

**PROCESS STANDARDIZATION AND APPRAISAL OF  
GULKAND YOGHURT**

By

**Mr. Navale Sachin Kundalik**

*(Reg. No. K-014/145)*

*A Thesis submitted to the*

**MAHATMA PHULE KRISHI VIDYAPEETH**

**RAHURI, DIST. AHMEDNAGAR,**

**(M.S.), INDIA**

*In partial fulfillment of the requirements for the degree  
of*

**MASTER OF SCIENCE (AGRICULTURE)**

*in*

**DAIRY SCIENCE**

**DIVISION OF ANIMAL HUSBANDRY AND DAIRY SCIENCE**

**COLLEGE OF AGRICULTURE, KOLHAPUR**

**MAHATMA PHULE KRISHI VIDYAPEETH**

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**DAIRY SCIENCE**

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**2016**

## CANDIDATE'S DECLARATION

*I hereby declare that this thesis or part  
there of has not been submitted by  
me or other person to any other  
University or Institute  
for a Degree or  
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**Date:** / /2016

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### **CERTIFICATE**

This is to certify that the thesis entitled, “**PROCESS STANDARDIZATION AND APPRAISAL OF GULKAND YOGHURT**”, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **Dairy Science**, is a record of a bonafide research work carried out by **Mr. Sachin Kundalik Navale**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree, diploma or publication in other form.

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*Place: Kolhapur*

*Date:     /     /2016*

*(Navale Sachin Kundalik)*

<b>CONTENT</b>
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Chapter	Title	Page No.
	<b>CANDIDATE'S DECLARATION</b>	iii
	<b>CERTIFICATES</b>	
	1. Research guide	iv
	2. Associate Dean (ACK)	v
	<b>ACKNOWLEDGEMENT</b>	vi
	<b>LIST OF TABLES</b>	xi
	<b>LIST OF FIGURES</b>	xiii
	<b>LIST OF PLATES</b>	xiv
	<b>LIST OF ABBREVIATIONS</b>	xv
	<b>ABSTRACT</b>	xvi
<b>1.</b>	<b>INTRODUCTION</b>	1
<b>2.</b>	<b>REVIEW OF LITERATURE</b>	4
	2.1 Importance of fermented milk products	
	2.2 Technology for preparation of yoghurt	
	2.3 Utilization of fruits pulp/ nuts/ cereals etc. in yoghurt	
	2.4 Gulkand – its importance and utilization	
<b>3.</b>	<b>MATERIALS AND METHODS</b>	20
	<b>3.1 Material</b>	20
	3.1.1 Milk	20
	3.1.2 Yoghurt cultures	20
	3.1.3 Gulkand	20
	3.1.4 Sugar	20
	<b>3.2 Equipments / Utensils</b>	20
	3.2.1 Hand Blender	20
	3.2.2 Incubator	20
	3.2.3 pH meter	20
	3.2.4 Stirrer	21
	3.2.5 Glassware	21

	3.2.6 Chemicals	21
	3.2.7 Cream Separator	21
	3.2.8 Media for microbiological analysis	21
	<b>3.3 Methodology</b>	21
	3.3.1 Preparation of gulkand yoghurt	21
	3.3.2 Optimization of ingredients in gulkand yoghurt	22
	3.3.3 Optimization of fat level of milk for preparation of yoghurt	23
	<b>3.4 Analytical Methods</b>	23
	<b>3.4.1 Physico-chemical analysis of milk</b>	23
	3.4.1.1 pH	23
	3.4.1.2 Acidity	24
	3.4.1.3 Specific gravity	24
	3.4.1.4 Fat	24
	3.4.1.5 Protein	24
	3.4.1.6 Lactose	24
	3.4.1.7 Ash	24
	3.4.1.8 Total solids	24
	<b>3.4.2 Physico-chemical analysis of gulkand yoghurt</b>	24
	3.4.2.1 Titratable acidity	24
	3.4.2.2 pH	25
	3.4.2.3 Fat	25
	3.4.2.4 Protein	25
	3.4.2.5 Reducing sugar	26
	3.4.2.6 Non-reducing sugar	27
	3.4.2.7 Total sugar	27
	3.4.2.8 Ash	28
	3.4.2.9 Total solid	28
	<b>3.4.3 Microbial analysis of yoghurt</b>	28
	3.4.3.1 Preparation of dilutions	28
	3.4.3.1.1 Standard Plate Count	29
	3.4.3.1.2 Coliform Count	29
	3.4.3.1.3 Yeast and Mould Count	30
	3.4.4 Sensory evaluation of gulkand yoghurt	30
	3.4.5 Consumer acceptability study	30
	3.4.6 Statistical Design	31
	3.4.7 Facilities required	31

<b>4.</b>	<b>RESULTS AND DISCUSSIONS</b>	32
	4.1 Physico-chemical properties of milk	32
	<b>4.2 Effect of gulkand and sugar on sensory qualities of yoghurt.</b>	33
	4.2.1 Effect on colour and appearance score	33
	4.2.2 Effect on flavour score	34
	4.2.3 Effect on consistency score	35
	4.2.4 Effect on sweetness score	36
	4.2.5 Effect on overall acceptability score	37
	<b>4.3 Effect of gulkand and sugar on physico-chemical properties of yoghurt</b>	38
	4.3.1 Effect on pH	38
	4.3.2 Effect on titratable acidity	39
	4.3.3 Effect on fat content	39
	4.3.4 Effect on protein content	40
	4.3.5 Effect on reducing sugar content	41
	4.3.6 Effect on non-reducing sugar content	42
	4.3.7 Effect on total sugar content	43
	4.3.8 Effect on ash content	43
	4.3.9 Effect on total solid content	44
	<b>4.4 Effect of gulkand, sugar and their interaction on microbial quality of yoghurt</b>	45
	4.4.1 Effect on standard plate count (SPC)	45
	4.4.2 Effect on coliform count (C.C.)	46
	4.4.3 Effect on yeast and mould count	46
	<b>4.5 Effect of fat level on sensory quality</b>	47
	<b>4.6 Consumer acceptance of gulkand yoghurt</b>	48
<b>5.</b>	<b>SUMMARY AND CONCLUSIONS</b>	50
<b>6.</b>	<b>LITERATURE CITED</b>	55
<b>7.</b>	<b>ANNEXTURE</b>	64
<b>8.</b>	<b>VITA</b>	66

## LIST OF TABLES

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
4.1	Physico-chemical properties of milk	33
4.2	Effect of gulkand, sugar and their interaction on colour and appearance (score) of yoghurt	33
4.3	Effect of gulkand, sugar and their interaction on flavour (score) of yoghurt.	34
4.4	Effect of gulkand, sugar and their interaction on consistency (score) of yoghurt.	35
4.5	Effect of gulkand, sugar and their interaction on sweetness (score) of yoghurt	36
4.6	Effect of gulkand, sugar and their interaction on overall acceptability (score) of yoghurt	37
4.7	Effect of gulkand, sugar and their interaction on pH of yoghurt	38
4.8	Effect of gulkand, sugar and their interaction on titratable acidity of yoghurt	39
4.9	Effect of gulkand and sugar and their interaction on fat content (%) of yoghurt	40
4.10	Effect of gulkand and sugar and its interaction on protein content (%) of yoghurt	40
4.11	Effect of gulkand, sugar and their interaction on reducing sugar content (%) of yoghurt	41
4.12	Effect of gulkand, sugar and their interaction on non-reducing sugar content (%) of yoghurt	42
4.13	Effect of gulkand, sugar and their interaction on total sugar content (%) of yoghurt	43
4.14	Effect of gulkand, sugar and their interaction on ash content (%) of yoghurt	43
4.15	Effect of gulkand, sugar and their interaction on total solid content (%) of yoghurt	44
4.16	Effect of gulkand, sugar and their interaction on standard plate count ( $\log_{10}/g$ )* of yoghurt	45
4.17	Effect of gulkand, sugar and their interaction on coliform count (cfu/g) of yoghurt	46
4.18	Effect of gulkand , sugar and their interaction on yeast and mould count ( $\log_{10}/g$ ) of yoghurt	47
4.19	Effect of fat level on sensory qualities (score) of yoghurt	48
4.20	Distribution of consumers on the basis of acceptance of gulkand yoghurt	49

## LIST OF FIGURES

<b>Fig. No.</b>	<b>Title</b>	<b>Between Page No.</b>
4.1	Radar diagram for colour and appearance score of yoghurt	33-34
4.2	Radar diagram for flavour score of yoghurt	33-34
4.3	Radar diagram for consistency score of yoghurt	35-36
4.4	Radar diagram for sweetness score of yoghurt	35-36
4.5	Radar diagram for overall acceptability score of yoghurt	37-38
4.6	Radar diagram for pH of yoghurt	38-39
4.7	Radar diagram for titratable acidity of yoghurt	38-39
4.8	Radar diagram for fat content (%) of yoghurt	40-41
4.9	Radar diagram for protein content (%) of yoghurt	40-41
4.10	Radar diagram for reducing sugar content (%) of yoghurt	41-42
4.11	Radar diagram for non-reducing sugar content (%) of yoghurt	41-42
4.12	Radar diagram for total sugar content (%) of yoghurt	43-44
4.13	Radar diagram for ash content (%) of yoghurt	43-44
4.14	Radar diagram for total solid content (%) of yoghurt	44-45
4.15	Radar diagram for standard plate count ( $\log_{10}/g$ ) of yoghurt	45-46
4.16	Radar diagram for coliform count (cfu/g) of yoghurt	45-46
4.17	Radar diagram for yeast and mould count ( $\log_{10}/g$ ) of yoghurt	47-48

**LIST OF PLATES**

<b>Plate No.</b>	<b>Title</b>	<b>Between Pages</b>
1.	Microbiological analysis of gulkand yoghurt	45-46

## ABBREVIATION

et al.	Any other
@	At the rate of
Avg	Average
°C	Degree Celsius
etc.	Excreta
ml	Milli litre
g	Gram
mg	Milli gram
%	Per cent
% LA	Per cent lactic acid
Fig.	Figure
CD	Critical difference
NS	Non-significant
SE	Standard Error
IS	Indian Standards
i.e.	That is
Wt.	Weight
w/w	Weight per Weight
v/v	Volume per volume
w/v	Weight per volume
<	Less than
hrs	Hours
psi	per square inch

**ABSTRACT**

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**PROCESS STANDARDIZATION AND APPRAISAL OF  
GULKAND YOGHURT***By***Mr. Sachin Kundalik Navale***A candidate for the degree of*  
**MASTER OF SCIENCE (AGRICULTURE)***in***DAIRY SCIENCE****2016**

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**Research Guide : Dr. D.D. Patange****Department : Animal Husbandry and Dairy Science**

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Yoghurt is a fermented milk product with custard like consistency. The flavor and texture of yoghurt is important aspects of its marketable quality. Recently popularity of yoghurt is due to the fortification of it with sugar of fruits and plant parts (leaves, flowers etc.). Gulkand is commercially used in milk shakes, filling for cakes and pastries to improve the flavor and the appearance of the products. Considering the demand for yoghurt and nutritional as well as aesthetic importance of gulkand, here the attempt was made to prepared yoghurt by utilizing gulkand.

For optimization gulkand was added @ 5 (G<sub>1</sub>), 7 (G<sub>2</sub>) and 9 (G<sub>3</sub>) per cent whereas, sugar was added at 2 (S<sub>1</sub>) and 4 (S<sub>2</sub>) per cent of milk. The product was evaluated for sensory and physico-chemical qualities.

The maximum score (8.20) for colour and appearance was recorded to the product containing 9 per cent gulkand and 4 per cent sugar. The maximum flavour, sweetness and overall acceptability score was recorded in the product containing 2 per cent sugar and 9 per cent gulkand and the scores were 8.15, 8.42 and 8.05, respectively. At excessive quantity of

sugar, yoghurt containing 9 per cent gulkand. The optimum consistency score (8.52) was recorded to the yoghurt prepared by the addition of sugar and gulkand at 2 and 5 per cent, respectively. The yoghurt containing 2 per cent sugar and 9 per cent gulkand was found as best product on the basis of sensory evaluation.

The pH of yoghurt was significantly ( $p < 0.05$ ) decreased with increasing level of gulkand and it was also found that the effect of gulkand and sugar was significant ( $p < 0.05$ ) on acidity of the products. The best quality product i.e. prepared by addition of gulkand and sugar at 9 and 2 per cent, respectively had acidity 0.93 (% LA). The maximum fat content (4.19 per cent), protein content (3.46 per cent) and reducing sugar content (4.52 per cent) was found in the yoghurt prepared by the addition of sugar and gulkand at 2 and 5 per cent, respectively.

The SPC of yoghurt blended with 2 per cent and 5 per cent gulkand was recorded lowest count whereas yoghurt prepared by addition of 4 per cent sugar and 9 per cent was recorded highest count with an average of 6.39 ( $\log_{10}/g$ ). The mean coliform count was 4.13cfu/g and it was ranged from 2 to 7.40 cfu/g. The mean yeast and mould count was 2.58 cfu/g. While knowing the effect of fat content of milk on gulkand yoghurt preparation, it was found that use of milk containing 3.0 per cent fat had maximum overall acceptability score (8.23).

## 1. INTRODUCTION

India has emerged as the highest milk producing country in the world. In the year 2014-15, India achieved a record production of 146 million tonnes of milk (Ayyappan, 2016). Out of the total milk produced, nearly 46 per cent consumed as liquid milk and 54 per cent is converted into various products in India. Milk is the most nutritionally essential food to human and contains nearly all nutrients including proteins, fats, minerals, lactose and vitamins. (Hatirli *et al.*, 2004).

Fermented dairy products find a very predominant position in the Indian culture. Around 9 per cent of total milk produced in India is converted into fermented milk product and this sector is showing an annual growth rate of more than 20 per cent per annum (Singh, 2006).

*Dahi*, *lassi*, *makkhan* (butter) and *chakka* are the important fermented milk products in India. Yoghurt a western curd is also getting popularity in India. It is just like Indian *dahi*. The origin of yoghurt is in Turkistan. Very few food items can claim to be over 4500 years old one of these is yoghurt, a food universally known and eaten worldwide. It is healthy and popular food is produced with lactic acid fermentation of milk by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. High functionality of this product is attributed to the presence of living microorganisms such as lactic acid bacteria (LAB), streptococci, bifidobacteria or their combinations, which originate from the starter cultures (Amerinasab *et al.*, 2015).

In India, according to FSSR (2011) yoghurt can be defined as a food produced by culturing cream, milk, partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yoghurt should contain at least 3.0 per cent of milk fat and 8.5 per cent of milk solids not fat with a titrable acidity of not less than 0.9 per cent, expressed as lactic acid. Traditionally yoghurt is made

from cow, buffalo, goat and sheep milk. Yoghurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and aids in immune function and weight control. Because of these health-benefits associated with yoghurt, there is an increasing trend for consumption of yoghurt and is the fastest growing dairy category.

Yoghurt has custard like consistency and the flavour and texture of yoghurt constitute important aspects of its marketable quality. The characteristics of sharp acid flavor with adequate firmness and smooth body and texture without syneresis are essential for top quality products. There are many varieties of yoghurt marketed in the world some of these are plain, acidophilus, flavored with fruits, with nuts, frozen stricks drinks, fruit topped and mixed with vegetables. For many years, only natural or plain yoghurt was available in the world market, but now there has been a good demand for fruit yoghurt. The fruit enriched yogurt adds variety to the consumer to select. Recently popularity of yoghurt is due to the fortification with sugar and fruits (Sundaram and Kumaran, 2015). Sweet fruit yoghurt is preferred by children, adolescents and the aged. Through we eat *dahi*, fruits are rarely added into it. Hence, there is great scope to popularize yoghurt, popularity fruit yoghurt in India in place of *dahi*. So far there is very limited work on yoghurt in our country. On organoleptic evaluation it is seen fruity yoghurt is preferred markedly over plain yoghurt (Raut *et al.*, 2015).

Rose has influenced cultures aesthetically, economically, medically and spiritually since humankind could smell and appreciate its fragrance. The high concentration of anthocyanin in the petals of rose provided protection against the infectious agents (Hirulkar, 2010). According to ayurvedic wisdom, the rose has a cooling effect on our mind, body and emotions. The flowers are renowned for their fine fragrance, and are commercially harvested for rose oil used in perfumery and to make rose

water. The flower petals are also edible. They may be used to flavor food, as a garnish, as a tisane and preserved in sugar as rose petal jam. Rose-petal jam is a Persian treat called “Gulkand”, which simply means flower and sugar. The National Institute of Ayurveda Medicine provides a list of the benefits obtained from eating gulkand. This includes reduction of acidity and heat in the body, a reduction in eye inflammation and redness, strengthening of the teeth and gums, and the treatment of acidity. Gulkand has cooling properties, thus it is beneficial in alleviating all heat related problems like tiredness, lethargy, itching, aches and pains. The gulkand is a powerful antioxidant and a very good rejuvenator and also it has a calming effect on the nervous system, thus helping in reducing stress (Bors *et al.*, 2014). Traditionally it has been used as cooling tonic to combat fatigue, latherly, muscular aches, and biliousness itching and heat-related conditions. It is rich in calcium and also called as antioxidant and good blood purifier. Gulkand is commercial used in milk shakes, filling for cakes and pastries to improve the flavor and the appearance of the products (Nadaf *et al.*, 2012). Considering the demand for value added yoghurt and nutritional as well as aesthetic importance of gulkand it was planned to utilize gulkand in the yoghurt with following objectives.

### **OBJECTIVES**

1. To optimize the level of gulkand in yoghurt.
2. To examine the sensorial properties of gulkand yoghurt.
3. To examine the physico-chemical properties of gulkand yoghurt.

## 2. REVIEW OF LITERATURE

In cultured milk products, yoghurts are well known and most popular worldwide. Like milk, yoghurt is a healthy and delicious food due to its high nutritive and therapeutic value. Due to low lactose content yoghurt is easily digestible and palatable than milk. Yoghurt is valued for controlling the growth of bacteria and in curing of intestinal disease like constipation, diarrhea and dysentery, anti-carcinogenic effect and lowering of blood cholesterol. Flavour, texture and aroma of yoghurt depend upon the country of origin as well as other factors including raw materials quality, manufacturing process and the culture strains involved. Good quality yoghurt should be smooth, glossy surface, no cracks or holes on the top of yoghurt, no whey syneresis, no off flavour or odor, clean layer on the surface of yoghurt. Yoghurt is generally considered as a safer product and its unique flavor appeals to so many that consideration is being given by nutritionists to incorporate inexpensive source of nutrients to make it an almost complete food. Preparation of fruit yoghurt has been investigated by a number of researchers in different parts of world. The research works carried out by various workers on different aspects of production of yoghurt are reviewed here under following heads;

2.1 Importance of fermented milk products

2.2 Technology for preparation of yoghurt

2.3 Utilization of fruits pulp/ nuts/ cereals etc. in yoghurt

2.4 Gulkand- its importance and utilization

### **2.1 Importance of fermented milk products**

Singh (2006) reported that cultured dairy products constitute a vital component of the human diet in India as well as in many other regions of the world. *Dahi*, *mishti dahi* and *shrikhand* have unique position in people's diet in different parts of India. Besides imparting nutrition and novelty, these products help preserve the precious nutrients in fluid milk which is prone to quick deterioration. There are numerous references to *dahi* in the ancient Vedas. Milk

was fermented with green leaves, palasha bark (*Butea monosperma*) and putica creeper (*Combretum indicum*). *Dahi* was eaten with barley or rice. Churning of *dahi* to make butter at home and utilize the refreshing buttermilk with leftover grains of butter in it, as a refreshing drink, has been practiced for several centuries. Lord Krishna as a child was fond of *dahi* and *makhan* (indigenous cultured white butter). As a playful child, he also teased the curd and butter selling maids by targeting their earthen pots of *dahi* and *butter* with small pellets. In other words cultured dairy products find a very prominent position in the Indian culture, food habit and religious ethos.

Ebringer *et al.* (2008) reviewed the beneficial health effects of milk and fermented dairy products. They stated that fermented dairy products decreased the absorption of cholesterol especially low density lipid. So far the mechanism of this effect has not been explained. However, it is assumed that fermented dairy product bacteria can metabolized cholesterol and thus reduce its re-absorption in gastrointestinal tract.

Matkar and Khedkar (2008) reported that fermented milk products like *dahi*, *shrikhand* and *lassi* are manufactured by employing selected microorganisms to develop characteristic flavour and /or body and texture. These authors reported that fermented dairy products are specifically known for their increased nutritional and therapeutic value, more palatability and easier assimilation than milk.

Kumbhar *et al.* (2009) mentioned that traditionally for several centuries man has adopted fermentation as a mean of food preservation and also found them have nutritional and therapeutic advantages. Fermented milk products constitute a vital component of the human diet in many regions of the world. In the Indian sub-continent products like *dahi*, *lassi*, *shrikhand*, etc. are prominent in people diet.

Khetra *et al.* (2011) described the health benefits of fermented dairy products in terms of nutritional functions. They reported that nutritional function is the attribute of supplying sufficient nutrition while the physiological function

refers to prophylactic and therapeutic functions beyond nutrition. Nutritional function of fermented milks involve supply of macro nutrients including carbohydrate, fat and protein in easily digestible form and micronutrient including calcium, phosphorous, magnesium, zinc and certain water soluble vitamins. Fermentation enhances the absorption of vitamins and minerals. They also stated that the calcium, phosphorous and iron are better utilized by consuming fermented milks.

Hati *et al.* (2012) reported that fermented milk products have therapeutic properties as they are anti-cholesterolemic, anti-carcinogenic in nature. Fermented products are add variety to our gustative desires and provide important nutrients. They are considered superior to non fermented dairy products in terms of nutritional attributes as the microflora presents produce simple compounds like lactic acids, amino acids and free fatty acids that are easily assimilable.

Chauhan *et al.* (2013) also put-up their views on importance of fermented dairy products. They reported that fermentation enrich the human diet through development of a wide diversity of flavours, aromas and textures in foods. This process also preserves substantial amounts of foods through lactic acid, alcoholic, acetic acid and alkaline production. It also leads to detoxification, destruction of undesirable factors present in raw foods and removal of lactose.

Shiby and Mishra (2013) concluded that the lactic acid bacteria (LAB) play a major role in determining the positive health effects of fermented milks and related products. The *L. acidophilus* and bifid bacteria species are known for their use in probiotic dairy foods. Several food powders like yoghurt powder and curd (*dahi*) powder are manufactured taking into consideration the number of organisms surviving in the product after drying. Such foods, beverages and powders are highly acceptable to consumers because of their flavor and aroma and high nutritive value. Antitumor activity is associated with the cell wall of starter bacteria and so the activity remains even after drying. Other health benefits of fermented milks include prevention of gastrointestinal infections,

reduction of serum cholesterol levels and anti mutagenic activity. The fermented products are recommended for consumption by lactose intolerant individuals and patients suffering from atherosclerosis.

Naggar *et al.* (2014) reported that intake of yogurt has been linked to a reduced risk of developing bladder cancer, a lower risk of heart attack and heart disease and decrease in blood pressure. The study was done to determine the effectiveness of low fat yogurt in reducing body weight among Malaysian university students. This study showed significant differences between the weight of those who consumed 140 ml of low fat yogurt and those who did not. Consuming 140 ml of low fat yogurt for 14 days is significantly reducing the body weight.

Pinto *et al.* (2014) assessed the consumption of yogurt containing bifido bacterium animalis subsp. Lactis DN-173010 probiotic for a period of 2 weeks affects salivary and dental plaque levels of mutans streptococci and lactobacilli in patients undergoing orthodontic treatment. Daily ingestion of yogurt with or without B. animalis subsp. Lactis for a period of 2 weeks was beneficial in reducing total microbial counts in dental plaque.

Weerathiiake *et al.* (2014) were studied the evolution, processing, varieties and health benefits of yoghurt. According to them yoghurt improves lactose tolerance, immune enhancement and prevention of gastrointestinal disorders. Because of these known health benefits of yoghurt, consumer demand for yoghurt and yoghurt related products has been increased and became the fastest growing dairy category in the global market. Yoghurts are now being manufactured in a numerous styles and varieties with different fat contents, flavors and textures suitable for different meal occasions and plates as a snack, dessert, sweet or savory food.

## **2.2 Technology for preparation of yoghurt**

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. thermophilus* and *L. bulgaricus*.

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.

Huhn *et al*, (1982) used buffalo milk for preparation of yoghurt. Milk was heated for 30 min at about 90°C, then cool to 45°C before addition of yoghurt culture (2-3 per cent by weight). When coagulum was firm, it was broken, passed through a blender or liquefier and refrigerated for 12-18 hr to improve consistence and viscosity.

Warren (1982) reported that sweetened yoghurts were prepared by mixing skim solids from powder, concentrate cream and sugar to give a base of 12-15 per cent TS, 1 per cent fat and 5 per cent sugar. Mixed ingredients was pasteurized and cooled to incubation temperature. When bulk starter (*L. bulgaricus* and *S. thermophilus*) was injected before incubation at 32-34°C/ 12-16 h long sets and 40°C /4-5 h for short sets. For stirred yoghurt the product was cooled through a plate heat exchanger to 8-19°C.

Singh (1983) prepared the yoghurt from standardized and homogenized milk which was heated to 85-90°C, held for 15-30 min, cooled to 45°C and inoculated at the rate of 3 per cent with a mixture of *Lactophilus bulgaricus* and *Streptococcus thermophilus* followed by incubation at 42°C for 3-4 hr. In general, the final acidity of yoghurt ranged between at 0.8 to 1.5 per cent and pH between 4.4 and 4.9. The yoghurt was held at 5°C in a refrigerator. He recorded that the mixed cultures showed more acid and flavor production and higher proteolytic activity as compared to the pure cultures. He also noticed that yoghurt from buffalo milk scored more points as compared to yoghurt from cow milk.

Malton and Sandine (1986) review microbiology of yoghurt related bacteria, viz. *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. They stated that yoghurt was manufactured from milk with fat content of 2 – 3.5 per cent fortified by addition of 1 -6 per cent milk solids non fat or conc. to 10-15 per cent by evaporation. The basic mix was then homogenized and heat treated at 85<sup>0</sup> c for 30 min, 90-95<sup>0</sup> c for 5 min or other suitable time/ temp combination. Heat treated milk was cooled to 40-45<sup>0</sup>c inoculated with 2 per cent yoghurt bulk culture and incubated until sufficient acidity was reached (usually pH 4.6- 4.7). Then it was cooled to 4-5<sup>0</sup>c. They further reported that with advances in genetic engineering of lactic starters, it would be possible to eliminate the need for use *S. thermophilus* and *L. bulgaricus* in starters. Cloning appropriate protease genes in *S. thermophilus* may eliminate need for use of *L. bulgaricus*. Therapeutic and healthful properties may be enhanced through wider use *bifidobacteria* and *L. acidophilus* in yoghurt starters.

Prasad and Tommer (1992) studied the behavior and involvement of yoghurt bacteria in the gel micro structure. Fresh unfortified cow milk (fat 4.1 per cent, TS 13.2 per cent) was used for yoghurt preparation. Milk was first preheated 90°C for 30 mins, cool to 45°C and inoculated with 2 per cent starter, of *S. thermophilus* and *L. bulgaricus*. After inoculation at 42°C for 4 hrs yoghurt samples were stored at 4- 6°C. It was found that streptococci predominated throughout in the form of bundles of long coccial chains either localized as micro colonies or oriented along the micellar chains. Lactobacilli appeared less frequently in close injection with casein aggregated. Thus yoghurt bacteria merely in corporate in the gel but were truly associated in microstructure formation.

Kaytanli (1994) studied effect of different heat treatments on rheological and organoleptical properties of yoghurt. Yoghurt was prepared from homogenized 3 per cent fat raw whole milk that had been fortified with 3 per cent dried skim milk, then heat treated by batch pasteurization at 80 or 90°C for 10, 20, 30 mins, Inoculated with 3 per cent yoghurt starter and incubated at 43°C

to pH 4.6 to 4.7. It was found that protein denaturation was highest during 80°C per 20 mins. Viscosity increased, syneresis decreased and water holding capacity increased ( $P < 0.05$ ). During storage no other property was significantly influenced by severity of heat treatment. Yoghurt prepared from milk heated at 80°C per 20 min received highest scores for organoleptic properties.

Park *et al.* (2005) prepared yoghurt from a combination of skim milk and soya milk (100:0, 75:25, 50:50, 25:75, and 0:100) containing saccharified-rice solution by lactic fermentation of 4 different cultures. Lower syneresis was observed in soya-based yoghurt and both the hardness and springiness of curd increased as the proportion of soymilk in the substrate increased. Skim milk-based yoghurt had higher resistance to shear force with higher yield stress. The sensory quality of yoghurt produced from mixed culture had higher preference compared with that produced from a single culture (*Streptococcus thermophilus*). There was no significant difference in texture and overall acceptability among yoghurts produced from mixed substrates and skim milk-based yoghurt.

Silva *et al.* (2013) evaluate the microbiological aspects of strawberry flavored yoghurts produced with goat milk with water-soluble soy extract and *bifidobacterium animalis* species. *lactis* probiotic culture during processing. Determination of the viable cell count during yoghurt storage showed that the number of lactic bacteria found was below the expected only in the treatment in which there was water-soluble soy extract addition, not presenting microbiological viability. The other yoghurt treatments were viable during storage. The enumeration of *bifidobacterium animalis* ssp. *Lactis* showed that the yoghurt treatments presented microbiological viability during storage. they concluded that the addition of water-soluble soy extract interfered negatively with the production of goat milk based yoghurts.

Ehirim and Onyeneke (2013) investigated the proximate composition, microbiological and sensory properties of yoghurt produced from cow milk and goat milk blend. Proximate composition of yoghurt samples with goat milk (100

per cent, 75, 50 per cent, and 25 per cent) substitution blend with cow milk revealed that goat milk yoghurt samples (100 per cent) had the highest protein content (4.2 per cent), Fat content (4.27 per cent) and caproic (C<sub>6</sub>), caprylic (C<sub>8</sub>), Capric (C<sub>10</sub>) and total solids (16.22 per cent). Yoghurt of 25 per cent goat milk sample had the least protein content (3.75 per cent), while (50 per cent and 75 per cent goat milk yoghurt samples had had their values 3.49 per cent and 3.29 per cent respectively. However, there were low carbohydrate content with (100 per cent) goat milk yoghurt samples (7.20 per cent) and moisture content (83.78 per cent) than the (25 per cent) goat milk yoghurt with (9.20 per cent) carbohydrate, (85.14 per cent) moisture content (14.86 per cent) total solids, respectively. Generally, goat milk yoghurt samples (100 per cent), (75 per cent), (50 per cent) were mostly significantly preferred to (25 per cent) goat milk yoghurt sample at ( $p > 0.05$ ).

Gallo *et al.* (2013) studied the production, sensory, physical-chemical and microbiological examination of goat yogurt by the staff of the institute of hygiene and technology of milk. The results of sensory evaluation of yoghurt were important, especially from the consumers' point of view. The most important were the appearance, aroma of the product, and taste above all. Taste and smell of goat milk is different as compared to cow's milk, which has significant impact on the final yogurt. The evaluators found differences in the sensory attributes of yoghurt made at the institute compared with commercially produced yoghurt. Up to (80 per cent) of the evaluators preferred taste and pleasantness of taste of yogurt made at the institute compared to goat yogurt purchased commercially.

### **2.3 Utilization of fruits pulp/ nuts/ cereals etc. in yoghurt**

Arnold (1963) outlined the manufacture of fruit yoghurt in Switzerland. Fruit yoghurt was made with the addition of about 10 per cent (bilberries, raspberries, pineapple, blackcurrants) and the same amount of sugar, the ingredients stirred in after the completion of incubation which was carried out in milk.

Schulz *et al.* (1965) described that Japanese yoghurt was made mainly from skim milk with the addition of binding agents (gelatin and agar-agar), sugar and fruits (Strawberry, apple, banana, apricot, pineapple and orange). The product contained gelatin (0.1-0.5 per cent) and agar-agar (0.14-0.30 per cent) and was said to have a smooth, firm consistency, stable on shaking and resistant to whey separation.

Fekete and Buchi (1972) prepared vegetables and/or fruit yoghurt by mixing fresh milk (whole or skim milk) with vegetables or fruits or components of these, heating the mixture for preferably 10-16 min and then cooling and inoculating it. Sugar (0.5–3.0 per cent) or an artificial sweetener, as well as stabilizers, added before fermentation. The sterilization at 110<sup>0</sup>C, a skim milk yoghurt containing shredded carrots and additives (maize starch, locust bean flour, sugar and a small amount of lemon juice) had a shelf life of < 3-6 months at room temperature.

Mann (1976) studied chemical and bacteriological quality of yoghurt marketed in the U.K included 15 types of strawberry yoghurt and 14 types of blackcurrant fruit yoghurt which had a pH of 3.7- 4.1 and titrable acidities of 0.88-1.30 per cent (strawberry) and 0.75-1.68 per cent (blackcurrant).The chemical composition of both types was similar (approx.) 18-25 per cent TS, 0.2 to 3.4 per cent fat, 3.3-5.0 per cent protein, 5.2- 15 per cent reducing sugars and 4.6-13 per cent non reducing sugars.

Lal *et al.* (1980) prepared fruit yoghurt by dissolving skim milk powder at the rate of 4 per cent and sugar 5 per cent in warm standardized milk with 3 per cent fat. The milk was boiled for 12-20 min and cooled to luke warm temperature (24<sup>0</sup>C-45<sup>0</sup>C). Yoghurt starter *S.themophilus* (H) and *L.bulgaricus* (W) in the ratio of 1:2 well cleaned orange fruit pulp was added. The contents well mixed, poured into paper cups and incubated at a room temperature of 42<sup>0</sup>C-45<sup>0</sup>C for 3.30 hr. The lot was transferred to refrigerator for overnight. The final product was evaluated the next day by the various judges for appearance, acidity, consistency, taste, general quality, fruit, sugar and flavor. The scores

were obtained from each judge on the specified schedule. There was no significant difference between the mean scores of various items except for consistency and fruit and sugar wherein it was significant. The average score for flavor was 2.57 out of 4.

Sharma and Singh (1981) studied sweetened fruit yoghurt prepared from cow and buffalo milk. The milk was standardized to 0.5 per cent fat, added 3 per cent SNF, 0.5 per cent gelatin, stirred and homogenised. After adding sugar at the rate of 5 per cent it was heated by steaming for 30 min, cooled to 40°C, incubated with 3 per cent culture (with 1:1 proportion). Fruit juice could be added at 10 per cent,

Panday (1985) studied the fruit yoghurt made from reconstituted milk from milk powder (skim or whole). Milk was preheated (60-65°C), homogenized, pasteurized (90-98°C / 6 min) and cooled to 45-46°C. It was then inoculated with 4 per cent culture in ripening tank till lactic acidity to 1.05 to 1.10 per cent. At the end it was added with 9 per cent fruit, filled in cups, chilled down and stored at 4°C till distribution.

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.

Caric (1986) studied the yoghurt made from reconstituted milk having 11 to 14 per cent total solids. The milk was pasteurized for 30 min at 85°C, 20 min at 90°C and 5 to 10 min at 95°C. The resulting products were tested for TS, fat, ash, lactic acid, titrable acidity and organoleptic properties. The result showed that the optimal procedure was to use the reconstituted milk pasteurized at 90°C for 30 min with 11 per cent TS and 1:1 mixture of culture.

Baker *et al.* (1989) prepared a low calorie, low fat, low carbohydrate, fruit-containing yoghurt product by a process of admixing a butterfat-containing milk product in sufficient amount. To provide a butterfat content in the yoghurt product of less than about 0.2 per cent by weight, a stabilizer, a nutritive

sweetener, and non-heat modified nonfat dry milk solids and processing the mixture by homogenizing, vat pasteurizing, fermenting with a three component bacterial yoghurt culture mixture consisting essentially of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, blending with low calorie, nutritive sweetener-containing fruit preserves and cooling. The resultant yoghurt product has the body, texture and taste of conventional fruit containing yoghurt and a consumer acceptable appearance characterized by the absence of free moisture on the product surface and within the packaging.

Rajasekaran and Rajor (1989) manufactured frozen yoghurt by combination of soy milk and skim milk solids (50:50) and adjusted to predetermined levels of 9, 11 and 13 per cent serum solids. In final product, the flavour, colour and appearance and overall acceptability was found with 11 per cent levels of serum solids.

Ryckeboer and Louis (1992) described process of manufacturing yoghurt by using carrot pulp. At the culturing stage 5 to 50 per cent weight of very finely ground carrot was added to the inoculated milk and the mixture was strongly agitated before culturing continued. Thus they standardized process for manufacture of yoghurt type food product with carrot pulp.

Zekai and Erdoğan (2003) prepared yoghurt with different fruit-flavors (Cornelian, Morello Chery and Rose hip marmalade, grape molasses, date pulp, and control (without additive) and stored up to 10 days at 5°C. The fruit flavors were added at the rate of 7 per cent w/w. There were significantly differences in the fat, ash, protein, total solids (TS) content and titratable acidity (TA) for samples due to different flavor additives. The yoghurt containing Grape molasses and Morello had higher flavor scores than using other flavoring.

Mishra and Kumar (2003) were studied the formulation of mango soy fortified yoghurt (MSFY) by using response surface methodology (RSM). The independent variables were proportions of mango pulp (1.2–13.8 per cent), soymilk (2.39–27.61 per cent), and fat content (0.48–5.52 per cent) of buffalo milk. The responses were recorded for overall acceptability rating, counts of

*Streptococcus thermophilus* and *Lactobacillus bulgaricus* and hardness of the product. The optimum formulation conditions of 7.1 kg mango pulp (18 per cent total solids), 14.7 kg soymilk (8.2 per cent total solids), and 78.2 kg buffalo milk (2.95 percent fat content and 9 per cent solid not fat) per 100 kg was recommended for the blend formulation yielding an acceptable and good quality MSFY.

Mahmood *et al.* (2008) were prepared plain, apple and banana fruit stirred yoghurt by using buffalo milk at household level. They investigated that yoghurt with 8 percent apple and 8 percent banana pulp were liked in all sensory attributes. There were significant changes in pH, acidity, moisture, protein and carbohydrate (lactose) contents. An increase in protein and carbohydrate (lactose) was observed when addition of fruit pulp was made. Highest values for protein and carbohydrate (lactose) were 3.57 and 6.70 percent, respectively, which were observed in case of fruit stirred banana yoghurt at 10 percent level. All the types of yoghurt maintained the physiochemical quality during 2 days short storage. Viable bacterial count decreased during short storage. They concluded that with the addition of fruit pulp the quality of yoghurt can be improved.

Patil *et al.* (2009) observed that addition of guava in pulp form, @ 5- 15 per cent, after incubation in the stirred form with addition of sugar from 6 to 9 per cent was more acceptable. The guava yoghurt prepared with 5 per cent pulp and 9 per cent sugar was sensorily superior to that of control. The final product contained 3.41 per cent fat, 21.54 per cent TS, 1.06 per cent acidity, 5.65 per cent reducing sugar, 7.93 per cent non-reducing sugar, 13.58 per cent total sugar with pH 4.41. Higher levels of guava pulp adversely affected sensory characteristics of yoghurt; the cost of production was Rs. 26.77 for the most acceptable guava yoghurt containing 5 per cent pulp and 9 per cent sugar. After physico- chemical analysis of yoghurt they observed that fat per cent decreased with increased in levels of fruit pulp. Total solid & acidity per cent increased with increased in fruit pulp levels. Highest score for color & appearance (7.2),

flavor (44.3), body & texture 27.9 given by panel to 5 per cent treatment combination.

Hossain *et al.* (2012) conducted the research to prepare fruit yoghurt fortified with different level of fruit juice (5, 10 and 15 per cent of different fruits (strawberry, orange and grape). Quality of the yoghurts was improved due to incorporation of low level of fruit juice. Yoghurts fortified with 10 per cent orange juice was the best in quality among the others. The smell and taste, body and consistency and color and texture of the fruit yoghurts were equally acceptable. 10 and 15 per cent strawberry fruit yoghurt contain more acid and its texture was cracked down in refrigeration temperature. The moisture and acidity content of fruit yoghurts were increased than plain yoghurt because of high content of these in the fruits. The fat, protein, carbohydrates and ash content of strawberry and orange fruit yoghurt were decreased than normal plain yoghurt. But the carbohydrates content of grape yoghurt were increased because grape contains more sugar than milk and other two fruits. Statistical analysis showed that yoghurt fortified with 10 per cent orange juice was more acceptable than others comparing all quality characteristics. In case of strawberry yoghurt, fruit juice concentration more than 5 per cent was not suitable for fruit yoghurt because that are highly acidic and curd was cracked down at refrigerated condition.

Sawant (2012) prepared yoghurt by using different levels of pineapple fruit pulp i.e. 3, 6, 9 per cent .He observed that the fat, protein, contents of yoghurt decreased with increasing levels of fruit pulps. Total solid, acidity and S.N.F. contents of yoghurt increased with increasing levels of fruit pulps. Yoghurt with 6 per cent pineapple pulp was found highest score in respect of flavour, body & texture, color and appearance as compared to other treatments.

Agarwal and Prasad (2013) were prepared low-fat frozen yoghurt incorporated with carrot pulp. The low fat frozen yoghurt samples of different treatments were analyzed for organoleptic characteristics (flavour, body & texture, general appearance and overall acceptability) and nutritional

characteristics (moisture, ash, fat, protein, acidity, pH & total solids). They concluded that the low fat frozen flavoured yoghurt with 3 per cent carrot pulp, 3.0 per cent fat (T<sub>3</sub>F<sub>3</sub>) was high as comparable with other treatments in the organoleptic characteristics (Flavour and taste, body and texture, colour and appearance and overall acceptability) and nutritional characteristics (moisture, ash, fat, protein, acidity, pH & total solids).

Amerinasab *et al.* (2015) developed novel yoghurt based on date liquid sugar. The addition effect of date liquid sugar (DLS, 1–9 per cent v/v) to yoghurt milk on the physical (colour, firmness and syneresis), chemical (pH, total titratable acidity (TTA), total phenolic content (TPC) and antioxidant activity), rheological (viscosity and flow behaviour) and sensory attributes was scrutinized. Results showed that the pH value decreased by increasing DLS concentration from 1 to 6 per cent, while the TTA decreased with an increase in DLS from 6 to 9 per cent ( $p < 0.05$ ). The whey syneresis, firmness and viscosity values were considerably influenced by the DLS content and acidity of the yoghurts ( $p < 0.05$ ). A noticeable increase in antioxidant activity and TPC was found by the increasing DLS content ( $p < 0.05$ ). Yoghurts containing 6 per cent v/v DLS also had the lowest syneresis and the highest firmness among the different samples. Moreover, a pseudo plastic rheological behavior was detected for all the produced yoghurts. An increased in DLS concentration of manufactured yoghurts led to an increase in a, b and total colour difference (TCD) values and a decrease in L value ( $p < 0.05$ ). The sensory evaluation revealed that there was no significant different in the colour scores. However, the used panelists determined the yoghurt supplemented with 6 per cent DLS had the highest scores for other investigated attributes.

Raut *et al.* (2015) were prepared the three yoghurt drink-mango pulp blends (97:3, 94:6, and 91:9) and a control yoghurt drink was prepared with sugar and chilled water at the rate of 10 per cent each. The physico-chemical composition of yoghurt drink was affected due to addition of mango pulp, fat and protein content decreased with increased in the amount of mango pulp while

that of total solids, acidity and solid not fat increased. The mango yoghurt drink prepared with 6 per cent mango pulp in treatment T<sub>2</sub> showed maximum sensory score.

Srivastava *et al.* (2015) were analyzed antioxidant activity of herbal yoghurt prepared from different milk. They prepared herbal yoghurt of cow, buffalo and goat milks by using different levels of *Zingiber officinale* and *beta vulgaris*. The samples were analyzed by using 2, 2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP). The result indicated that highest antioxidant activities with DPPH & FRAP methods were found in 2 percent level of ginger rhizome goat milk yoghurt and 2 percent level of beet root extracts of goat milk yoghurt followed by 2 percent level of ginger extract in cow milk yoghurt the lowest antioxidant activity were found in buffalo milk herbal yoghurt.

Sundaram and Kumaran (2015) studied on optimization of seabuckthorn fruit pup and SMP level in preparation of yogurt using response surface methodology. The responses were counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, taste and viscosity of the product. Statistical analysis of this study revealed that fruit syrup and skimmed milk powder significantly affected all the responses. Optimum formulation conditions of fruit syrup (15 per cent) and skimmed milk powder (12.5 per cent) are recommended for the blend formulation yielding an acceptable and good quality seabuckthorn fruit yogurt. The resultant product also exhibited more amounts of fat, protein, carbohydrate and antioxidants viz., vitamin C, E, carotenoids, phenols and anthocyanins when compared to a commercial one.

#### **2.4 Gulkand – its importance and utilization**

Yayun (2005) reported the processing techniques for preparation of gulkand using fresh rose petal as the raw material. Three optimal formulas were developed viz. a) 50 g rose petals, 120 g sugar, 0.35 per cent citric acid, 150 ml pure water and 0.6 per cent fruit pectin. b) 50 g rose petal, 120 g sugar, 0.35 per cent citric acid, 30 ml rose water, 120 ml pure water, 0.6 per cent fruit pectin c)

50 g rose petal, 120 g sugar, 0.35 per cent citric acid, 150 ml mix water (or 30 ml rose water, 120 ml pure water), 0.6 per cent fruit pectin.

Hanan *et al.*, (2012) prepared low-calorie baladi gulkand and reported its composition, which contents crude proteins ranged between 11.86 - 22.42 per cent, 2.71-15.50 per cent crude fats, 0.89-1.53 per cent ash, 6.39-7.42 per cent crude fibre, 66.36-68.18 per cent carbohydrates, 20.92-24.30 per cent moisture, and 381.65 - 452.38 caloric value (k.cal/100gm).

Nadaf *et al.*, (2012) prepared shrikhand by adding gulkand and rose petal powder and studied its effects, chemical and sensory quality and properties of shrikhand. In their study the various compositions of gulkand and rose petal powder viz. 1:1.5, 1.5:1.5, 2:1.5 and 2.5:1.5 (gm) was added in chakka during shrikhand preparation the results of their study revealed that the protein and fat per cent of the shrikhand decreased whereas, carbohydrate, ash and moisture per cent increased with increasing level of gulkand and rose petal powder. On the basis of various sensory parameters, shrikhand containing 2:1.5 g of gulkand and dried rose petal powder was most liked by panel members.

Roman *et al.*, (2013) identified bioactive compound and antioxidant activity of rose hips. They reported that rose hips comprised several biologically active compounds such as sugars, organic acids, pectin, flavonoids, tannins, carotenoids, fatty acids, vitamins (particularly vit C and also Vitamins B<sub>1</sub>, B<sub>2</sub>, K, E), macro and micro elements. They also reported that the vitamin C in rose hips is far exceeding (30-1300 mg/g) the one found in citrus fruits.

Bors *et al.*, (2014) studied regarding the production and characterization of rose petal jam (gulkand). They reported the gulkand had moisture 69.85 per cent, ash 4.86 per cent, total acidity 0.2 per cent malic acid, total sugar 53.31 per cent, ascorbic acid 54.18 mg/g, total flavonoids 16.65 mg/100g and antioxidant capacity 18 min.

### **3. Material and Methodology**

The present investigation entitled “Process standardization and appraisal of gulkand yoghurt” carried out in the Laboratory of Animal Husbandry and Dairy Science, College of Agriculture, Kolhapur. The material used and methodology adopted during the course of investigation is discussed as under.

#### **3.1 Material**

##### **3.1.1 Milk**

Fresh buffalo milk was procured from Dairy Farm, College of Agriculture, Kolhapur and standardized to 4.5 per cent fat and was analyzed for pH, acidity, specific gravity, protein, lactose, ash and total solid content.

##### **3.1.2 Yoghurt cultures**

The freeze dried pure cultures of *S. thermophilus* and *L. bulgaricus* was procured from the National Collection of Dairy Culture, National Dairy Research Institute, Karnal (Haryana) and used for fermentation of milk @ 1 per cent of milk with ratio of 1:1. In order to keep the cultures active, they were propagated in skim milk at frequent intervals and preserved in refrigerator.

##### **3.1.3 Gulkand**

Gulkand was procured in single lot from local market of Kolhapur city.

##### **3.1.4 Sugar**

Sugar was procured in single lot from local market of Kolhapur city

#### **3.2 Equipments/ Utensils**

##### **3.2.1 Hand Blender**

Philips make hand blender was used to mix the milk and gulkand.

##### **3.2.2 Incubator**

Metalab make incubator was used for incubation of milk for preparation of yoghurt.

##### **3.2.3 pH meter**

Orion Star of Thermo Scientific make Single electrode pH meter was used to adjust the pH of media and to measure the pH of yoghurt.

### **3.2.4 Stirrer**

Long handled stirrer with flattened end made up of Stainless steel was used for stirring the milk during preparation of yoghurt.

### **3.2.5 Glassware**

All glassware's viz. beakers conical flasks, pipettes and burettes etc. were used Borosil make for analytical work. All the glassware's were cleaned and sterilized properly before their use.

### **3.2.6 Chemicals**

All the chemicals required for the analytical work were used of analytical (AR) or guaranteed reagent (GR) grade manufactured by Merk, India Ltd/Glaxo India Ltd.

### **3.2.7 Cream Separator**

Madhur Engineering Ltd. Kolhapur Make cream separator machine was used for separation of milk to standardize desired fat percentage in yoghurt.

### **3.2.8 Media for microbiological analysis**

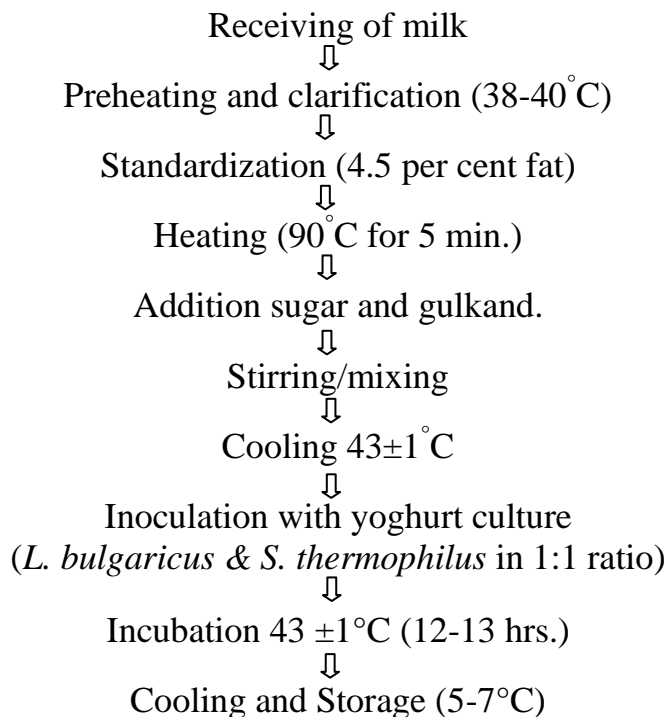
The media for different microbiological analysis were of M/S Hi-media laboratories, make namely plate count agar, violet red bile agar and potato dextrose agar.

## **3.3 Methodology**

### **3.3.1 Preparation of gulkand yoghurt**

The gulkand yoghurt was prepared by using standard techniques described by Agarwal and Prasad (2013) low- fat frozen yoghurt incorporated with carrot pulp with suitable modification (Fig- 3.1). Initially the buffallo milk was standerdize to 4.5 per cent fat and heated to 90<sup>0</sup>C for 5 min fallowed by addition of sugar and gulkand as per treatment combination and stirred the content. After cooling, the mix was inoculated with yoghurt culture and filled in the sterilized plastic cups of 100 ml capacity and covered with plastic lid. The filled cups were incubated at 42°C for 12-13 hrs. in incubator to obtain good quality of yoghurt. The set

gulkand yoghurt was allowed to cool at 5-7°C and evaluated for sensory and physico-chemical properties.



**Fig. 3.1 Flow diagram for manufacture of gulkand yoghurt.**

### 3.3.2 Optimization of gulkand and sugar quantity in yoghurt.

On the basis of beaker trial the quantity of gulkand and sugar was added at different levels of milk before inoculation of milk with yoghurt culture. The level and treatment combination for gulkand and sugar were as below.

Gulkand Level: G<sub>1</sub> (5%), G<sub>2</sub> (7%) and G<sub>3</sub> (9%)

Sugar Level: S<sub>1</sub>(2%) and S<sub>2</sub> (4%)

The yoghurt formulation in all treatment was evaluated for sensory and chemical qualities.

### Treatment combinations

Treatment no.	Treatment combination	Gulkand level (%)	Sugar level (%)
1	G <sub>1</sub> S <sub>1</sub>	5	2
2	G <sub>1</sub> S <sub>2</sub>	5	4
3	G <sub>2</sub> S <sub>1</sub>	7	2
4	G <sub>2</sub> S <sub>2</sub>	7	4
5	G <sub>3</sub> S <sub>1</sub>	9	2
6	G <sub>3</sub> S <sub>2</sub>	9	4

One best combination gulkand and sugar was optimized on the basis of sensory qualities of gulkand yoghurt.

#### 3.3.3 Optimization of fat level of milk for preparation of yoghurt

The quality of gulkand yoghurt prepared from the best combination of gulkand and sugar was further evaluated for suitable fat level. For that 1.5 (F<sub>1</sub>), 3 (F<sub>2</sub>), and 4.5 (F<sub>3</sub>) per cent fat containing buffalo milk was used for preparation of gulkand yoghurt. Obtaining gulkand yoghurt samples was examined for sensory quality and one best level of fat was optimized.

The treatment details are as under

F<sub>1</sub>- Gulkand yoghurt prepared from milk contained 1.5per cent fat

F<sub>2</sub>- Gulkand yoghurt prepared from milk contained 3 per cent fat

F<sub>3</sub>- Gulkand yoghurt prepared from milk contained 4.5 per cent fat

### 3.4 Analytical Methods

Fresh milk and gulkand yoghurt was analyzed for their physico-chemical and sensory qualities. The optimized gulkand yoghurt was also evaluate for microbiological quality.

#### 3.4.1 Physico-chemical analysis of milk

##### 3.4.1.1 pH

The pH of sample was measured by using Oroion 3 star pH benchtop pH meter. Firstly the pH meter was standardized at 25°C by using standard buffer solution. The pH of the sample was observed on the dial and recorded.

#### **3.4.1.2 Acidity**

It was determined as per method given in IS: 1479, Part I (1960).

#### **3.4.1.3 Specific gravity**

It was determined as per method suggested by Thompkinson and Sabhikhi (2006).

#### **3.4.1.4 Fat**

Fat of milk was determined by Gerber method as described in IS: 1224 Part-I (1977)

#### **3.4.1.5 Protein**

Protein was determined by the Micro-kjeldhal method as described in IS: SP: 18 part XI (1981).

#### **3.4.1.6 Lactose**

It was as estimated as per BIS, handbook of food analysis, SP: 18 (part II), 1981.

#### **3.4.1.7 Ash**

It was as estimated as per BIS, handbook of food analysis, SP: 18 (part II), 1981.

#### **3.4.1.8 Total solids**

Total solids was determined as per method given in IS: 1479, Part-II (1961)

### **3.4.2 Physico-chemical analysis of gulkand yoghurt.**

#### **3.4.2.1 Titratable acidity (%)**

Determined by the procedure recommended in IS: 1479 (Part -I), 1960. About 10 g of yoghurt sample was taken into beaker and 2-3 drops of phenolphthalein indicator was added in it. The content in the beaker was titrated with 0.1 N NaOH solutions till to get end point of titration i.e. appearance of faint pink colour and it should persistent observed upto 8-10 second in beaker. Burette reading was taken and acidity of yoghurt sample was calculated as lactic acid (%).

$$\text{Titrateable acidity (\%)} = \frac{\text{No. of ml of 0.1 N NaOH required} \times 0.009}{\text{Wt. of yoghurt sample taken}} \times 100$$

#### **3.4.2.2 pH**

Ten g of gulkand yoghurt sample was weighed in a 50 ml beaker and the pH was measured by Oroion 3 star pH benchtop pH meter.

#### **3.4.2.3 Fat**

Fat of gulkand yoghurt was determined by Gerber method as described in IS: 1224 Part-I (1977).

#### **3.4.2.4 Protein**

Total protein in gulkand yoghurt samples was determined by Micro-Kjeldahl method (SP: 18 (Part XI), 1981).

Three grams of the gulkand yoghurt sample was accurately weighed and transferred carefully to the Kjeldahl flask, taking precaution to see that particles of sample do not stick in the neck of the flask. Ten g of potassium sulphate, 0.5 g of mercuric oxide and 20 ml of sulphuric acid were added to the flask. The content was mixed and boiling chips added in it and heated the flask carefully on the digestion apparatus until foaming stops and the contents have become liquid. The digestion was continued with more vigorous heating until the contents of the Kjeldahl flask became completely clear and colourless. During heating, the content was mixed from time to time. The liquid was boiled vigorously for 1 ½ hours after it has become clear. Local over heating was avoided. The contents of the flask were cooled to room temperature and made up volume to 100 ml in volumetric flask with distilled water. Ten ml of aliquot of the solution was pipette out to the micro-Kjeldahl distillation apparatus and the solution was made alkaline by 8 ml of sodium hydroxy-sodium thiosulphate solution and steam distilled. The evolved ammonia was absorbed in 5 ml of boric acid solution containing 2 to 3 drops of indicator. About 25 ml of distillate was collected

and titrated against hydrochloric acid (0.02 N) till violet colour appeared. Blank was carried out using all reagents except the sample material.

$$\text{Protein (\% by weight)} = \frac{89.32 (V_2 - V_1) N}{W}$$

Where,

$V_2$  = Number of ml of hydrochloric acid used in distillation

$V_1$  = Number of ml of hydrochloric acid used in the blank test

$N$  = Normality of the hydrochloric acid

$W$  = Weight of sample taken for analysis

#### 3.4.2.5 Reducing sugars

The reducing sugars of gulkand yoghurt were estimated by Lane and Eynon method (1923). Ten gm of gulkand yoghurt sample was extracted using distilled water. Neutral lead acetate (45 per cent) was added drop by drop till no further precipitate formed. The solution was deloaded with potassium oxalate solution (22 per cent) and filtered through Whatman No. 1 filter paper and volume was made 250 ml ( $V_1$ ) with distilled water. The burette was filled with extracted reducing sugar. Five ml of each Fehling solution A and B was pipetted in 250 ml conical flask and 40 ml distilled water was added. The contents were boiled and under vigorous boiling condition, the solution from burette was added to solution in conical flask till bluish colour of solution was discharged and yellowish brown colour appeared. Later, 3-4 drops of methylene blue indicator were added and titration was continued till blue colour was completely discharged and permanent brick red colour was obtained. The reading was noted. The titration was repeated twice and main burette reading was calculated. Standard lactose solution was used as standard. Factor (F) was determined by titrating standard lactose solution (0.3%) with Fehling solution.

$$\text{Reducing sugar (\%)} = \frac{F \times V_1 \times 100}{\text{MBR} \times W}$$

Where,

F	=	Factor
MBR	=	Mean burette reading
V <sub>1</sub>	=	Volume made of sample
W	=	Weight of sample

#### 3.4.2.6 Non-reducing sugar

Non-reducing sugar of gulkand yoghurt were determined by subtracting reducing sugars from total sugars. The procedure used for inversion and estimation of total sugar was as under.

Deleaded extract 50 ml (V<sub>2</sub>) prepared under 3.3.3.4 was taken in 250 ml conical flask. It was diluted with 25 ml distilled water. Ten ml of HCL solution (6.34 N) was added and heated at 49°C for a half hour. After cooling, it was neutralized with normal sodium hydroxide solution using methyl red indicator and volume was made up to 250 ml (V<sub>3</sub>). The sugar was determined as mentioned in the procedure above (3.4.2.5).

$$\text{Total sugar (\%)} = \frac{F}{W} \times \frac{V_1}{V_2} \times \frac{V_3}{\text{MBR}} \times 100$$

Where,

F	=	Factor
W	=	Weight of sample
V <sub>1</sub>	=	Volume made of sample
V <sub>2</sub>	=	Volume taken for inversion
V <sub>3</sub>	=	Volume made after inversion
MBR	=	Mean burette reading

#### 3.4.2.7 Total sugar

Total sugar of gulkand yoghurt was determined by procedure used for inversion and estimation of total sugar was as mentioned in the procedure above (3.4.2.6).

### 3.4.2.8 Ash

The ash content of gulkand yoghurt was determined as per method of A.O.A.C. (1995) for milk with slight modifications as under.

Five g of gulkand yoghurt was weighed into a silica dish and ignited on a laboratory Bunsen burner. The final incineration was done in a muffle furnace at not more than 550°C temperature for 2 hrs until the ash was free from carbons, cooled into desiccators and weighed. Constant weight of ash considered to have reached when the difference in two consecutive weighing after repeated ignition was less than 0.5 mg. The ash content was expressed as per cent of the gross product.

$$\% \text{ Total ash} = \frac{W_2 - W_1}{W} \times 100$$

Where,

$W_1$  = Weight of silica dish

$W_2$  = Weight of silica dish + total ash (after final ignition)

$W$  = Weight of gulkand yoghurt taken

### 3.4.2.9 Total Solids (%)

The total solids content of gulkand yoghurt was determined by subtracting moisture content of sample from weight of sample, which was determined as per gravimetric method as per IS: 1479 (Part-II), 1961.

### 3.4.3 Microbial analysis of yoghurt

The standard plate count, coliform count and yeast and mould count was enumerated as per the procedure given in ICAR Bacteriological laboratory manual (1982).

#### 3.4.3.1 Preparation of dilutions

Eleven g of gulkand yoghurt was weighed in sterile aluminum dish, in the balance using a sterile spatula. The content of the aluminium dish was then transferred to a sterile glass mortar. Then about 20 ml of sterile buffer solution (at 45°C) from 99 ml dilution blank was added and smooth paste

was made using the pestle which was then transferred into a sterile 250 ml conical flask. Another 20 ml of the buffer was poured into the mortar, rinsed thoroughly and transferred into the flask. Rest of the buffer solution in 99 ml dilution blank was poured into the mortar, rinsed and transferred into the flask. The content of the flask was mixed well. This gives a dilution of 1: 10. From this initial dilution, further dilutions were prepared by transferring 1 ml into 9 ml blanks.

#### **3.4.3.1.1 Standard plate count (SPC)**

Plate Count Agar (Hi-media) was used to enumerate the standard plate counts in the gulkand yoghurt sample.

To rehydrate this medium, 23.5g of the dry medium was suspended in 1000 ml distilled water. The mixture was then boiled to dissolve the medium completely. It was then filled in conical flask and the mouths of the conical flasks were closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 min. One ml of the diluted sample (suitable dilution) was transferred in each of the duplicate petri dishes. Ten to fifteen ml of the melted agar (at 45°C) was then poured and the contents were mixed well by rotating in a horizontal position. The contents were allowed to solidify. The plates were then inverted and incubated at 37°C for 48 hrs.

#### **3.4.3.1.2 Coliform count (CC)**

Violet Red Bile Agar (VRBA) of Hi-Media was used to enumerate the coliform counts in gulkand yoghurt samples.

To rehydrate this medium, 41.5g of V.R.B.A. powder was suspended in 1000 ml distilled water. Then the mixture was brought to boil to dissolve completely. The media was then cooled to 45°C and poured into conical flasks (150 ml). This media was not autoclaved.

To each of the duplicate sterile petridishes were added 1 ml (1 : 10 dilution) of the sample. To each of these petridishes 10-15 ml of the melted (45°C) VRBA was added and the contents of the petridishes were mixed

well by rotating the plates by placing them horizontally on a table. The media was then allowed to solidify and then a second layer of agar was made by adding 5-10 ml of melted agar. The media was allowed to solidify and then incubated after inverting the plates at  $37 \pm 0.5^\circ\text{C}$  for 2 days.

#### **3.4.3.1.3 Yeast and mould count (YMC)**

Potato Dextrose Agar (PDA) of Hi-media was used to enumerate yeast and mould counts in the gulkand yoghurt samples. To rehydrate this medium, 39g of PDA powder was suspended in 1000 ml distilled water and then boiled to dissolve the medium completely. It was then filled in conical flasks and the mouths of the flasks were closed with cotton plugs. The flasks were then sterilized by autoclaving at 15 psi pressure ( $121^\circ\text{C}$ ). The pH of the media was adjusted to 3.5 at the time of plating by using 10 per cent sterile tartaric acid solution.

One ml of the diluted sample (suitable dilution) was added to each of the duplicate sterile petridishes. Ten to fifteen ml of the melted potato Dextrose Agar (at  $45^\circ\text{C}$ ) was added to each petridish and 1 drop of 10 per cent tartaric acid (sterile) was added to them. The contents of the petridishes were mixed by rotating the plates in horizontal position placing them on a table. The media was allowed to solidify and then inverted and incubated at  $22 \pm 2^\circ\text{C}$  for 3 to 5 days.

#### **3.4.4 Sensory evaluation of gulkand yoghurt.**

Sensory evaluation of gulkand yoghurt was assessed by the panel of judges from Division of Animal Husbandry and Dairy Science, College of Agriculture, Kolhapur using 9-point Hedonic scale (Amerine *et al.*, 1965). The colour and appearance, flavour, sweetness, consistency and overall acceptability were attributes for this study.

#### **3.4.5 Consumer acceptability study**

The developed gulkand yoghurt during the investigation was also subject to consumer acceptance. The test was carried out with 200

respondents from local area. The information for the same was collected on prescribed format. (Appendix-II)

#### **3.4.6 Statistical design:**

The data generated during optimization of level of gulkand and sugar were analyzed statistically using factorial complete randomized design (Snedecor and Cochran, 1967)

#### **3.4.7 Facilities required:**

The necessary facilities available in the Division of Animal Husbandry and Dairy science, College of Agriculture, Kolhapur were utilized.

## 4. RESULT AND DISCUSSION

Yoghurt is an important fermented dairy product. It is highly acidic in taste and harsh acidic flavour. So, it is rarely consumed plain. It comes with a variety of added flavour including fruits in various forms. Rose-petal jam is a Persian treat called “Gulkand”, which simply means flower and sugar. According to ayurvedic wisdom; the rose has a cooling effect on our mind, body and emotions. The flower petals are also edible. They may be used to flavor food, as a garnish, as a tisane and preserved in sugar as rose petal jam. Gulkand has cooling properties, having beneficial in alleviating all heat related problems like tiredness, lethargy, itching, aches and pains. It also helps in reducing burning sensations in the soles and palms. By considering these aspect here attempt was made to prepare yoghurt by blending with gulkand. The results obtained during this investigation are presented in following headings.

4.1 Physico-chemical properties of milk

4.2 Effect of gulkand and sugar on sensory qualities of yoghurt

4.3 Effect of gulkand and sugar on physico-chemical properties of yoghurt

4.4 Effect of gulkand and sugar on microbial properties of yoghurt

4.5 Effect of fat level on sensory quality of yoghurt

4.6 Consumer acceptance of gulkand yoghurt

### **4.1 Physico-chemical properties of milk**

The buffalo milk was procured from Dairy Farm, College of Agriculture, Kolhapur. The obtained milk was standardized to 4.5 per cent fat and analyzed for various physico- chemical properties (Table 4.1) From the table it is seen that the acidity, pH and specific gravity of the milk sample was 0.16 (% LA), 6.62 and 1.033, respectively. The milk was composed by means of fat 4.5 per cent, protein 3.89 per cent, lactose 5.02 per cent, ash 0.79 per cent and total solid 14.20 per cent. The present findings are in agreement with the reports of David (2006) and De (2008).

**Table 4.1 Physico-chemical properties of milk**

Constituents	Values
Acidity (% LA)	0.16 ± 0.02
pH	6.62 ± 0.02
Specific gravity	1.033 ± 0.007
Fat (%)	4.5 ± 0.05
Protein (%)	3.89 ± 0.04
Lactose (%)	5.02 ± 0.01
Ash (%)	0.79 ± 0.01
Total solid (%)	14.20 ± 0.05

#### 4.2 Effect of gulkand and sugar on sensory qualities of yoghurt

Preliminary trials were conducted to know the suitability of level of gulkand and sugar in yoghurt. It was found that the best qualities of yoghurts can be made by addition of gulkand at the rate 5 to 9 per cent and sugar at the rate 2 to 4 per cent. Therefore, the experiment was conducted wherein gulkand added @ 5 ( $G_1$ ), 7 ( $G_2$ ) and 9 ( $G_3$ ) per cent and sugar added @ 2 ( $S_1$ ) and 4 ( $S_2$ ) per cent. The individual and interactive effect of the gulkand and sugar was determined by factorial completely randomized design (FCRD). The effect of gulkand and sugar level on sensory qualities of yoghurt is presented in following paragraphs.

##### 4.2.1 Effect on colour and appearance score

The effect of gulkand and sugar on colour and appearance score of yoghurt is presented in Table 4.2.

**Table 4.2 Effect of gulkand, sugar and their interaction on colour and appearance (score)\* of yoghurt**

Treatments	$G_1$	$G_2$	$G_3$	Mean
$S_1$	7.24	7.62	8.12	<b>7.66</b>
$S_2$	7.30	7.69	8.20	<b>7.73</b>
<b>Mean</b>	<b>7.27</b>	<b>7.66</b>	<b>8.16</b>	<b>7.70</b>
	G	S	G×S	
<b>SEm</b>	0.034	0.028	0.048	
CD (P0.05)	0.098	NS	NS	

\* Mean ±SE of five replication    NS= Non Significant

From the above table, it is seen that the colour and appearance score of yoghurt was ranged from 7.24 to 8.20. The maximum score (8.20) for colour and appearance was recorded to the product containing 9 per cent gulkand and 4 per cent sugar. The effect of gulkand was significant ( $p < 0.05$ ) on colour and appearance score of yoghurt whereas, its interaction with sugar exerted a non significant effect. The judges reported that at higher level of gulkand the product shows slightly pinkish with appreciable appearance of rose petals in the form of jam. However, sedimentation of gulkand was also appeared at increasing concentration of gulkand. Cliff *et al.* (2013) were reported that the colour and appearance score of yoghurt was increased with level of carrot juice added in it.

#### 4.2.2 Effect on flavour score

**Table 4.3 Effect of gulkand, sugar and their interaction on flavour (score)\* of yoghurt.**

Treatments	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	7.61	7.80	8.15	<b>7.85</b>
S <sub>2</sub>	7.67	7.92	7.94	<b>7.84</b>
<b>Mean</b>	<b>7.64</b>	<b>7.86</b>	<b>8.05</b>	<b>7.85</b>
	G	S	G×S	
SEm	0.028	0.023	0.040	
CD( $p < 0.05$ )	0.08	NS	0.12	

\* Mean  $\pm$ SE of five replication    NS= Non Significant

From the data (Table 4.3) it is revealed that the level of gulkand and its interaction with sugar were significantly ( $p < 0.05$ ) affected on the flavour score of yoghurt. The maximum flavour score (8.15) was recorded in the G<sub>3</sub>S<sub>1</sub> product (fig 4.2). The overall mean of flavour score was 7.85. It was also observed that with increasing level of gulkand, the flavour score was also increased irrespective of sugar. This might be because of the richness of flavour of gulkand and exerted cooling effect of mind and emotions (Bors *et al.*, 2014).

Nadaf *et al.* (2012) also found maximum score for aroma of shrikhand when gulkand and rose petal powder were added at the

concentration of 2 per cent and 1.5 per cent respectively. The present findings are in accordance with the report of Cliff *et al.* (2013), who found that the perceived intensity of carrot flavour increases as carrot juice level increased in yoghurt preparation. Zekai and Erdogan, (2003) compare the yoghurt prepared with addition of various fruit flavours including rosehip marmalade. They found that the flavour score (4.10 out of 5) was maximum to the yoghurt prepared by addition of rosehip marmalade over the other flavours used in their study. Desai *et al.* (1994) who found that smell and taste of mango and pineapple yoghurt were higher than that of control yoghurt.

#### 4.2.3 Effect on consistency score

Consistency is an important parameter in evaluating the quality of fruit yoghurt. The effect of gulkand and sugar on consistency score of yoghurt is shown in Table 4.4 and Fig 4.3

**Table 4.4 Effect of gulkand, sugar and their interaction on consistency (score)\* of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	8.52	8.11	7.49	<b>8.04</b>
S <sub>2</sub>	8.30	7.76	7.28	<b>7.78</b>
<b>Mean</b>	<b>8.41</b>	<b>7.94</b>	<b>7.39</b>	<b>7.91</b>
	G	S	G×S	
SEm	0.019	0.016	0.027	
CD(p<0.05)	0.055	0.045	0.078	

\* Mean ±SE of five replication NS= Non Significant

From the above Table 4.4 it is clear that optimum consistency score 8.52 were found in the yoghurt prepared by the addition of sugar and gulkand at 2 and 5 per cent, respectively. There was significant (P<0.05) difference among the level of gulkand, sugar and their interaction. The overall consistency score was ranged from 7.28 to 8.52 with mean score of 7.91. The decreased in consistency score with increasing level of sugar might be because of dissolving the sugar in the moisture content of yoghurt and made it more soluble. Similarly as gulkand level increased the

consistency score was also decreased this trend reflected because of non binding power of gulkand and containing more amount of sugar in it.

The present finding are accordance with the study of Hossain *et al.* (2012) who reported that addition of 15 per cent strawberry, orange and grape juice in yoghurt preparation resulted in decreased its consistency score. Contradictory report was also observed in literature where Raut *et al.* (2015) reported that the body and texture score of yoghurt drink were increased due to addition of mango pulp. Similarly contradictory observation was also reported by Agarwal and Prasad, (2013) in the low fat frozen yoghurt incorporated with carrot pulp.

#### 4.2.4 Effect on sweetness score

Human beings have an innate preference for sweet taste due to its relevance as a natural cue for edibility and energy-rich foods. Progress in food technology made it possible to add sweeteners to foods to impart sweet taste and increase the pleasure of eating, which has made sugar one of the most important hidden sources of calories. Considering this fact sugar was added in yoghurt at the rate 2 per cent and 4 per cent whereas, gulkand was added at 5, 7 and 9 per cent, the effect of these factors was studied on sweetness score of yoghurt.

**Table 4.5 Effect of gulkand, sugar and their interaction on sweetness (score)\* of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	7.12	7.62	8.42	<b>7.72</b>
S <sub>2</sub>	7.36	7.84	8.14	<b>7.78</b>
<b>Mean</b>	<b>7.24</b>	<b>7.73</b>	<b>8.28</b>	<b>7.75</b>
	G	S	G×S	
SEm	0.034	0.028	0.049	
CD(p<0.05)	0.099	NS	0.140	

\* Mean ±SE of five replication

NS= Non Significant

The data depicted in Table 4.5 indicate that the yoghurt had sweetness score ranged from 7.12 to 8.42 with a mean score of 7.75. The

maximum sweetness score was recorded in the product containing 2 per cent sugar and 9 per cent gulkand. Here it is worthy to report that at excessive quantity of sugar the sweetness score was decreased from 8.42 to 8.14 in the yoghurt containing 9 per cent gulkand. This might be because of excessive sweetness of the product, which was not preferred by panelist. The influence of sugar reduction on the sensory characteristics of products depends on the type of product and the level of reduction (Oliveira *et al.*, 2015)

The effect of gulkand and its interaction with sugar on sweetness score was significant ( $P < 0.05$ ). The effect of level of date liquid sugar on sweetness score of yoghurt was studied by Amerinasab *et al.* (2015) and recorded sweetness score of yoghurt was in range of 6.60 to 7.40 out of 9.0.

#### 4.2.5 Effect on overall acceptability score

The effect of gulkand and sugar on overall acceptability score of yoghurt is presented in Table 4.6 and fig 4.5.

**Table 4.6 Effect of gulkand, sugar and their interaction on overall acceptability (score)\* of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	7.62	7.79	8.05	<b>7.82</b>
S <sub>2</sub>	7.66	7.80	7.89	<b>7.78</b>
<b>Mean</b>	<b>7.64</b>	<b>7.80</b>	<b>7.97</b>	<b>7.80</b>
	G	S	G×S	
SEm	0.017	0.014	0.024	
CD(p<0.05)	0.049	NS	0.070	

\* Mean ±SE of five replication NS= Non Significant

From the Table 4.6 it is revealed that the addition of gulkand had significant effect on overall acceptability score of yoghurt. The score for overall acceptability of G<sub>1</sub>S<sub>1</sub>, G<sub>2</sub>S<sub>1</sub>, G<sub>3</sub>S<sub>1</sub>, G<sub>1</sub>S<sub>2</sub>, G<sub>2</sub>S<sub>2</sub>, and G<sub>3</sub>S<sub>2</sub> treatment combination were 7.62, 7.79, 8.05, 7.66, 7.80, and 7.89, respectively. The maximum score was recorded to the product contained 2 per cent sugar and 9 per cent gulkand. Raut *et al.*(2015) were reported that the overall

acceptability score were increased of yoghurt drink prepared with mango pulp up to certain level after that it starts to decline.

It was observed that the mean score for overall acceptability was increased with increased level of gulkand. From the above result, score was decreased at higher concentration of gulkand with increased level of sugar. Therefore it has been claimed that yoghurt containing 2 per cent sugar and 9 per cent gulkand was comparatively superior over the rest of combination studied.

### 4.3 Effect of gulkand and sugar on physico-chemical properties of yoghurt

#### 4.3.1 Effect on pH

**Table 4.7 Effect of gulkand, sugar and their interaction on pH of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	4.82	4.65	4.48	<b>4.65</b>
S <sub>2</sub>	4.73	4.55	4.42	<b>4.57</b>
<b>Mean</b>	<b>4.78</b>	<b>4.60</b>	<b>4.45</b>	<b>4.61</b>
	G	S	G×S	
SEm	0.009	0.007	0.012	
CD(p<0.05)	0.025	0.020	NS	

\* Mean ±SE of five replication NS= Non Significant

It could be seen from the Table 4.7 that the mean value of pH of yoghurt was significantly ( $p<0.05$ ) decreased from 4.82 to 4.42 with increasing level of gulkand. Similar effect on pH of yoghurt was shown by the level of sugar. The pH value recorded for product G<sub>1</sub>S<sub>1</sub>, G<sub>2</sub>S<sub>1</sub>, G<sub>3</sub>S<sub>1</sub>, G<sub>1</sub>S<sub>2</sub>, G<sub>2</sub>S<sub>2</sub> and G<sub>3</sub>S<sub>2</sub> was 4.82, 4.65, 4.48, 4.73, 4.55 and 4.42, respectively (fig 4.6). The decreased pH was observed with increasing level of gulkand might be because of acidic nature of gulkand (pH 5.4) as reported by Youssef and Mousa (2012).

Weerathinake *et al.* (2014) in their report pointed out that the pH of regular yoghurt was less than 4.50. However, pH less than 4.0 of fruit flavoured yoghurt was recorded by Zekai and Erdogan (2003).

### 4.3.2 Effect on titratable acidity

The titratable acidity of the yoghurt prepared by the addition of gulkand and sugar is presented in Table 4.8

**Table 4.8 Effect of gulkand, sugar and their interaction on titratable acidity of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	0.75	0.85	0.93	<b>0.84</b>
S <sub>2</sub>	0.80	0.89	0.96	<b>0.88</b>
<b>Mean</b>	<b>0.78</b>	<b>0.87</b>	<b>0.95</b>	<b>0.86</b>
	G	S	G×S	
SEm	0.004	0.004	0.006	
CD(p<0.05)	0.013	0.010	NS	

\* Mean ±SE of five replication NS= Non Significant

It is seen from the Table 4.8, that titratable acidity of yoghurt in treatments G<sub>1</sub>S<sub>1</sub>, G<sub>2</sub>S<sub>1</sub>, G<sub>3</sub>S<sub>1</sub>, G<sub>1</sub>S<sub>2</sub>, G<sub>2</sub>S<sub>2</sub> and G<sub>3</sub>S<sub>2</sub> was 0.75, 0.85 0.93, 0.80, 0.89 and 0.96 per cent LA, respectively (fig 4.7). It was observed that titratable acidity of yoghurt blended with 2 per cent sugar and 5 per cent gulkand was lowest whereas, highest titratable acidity was recorded in product, containing sugar at 4 per cent and gulkand at 9 per cent. It was also found that the effect of gulkand and sugar was significant (p<0.05) towards increasing the acidity of yoghurt. The highest acidity with higher level of sugar and gulkand might be because of higher amount of carbohydrate present in these factors. With this presence of malic acid in rose petal jam reflected in higher titratable acidity in yoghurt. The malic acid more than 0.2 per cent in gulkand was reported by Bors *et al.* (2014).

### 4.3.3 Effect on fat content

Fat is important component in development of food products. It gives richness as well as smoothness to the product.

From the Table 4.9, it was revealed that fat content in yoghurt was ranged from 3.20 to 4.19 per cent. The minimum fat content (3.2 per cent) was observed in yoghurt containing 9 per cent gulkand and 4 per cent sugar

level. Whereas, the maximum fat content (4.19 per cent) was recorded for yoghurt prepared by addition of 5 per cent gulkand and 2 per cent sugar (fig 4.8). As the sugar level increased the fat content was significantly ( $p < 0.05$ ) decreased. However, the interaction effect of gulkand and sugar was non-significantly recorded for fat content of yoghurt. This is because of both the factors are absence or negligible percentage of fat.

**Table 4.9 Effect of gulkand and sugar and their interaction on fat content (%) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	4.19	3.72	3.40	<b>3.77</b>
S <sub>2</sub>	4.16	3.60	3.20	<b>3.65</b>
<b>Mean</b>	<b>4.18</b>	<b>3.66</b>	<b>3.30</b>	<b>3.71</b>
	G	S	G×S	
SEm	0.025	0.021	0.036	
CD( $p < 0.05$ )	0.072	0.059	NS	

\* Mean  $\pm$ SE of five replication NS= Non Significant

Fat content in yoghurt decreased due to addition of fruit juice was reported by Hossain *et al.* (2012) and Raut *et al.* (2015) this might be because of negligible of fat in fruit juice.

#### 4.3.4 Effect on protein content

The protein content of yoghurt as affected by treatment combination is presented in Table 4.10

**Table 4.10 Effect of gulkand and sugar and its interaction on protein content (%) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	3.46	3.04	2.50	<b>3.00</b>
S <sub>2</sub>	3.30	2.79	2.30	<b>2.80</b>
<b>Mean</b>	<b>3.38</b>	<b>2.92</b>	<b>2.40</b>	<b>2.90</b>
	G	S	G×S	
SEm	0.024	0.020	0.034	
CD( $p < 0.05$ )	0.069	0.056351	NS	

\* Mean  $\pm$ SE of five replication NS= Non Significant

From the Table 4.10 it was observed that the protein content of the product  $G_1S_1$ ,  $G_2S_1$ ,  $G_3S_1$ ,  $G_1S_2$ ,  $G_2S_2$  and  $G_3S_2$  was recorded 3.46, 3.04, 2.50, 3.30, 2.79 and 2.30 per cent, respectively (fig 4.9). It show that the protein content ranged between 2.30 to 3.46 per cent. The highest protein content was recorded in yoghurt prepared by addition of 2 per cent sugar and 5 per cent gulkand. The data shows that as the sugar level increased the protein content were significantly ( $p<0.05$ ) decreased and the same effect of gulkand was also observed. However, the interaction of gulkand and sugar were non-significantly affected on protein content of yoghurt.

The findings in present study was supported by Raut *et al.* (2015) who reported that protein content of yoghurt drink was decreased due to addition of varied proportion of mango pulp because of protein content of mango pulp is less than milk. Similarly Hossain *et al.* (2012) also reported that protein content of fruit yoghurt decreased because protein content of fruit juice is less than milk.

#### 4.3.5 Effect on reducing sugar content

Reducing sugar is important for growth and development of micro organism that is *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The reducing sugar in yoghurt is shown in Table 4.11

**Table 4.11 Effect of gulkand, sugar and their interaction on reducing sugar content (%) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	4.52	4.25	4.02	4.26
S <sub>2</sub>	4.32	4.12	3.87	4.108
Mean	4.42	4.18	3.94	4.18
	G	S	G×S	
SEm	0.006	0.005	0.008	
CD( $p<0.05$ )	0.016	0.013	0.023	

\* Mean  $\pm$ SE of five replication NS= Non Significant

From the Table 4.11 it observed that reducing sugar was ranged from 3.87 to 4.52 per cent. The maximum reducing sugar content was recorded in the yoghurt prepared by addition of 5 per cent gulkand and 2 per cent sugar

whereas, the minimum content of reducing sugar recorded in yoghurt prepared by addition of 9 per cent gulkand and 4 per cent sugar. It was observed that as the sugar level increased the reducing sugar were decreased significantly ( $p < 0.05$ ), similar effect was observed for gulkand level. The interaction effects of gulkand and sugar on reducing sugar were significantly ( $p < 0.05$ ) affected.

The present finding was in contradictory result of Hossain *et al.* (2012), Raut *et al.* (2015) who found that increasing level of fruit pulp also increased in reducing sugar content of yoghurt. This was because of content of fructose in fruits used by them.

#### 4.3.6 Effect on non-reducing sugar content

Non-reducing sugar content of the yoghurt is present in Table 4.12

**Table 4.12 Effect of gulkand, sugar and their interaction on non-reducing sugar content (%) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	5.27	6.55	7.55	6.46
S <sub>2</sub>	7.25	8.54	9.55	8.45
Mean	6.26	7.55	8.55	7.45
	G	S	G×S	
SEm	0.010	0.008	0.014	
CD(p<0.05)	0.03	0.024	NS	

\* Mean ±SE of five replication NS= Non Significant

From the Table 4.12, it is clear that the non-reducing sugar content of the yoghurt containing sugar and gulkand were ranges from 5.27 to 9.55 per cent. It was expected and found that the effect of sugar, gulkand and in its interaction had significant ( $p < 0.05$ ). Non-reducing sugar content of yoghurt was increased as the level of gulkand increased. Similarly the increased in the sugar content also increased the non reducing sugar content yoghurt. The carbohydrate content in shrikhand prepared without addition of gulkand was significant lower than the other gulkand added sample (Nadaf *et al.* 2012). More than 65 per cent total sugar in gulkand was reported by Yousuff and Mousa (2012).

#### 4.3.7 Effect on total sugar content

From the Table 4.13, it is revealed the level of sugar, gulkand and their interaction significantly ( $p < 0.05$ ) affected on the total sugar content of yoghurt. This might be because of both the factors contained sugar as a base material.

**Table 4.13 Effect of gulkand, sugar and their interaction on total sugar content (%) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	9.79	10.80	11.57	10.72
S <sub>2</sub>	11.58	12.67	13.42	12.56
Mean	10.68	11.73	12.50	11.64
	G	S	G×S	
SEm	0.013	0.010	0.018	
CD( $p < 0.05$ )	0.037	0.030	NS	

\* Mean ±SE of five replication NS= Non Significant

The overall total sugar content was in the ranged of 9.79 to 13.42 per cent. The maximum total sugar was recorded in the product containing 9 per cent gulkand and 4 per cent sugar (G<sub>3</sub>S<sub>2</sub>).

The present findings are in agreement with Mohamood *et al.* (2008) who reported the total carbohydrate contain of yoghurt increased with increasing the level of fruits in yoghurt.

#### 4.3.8 Effect on ash content

The ash contained in yoghurt as affected by treatment is shown in Table 4.14.

**Table 4.14 Effect of gulkand, sugar and their interaction on ash content (%) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	0.85	0.88	1.03	<b>0.92</b>
S <sub>2</sub>	0.88	0.93	1.06	<b>0.95</b>
Mean	<b>0.87</b>	<b>0.90</b>	<b>1.05</b>	<b>0.94</b>
	G	S	G×S	
SEm	0.006	0.005	0.008	
CD( $p < 0.05$ )	0.017	0.014	NS	

\* Mean ±SE of five replication NS= Non Significant

From the Table 4.14, it is observed that the ash content was increased from 0.85 to 1.06 per cent. The product containing ash prepared by  $G_1S_1$ ,  $G_2S_1$ ,  $G_3S_1$ ,  $G_1S_2$ ,  $G_2S_2$  and  $G_3S_2$  was recorded 0.85, 0.88, 1.03, 0.88, 0.93 and 1.06 per cent, respectively. Further, it was also observed that as the gulkand level increased ash content was also increased significantly ( $p<0.05$ ). This might be because of presence of mineral matter in gulkand (Hossain *et al.*, 2012). Similarly, the sugar level increased ash per cent was also increased significantly ( $p<0.05$ ) whereas, the interaction effect of gulkand and sugar on ash non-significantly affected.

#### 4.3.9 Effect on total solid content

The total solid content in yoghurt as affected by the level of gulkand and sugar is depicted in Table 4.15

**Table 4.15 Effect of gulkand, sugar and their interaction on total solid content (%) of yoghurt**

Treatment	$G_1$	$G_2$	$G_3$	Mean
$S_1$	18.30	18.44	18.51	18.42
$S_2$	19.91	19.94	19.98	19.96
Mean	19.10	19.21	19.25	19.19
	G	S	G×S	
SEm	0.035	0.028	0.049	
CD( $p<0.05$ )	0.10	0.082	NS	

\* Mean  $\pm$ SE of five replication NS= Non Significant

From the Table 4.15 it observed that the total solid content of yoghurt was ranged from 18.30 to 19.98 per cent. The yoghurt prepared  $G_1S_1$ ,  $G_2S_1$ ,  $G_3S_1$ ,  $G_1S_2$ ,  $G_2S_2$  and  $G_3S_2$  were recorded total solid 18.30, 18.44, 18.51, 19.91, 19.94 and 19.98 per cent, respectively. It is clear that as the sugar level increased the total solid content in yoghurt was also increased significantly ( $p<0.05$ ). The total solid content was also increased significantly ( $p<0.05$ ) with increased in gulkand level. The interaction effect of gulkand and sugar level on total solid was significant ( $p<0.05$ ). Raut *et al.* (2015) were reported that the total solid content increased in yoghurt drink prepared with addition mango pulp.

Hossain *et al.* (2012) who found that total solid content of yoghurt prepared with addition of grape juice decreased because of grape juice contain lower amount of total solid.

#### 4.4 Effect of gulkand, sugar and their interaction on microbial quality of yoghurt

##### 4.4.1 Effect on standard plate count (SPC)

The SPC of yoghurt prepared by the addition of gulkand and sugar is presented in Table 4.16

**Table 4.16 Effect of gulkand, sugar and their interaction on Standard plate count ( $\log_{10}/g$ )\* of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	6.12	6.26	6.39	<b>6.26</b>
S <sub>2</sub>	6.14	6.33	7.10	<b>6.52</b>
<b>Mean</b>	<b>6.13</b>	<b>6.30</b>	<b>6.75</b>	<b>6.39</b>
	G	S	G×S	
SEm	0.076	0.062	0.108	
CD(p<0.05)	0.220	0.180	0.312	

\* Mean  $\pm$ SE of five replication NS= Non Significant

It is seen from the table 4.16 that SPC of yoghurt in treatment G<sub>1</sub>S<sub>1</sub>, G<sub>1</sub>S<sub>2</sub>, G<sub>2</sub>S<sub>1</sub>, G<sub>2</sub>S<sub>2</sub>, G<sub>3</sub>S<sub>1</sub> and G<sub>3</sub>S<sub>2</sub> were 6.12, 6.14, 6.26, 6.33, 6.39 and 7.10  $\log_{10}/g$ , respectively. There was significant (P<0.05) difference in the SPC of the sample prepared by addition of varying level of gulkand and sugar. It was observed that the SPC of yoghurt blended with 2 per cent sugar and 5 per cent gulkand was recorded lowest count and 4 per cent sugar and 9 per cent gulkand was recorded highest count with an average of 6.39 ( $\log_{10}/g$ ).

Comparatively lower number of standard plate count (5.29 to 5.87  $\log_{10}$  cfu/g) in day 1 samples sample of fruit yoghurt was observed by Zekai and Erdogan (2003). The SPC count of  $9.50 \times 10^2$  and 5.60 cfu/g reported by Hossain *et al* (2012) in yoghurt which were higher than obtained count in experimental yoghurt.

#### 4.4.2 Effect on coliform count (C.C.)

The coliform count of yoghurt prepared with the different level of sugar and gulkand is presented in the Table 4.17 and fig 4.16

**Table 4.17 Effect of gulkand, sugar and their interaction on coliform count (cfu/g)\* of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	2.00	2.60	7.40	<b>4.00</b>
S <sub>2</sub>	2.60	5.00	5.20	<b>4.27</b>
<b>Mean</b>	<b>2.30</b>	<b>3.80</b>	<b>6.30</b>	<b>4.13</b>
	G	S	G×S	
SEm	0.852	0.696	1.206	
CD(p<0.05)	2.456	NS	NS	

\* Mean ±SE of five replication NS= Non Significant

From the above table 4.17, it is revealed that the addition gulkand had a significant effect on coliform count. For G<sub>1</sub>S<sub>1</sub>, G<sub>2</sub>S<sub>1</sub>, G<sub>3</sub>S<sub>1</sub>, G<sub>1</sub>S<sub>2</sub>, G<sub>2</sub>S<sub>2</sub> and G<sub>3</sub>S<sub>2</sub> treatment, the coliform count was recorded to be 2.00, 2.60, 7.40, 2.60, 5.00 and 5.20 cfu/g, respectively (fig 4.16). It observed that the mean coliform count was 4.13cfu/g and was ranged from 2 to 7.40 cfu/g and maximum count was recorded in the sample G<sub>3</sub>S<sub>1</sub>.

It can be seen from table that all of the yogurt samples had <8.0 cfu/g coliform bacteria. However, Zekai and Erdogan (2003) recorded < 1 coliform bacterial count in fruit flavoured yoghurt.

The coliform count of 19×10<sup>5</sup> and 10 MPN/ g, was reported by Hossain *et al.* (2012), respectively in yoghurt and which also supported to the present finding.

#### 4.4.3 Effect on yeast and mould count

From the Table 4.18 it is revealed that the addition gulkand had a significant effect on yeast and mould count. The yeast and mould count in G<sub>1</sub>S<sub>1</sub>, G<sub>2</sub>S<sub>1</sub>, G<sub>3</sub>S<sub>1</sub>, G<sub>1</sub>S<sub>2</sub>, G<sub>2</sub>S<sub>2</sub> and G<sub>3</sub>S<sub>2</sub> sample was recorded to be 2.41, 2.51, 2.71, 2.47, 2.62, and 2.77 log<sub>10</sub>/g, respectively (fig 4.17). It was observed that the mean yeast and mould count was 2.58 log<sub>10</sub>/g and in the

yoghurt sample, it was recorded that maximum count was obtained by G<sub>3</sub>S<sub>2</sub> i.e. 9 per cent gulkand and 4 per cent sugar. According to FSSR (2011), yogurt Standards a maximum of 100 cfu/ g of mold is allowed in yoghurt.

**Table 4.18 Effect of gulkand, sugar and their interaction on yeast and mould count (log<sub>10</sub>/g) of yoghurt**

Treatment	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	Mean
S <sub>1</sub>	2.41	2.51	2.71	<b>2.54</b>
S <sub>2</sub>	2.47	2.62	2.77	<b>2.62</b>
<b>Mean</b>	<b>2.44</b>	<b>2.56</b>	<b>2.74</b>	<b>2.58</b>
	G	S	G×S	
<b>SEm</b>	0.043	0.035	0.061	
<b>CD(p&lt;0.05)</b>	0.125	NS	NS	

\* Mean ±SE of five replication NS= Non Significant

The higher organism of yeast and mould count in yoghurt blended with the gulkand was might be due to post contamination in the sample. The high yeast and mould count could be attributed to contamination from air, gulkand, sugar and one day old culture used for yoghurt manufacture (Con *et al.*1996).

The yeast and mould count of 25 cfu/gm and 2.65 log cfu/g was reported by Hossain *et al.* (2012) and Zekai and Erdogan (2003) in yoghurt.

#### **4.5 Effect of fat level on sensory quality**

Effect of fat level on sensory qualities of yoghurt is presented in Table 4.19. To know the suitability of level of fat, yoghurt was to prepare with varying level of fat in milk (1.5 to 4.5 per cent). During the selection of fat level in milk the gulkand and sugar was added at the rate of 9 and 2 per cent, respectively.

From the Table 4.19, it is observed that maximum colour and appearance score of yoghurt was observed for F<sub>3</sub> treatment (4.5 per cent fat) and it was 8.28±0.07. The colour and appearance score of yoghurt was increased significantly (P<0.05) as the fat level increased.

**Table 4.19 Effect of fat level on sensory qualities (score\*) of yoghurt**

Treatment	Colour and Appearance	Flavour	Consistency	Sweetness	Overall acceptability
F <sub>1</sub>	7.84 <sup>a</sup> ±0.09	7.84 <sup>a</sup> ±0.09	7.63 <sup>a</sup> ±0.10	8.04±0.10	7.81 <sup>a</sup> ±0.05
F <sub>2</sub>	8.16 <sup>b</sup> ±0.10	8.32 <sup>c</sup> ±0.10	8.31 <sup>c</sup> ±0.10	8.09±0.12	8.23 <sup>c</sup> ±0.04
F <sub>3</sub>	8.28 <sup>b</sup> ±0.07	8.06 <sup>b</sup> ±0.11	8.05 <sup>b</sup> ±0.12	8.11±0.10	8.13 <sup>b</sup> ±0.04
SE	0.06	0.06	0.06	0.06	0.02
CD (p<0.05)	0.14	0.17	0.17	NS	0.07

\* Mean ±SE of five replication NS= Non Significant

The flavour score is also depicted in Table 4.19, from the table, it is observed the maximum flavour score was obtained for F<sub>2</sub> treatment (3 per cent fat). The flavour score was increased up to certain point of fat level and beyond that the flavour score was decreased significantly (P<0.05).

The consistency score was maximum (8.31± 0.10) observed for F<sub>2</sub> treatment containing 3 per cent fat of yoghurt. It was observed that the consistency score of yoghurt increased with fat level up to F<sub>2</sub> treatment and beyond that it decreased for F<sub>3</sub> treatment. The sweetness score of yoghurt were non-significantly affected by the fat level of milk.

The overall acceptability score of gulkand yoghurt was depicted in Table 4.19. The maximum score 8.23 ± 0.04 of overall acceptability for yoghurt was observed for F<sub>2</sub> treatment followed by F<sub>3</sub> treatment (8.13±0.04) and lastly to F<sub>1</sub> treatment (7.81± 0.05).

From the foregoing study it could be concluded that the best quality of yoghurt can be prepared from buffalo milk having 3 per cent fat with addition of 9 per cent gulkand and 2 per cent sugar.

#### **4.6 Consumer acceptance of gulkand yoghurt**

The frequency distribution of the consumer perception of the gulkand yoghurt is given in Table 4.20 and found that 56.5 per cent of the consumers reported excellent quality of gulkand yoghurt and 23.5 per cent reported very

good remark about quality of gulkand yoghurt. Further 17.5 and 7.5 per cent of the consumer expressed good and fair status of gulkand yoghurt, respectively.

**Table 4.20 Distribution of consumers on the basis of acceptance of gulkand yoghurt**

<b>Preference</b>	<b>Score</b>	<b>No. of respondent</b>	<b>Percentage</b>
Fair	1	15	7.5
Good	2	35	17.5
Very good	3	47	23.5
Excellent	4	113	56.5

## 5. SUMMARY AND CONCLUSION

The results obtained during the course of investigation of process standardization for preparation of gulkand yoghurt are summarized in following sub heading.

### 5.1 Physico-chemical properties of milk

The standardized buffalo milk used for preparation of yoghurt had 0.16 (% LA) titratable acidity, 6.62 pH and 1.033 specific gravity. The milk used for experiment was composed by means of fat 4.5 per cent, protein 3.89 per cent, lactose 5.02 per cent, ash 0.79 per cent and total solid 14.20 per cent.

### 5.2 Effect of gulkand and sugar level on sensory qualities of yoghurt

Based on the preliminaries trial the quantity of gulkand and sugar to be optimized was finalized. Accordingly gulkand was added @ 5 ( $G_1$ ), 7 ( $G_2$ ) and 9 ( $G_3$ ) per cent whereas, sugar was added at 2 ( $S_1$ ) and 4 ( $S_2$ ) per cent of milk. The experiment was conducted in factorial completely randomized design (FCRD).

#### 5.2.1 Effect on colour and appearance score of yoghurt

The maximum score (8.20) for colour and appearance was recorded to the product containing 9 per cent gulkand and 4 per cent sugar. The effect of gulkand was significant ( $p < 0.05$ ) on colour and appearance score of yoghurt.

#### 5.2.2 Effect on flavour score of yoghurt

The maximum flavour score (8.15) was recorded in the  $G_3S_1$  product. It was also observed that with increasing level of gulkand, the flavour score was also increased irrespective of sugar.

#### 5.2.3 Effect on consistency score of yoghurt

The optimum consistency score (8.52) were found in the yoghurt prepared by the addition of sugar and gulkand at 2 and 5 per cent, respectively. There was significant ( $P < 0.05$ ) difference among the level of

gulkand, sugar and their interaction. The consistency score was decreased with increasing level of ingredients.

#### **5.2.4 Effect on sweetness score of yoghurt**

The maximum sweetness score was recorded in the product containing 2 per cent sugar and 9 per cent gulkand. At excessive quantity of sugar the sweetness score was decreased from 8.42 to 8.14 in the yoghurt containing 9 per cent gulkand.

#### **5.2.5 Effect on overall acceptability score of yoghurt**

The addition of gulkand had significant effect on overall acceptability score of yoghurt. The maximum score was recorded to the product contained 2 per cent sugar and 9 per cent gulkand. It was observed that the mean score for overall acceptability was increased with increased level of gulkand. The score was decreased at higher concentration of gulkand with increased level of sugar.

### **5.3 Effect of gulkand and sugar on physico-chemical properties of yoghurt**

#### **5.3.1 Effect on pH of yoghurt**

The pH of yoghurt was significantly ( $p < 0.05$ ) decreased from 4.82 to 4.42 with increasing level of gulkand. Similar effect on pH of yoghurt was shown by level of sugar.

#### **5.3.2 Effect on titratable acidity of yoghurt**

Yoghurt blended with sugar at 4 per cent and gulkand at 9 per cent was recorded the highest titratable acidity in product. It was also found that the effect of gulkand and sugar was significant ( $p < 0.05$ ) in positive term.

#### **5.3.3 Effect on fat content (%) of yoghurt**

The maximum fat content (4.19 per cent) was recorded for yoghurt prepared by addition of 5 per cent gulkand and 2 per cent sugar. As the sugar level increased the fat content were significantly ( $p < 0.05$ ) decreased. However, the interaction effect of gulkand and sugar was non-significantly recorded on fat content of yoghurt.

#### **5.3.4 Effect on protein content (%) of yoghurt**

The highest protein content was recorded in yoghurt prepared by addition of 5 per cent gulkand and 2 per cent sugar. It was found that as the sugar and gulkand level increased the protein content were significantly ( $p < 0.05$ ) decreased.

#### **5.3.5 Effect on reducing sugar content (%) of yoghurt**

The maximum reducing sugar content was recorded in the yoghurt prepared by addition of 5 per cent gulkand and 2 per cent sugar. As the sugar level increased the reducing sugar content was decreased significantly ( $p < 0.05$ ), same effect was observed for gulkand level.

#### **5.3.6 Effect on non-reducing sugar content (%) of yoghurt**

The non-reducing sugar content of the yoghurt containing sugar and gulkand were ranges from 5.27 to 9.55 per cent. It was expected and found that the effect of sugar, gulkand and in its interaction had significant ( $p < 0.05$ ). Non-reducing sugar content of yoghurt was increased as the level of gulkand increased.

#### **5.3.7 Effect on total sugar content (%) of yoghurt**

The level of sugar, gulkand and their interaction significantly ( $p < 0.05$ ) affected on the total sugar content of yoghurt. The maximum total sugar was recorded in the product containing 9 per cent gulkand and 4 per cent sugar ( $G_3S_2$ ).

#### **5.3.8 Effect on ash content (%) of yoghurt**

As the gulkand level increased ash content was also increased significantly ( $p < 0.05$ ) similarly, as the sugar level increased ash per cent was also increased significantly ( $p < 0.05$ ) whereas, the interaction effect of gulkand and sugar on ash were non-significant.

### **5.3.9 Effect on total solid content (%) of yoghurt**

The total solid content of yoghurt was ranged from 18.30 to 19.98 per cent. As the sugar level increased the total solid was also increased significantly ( $p < 0.05$ ). The total solid content was also increased significantly ( $p < 0.05$ ) with increase in gulkand level. The interaction effect of gulkand and sugar level on total solid was significant ( $p < 0.05$ ).

## **5.4 Effect of gulkand, sugar and their interaction on microbial quality of yoghurt**

### **5.4.1 Effect on standard plate count (SPC) of yoghurt**

The SPC of yoghurt blended with 2 per cent sugar and 5 per cent gulkand was recorded lowest count while 4 per cent sugar and 9 per cent gulkand was recorded highest count with an average of 6.39 ( $\log_{10}/g$ ).

### **5.4.2 Effect on coliform count (C.C.) of yoghurt**

In the yoghurt sample, mean coliform count was 4.13 cfu/g and it was ranged from 2 to 7.40 cfu/g. The maximum count was recorded in the sample G<sub>3</sub>S<sub>1</sub>.

### **5.4.3 Effect on yeast and mould count of yoghurt**

In the yoghurt sample mean yeast and mould count was 2.58 cfu/g and maximum count was observed in G<sub>3</sub>S<sub>2</sub> product i.e. 9 per cent gulkand and 4 per cent sugar.

## **5.5 Effect of fat level on sensory quality of yoghurt**

The maximum score of colour and appearance and sweetness was recorded for the yoghurt prepared from the milk containing 4.5 per cent fat and the score was  $8.28 \pm 0.07$  and  $8.11 \pm 0.10$ , respectively. Whereas, the flavour, consistency and overall acceptability scores were recorded maximum for the yoghurt prepared from the milk containing 3.0 per cent fat and score was  $8.32 \pm 0.10$ ,  $8.31 \pm 0.10$ , and  $8.23 \pm 0.04$ , respectively.

## **5.6 Conclusion**

1. The yoghurt prepared by addition of 9 per cent gulkand and 2 per cent sugar of milk was rated highest sensory score for flavor (8.15), sweetness (8.42) and overall acceptability (8.05).
2. The product with optimized formulation had 4.48 pH, 0.93 (%LA) titratable acidity, 3.40 per cent fat, 2.50 per cent protein, 11.57 per cent total sugar and 18.51 per cent total solid.
3. The quality of the product further improved by reduction in the fat percentage and it was observed that the milk of 3.0 per cent fat was found to be most acceptable for preparation of optimized level of gulkand yoghurt.

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