was run simultaneously. The per cent nitrogen in the sample was calculated by using following formula.

$$\% \text{ Nitrogen in sample} = \frac{(A-B) \times N \times 0.014 \times \text{vol.made} \times 100}{\text{Weight of sample}}$$

Where A = burette reading B = blank reading N= Normality of working solution

% protein = % of Nitrogen in fermented drink sample x 6.25

3.6.3 Lactose

The lactose content of yoghurt was determined as per procedure of Lane-Eynon in SP: 18 (Part-XI, 1981).

Reagents

1. Fehling 'A' solution: It was prepared by dissolving 35 g copper sulphate ($CuSO_4$) in 500 ml distilled water.
2. Fehling 'B' solution : It was prepared by dissolving sodium potassium tartarate (173g) and potassium hydroxide (120g) in small quantity of water and then the final volume was made upto 500 ml with distilled water.

3. Acetic acid 10% solution
4. Methylene blue indicator (1% in ethyl alcohol)
5. Saturated sodium dihydrogen phosphate (saturated solution)

Procedure

The 10 g of samples of yoghurt was transferred into a 250 ml conical flask and diluted with 150 ml of water. The content of the flask was mixed very thoroughly. Added 6 ml acetic acid (10 %) drop by drop, until no further precipitate was formed. Then it as allowed to stand for a few minutes. The flask was rotated at intervals to ensure complete precipitation of protein. All content of flask were filtered through a fluted 18 cm No. 1 filter paper into a 250 ml graduated flask, then washed the precipitate and the paper thoroughly with hot water collecting the washings in the conical flask. Then volume was made up to mark. The titration was carried out against Fehling solution. Then the percentage of lactose was calculated with help of following formula.

$$\text{Lactose (\% by weight)} = \frac{67.84}{\text{Burette reading}}$$

3.6.4 Ash

The ash content of yoghurt was determined as per the procedure given in ISI (1981).

10 g of sample of yoghurt was weighed in crucible, heated gently until moisture was almost evaporated and then it was placed in muffle furnace maintained at 550^{0}C till the ignited material was free of carbon (4 to 5 hrs).

$$\text{Ash (\% by weight)} = \frac{W_2 - W}{W_1 - W}$$

Where,

W = weight of crucible

W_1 = weight of crucible + sample

W_2 = weight of crucible + Ash

3.6.5 Total solids and moisture

The total solids content of sample of yoghurt was determined as per procedure given in IS: 1966 (Part-II, 1983). The aluminum dishes were heated, cooled and weighed. The 5 g of sample of yoghurt in duplicate was weighed accurately and dried in oven at 105 ^{0}C for 5 hrs. After cooling in desiccators, it was reweighed. The drying was repeated for one more hour and cooled in desiccators as above and reweighed until constant weight was obtained. The mean loss in weight was taken as moisture content. Total solid and moisture content calculated by following formula.

Total solids (%) = 100- Moisture (%)

$$\text{Moisture (\% by weight)} = \frac{100\,(W_1 - W_2)}{W_1 - W}$$

Where,

W_1 = Weight in gm of the dish with yoghurt before drying

W_2 = Weight in gm of the dish with yoghurt after drying

W = Weight in gm of the empty dish

3.6.6 Acidity (% lactic acid)

The acidity of sample yoghurt was determined by using method prescribed in IS: 1479 (Part I), 1960.

The 10 g of yoghurt was weighed into a conical flask. The product was mixed properly by using 3 ml of warm distilled water (65 ^{0}C). Then 10 ml of warm distilled water was added. After adding 1 ml of 0.5 per cent phenolphthalein indicator, the contents were titrated against 0.1 N sodium hydroxide with continuous stirring till the pink colour appears. The acidity was expressed as lactic acid per 100 g of drink.

$$\text{Acidity (\% LA)} = \frac{\text{B.R} \times \text{Normality of NaOH}}{\text{W}} \times 0.091 \times 100$$

Where,

V= Volume of 0.1 N NaOH required for titration

W= Weight of the sample of the yoghurt

3.6.7 pH

The pH of the yoghurt was determined by using Systronic digital pH meter.

3.7 Microbiological analysis

3.7.1 Standard plate count

The standard plate count was prepared by using following ingredients in their respective quantities as follows:

Ingredients	**Weight**
Tryptophan (g)	5.0
Yeast extract (g)	2.5
Glucose (g)	1.0
Distilled water (ml)	1000
Agar (g)	15.0
pH (at 25^{o}C)	7

Mix the sample thoroughly by shaking vigorously / shredding so that uniform consistency is obtained. Transfer 1 ml of suspension from this to 9 ml dilution blank to have 1:10 dilutions. Similarly prepare further serial dilutions. Pour 1 ml portion from 10^{-6} dilution into sterile petri dishes in duplicates. Add to each plate 10-15 ml SPCA previously melted and cooled to $45^{0}C$. Mix the contents thoroughly by tilting and rotating the plates and allow the agar to set. Invert and incubate the plates at $42^{0}C$ for 24-48 hrs. After incubation, remove the plates and count all the colonies. Express the results as total viable count per gram of the product.

3.7.2 Coliform count

VRBA media Ingredients	**Weight**
Yeast extract (g)	3.00
Peptone (g)	7.0
Bile salt (g)	1.50
Lactose (g)	10.00
Sodium chloride (g)	5.00
Neutral red (g)	0.03
Crystal Violet (g)	0.002
Agar (g)	15.00
Distilled water (ml)	1000
pH (at $25^{0}C$)	7

The first and second dilutions of samples of yoghurt which were prepared has been used for enumeration of coliforms. The 1 ml from first and second dilution was taken in duplicate into petriplates and the violet red bile agar (VRBA, Appendix-II) was added and mixed well. The plates were allowed to solidify. The plates were again over layered with the same agar and allowed to solidify. These plates were incubated at 42^{0}C for 24 hrs and numbers of coliform colonies developed were counted as colony forming units (c.f.u.) per gram.

3.7.3 Yeast and mould count

The media (Potato Dextrose Agar) was prepared by using following ingredients in their respective quantities as follows,

Ingredients	**Weight**
Potato extract (g)	200
Dextrose (g)	20
Agar (g)	5.20
Water (ml)	1000

The 1st and 2nd dilutions of all samples of yoghurt were taken in duplicate into petriplates and then potato dextrose agar (PDA, Appendix-II) was added by adjusting pH 3.5 by adding 5 ml of 10 % sterilized tartaric acid. The plates were allowed to solidify and incubated at 42˚C for 5 days. Numbers of yeast and mould colonies developed were counted as colony forming units/g.

3.8 Statistical Analysis

The data obtained in the present investigation was statistically analyzed by Completely Randomized Design (CRD) as per suggested by Gomez and Gomez (1984).

CHAPTER IV

RESULT AND DISCUSSION

The results obtained in present investigation entitled "Effect of lemongrass distillate on physico-chemical properties of yoghurt" in relation to the chemical, microbiological and organoleptic attributes for different treatments are presented, collected data is tabulated, statistically analyzed and results of the present investigation have been discussed in this chapter under following headings.

4.1 Chemical analysis of cow milk

The cow milk was procured from Research cum Development Project (RCDP) On Cattle, Rahuri and analyzed for its chemical composition. The results obtained are given in Table 4.1.

Table 4.1 Chemical analysis of cow milk

Parameter	Replications				Mean ± SE
	I	II	III	IV	
Fat (%)	3.80	3.70	3.70	3.90	3.77 ±0.047
Protein (%)	3.65	3.70	3.65	3.63	3.65 ±0.014
Lactose (%)	4.39	4.30	4.44	4.46	4.39 ±0.035
Ash (%)	0.69	0.66	0.70	0.72	0.69 ±0.012
TS (%)	12.53	12.56	12.49	12.50	12.52 ±0.015
Acidity (% LA)	0.141	0.139	0.144	0.141	0.141 ±0.001

The data obtained from Table 4.1 infers that milk used for preparation of lemongrass added yoghurt had an average 3.77 per cent milk fat, 3.65 per cent protein, 4.39 per cent lactose, 0.69 per cent ash, 12.53 per cent total solids and 0.141 per cent LA acidity.

4.2 Chemical composition of Lemongrass distillate

Lemongrass contain various chemical compound, which has antioxidant, antimicrobial effect.

Table 4.2 Chemical composition of Lemongrass distillate

Sr.no	Compound	%
1	Geranial	41.4
2	Neral	33.4
3	Geraniol	6.3
4	Geranyl acetate	4.7

4.3 Chemical analysis of yoghurt

The chemical analysis of yoghurt was done for fat, protein, lactose, total solids, ash, moisture and titrable acidity etc. as per the standard procedure and results obtained are given in tabular form as follows,

4.3.1 Fat

The fat content of yoghurt was analyzed and results obtained are tabulated in Table 4.3.1.

Table 4.3.1 Fat content (%) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	2.80	2.70	2.70	2.60	2.70^a
T_1	2.50	2.50	2.40	2.30	2.43^b
T_2	2.00	2.10	2.20	2.10	2.10^c
T_3	2.08	2.08	2.07	2.09	2.08^c
SE	0.0375				**
CD at 5%	0.120				

Data obtained on fat analysis of yoghurt revealed that the influence of experimental treatments on fat content of yoghurt was significant. The mean values of fat content of treatment T_0, T_1, T_2 and T_3 were 2.70, 2.43, 2.10 and 2.08, respectively. It was observed that fat content is varied between the range of 2.08 to 2.70 per cent. The highest fat content was recorded for treatment T_0 i.e. 2.70 and lowest fat content was observed for T_3 i.e. 2.08 per cent. Fat percentage decreased as concentration of Lemongrass distillate increased. This is attributed to decreasing acidity percentage from T_0 to T_3.

These findings are in agreement with Hasan and Amjad (2010) and Kirti and Sangita (2014), who reported similar trend of decreasing fat percentage in yoghurt.

4.3.2 Protein

Protein content of the yoghurt is presented in Table 4.3.2.

Table 4.3.2 Protein content (%) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	3.15	3.19	3.16	3.13	3.16[a]
T_1	3.06	3.09	3.05	3.11	3.08[a]
T_2	2.71	2.95	2.87	2.91	2.86[b]
T_3	2.33	2.35	2.34	2.33	2.34[c]
SE	0.0279				**
CD at 5%	0.086				

It was revealed from the results presented in Table 4.3.2 that the protein content of yoghurt was significant. The mean values of protein content of treatment T_0, T_1, T_2 and T_3 were 3.16, 3.08, 2.86 and 2.34 per cent, respectively and it ranged from 2.34 to 3.16 per cent. The highest value was reported for treatment T_0 (3.16 per cent) while lowest value observed for T_3

(2.34 per cent). There was decreasing trend observed as Essential oil concentration increases in yoghurt due to decreased proteolytic activity of LAB as a number of LAB decreases.

Lutchmedial *et al.* (2004) and Ghalem and Zouaoui (2013) observed similar results with respect to protein content of yoghurt.

4.3.3 Lactose

Lactose is a major reducing sugar in milk. These lactose converted to lactic acid during fermentation. The results obtained after analysis of yoghurt were tabulated in Table 4.3.3.

Table 4.3.3 Lactose content (%) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	5.47	5.46	5.45	5.47	5.46^{a}
T_1	5.34	5.35	5.34	5.33	5.34^{ab}
T_2	5.51	5.50	5.20	5.10	5.33^{ab}
T_3	5.17	5.18	5.19	5.18	5.18^{b}
SE	0.0523				**
CD at 5%	0.161				

It was noted that the average lactose content of yoghurt found to be 5.46, 5.34, 5.33 and 5.18 for treatment T_0, T_1, T_2 and T_3, respectively. It was ranged from 5.18 to 5.46 per cent with significant difference in between them. The highest lactose content was observed for treatment T_0 (5.46 per cent) and lowest for treatment T_3 (5.18 per cent). There was decreasing trend observed with addition of EO increases. This may be attributed to the fermentation action by LAB.

The similar observations were reported by Shaaban *et al.* (2010) and Sutariya *et al.* (2013) while preparing yoghurt enriched with lemongrass extract.

4.3.4 Ash

The ash content in yoghurt includes all the major and minor mineral present in milk. The ash content of yoghurt was estimated by gravimetric method and results obtained are given in Table 4.3.4.

Table 4.3.4 Ash content (%) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	0.33	0.33	0.33	0.33	0.33[b]
T_1	0.33	0.33	0.33	0.33	0.33[b]
T_2	0.66	0.33	0.66	0.66	0.58[a]
T_3	0.66	0.66	0.66	0.66	0.66[a]
SE	0.0412				**
CD at 5%	0.1271				

From the data showed in Table 4.3.4 observed that the ash content of yoghurt was found to be significant statistically and ranged from 0.33 to 0.66 per cent. The highest ash content was reported for treatment T_3 (0.66 %) and lowest for treatment T_0 (0.33 %) treatment. The ash content of yoghurt had shown slightly increasing trend with increases in lemongrass EO concentration.

Ghalem *et al.* (2013) reported similar trend in ash content with addition of *Rosmarinus officinalus* in yoghurt.

4.3.5 Total solids

The total solids content of yoghurt were determined and tabulated in Table 4.3.5.

Table 4.3.5 Total solids content (%) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	12.03	12.05	12.51	12.04	12.16[d]
T_1	13.00	13.01	13.05	13.04	13.03[c]
T_2	13.10	13.42	13.09	13.20	13.20[b]
T_3	13.40	13.30	13.62	13.80	13.53[a]
SE	0.089				**
CD at 5%	0.27				

It is clearly indicated from Table 4.3.5 that the mean total solid content of yoghurt was12.16, 13.03,13.20 and 13.53 per cent for treatment T_0, T_1, T_2 and T_3, respectively with significant difference between the treatments. The highest total solid observed for treatment T_3 i.e.13.53 while lowest for treatment T_0 i.e. 12.16. The increasing trend of TS in yoghurt was observed with increasing content of lemongrass EO. Total solid percentage was increased due to decreased moisture percentage in subsequent treatments.

Similar results were reported by Abubakar *et al.* (2005); who conducted study on physico-chemical properties of yoghurt prepared from cow milk, whole milk and powdered milk. The results obtained in present investigation are in close agreement with the results obtained by Sutariya *et al.* (2013).

4.3.6 Titrable acidity

Titrable acidity of fermented product is important factor affecting quality of product. The acidity of yoghurt was estimated and results obtained were given in Table 4.3.6.

Table 4.3.6 Titrable acidity (%LA) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	0.67	0.66	0.67	0.65	0.66[a]
T_1	0.65	0.65	0.64	0.64	0.65[b]
T_2	0.64	0.63	0.63	0.63	0.63[b]
T_3	0.60	0.61	0.63	0.60	0.61[c]
SE	0.0046				**
CD at 5%	0.014				

From the data, it was inferred that the experimental treatments had significant effect on acidity of yoghurt. The acidity of the experimental treatments was in between 0.61 to 0.66 per cent. The acidity was highest for treatment T_0 (0.61 per cent) which had highest LAB count, which increased lactic acid production in yoghurt and observed lowest acidity for treatment T_0 (0.20 per cent) which had lowest LAB count. Titrable acidity decreased due to decreasing number of LAB in samples.

Sutariya *et al.* (2013) observed that there was decrease in acidity of yoghurt with increase in lemongrass extract level in yoghurt and lassi preparation. The similar results were also observed by Shabaan *et al.* (2010).

4.3.7 pH

The data on pH of yoghurt are given in Table 4.3.7.

Table 4.3.7 pH of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	4.13	4.12	4.13	4.15	4.13[d]
T_1	4.20	4.19	4.20	4.17	4.19[c]
T_2	4.21	4.23	4.21	4.22	4.22[b]
T_3	4.27	4.28	4.29	4.26	4.28[a]
SE	0.0062				**
CD at 5%	0.0191				

The pH of experimental treatments showed statistically significant difference and ranged from 4.13 to 4.28. The treatment T_0 showed highest pH while treatment T_3 showed lowest pH. This was due to decreasing percentage of acidity of yoghurt in subsequent treatments. Hence pH was increasing with decrease in acidity percentage.

The similar results were observed by Shabaan *et al.* (2010) and Sutariya *et al.* (2013); who reported that there was decrease in acidity of yoghurt with increase in lemongrass extract level in yoghurt and lassi preparation respectively.

4.3.8 Moisture content

Moisture content of different treatment combinations is given below in Table 4.3.8.

Table 4.3.8 Moisture percentage of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	88.00	88.01	88.05	88.00	88.03^a
T_1	87.20	87.00	87.00	87.02	87.01^b
T_2	86.90	86.98	86.99	86.04	86.53^c
T_3	86.00	86.20	86.30	86.20	86.15^d
SE	0.0922				**
CD at 5%	0.2843				

It was revealed that the moisture content of yoghurt ranges from 86.15 to 88.03 per cent and noted significant difference between the treatments. The decreasing trend in moisture content of yoghurt was observed with increase in concentration of lemongrass EO from treatment T_0 to T_3. Moisture percentage was decreased due to increased TS percentage in subsequent treatments.

Similar results were reported by Abubakar *et al.* (2005); who conducted study on physico-chemical properties of yoghurt prepared from cow milk, whole milk and powdered milk. The results obtained in present investigation are in close agreement with the results obtained by Sutariya *et al.* (2013).

4.4 Microbiological analysis of yoghurt

Microbiological analysis *viz.*, standard plate count, coliform count and yeast & mould count of yoghurt were studied and discussed below.

4.4.1 Standard plate count

The standard plate count is the colony of bacterial cells which is actively reproducing. It was estimated and results are given in Table 4.4.1.

Table 4.4.1 Standard plate count (c.f.u/ml × 10^{-6}) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	66.00	67.00	63.00	65.00	65.25[a]
T_1	57.00	59.00	55.00	53.00	56.00[b]
T_2	55.00	53.00	55.00	52.00	53.75[bc]
T_3	51.00	53.00	50.00	51.00	51.25[c]
SE	0.915				**
CD at 5%	2.822				

From the Table 4.4.1, it was inferred that the standard plate count of yoghurt showed significant difference among treatments. The range of SPC was between 51.25 to 65.25 c.f.u/ml × 10^{-6}. It was observed that the highest SPC recorded for treatment T_0 while lowest for treatment T_3. It also showed that, SPC may be dependent upon environmental conditions and pre-processing factors.

The standard plate count obtained was within the limits set by the IS: 10501 (1983) which infers that the product is safe for consumption.

4.4.2 Coliform count

From the data, it was observed that coliform count was found to be completely absent, which signifies that product was prepared in hygienic condition.

Table 4.4.2 Coliform count (x 10^1 c.f.u/g) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	0.00	0.00	0.00	0.00	ND
T_1	0.00	0.00	0.00	0.00	ND
T_2	0.00	0.00	0.00	0.00	ND
T_3	0.00	0.00	0.00	0.00	ND
SE	-				-
CD at 5%	-				

The similar result was observed by Osundahusi (2007) while preparing product.

4.4.3 Yeast and mould count

The yeast and moulds are the major food spoilage microbes in any product and need to be quantify in order to give quality assurance to consumer. The results obtained after analysis are presented in Table 4.4.3.

Table 4.4.3 Yeast and mould count (x 10^1 c.f.u/g) of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	0.00	0.00	0.00	0.00	ND
T_1	0.00	0.00	0.00	0.00	ND
T_2	0.00	0.00	0.00	0.00	ND
T_3	0.00	0.00	0.00	0.00	ND
SE	-				-
CD at 5%	-				

The data in Table 4.4.3 showed that the yeast and mould in yoghurt was completely absent, which was attributed to antimicrobial effect of lemongrass distillate.

Shaaban *et al.* (2010) showed that EO treatments caused marked reduction in yeast and mold production in yoghurt.

4.5 Sensory evaluation of yoghurt

Even though the product achieved chemical and microbiological standards, its sensory qualities needed to be assessed. Hence, the sensory analysis of yoghurt was done for its flavour, taste, body and texture and colour and results are tabulated as follows,

4.5.1 Flavour

The results regarding the flavour score of yoghurt is presented in Table 4.5.1.

Table 4.5.1 Effect of treatment combinations on flavour of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	7.35	7.33	7.34	7.33	7.33[c]
T_1	8.75	8.74	8.75	7.35	7.35[b]
T_2	8.95	8.94	8.95	8.90	8.90[a]
T_3	7.28	7.27	7.28	7.20	7.20[d]
SE	0.011				**
CD at 5%	0.035				

From the table, it was observed that the score obtained for flavour was found to be significantly differed from each other. The sensory score for flavour ranged from 7.20 to 8.90. The highest score obtained by T_2 (8.90) and lowest by the treatment T_3 (7.20). Harsh flavour may results into lower score for T_3 as the treatment contains higher concentration of lemongrass distillate.

These results are in agreement with results showed by Rajor *et al.* (1990) that as proportion of soymilk increased there was decrease in flavor score of yoghurt.

4.5.2 Body and texture

The sensory scores for body and texture was judged and given in Table 4.5.2.

Table 4.5.2 Body and texture of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	7.73	7.75	7.74	7.70	7.73[d]
T_1	8.28	8.27	8.28	8.27	8.28[c]
T_2	8.60	8.50	8.60	8.50	8.55[a]
T_3	8.50	8.40	8.50	8.50	8.48[b]
SE	0.0198				**
CD at 5%	0.0613				

From the Table 4.5.2, it was revealed that the effect of different treatments on body and texture score was found to be significant. The scores for body and texture of yoghurt were ranged from 7.73 to 8.55. The treatment T_2 had maximum sensory score for body and texture (8.55) and T_0 showed lowest sensory scores (7.73). The body and texture characteristics are govern by total solids and moisture content of yoghurt. As the total solid content in treatments increases subsequently body and texture gets firm and smooth.

The results are in agreement with Ranganatham and Gupta (1987); said that weak body may be due to low total solids content in milk, which is used for preparation of yoghurt.

4.5.2 Colour and appearance

The colour parameter of yoghurt was judged in Table 4.5.2.

Table 4.5.3 Effect on colour and appearance of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	8.00	8.10	8.00	8.00	8.03[c]
T_1	8.20	8.20	8.19	8.20	8.20[b]
T_2	8.90	8.89	8.90	8.90	8.90[a]
T_3	7.00	7.49	7.50	7.50	7.50[d]
SE	0.0126				**
CD at 5%	0.03908				

From given table, it is clear that the score obtained for different treatment combination was ranged 7.50 to 8.90. Treatment T_2 obtained highest score (8.90) for colour and appearance while T_3 obtained lowest score (7.50). This was due to large quantity of lemongrass distillate in yoghurt which has dominant effect over colour of yoghurt.

This results are in agreement with the results noted by Bire (1995) and Yadav (2003); who recorded the decreasing score of yoghurt for colour and appearance attribute due to increase in blending of soymilk.

4.5.4 Taste

The score obtained for taste of yoghurt enriched with lemongrass distillate is presented in Table 4.5.4.

Table 4.5.4 effect on taste of yoghurt

Treatments	Replications				Mean
	I	II	III	IV	
T_0	7.50	7.49	7.50	7.50	7.48[b]
T_1	7.30	7.35	7.29	7.30	7.31[c]
T_2	8.90	8.89	8.90	8.90	8.90[a]
T_3	7.20	7.19	7.22	7.20	7.20[d]
SE	0.0076				**
CD at 5%	0.0236				

From the table, it was observed that the score obtained for taste was found to be significant. The sensory score for taste ranged 7.20 to 8.90.The highest score obtained by T_2 (8.90) and lowest by the treatment T_3 (7.20). Grassy taste may results into lower score for T_3 as the treatment contains higher concentration of lemongrass distillate.

Similar results were noted by Yadav *et al.* (2014) with the addition of cinnamon extract in yoghurt.

4.5.5 Overall acceptability

The overall acceptability of the yoghurt was judged by considering all the sensory attributes. The results obtained are given in Table 4.5.4.

Table 4.5.4 Effect on overall acceptability of yoghurt added with lemongrass distilate

Treatments	Replications				Mean
	I	II	III	IV	
T_0	7.73	8.03	7.50	7.34	7.65^{b}
T_1	8.28	8.20	7.31	8.75	7.79^{ab}
T_2	8.55	8.90	8.90	8.94	8.81^{a}
T_3	8.48	7.50	7.20	7.28	7.60^{b}
SE	0.219				**
CD at 5%	0.675				

All the treatments had statistically significant effect on overall acceptability score of the yoghurt. The highest score was obtained by T_2 (8.81), which was most acceptable treatment combination. While score obtained by T_0, T_1, T_3 was 7.65, 7.79, 7.60, respectively. Basic reason behind this acceptability was lemongrass EO content of yoghurt, which has cumulative effect on every parameter i.e. chemical, microbiological and sensory.

This results are also in agreement with the results noted by Bire (1995), Rajor (1990), Krupal (2003) and Yadav (2003).

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation entitled, "Effect of lemongrass distillate on physico-chemical properties of yoghurt" was carried out to analyze potential of lemongrass to become potential food additive in milk product.

5.1 Summary

5.1.1 To optimize the levels of lemongrass distillate in yoghurt

Preliminary trials were conducted to finalize the levels of lemongrass distillate in yoghurt. The samples of product were subjected to sensory evaluation. On the basis of the results of sensory evaluation, treatments were finalized for experimental trials.

Acceptability of extent of lemongrass distillate level (1,0.8,0.6,0.3,0.1,0.05,0.04,0.03,0.02,0.01%) in the finished product by the judges was determined by preparing product samples having variable levels of lemongrass distillate. On the basis of results of sensory evaluation 0.01, 0.02, and 0.03% lemongrass distillate levels were selected for final experimental trials.

5.1.2 To study the chemical and organoleptic qualities of yoghurt

5.1.2.1 Chemical qualities of yoghurt

5.1.2.1.1 Fat

The fat analysis of yoghurt revealed that the influence of experimental treatments on fat content of yoghurt was significant. The mean values of fat content of treatment T_0, T_1,

T_2 and T_3 were 2.70, 2.43, 2.10 and 2.08, respectively. It was observed that fat content is varied between the range of 2.08 to 2.70 per cent. The highest fat content was recorded for treatment T_0 i.e. 2.70 and lowest fat content was observed for T_3 i.e. 2.08 percent. Fat percentage decreased as concentration of Lemongrass distillate increased. This is attributed to decreasing acidity percentage from T_0 to T_3 as lemongrass distillate slower down the Lactobacillus activity.

5.1.2.1.2 Protein

The protein content of different treatment combination of yoghurt was significant. The mean values of protein content of treatment T_0, T_1, T_2 and T_3 were 3.16, 3.08, 2.86 and 2.34 per cent, respectively and it was ranged from 2.34 to 3.16 per cent. The highest value was reported for treatment T_0 (3.16 per cent) while lowest value observed for T_3 (2.34 per cent). There was decreasing trend observed as EO concentration increases in yoghurt due to decreased proteolytic activity of LAB as a number of LAB decreases.

5.1.2.1.3 Lactose

The average lactose content of yoghurt found to be 5.46, 5.34, 5.33 and 5.18 for treatment T_0, T_1, T_2 and T_3, respectively. It ranged from 5.18 to 5.46 per cent with significant difference between them. The highest lactose content was observed for treatment T_0 (5.46 per cent) and lowest for treatment T_3 (5.18 per cent). There was decreasing trend observed with addition of EO increases. This may be attributed to the fermentation action by LAB.

5.1.2.1.4 Ash

The ash content of different treatment combination of yoghurt was found to be significant statistically and ranged from 0.33 to 0.66 per cent. The highest ash content was reported for treatment T_3 (0.66 %) and lowest for treatment T_0 (0.33 %) treatment. The ash content of yoghurt had shown slightly increasing trend with increases in lemongrass EO concentration.

5.1.2.1.5 Total solid

The mean total solid content of yoghurt showed significant difference between the treatments. The highest total solid observed for treatment T_3 i.e.13.53 while lowest for treatment T_0 i.e. 12.16. The increasing trend of TS in yoghurt was observed with increasing content of lemongrass EO. Total solid percentage was increased due to decreased moisture percentage in subsequent treatments.

5.1.2.1.6 Acidity

The experimental treatments had significant effect on acidity of yoghurt. The acidity of the experimental treatments was in between 0.61 to 0.66 per cent LA. The acidity was highest for treatment T_0 (0.61 per cent) which had highest LAB count, which increased lactic acid production in yoghurt and observed lowest acidity for treatment T_0 (0.20 per cent) which had lowest LAB count. Titrable acidity decreased due to decreasing number of LAB in samples.

5.1.2.1.7 pH

The pH of experimental treatments showed statistically significant difference and ranged from 4.13 to 4.28. The treatment T_0 showed highest pH while treatment T_3 showed lowest pH. This was due to decreasing percentage of acidity of yoghurt in subsequent treatments. Hence pH was increasing with decrease in acidity percentage.

5.1.2.1.8 Moisture

The moisture content of yoghurt ranges from 86.15 to 88.03 per cent and noted significant difference between the treatments. The decreasing trend in moisture content of yoghurt was observed with increase in concentration of lemongrass EO from treatment T_0 to T_3. Moisture percentage was decreased due to increased TS percentage in subsequent treatments.

5.1.2.2 Organoleptic quality of yoghurt

5.1.2.2.1 Flavour

The score obtained for flavour was found to be significant. The sensory score for flavour ranged from 7.20 to 8.90. The highest score obtained by T_2 (8.90) and lowest by the treatment T_3 (7.20). Harsh flavour may results into lower score for T_3 as the treatment contains higher concentration of lemongrass distillate.

5.1.2.2.2 Body and Texture

The effect of different treatments on body and texture score was found to be significant. The scores for body and texture of yoghurt were ranged 7.73 to 8.55. The treatment T_2 had maximum sensory score for body and texture (8.55) and T_0 showed lowest sensory scores (7.73).The body and texture characteristics are govern by total solids and moisture content of yoghurt. As the total solid content in treatments increases subsequently body and texture gets firm and smooth.

5.1.2.2.3 Colour and appearance

The score obtained for different treatment combination ranged from 7.50 to 8.90. Treatment T_2 obtained highest score (8.90) for colour and appearance while T_3 obtained lowest score (7.50). This was due to large quantity of lemongrass distillate in yoghurt which has dominant effect over colour of yoghurt.

5.1.2.2.4 Taste

The score obtained for taste was found to be significant. The sensory score for taste ranged from 7.20 to 8.90.The highest score obtained by T_2 (8.90) and lowest by the treatment T_3 (7.20). Grassy taste may results into lower score for T_3 as the treatment contains higher concentration of lemongrass distillate.

5.1.2.2.4 Overall Acceptability

All the treatments had statistically significant effect on overall acceptability score of the yoghurt. The highest score was obtained by T_2 (8.81), which was most acceptable treatment combination. While score obtained by T_0, T_1, T_3 was 7.65, 7.79 and 7.60, respectively. Basic reason behind this acceptability was lemongrass EO content of yoghurt, which has cumulative effect on every parameter i.e. chemical, microbiological and sensory.

5.1.3 To study the microbiological qualities of yoghurt

5.1.3.1 Standard plate count

The standard plate count of yoghurt showed significant difference among treatments. The range of SPC was between 51.25 to 65.25 c.f.u/g × 10^{-6}. It was observed that the highest SPC recorded for treatment T_0 while lowest for treatment T_3. It also showed that, SPC may be dependent upon environmental conditions and pre-processing factors. The standard plate count obtained was within the limits set by the IS: 10501 (1983) which infers that the product is safe for consumption.

5.1.3.2 Coliform count

Coliform count was found to be completely absent, which signifies that product was prepared in hygienic condition.

5.1.3.3 Yeast and mould count

The yeast and mould in yoghurt was completely absent, which was attributed to antimicrobial effect of lemongrass distillate.

5.2 Conclusion

It was observed that addition of lemongrass distillate had significant effect on physico-chemical properties of yoghurt and showed antimicrobial effect over coliform and YM production.

Overall acceptability score for all treatments was 7.65, 8.14, 8.82 and 7.62, respectively. Therefore, it is concluded that addition of 0.02% of lemongrass distillate in yoghurt found to be more desirable over other treatment.

CHAPTER VI

LITERATURE CITED

Abrahamsen, R. K. and T. B. Holmen (1981). Goat's milk yoghurt made from non-homogenized and homogenized milks concentrated by different methods. J. Diary Res. 48(3):457.

Abubakar, Adegola, Oyawoye, E. (2005).Determination of physiochemical, microbial and organoleptic properties of yoghurt.J.Texture Stud.36: pp.333.

Abu-seif, Feryala, A., Abdel-Fattah, M., Abo Sreia (2009). Properties of some medicinal plants against undesirable and mycotoxins-producing fungi. Mansuora Univ., 34(3):1745-1756.

Adejuwon, A. A. and Esther, O. A. (2007). Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of *Cymbopogon citratus* Stapf in rats. Journal of Ethnopharmacology 112: 440–444.

Adukwu, E. C., Allen, S. C. H., & Phillips, C. A. (2012). The anti-biofilm activity of lemongrass (*Cymbopogon flexuosus*) and grapefruit (*Citrus paradisi*) essential oils against five strains of Staphylococcus aureus. Journal of Applied Microbiology, 113, 1217-1227.

Amal, A. Matter, Eman, A. M. Mahmoud, and Nahla, S. Zidan (2016). Fruit Flavored Yoghurt: Chemical, Functional and Rheological Properties. International Journal of Environmental & Agriculture Research (IJOEAR), ISSN: [2454-1850], Vol-2, Issue-5.

Annual report (2014-15). GOI, Dept. Of Animal husbandry, Dairy and fishery, Govt.of India.

Anonymous (2012). Economic Survey. Economic Division, Ministry of Finance, Govt. of India. 348.

Anonymous (2014). www.indiastat.com. Utilization of pattern of milk in India.

Aradhana Gupta and D. N. Prasad (2000).Use of stabilizers in cultured milk products. Indian Dairyman, 52(9): 19-23.

Ashurst, P.R. (1999). Essential oils. In John Wright (Eds). Food flavoring. 3rd ed.pp. 23-24, Aspen Publishers, Inc., Maryland.

Bachir Raho Ghalem and Benattouche Zouaoui (2013). Evaluation of the quality of steamed yogurt treated by *Lavandula* and *Chamaemelum* species essential oils.Vol. 7(42), pp. 3121-3126.

Bachir Raho Ghalem and Benattouche Zouaoui (2013). Microbiological, physic chemical and sensory quality aspects of yoghurt enriched with *Rosmarinus officinalis* oil. African Journal of Biotechnology Vol. 12(2), pp. 192-198.

Balsubramanya, N. N., A. M. Natrayam and R. V. Rao (1984).Availability of calcium and phosphorus for albino rats from yoghurt. Asian J. Dairy Res. 3(3): 131.

Banerjee, G. C. (1960). Yoghurt. Indian Dairyman. 12(2): 357.

Bhandari, V (1983).Yoghurt - A health building food. Indian Dairyman, 35(11): 655.

Bire, P. V, (1995) .Blending of soy milk for preparation of different milk products. M. Sc. Thesis (Unpub.) Dr. PDKV, Akola, (M. S.)Campbell, J. R. and R. T. Marshall (1975).

The science of providing milk for man Mc. Grow HUl publ.: 607.

Bourlix, P. and Pochart, P. (1988).Nutritional and health properties of yoghurt. World Rev Nutr. Diet.56:217-58.

Chawla, A. K. and B. Balchandran (1994).Studies on yoghurt from buffalo milk. Effect of different levels on solids-not-fat levels on chemical, Rheological and Sensory characteristics. Indian J. Dairy Science, 47(9): 792-795.

Dari, L. (2013). Butternut squash yoghurt, benefit and consumer acceptance. Asian Journal of Science and Technology Vol. 4, Issue 04, pp.035-037.

Dave, J. M. (1984). Manufacture of yoghurt. Indian Dairyman. 36(7): 275.

Deeth (1984). Yoghurt a cultured products. The Aust. J. Dairy Technol., 39(1): 11.

Dharmendra, S., Suman, P. S. K., Atul, P. K., Subhash, C. G. and Sushil, K. (2001). Comparative antifungal activity of essential oils and constituents from three distinct genotypes of *Cymbopogon* spp. Current Science 80: 1264-1266.

Ejtajad, Mohtadi, Nilafer, .Asghari, Mofi and Mogharia (2011). Starter culture used in probiotic dairy drinks. Journal of Dairy science, Homayu uni.Rada.

F.N. Ehirim and E.N. Onyeneke (2013), Physico-chemical and organic properties of yoghurt manufactured with cow milk and goat milk. Academic Research International, vol.4 no.4.

FAO. (1976). Report of Joint Food and Agricultural Organization expert committee on the code of Principles concerning, milk and milk products.

FAO. (1998). Fermented fruits and vegetables- A global perspective. vol.134, FAO Agriscience bulletin Rome, Italy.

FAO. (1992). Codex Standard for Fermented Milks- CODEX STAN 243-2003. Rome: FAO/OMS.

Farinde, E.O., Obatolu, V.A., Fasoyiro, S.B., Adeniran, A.H. and Agboola, E.R, (2008). Use of alternative raw materials for yoghurt production. African Journal of Biotechnology, 7 (18): 3339-3345.

Fenwick, G.R.; Lutomski, L. and Nieman (1990). Licorice Glycyrriza global. Composition, Uses and analyses Food chem., 38,119-143.J. of Food Sci., 54(1): 74-76.

Gagan Shah, Richa, Panchal, Sharma, Singh, and A. S. Mann (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus, stapf* (Lemon grass). J Adv Pharm Technol. Res., 2(1): 3–8.

Geetha C. Jain and M. H. Khan (1994). Characteristics of Yoghurt like cultured milk. J. Food Sci. Technol., 31(5): 413-415.

Goodenough and Klein, D. H. (1976). Qualitative and quantitative changes in carbohydrates during the manufacture of yoghurt. Journal of Dairy Science. In: Modern Food Microbiology sixth edition, (ed.) Jay J.M. (2000). Aspen Publishers Inc., Maryland- USA: 121, 391.

Grace, O., Yisak, W. and Ogulana, EO. (1984). Antibacterial constituents in the essential of Cymbopogon citratus (DC) Stapf. Journal of Ethnopharmacology, 12: 279-286.

Grosser, V., (1978). How to make and sell hard frozen yoghurt. American Dairy Review, 40(5): 20.

Gupta, P. K. Bi. K. Mittal and S. K. Garg (1997). Preparation and evaluation of acidophilus yoghurt. J. Food Science Technol., 34(2): 168-170.

Gupta, S. K. and A. A. Patel (1984). Use of soy milk for Dahi and yoghurt manufacture. Indian Dairyman, 36(6): 313.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 86, 985-990.

Harin Sutariya and Jayraj Rao K. (2015). Utilization of lemongrass distillate in the preparation of yoghurt. Indian J.Dairy Sci., 68 (6)

Imele, H. (2001). Preliminary study on the utilization of coconut in yoghurt production. The Journal of Food Technology, Africa. 6(1): 11-12.

IS: 10501 (1983). Indian Standards Specification for *kulfi*, By Indian Standard Institute, Manak Bhavan, New Delhi.

Iyengar, M. K.K., A. T. Dudani and V. K. N. Namubudripad (1967). Effect of heat treatment on buffalo milk and cow milk in manufacture of yoghurt. Indian J. Dairy Science, 20(1): 8.

Jawalekar, S. D., U. M. Ingle, P. S. Waghmare, P. N. Zanjad (1993). Influence of Hydrocolloids on rheological and sensory properties of cow and buffalo milk yoghurt. Indian J. Dairy Science, 46(5): 217.

Jay, J.M., (2000). Modern Food Microbiology sixth edition. Aspen Publishers Inc., Maryland- USA: 121, 391.

Jayalalitha, V., Manoharan, Balasundaram, B. and Elango, A. (2015). Formulation of Value Enriched Yoghurt with Soy Milk and Mango Pulp. Nutr. Food Sci, 5:6.

Joel Ndife, Felicia Idoko, Rabiu Garba (2014). Production and quality assessment of functional yoghurt enriched with coconut, International Journal of Nutrition and Food Sciences, 3(6): 545-550.

Juárez-Rosete, C. R., Aguilar-Castillo, J. A., Juárez-Rosete, M, E., Bugarín-Montoya, R., Juárez-López, P., & Cruz, C. E. (2013). Hierbas aromáticas y medicinales en México: tradición e innovación. Revista Bio Ciencias, 2(3), 119-129.

Kanmani, P., R.S. Kumar, N. Yuvaraj, K.A. Paari, V. Pattukumar and Arul, (2013). Probiotics and its functionally valuable products: a review. Critical Reviews in Food Science and Nutrition, 53 (6): 641- 658.

Kasali, A.A., A.O. Oyedeji, and A.O. Ashilokun. (2001). Volatile leaf oil constituents of Cymbopogon citrates (DC) Stapf. Flavor and Fragrance Journal. 16: 377-378. DOI: 10.1002/ffj.1019.

Keerti Yadav, Sangeeta Shukla (2014). Microbiological, physicochemical analysis and sensory evaluation of herbal yogurt. The Pharma Innovation Journal, 3(10): 01-04.

Khanna, A. and J. Singh (1979). A comparison of yoghurt starter in cow and buffalo milk. J. Dairy Res., 46(1): 681.

Kongphoothorn, C. and Leelawatcharamas, V. (2005). Proceedings of 43rd Kasetsart University Annual

Conference, Thailand, 1-4 February, Subject: Animals; 2005. : 411-418. 10.

Krupal, R. G. (2003). Utilization of soy milk and cow skim milk in different combinations for yoghurt preparation. M.Sc. Thesis (Unpub). Dr. PDKV, Akola.

Kulkarni, S. and L. Vishweshwairaih (1983). Curd tension and its measurement. Indian Dairyman, 35(11): 671.

Labropoulous, A. K.; W. F. Collins and W. K. Stone (1984). Effect of U. H. T. and Vat Processes on heat induced rheological properties of yoghurt. J. Dairy Science, 67(2): 405.

Laura P. Tovar, Gláucia M. F. Pinto , Maria R. Wolf-Maciel , César B. Batistella, and Rubens Maciel-Filho (2011). Short-Path-Distillation Process of Lemongrass Essential Oil: Physicochemical Characterization and Assessment Quality of the Distillate and the Residue Products. L*Ind. Eng. Chem. Res.*, *50* (13), pp 8185–8194.

Laxminarayan, H. and P.S. Shankar, (1980). Fermented milk in human nutrition Indian Dairyman, 32(2):121.

Leder, K. H. and J. Thomason (1973). Use of gelatin in yoghurt of manufacture. Dairy Science Abstract, 37(9): 690-694.

Lee, W.J and Lucey, J.A (2010). Formation and physical properties of yoghurt.J.Anim.Sci. 23(9), 1127-1136.

Lucey, J. A. and Singh, H. (1998). Formation and physical properties of acid milk gels: A review. Food Research International, 30(7), 529–542.

Lucey J. A., Munro P. A., and Singh H. (1998). Whey separation in acid skim milk gels made with glucono-delta-lactone: Effects of heat treatment and gelation temperature.

Journal of Texture Studies. In: Formation and physical properties of acid milk gels: A review (Eds.).

Lucey, J. A., Tetteo, Munro P. A., and Singh, H. (1997). Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. Journal of Dairy Research. In: Formation and physical properties of acid milk gels: A review (eds.) Lucey, J.A. and Singh, H. (1998). Food Research International, 30(7), 529–542.

Lucey, J.A. and Singh H. (1998). Food Research International, 30(7), 529–542.

Lutchmedial, M., Ramlal, R., Badrie, N. and Chang-yen, I., (2004).Nutritional and sensory quality of stirred soursop (*Annona muricata L.*) yoghurt. International Journal of Food Sciences and Nutrition, 55 (5): 407 – 414.

Madanlal, K., Menna Sachdeva (1978). Studies on selection of starter culture for the manufacture of yoghurt. J. Food Sci Technology, 15 (1):20-21.

Malik, M. (1993). Trends in Dairy Science and Technology. Indian Dairyman, 45(3): 117.

Mallikarjun Chukki, K Jayaraj Rao, BV Balasubramanyam, Bikash C. Ghosh and S. Kulkarni (2013).Suitability of Concentrated Milk for Yoghurt Preparation. Journal of Food and Dairy Technology, Volume 1, Issue 2.

Maria, C. K., Leandros, P. V. and Efthymia, K., (2002). Manufacture of yoghurt from stored frozen sheep's milk. Food Chemistry, 77: 413–420.

María, Luis Ricardo & José Ángel (2015). Physicochemical and antioxidant properties of *Cymbopogon citratus* essential oil. Journal of Food Research, Vol. 4, No. 3.

Mbaeyi-Nwaoha, Ekere (2014). Production and evaluation of flavored yoghurt from graded levels of soursop (*Annona muricata*) pulp. Innovare journal of food science, Vol 2, Issue 1.

Mirghani, M. E. S., Liyana, Y., & Parveen, J. (2012). Bioactivity analysis of lemongrass (*Cymbopogan citratus*) essential oil. International Food Research Journal, 19(2), 569-575.

Mittai, B. K (1984). Growth and activity of Starter culture in soy milk. Indian Dairyman, 36(6): 3-9.

Moayednia, Ehsani, Emamdjomeh and Mazaheri (1999). Effect of refrigerated storage Time on the viability of probiotic bacteria in fermented probiotic milk drinks. International Journal of Dairy Technology, 62 (2): 204- 208.

Mohamed Nadjib Boukhatem, Mohamed Amine Ferhat, Abdelkrim Kameli, Fairouz Saidi, and Hadjer Tchoketch Kebir (2014). Lemon grass (Cymbopogon citratus) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan J Med., 9: 10.

Najafi NM, Koochki A, Rezaii Z (2008). Investigation of the effect of whey protein concentration on the properties of soft frozen yoghurt. The international Hydrocolloid conference, Singapore.

Nambudripad (1969). Yoghurt. Indian Dairyman. 21(9): 270.

Nazan Kavas and Gokhan Kavas (2016). Probiotic Frozen Yoghurt Production Using Camel Milk (*Camelus dromedarius*) with Improved Functions by Strawberry Guava (*Psidium littorale var. cattleianum*) Fortification British Journal of Applied Science & Technology, 14(5): 1-12.

Negrelle, R. R. B. and Gomes, E. C. (2007). Cymbopogon citratus (DC.) Stapf. Chemical composition and biological activities. Revista Brasileira de Plantas Medicinais 9: 80-92.

Nelson, J. A. and G. M. Trout (1964). Judging Dairy Products 4th Edition Olson Publ. Co. Nilwankee. P. 296.

Nelson, J. P, (1975). The importance of heat treatment of milk in the production of yoghurt with varied content of solid-not-fat. Dairy Science Abstract, 22(12): 625.

Nieto,Gorrido,A.,Ssanhaeza,J.,Loyala,L.A.,Marale,G.,Leighton, F.G.and Valenzul A. (1993). Flavonoid as stabilizers fish oil: an alternative to synthetic antioxidant.J.Am.Oil Chem., Soc., 70,773-778.

Nikonvin, N. (1960). Activity of various cultures in cultural milk products. Dairy Science Abstract. 22(12): 629.

Okuda, Tamura, Yamamoto, Inoue, Takahashi, Demizu, Kajiuama and Kinoshita (1989). Identification of antimicrobial and antioxidant constituents from licorice of Russian and xinjinf origin.chem.pharm.bull, 37, 2528-2530.

Olorunnisola, Asiyanbi, Hammed and Simsek (2014). Biological properties of lemongrass: An overview. International Food Research Journal, 21(2): 455-462.

Omer Erturk, Eda Demirkol (2014). The Effect of Some Medicinal Plant Extracts on Biochemical, Physicochemical and Antimicrobial Activity of Extract Added Yogurt, Harran Üniv. Vet. Fak Derg, 3(2) 78-83.

Osundahusi, O. F., D. Amousi, Ifesan (2007). Quality evaluation and acceptability of soy yoghurt with different colours and fruit flavours. American J.of food technology, 2(4), 273-280.

Parikh, J. K., & Desai, M. A. (2011). Hydrodistillation of essential oil from Cymbopogon flexuosus. International Journal of Food Engineering, 7(1), 1-9.

Patel, R. S. and Anna Renz – Schauen (1997). Lactic acid bacteria, Yoghurt and Heath benefits Report. Indian Dairyman, 49(9): 14.

Penna ALB, Subbarao G, Barbosa-Canovas GV (2007). High hydrostatic pressure processing on microstructure of probiotic low-fat yoghurt. Food Res Int., 40: 510-519.

Pradyuman Kumar, and Mishra, H. N. (2003). Journal of Texture Studies, 34(3):249-269.

Pranoto, Y., Salokhe, V. M., & Rakshit, S. K. (2005). Physical and antimicrobial properties of alginate-based edible film incorporated with garlic oil. Food Research International, 38, 267-272.

Pratt, D.E and Hudson, B.J.F. (1990). Antioxidant activity of phenolic compound in meat model system, in phenol compounds, in food and their effects on health. AC Symposium serious 506; American chemical society, Washington DC, 1991; pp 214-222.

Rajor, R. B. (1990). Soyyoghurt: The low cost nourishing food. Indian Dairyman, 42(9): 386.

Ranganatham, M. and S.K. Gupta (1987). Sensory evaluation of Dahi and yoghurt. Indian Dairyman, 39(10): 793.

Ranganatham, M. and S.K. Gupta (1987). Sensory evaluation of Dahi and yoghurt. Indian Dairyman, 39(10): 793.

Robinson, R. K. and Tamime A.Y. (1975). Yogurt-a review of the product and its manufacture. Soc. Dairy Technol., 28:149-163.

Robinson, D. K. (1981). Yoghurt manufacture- some considerations of quality. Dairy Industries International, 46(3), 31–35.

Rossi, E. A., J. B. Faria, Borsato and Baldochi (1990). Optimization of a stabilizer system for soy-whey yoghurt. J.Dairy Sci.

Sahana N, Yasarb K, Hayaloglu AA (2008). Physical, chemical and flavor quality of non-fat yoghurt as affected by ab-glucans hydro colloidal composite during storage. Food hydrocolloid, 22:1291-1297.

Sanful, R.E., (2009a). Promotion of Coconut in the Production of Yoghurt. African Journal of Food Science 3 (5): 147-149.

Sanful, R.E., (2009b). The Use of Tiger-Nut (*Cyperus esculentus*), Cow Milk and Their Composite as Substrates for Yoghurt Production. Pakistan Journal of Nutrition, 8 (6): 755-758.

Sarkar, S. (1995). Potential of yoghurt for its commercialization in India. Indian Dairyman, 67(4): 35-42.

Sarkar, S., A. K. Misra (2003). Characteristics of dietetic yoghurt. Dairy Set Abstract, 65(1): 45.

Sawyerr, E. (1982). Traditional medicine in Sierra Leone - a critical appraisal. Nigerian Journal of Pharmacy, 13: 28-33.

Schmalstieg, F. C. and Goldman, A. S. (2008). "IlyaIlich Metchnikoff (1845-1915) and Paul Ehrlich (1854-1915):

the centennial of the 1908 Nobel Prize in Physiology or Medicine". Journal of Medical Biography, 16 (2): 96–103.

Shaaban, M. Abd-El Fattah, Abo sree, Yahia Hassan, Hala M. Bayoum and Hesham Eissa (2011). The Use of Lemongrass Extracts as Antimicrobial and Food Additive Potential in Yoghurt. Journal of American Science, 6(11).

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of Cymbopogon citratus, Stapf (Lemon grass). Journal of Advanced Pharmaceutical Technology and Research, 2, 3-8.

Shaneberg, B.T., and I.A. Khan. (2002). Comparison of extraction methods for Marker compounds in the essential oil of lemon grass by GC. Journal of Food Chemistry. 50: 1345-1349.

Shukla, F. C: S. C. Jain and K. S. Sandhu (1986). Effect of stabilizers and additives on the diacetyl and volatile fatty acids content of yoghurt. Indian J. Dairy Science, 39(4): 486.

Siham A. Rahmatalla, Lina Abd Aazeem, Mohamed Osman Mohamed Abdallah (2014). Quality Evaluation of Set Yoghurt Supplemented with Turmeric Powder (*curcoma longa.L*) during Storage Period.

Sinha, R. N. (1984). Factors influencing quality of yoghurt. Indian Dairyman. 36(6): 285.

Sinha, R. N. (1984). Factors influencing quality of yoghurt. Indian Dairyman. 36(6): 285.

Sirinat Natisri, Kanjana Mahattanatawee and Siwatt Thaiudom (2014). Improving the Flavor of Soy Ice Cream by Adding

Lemongrass or Pandan Leaf Extracts.CMUJ NS Special Issue on Food and Applied Bioscience, Vol.13 (1) 469.

Smits, P., and van Brouwershaven, J. H. (1980). Heat-induced association between beta-lactoglobuline and casein micelles. Journal of Dairy Research, 47(3), 313–325.

Sun-Young Lee, Joungjwa, and Hae-Soo Kwak (2011). Effects of the Extract Yam Powder Addition on Yogurt Properties during Storage. Korean J.Food Sci.Ani. Resour. Vol. 31, No. 1, pp. 66-73.

Tajidin, Ahmad, Rosenani, Azimah and Munirah (2012). Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. African Journal of Biotechnology, Vol. 11(11), pp. 2685-2693.

Tamime, A. Y., R. K. Robison (1988). Fermented milk and their future trend. Part-II. J. Dairy Res., 55(2): 287.

Tatiana, F. B., José, M. S. (2011). Lemongrass and citral effect on cytokines production by murine macrophages. Journal of Ethnopharmacology, 137: 909– 913.

Tomar, S. K. (1988). Nutritional value of yoghurt. Indian Dairyman, 40(8):429.

Tomar, S. K., D. N. Prasad (1989). Therapeutic value of yoghurt. An assessment. Indian Dairyman, 41(9): 483.

Ukwo (2015). Physicochemical profile and sensory attribute of plain yoghurt from cow and soymilk blends. Nigerian Journal of Agriculture, Food and Environment, 11(2):20-23.

Viana, G. S. B., Vale, T. G., Pinho, R. S. N. and Matos, F. J. A. (2000). Antinociceptive effect of the essential oil from

Cymbopogon citratus in mice. Journal of Ethnopharmacology, 70: 323–327.

Yadav, D. N. Chauhan, G. S. Chauhan, O. P. Sharma and P. Bajpai, A. (2003). Journal of Food Science and Technology (Mysore), 40(4):403-405.

Yadav, J. S. S. Grover and V. K. Batish (1993). A Comprehensive Dairy Microbiology. Metropolitan Co. New Delhi, pp: 433-436.

Yang and Li (2010). Characteristics of Probiotic Soy Yogurt, Food Technol. Biotechnology, 48 (4): 490–496.

Yilmaz-Ersan and E. Kurdal (2014). The Production of Set-Type-Bio-Yoghurt with Commercial Probiotic Culture. International Journal of Chemical Engineering and Applications, Vol. 5, No. 5.

Younus S., Masud T. and Aziz T. (2002).Quality Evaluation of Market Yoghurt /Dahi. Pakistan Journal of Nutrition, 1 (5): 226-230.

Ziemer & Gibson (1998). An overview of probiotics, prebiotics and symbiotic: In functional food concept, perspective and future strategies, International Dairy Journal, vol.8, 473-479.

8. VITA

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Candidate for the degree of

MASTER OF SCIENCE

IN

DAIRY SCIENCE

2017

Title of the thesis	:	Effect of lemongrass distillate on physico-chemical properties of yoghurt
Major field	:	Dairy Science

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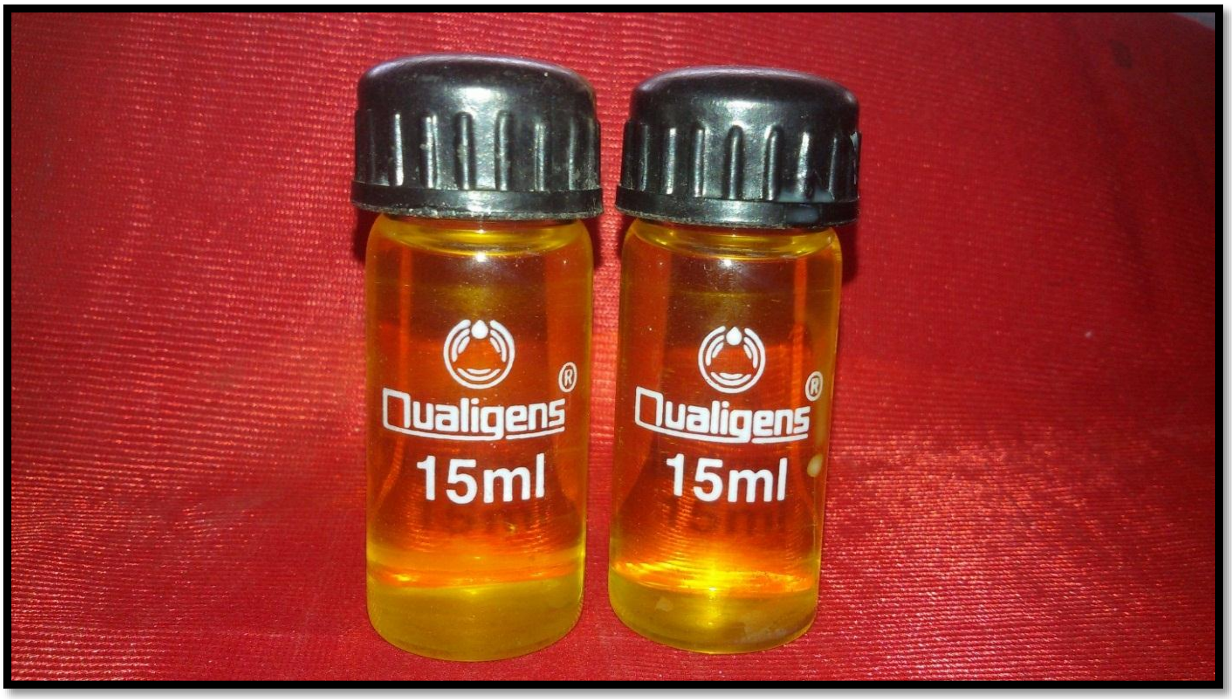
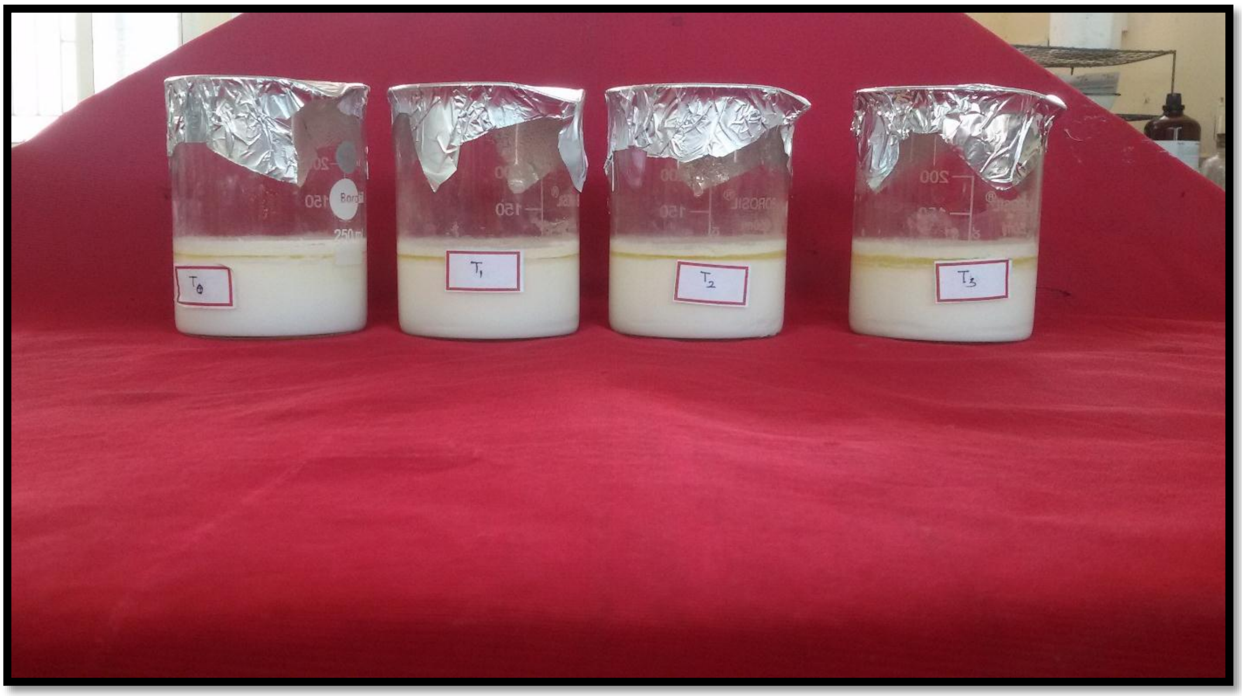
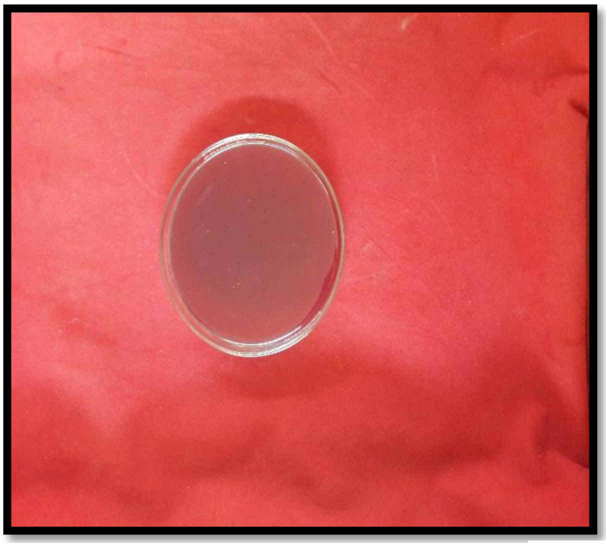


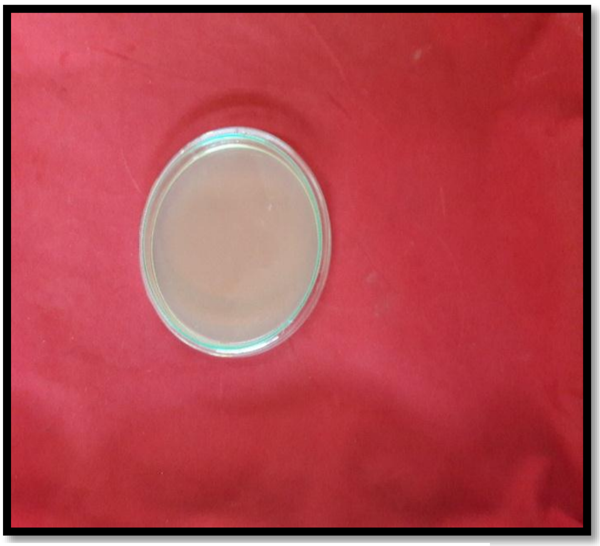
Lemongrass distillate



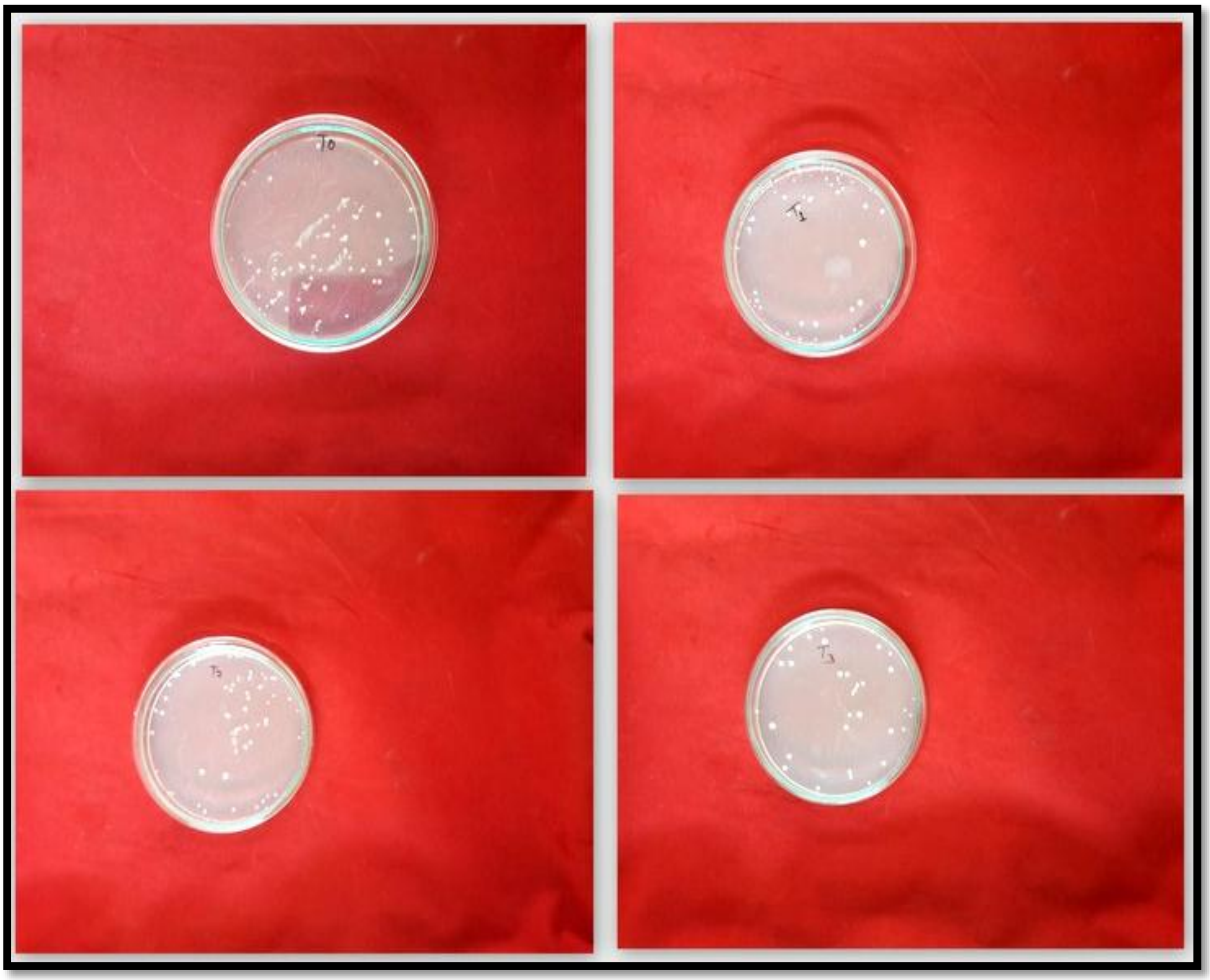
Yoghurt samples of T_0,T_1,T_2,T_3



Coliform count



Yeast and Mold count



Standard Plate Count of T_0,T_1,T_2,T_3