COMPARATIVE STUDY OF LOW-FAT SPREAD PREPARATION FROM COW AND BUFFALO MILK GHEE

By

Mr. DHANAKE VRISHASEN VINAYAK

(*Reg. No. K-15/148*)

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

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Approved by

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2017

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 Diploma.

Place: Kolhapur **(V. V. Dhanake)**
Date: / /2017

Dr. D. D. Patange
Assistant Professor,
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College of Agriculture, Kolhapur

CERTIFICATE

This is to certify that the thesis entitled, **"COMPARATIVE STUDY OF LOW-FAT SPREAD PREPARATION FROM COW AND BUFFALO MILK GHEE",** 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**, embodies the result of the piece of bonafide research work carried out by **Mr. VRISHASEN VINAYAK DHANAKE,** 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.

Place: Kolhapur. **(D. D. Patange)**

Date: / / 2017 Chairman and Research Guide

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Associate Dean,
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Maharashtra State, (India).

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Place : Kolhapur. (**G. G. Khot**)
Date: / / 2017 Associate Dean

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Place: Kolhapur

Date: / /2017 *(Dhanake Vrishasen Vinayak)*

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LIST OF ABREVIATIONS

et al.	Any other
@	At the rate of
AMF	Anhydrous milk fat
β	Beta
B.G.S	Buffalo ghee spread
BR	Butyro-refractometer
CD	Critical difference
C.G.S	Cow ghee spread
CV	Coefficient of variation
°C	Degree Celsius
etc.	Et cetera
FFA	Free fatty acid
Fig.	Figure
g.	Gram(s)
IS	Indian standards
Kg	Kilo gram(s)
LFS	Low-fat spread
ml.	Milli litre
min.	Minute(s)
MP	Melting point
viz.,	Namely
N.D.R.I.	National Dairy Research Institute
NS	Non-significant
o/w	Oil in water
%	Per cent
ppm	Parts per million
rpm	Revolution per minute
SFC	Solid fat content
SFO	Sunflower 0il

SMP	Skim milk powder
SNF	Solid not fat
SE	Standard error
Sec	Second
SEm	Standard error mean
i.e.	That is
w/o	Water in oil
Wt.	Weight
>	More than
<	Less than
±	Plus or minus
lit.	Litre
TSS	Total soluble solid

ABSTRACT

COMPARATIVE STUDY OF LOW-FAT SPREAD PREPARATION FROM COW AND BUFFALO MILK GHEE
by
DHANAKE VRISHASEN VINAYAK
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2017

Research Guide : Dr. D. D. Patange
Division : Animal Husbandry and Dairy Science
Major Field : Dairy Science

Milk fat has unique universal appeal which is well established in the history of culinary science and has not been matched by any other food component. Fat plays a key role in majority of food products because it not only produces creamy texture and flowing mouth feel to foods but also imparts a delicate nutty pleasant flavour to product. Importance of butter has been fully realized and utilized as spread. However butter loses its spreadability and forms crystals at refrigerator temperature. At ambient temperature it loses its plasticity and becomes sloppy which is too undesirable. Therefore it is necessary for dairy manufactures to introduce low-energy and low-priced fat products with improved functional properties. Low-fat spread is one of the dairy product in such category.

Low-fat spread (LFS) are the product harmonizing with the idea of healthy nutrition. At the same time it has a good taste and flavour as well as very good spreadability at refrigerator temperature. It is also blends easily with other foods. LFS provides both dietary and convenience requirements of consumer.

Number of fat source are used for preparation of LFS including ghee. Indian ghee market is known by identical characteristic of cow and buffalo milk ghee. Present study was emerged with idea of LFS preparation from

cow and buffalo milk ghee and compare them for sensory and physico-chemical qualities. Rapidly and slowly precooled cow and buffalo ghee were evaluated for sensory and textural studies. In preparation of LFS ghee, SMP, Tween-80, carrageenan, glycerol, salt and citric acid were used. For improving colour and appearance and flavour of LFS butter annatto colour and diacetyl flavour was used. Both cow and buffalo ghee LFS was judged for sensory and physico-chemical analysis including oiling and wheying off percentage. LFS was also exposed for 10, 20 and 30 min to see the effect of environmental temperature on sensory properties of LFS. For sensory analysis 9-point Hedonic scale was used.

Slowly pre-cooled cow ghee had intense yellow colour than rapidly pre-cooled cow ghee, whereas slowly pre-cooled buffalo ghee has creamish white colour whereas rapidly pre-cooled buffalo ghee had white colour. Rapidly pre-cooled cow ghee had very smooth and pasty texture (score 5.28) than rapidly pre-cooled buffalo ghee (score 11.88). The consistency score appeared to be solid hard (score 94.16) for rapidly pre-cooled buffalo ghee than rapidly pre-cooled cow ghee (score 85.12). The LFS of cow ghee had shown maximum sensory scores for colour and appearance (7.70), body and texture (7.90), spreadability (7.98) and overall acceptability (7.95) as compare to buffalo ghee LFS. However buffalo ghee LFS recorded higher score for flavour (7.18). Chemically it was observed that both the LFS was more or less similar in fat, protein, carbohydrate, ash, T.S and pH except FFA content. Oiling off (4.04 %) and wheying off (7.67%) was found higher in cow ghee LFS over buffalo ghee LFS. Colour and appearance and flavour score was found improved by addition of butter annatto colour (0.1%) and diacetyl flavour (4 ppm) respectively. When both the ghee LFS was exposed for 10, 20 and 30 min at 35°C it was observed that cow ghee LFS best for colour and appearance, body and texture, spreadability and the overall acceptability scores up to 10 minutes. Buffalo ghee spread was well maintained colour and appearance, body and texture and spreadability. Both categories of LFS obtained from ghee maintained their physical properties up to 10 minutes at treatment of 35°C which all the sensory properties decreased with increasing time of exposure.

1. INTRODUCTION

Milk fat is secreted from the mammary gland of the female mammals containing all the essential nutrients except for iron and vitamin C and widely used food ingredients due to their unique flavour, high nutritional value and rheological properties. Milk fat occurs naturally in milk and present in oil in water emulsion (Ronholt *et al.*, 2013). These attributes of milk fat contribute unparallel richness to the food products resulting in better market value and consumer acceptability (Sharma *et al.,* 1998). Because of these properties, some cultures have historically regarded various forms of milk fats as luxuries. Butter one of these foods.

For dairy industry butter and other milk fat based product are valuable product due to their unique test, their textural characteristics and nutritional value (Ronholt *et al.*, 2013). The importance of butter in the diet has been fully realized. In developed countries butter is utilized as a spread. It has poor spreadability when stored under refrigeration (below 10^{o}C) it becomes hard and brittle and lose its spreadability. At ambient temperature it loses its plasticity which is too undesirable. The ease with which butter may be used as spread over a bread slice is perhaps the only rheological property of significance nowadays. This opens up a new avenue for dairy manufactures to introduce low-energy, low-priced fat products with improved functional properties. Low-fat spreads comprise one such category of dairy products that have thus emerged (Patange, 2006).

Spreads are the products harmonizing with the idea of healthy nutrition. At the same time it has good taste and flavor as well as very good spreadability at refrigerator temperature and retain its stand up property even at high ambient temperature (Dostalova, 2003). Spreads have low caloric content than butter and blends easily with other foods for convenience in cookery and serving. Both the dietary and convenience requirements of the consumer have been required by table spreads. Commercial table spreads now exists that contain fat level ranging from a high of 80 per cent all the way down to 0 per cent. Products resembling margarine containing less than 80 per cent fat are usually called spreads. As per regulations in some countries, only products containing less than 80 per cent but more than 40 per cent fat, 40-70 per cent fat, 62-80 per cent fat, or less than 75 per cent fat are labeled as spreads. Products with 60-80 per cent fat or with 41-60 per cent fat are 'reduced-fat' spreads and products containing less than 40 per cent are referred to as 'low-fat' spreads. The term 'very low-fat' spreads is used for spreads of 5-15 per cent fat and even less. The spreads with extremely low-fat content are sometime called 'Ultra low-fat' spreads (Dostalova, 2003).

Low-fat spread, generally contain 30-50 per cent moisture, 30-50 per cent fat and 8-12 per cent solids-not-fat (SNF). It can be manufactured from different types of fat (viz. butterfat, vegetable fat or other animal fat), protein (milk proteins e.g. skim milk, buttermilk, whey or their concentrated forms, sodium caseinate, calcium reduced skim milk powder, ultrafiltered protein concentrate, whey protein concentrate etc.) and using additives like stabilizers, emulsifiers, plasticizers, emulsifying salts, vitamins, colorants and flavoring material.

Considerable efforts have been made in India for development of fat spreads of dairy and non-dairy type using a variety of ingredients viz, butter, butter oil, cream, paneer, channa, cheese, vegetable fat and ghee. The exploitation of ghee in the manufacture of low fat spread is the need of today's dairy industry due to its easy availability and better shelf life at ambient temperature. Patange *et al.* (2015) utilized ghee in general as a source of fat in the manufacture of low fat spread.

Ghee, a heated and clarified butter is one of the most important products in Indian market. It is produced from cream or butter by removal of water, protein and other minor components. It contains, by definition, a minimum of 99.8 per cent of milk fat, of which at least 98.8 per cent is triglycerides and not more than 0.1 per cent water (World Health Organization, 2010). Of the total milk production, 28 per cent milk is converted into ghee. Thus on an average 22.68 million tons of milk gets converted in to ghee annually. The per capita consumption of ghee in 1992 was 1.22 kg, which increased 2.23 kg in 2003 (Parekh, 2003). In Indian diet ghee is considered as superior fat food over other fats and is preferred for cooking and other food preparations. It is the most widely used milk product in the Indian sub-continent. Ghee contributes significantly towards nourishment of people of all age groups. It is a good source of fat-soluble vitamins (A, D, E and K) and essential fatty acids (Chand *et al.*, 1986). Ghee lipids contain saturated fatty acids to an extent of approximately 60 per cent of total fatty acids. It contains 3 per cent linoleic acid which has anti-oxidant properties (Kumar, 1999). Ghee also contains about 0.2 to 0.4 per cent cholesterol (Nath *et al.*, 1996). Among all natural fats, milk fat is the most varied in its chemical characteristics and functional properties. Milk fat is a very complex mixture containing more than 437 fatty acids of different chain lengths and un-saturation, formulating varieties of triglycerols having wide melting ranges from - 40 to 40°C. This characteristic melting behavior of milk fat lends itself to easy fractionation, having different chemical and physical properties.

Indian dairy industry marketed ghee either as cow ghee or buffalo ghee or only as ghee. Both the ghee has differed in respect to color, texture and flavor due to differentiate in fatty acid profile. Buffalo milk fat is distinctly harder than cow milk fat. It could due to it contains large amounts of long chain saturated fatty acids (16:0 and 18:0) as compared to cow milk fat. Buffalo milk ghee contain high amount of melting triglycerides (8.7%) than in cow milk fat (4.9%). Due to this difference, the triglycerides crystallize much earlier in buffalo milk fat than in cow milk fat and at a given temperature the amount of crystallized fat is much higher in case of buffalo milk fat than cow milk fat (Bector, 2002). All over the world anhydrous milk fat or ghee is mostly used for recombination of various dairy products including chocolate and ice cream etc. In India traditionally ghee is consumed by spreading over the *Paratha* and *Puran poli* also serve along with the number of food dishes.

In view of this light background of properties of cow and buffalo milk ghee and importance of ghee in manufacture of low fat spread the study was planned with following objective.

1. To study the effect of pre-cooling method on sensory and textural properties of cow and buffalo milk ghee.

2. To study sensory qualities of low-fat spread prepared from cow and buffalo milk ghee.

3. To study physico-chemical qualities of low-fat spread prepared from cow and buffalo milk ghee.

2. REVIEW OF LITERATURE

2.1 Definition of low- fat spread:

Bullock (1966) defined the term `low fat dairy spread' as a product which contain only dairy ingredients and has less fat than commonly used spreads such as butter and margarine.

Frede (1990) also provides general definition of low-fat spread as food in the form of spreadable emulsion, intended for spreading which is mainly of the type of water in oil, comprising principally of an aqueous phase and an edible fat or oil phase.

Moran (1993) define the term spread is an emulsion of water in oil above about 15 percent fat and normally emulsion of oil in water at lower level of fat.

Dostalova (2003) proposed the term very low fat spread for spreads with 5-15 per cent fat and even less. The spreads with extremely low-fat content are sometime called 'ultra-low –fat' spreads.

CAC (2004), proposed standard for spread according to this standard low- fat dairy spread containing 39-41 per cent fat are sometimes termed as `half-fat butter' while those in which caloric reduction is at least 33 per cent are termed as `reduced calorie spreads'.

According to FSSAI (2011) fat spread means a product in the form of water in oil emulsion, of an aqueous phase and a fat phase of edible oils and fats excluding animal body fats. The individual oil and fat used in the spread shall conform to the respective standards prescribed by these regulations. According to regulation Fat spread shall be classified into the following three groups viz. (a) Milk fat spread: Fat content will be exclusively milk fat; (b) Mixed fat spread: Fat content will be a mixture of milk fat with any one or more of hydrogenated, unhydrogenated refined edible vegetable oils or interesterified fat.(c) Vegetable fat spread.

2.2 Preparation of the component phases for low fat spread preparation

2.2.1 Preparation of aqueous phase:

It involves dissolving soluble or dispersible dairy and non-dairy ingredients like milk protein/SNF, stabilizer, etc. in water.

Christensson and Lofgren (1992) suggested that the cultured milk or synthetic flavour or starter should be incorporated at the end of aqueous phase. Tossavainen *et al.* (1996) added stabilizer, protein powder and salt were to water (at 50^0C). A hand beater was used after which the suspension was allowed to stand (1h at 75^oC). Then it was stirred for few minutes until it was quite homogenous. It was subsequently tempered to 45^oC.

Steeg *et al.* (2001) adjusted pH before sterilization of the aqueous phase. For faster dispersion and solubilization of ingredients the blending temperature may be between 40^oC to 80^oC. After pasteurization the aqueous phase is cooled to 27^oC or below, before being combined with fat phase.

Patange (2006) prepared aqueous phase wherein skim milk powder or whey protein concentrate as a source of MSNF was dispersed in water together with soluble ingredients followed by mixing with an electric blender (Yamaha Blender Company), preheating (55^oC), filtration (2- fold muslin cloth), pasteurization (72^oC for 15-20 sec), cooling in an ice water-bath to 20^oC and holding overnight in a refrigerator. Finally, when required this aqueous phase was acidulated using a dilute acid to the desired pH added with flavoring and warmed it to blending temperature.

2.2.2 Preparation of fat phase:

Bullock (1966) suggested that butter/butter oil based processes involve overnight tempering of conventionally churned butter/butter oil at an appropriate temperature (21^oC) before converting into spread.

Robinson (1986) reviewed that, the flavor and melting characteristics are very important in spread making. Preparation of fat phase influences one of these attributes. Blending involves melting of fat, mixing it with minor quantities of emulsifier, fat soluble vitamins and color.

Crabtree (1989) reviewed that, this phase of the spread is composed of crystal matrix of solid fat which retain the liquid oil in suspension. The solid fat is capable of existing in different crystal forms depending on how they are crystallized and the orient themselves in the crystal matrix and the fatty acid arrangement around the triglyceride molecule.

Rodrigues and Gioielli (2003) advocated the chemical intersterification process to oil fat blend to increase the softening point of blend. Functional properties of butter can be improved by number of treatments aimed at improving cold spreadability while maintaining stability at room temperature.

Patange (2006) prepared low fat spread in which ghee was heated to 50^{o}C, and then added with the emulsifier and color. It was mixed then heated (in a water-bath) to 70^{o}C before being rapidly cooled to 20^{o}C (rate of cooling, 12^{o}C/min) with continuous agitation in a chilled water-bath (2.5^{o}C ± 1^{o}C) and subsequently to 5^{o}C by quiescent holding in a refrigerator for an overnight period. The cooled fat phase was then tempered to the blending temperature of 25^{0}C ± 1^{o}C by holding in water bath for 6 h before use.

2.3 Emulsification

An emulsion is a phenomenon in which two phases are mixed together. It is the next step after preparation of the phases in spread-making. The emulsification carried out by various means including blending in planetary mixer, homogenization, churning, shearing action etc.

Ramchandra and Hemantha (1998) defined emulsion is a phenomenon in which two phases of a liquid system consisting of fairly dispersion coarse dispersion of one liquid into another with which it is not miscible.

2.4 Blending

Bullock (1966) manufactured a w/o type low-fat dairy spread using a model 120 A Hobart Food Mixer wherein tempered butter (21^{o}C) was creamed followed by addition of serum in 300 ml increments. Blending was carried out after each addition of serum using medium speed for 30 sec. A final blending for 15 sec at high speed was used to facilitate fine dispersion of serum in fat phase.

Prajapati *et al.* (1991) standardized the method of manufacture of low-fat spread based on vegetable fats wherein the two separate phases were blended together in a Hobart mixer with slightly modified processing protocol as compared to the above.

Patange (2006) transferred tempered fat phase to the bowl of Hobart Food Mixer and creaming was carried out using the flat beater attachment of the mixer for 30 sec at ‘medium’ speed (285 rpm for agitator and 125 rpm for attachment). The serum phase was added in three equal installments. Blending was carried out after each addition of the serum phase using medium speed for 30 sec. It was continued further for 150 sec at same speed, and finished with a 30 sec, high-speed (591 rpm for agitator and 259 rpm for attachment) run of the mixer. The spread was packed in 200 g polypropylene tubs before being transferred to refrigerator (5^{o}C).

2.5 Physico-chemical properties of cow and buffalo ghee

Frankel *et al.* (1958) reported that a bright yellow colour caused by the presence of carotenoid pigments is associated with cow ghee. Buffalo ghee lacks carotene, and is whitish with slight greenish tinge or pale-cream in colour. The colour of fats always appears deeper in eyes when melted than when in solid form. The natural colour in milk fat is due to the presence of carotenoids and similar compounds. Nearly 85-95% of total fat-soluble pigment is β-carotene.

Armugham and Narayanan (1979) found that at 29°C the average per cent of the liquid fraction was 62 % for buffalo and 83 % for cow milk fat using thermal expansion dilatometer and reported that buffalo, cow and goat milk fat at 28°C contained solid fat at the levels of 84.4, 66.4 and 22.8 % respectively. It was also observed that 50 % solid fat was obtained at 33°C, 30°C and 25°C in cow, buffalo and goat milk fats respectively.

Angelo and Jain (1982) summarized physico-chemical properties of ghee made from cow and buffalo milk (Table no.2.1).

Table 2.1 properties of cow and buffalo milk ghee

Properties	Cow ghee	Buffalo ghee
Reichert-Meissl value	16.9-29.7	14.5-39.9
Polenske value	0.9-3.2	0.4-5.3
Iodine value (mg/gm)	31.0-45.6	21.4-39.9
BR reading (40 °C)	42.5-47.7	40.9-46.9
Melting point (°C)	28.8-35.7	26.6-36.1
Unsaponifiable matter (g 100 g^{-1})	0.42-0.49	0.45-0.54
Saponification value	212.8-232.8	198.0-239.3
Kirschner value	22.1	28.4
Softening point (°C)	33.5-33.9	33.5-34.6
Smoking point (°C)	252.0	Not reported

Lakshminarayana and Rama Murthy (1985) reported the MP of three solid fractions at 31, 23, 15°C and the remaining liquid fraction at 15°C of buffalo ghee as 37.5 (S31), 31.2 (S23), 19 (S15) and 14.5 % (L 15), and for cow as 34.2 (S31), 36.5 (S23), 30.5 (S15) and 14 (L15), respectively.

Bindal and Wadhwa (1993) observed that the average MP of goat ghee (28.1-30.2°C) was significantly lower ($P < 0.01$) than that of cow ghee (32.7-35.8°C) and much lower than that of buffalo ghee (33.4-38.8°C), while solid fractions of these ghee showed a similar trend but in liquid fractions, goat ghee had highest MP followed by buffalo and cow.

Bector (2002) revealed the chemical characteristics of cow and buffalo milk fat, The short chain fatty acid (4:0 to 12:0) and the unsaturated fatty acids contribute to the softness of fat, the long chain saturated fatty acid contribute to its hardness. Buffalo milk fat is distinctly harder than cow milk fat. This is because of it contains large amount of long chain of saturated fatty acid (16:0 and 18:0) as compare to cow milk fat. For the same reason the amount of high melting triglycerides is significantly higher in buffalo (8.7 %) than in cow milk fat (4.9 %). Due to this difference, the triglycerides crystalize much earlier in buffalo milk fat than in cow milk fat and at a given temperature the amount of crystalized fat is much higher in case of buffalo milk fat than in cow milk fat. He also summarized the average fatty acid composition of milk fat as in below table

Table 2.2 Fatty acid composition of cow and buffalo milk fat

Fatty acid	Cow milk fat	Buffalo milk Fat
4:0	3.2	4.4
6:0	2.1	1.5
8:0	1.2	0.8
10:0	2.6	1.3
10:1	0.3	-
12:0	2.8	1.8
14:0	11.9	10.8
14:1	2.1	1.3
15:0	1.2	1.3
16:0	29.9	33.1
16:1	1.8	2.0
17:0	0.3	0.6
18:0	10.0	11.9
18:1	28.4	27.1
18:2	1.5	1.5
18:3	0.6	0.5

Kanawjia and Makhal (2005) summarized major and minor constituent of ghee as table 2.2.

Table 2.3. The major and minor constituents of cow and buffalo milk ghee

Constituent	Buffalo Milk ghee	Cow milk ghee
Saponifiable constituent		
Tryglycerides*		
Short chain (%)	45.3	37.6
Long chain (%)	54.7	62.4
Trisaturated (%)	40.7	39.0
High melting (%)	8.7	4.9
Partial glycerides*		
Diglycerides (%)	4.5	4.3
Monoglycerides (%)	0.6	0.7
Phospholipids (mg %)	42.5	38.0
Unsaponifiableconstituents		
Total cholesterol (mg %)	275.0	330.0
Lanosterol (mg %)	8.27	9.32
Lutein (µg/g)	3.1	4.2
Squalene (µg/g)	62.4	59.2
Carotene (µg/g)	0.0	7.2
Vitamin A (µg/g)	9.5	9.2
Vitamin E (µg/g)	26.4	30.5

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Changade *et al.* (2006) reported that ghee samples showed an average moisture of 0.24% in cow and 0.21% buffalo milk ghee. Insoluble impurities were ranging from 2.1 to 2.0 per cent. Color units of cow ghee (15.90) were about five times higher than those from buffalo ghee (3.56). Comparatively higher liquid portion was observed in desi ghee (64.4%). Smallest grain size was observed in ghee from direct boiling of ripened cream (200.66m). Significantly higher melting point (33.64°C) was observed in buffalo ghee than cow ghee (32.20°C). Specific gravity demonstrated that, cow ghee is lighter than buffalo ghee. Higher viscosity value was recorded with cow ghee (33.893 cP.) irrespective of methods of preparation.

El-Zeini *et al*. (2006) reported much average globule sizes (8.7µm) in buffalo milk as compared to 3.8, 3.8, 3.2 and 3.0 µm for cow, sheep, goats and camel milks. Higher percentage (20.34%) of large fat globules (16-18μm) has been found in buffalo milk but not in the milk of other ruminants.

Ahmad *et al.* (2008) reported that the fat globule in buffalo milk is coarse and bigger than in cow milk (1 ml buffalo milk contains about 2.7 million fat globules), with 60% having a size between 3.5 to 7.5 μm.

Kumar (2013) reported that the critical temperature of dissolution (CTD) value (°C) for pure cow ghee ranged from 51.6 to 54.6 with an average of 53.3, whereas that for pure buffalo ghee ranged from 52.4 to 56.2 with an average of 54.3. Cow milk fat was found to be more unsaturated than buffalo milk fat on account of its higher percentage of unsaturated fatty acids (28.17) than buffalo milk fat (22.96). The butyro–refractometer (BR) reading for buffalo milk fat ranged from 40.4 to 41.2 (mean 40.7± 0.17), while for cow milk fat ranged from 41.4 to 42.6 (mean 42.2±0.11). The iodine values for buffalo and cow milk fats ranged from 27.41 to 34.13 (mean 30.60±0.44) and 30.82 to 35.77 (mean 34.30±0.49), respectively. He also reported that seasons exerted slight influence on the fatty acid make up as well as on the physico-chemical characteristics studied.

Suwarat and Tungjaroenchai (2013) studied characteristic of ghee obtained from different post-clarification temperatures. Fresh cream from buffalo's milk was inoculated with freeze dried mixed cultures of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis* subs*p. lactis biovar diacetyllactis* and *Leuconostoc mesenteroides subsp. cremoris,* and incubated at 32±0.5°C for 4 hr. Fermented cream samples were heated to render butter oil (ghee), and post-clarification heatings were conducted at temperatures of 100±5, 110±5, or 120±5°C, for 5, 10, or 15 min, respectively. Free fatty acid values (FFA) and oxidative stability in hours significantly increased ($P < 0.05$) with increasing temperature and prolonged periods of time. The oxidative stability values increased from 15.45±0.17 to 28.60±0.34 h., while FFA values increased from 0.22±0.12 to 0.36±0.03% (expressed as oleic acid). Changes in moisture content of ghee samples were not affected (P >0.05) by post-clarification heating. Both temperature and time of post-clarification resulted in changes of l, a, and b values. Values of lightness (l) ranged 100.95±0.07 to 94.91±0.05, yellow (b) Ranged 23.17±0.05 to 48.32±0.07, and green (a) ranged -6.46±0.02 to -7.44±0.03, respectively.

2.6 Cooling and crystallization behavior of milk fat:

Paul and Suri (1949) reported that greatest number of large grains in ghee was obtained when the ghee was melted at 60 to 100ºC and maintained at 29ºC for cow ghee and 31ºC for buffalo ghee. The maximum size of grains in case of buffalo and cow ghee were found to be 0.54 mm and 0.34 mm respectively.

Joshi and Vyas (1976) reported that, composition, processing and season also exert profound influence on liquid portion of ghee. The grain size showed higher crystal size in buffalo milk ghee especially those prepared by desi method. The smallest grain size was observed in ghee samples prepared from direct boiling of cream.

Arumughan and Narayanan (1979) observed that the crystal size in the liquid fraction were very small. This may explain why low melting triglycerides in liquid portion will produce smaller size grain. They found support in these observation by studying the fatty acid composition of different fractions.

Farag *et al*, (1983) carried out the fractional crystallization of fat (pure and adulterated ghee samples) dissolved in silver nitrate-saturated methanol /acetone mixture (70:30) in a ratio of 1:10 at 22, 7 and -8ºC and three fractions obtained were subjected to gas liquid chromatography (GLC) for their fatty acid profile. They reported that fat detecting adulteration, $C_{18:0}$ and $C_{18:1}$ fatty acids are great importance in first fraction, whereas $C_{22:0}$ is important in second and third fraction.

Panda and Bindal (1997) studied the crystallization behaviour at 17°C of fat dissolved in a solvent mixture of acetone and benzene (3.5:1) and reported that ghee adulterated with animal body fats (10%) and ghee adulterated with vegetable oils and fats (10% level) took 19 min, 3 to 15 min and 22 to 23 min to crystallize, respectively. They concluded from the study that even low level adulteration of animal body fats and vegetable oils and fats could be detected in ghee.

Panda and Bindal (1998) developed a test known as crystallization test to detect the adulteration of ghee with animal body fats and vegetable oils separately or in combination. This test measures the time taken for the crystallization of melted, filtered fat (0.8 ml), which is treated with 2.5 ml of solvent mixture (acetone: benzene 19 3.5:1) at 17 OC. The crystallization time for normal ghee was 19 min., while adulterated samples showed lower or higher values. Ghee adulterated with body fats took 4-12 min while that with vegetable oils took 23-27 min for crystallization. However, ghee from cotton tract area was being suspected for adulteration with animal body fat when the test results were applied. Accordingly they have modified the test to remove this ambiguity. They concluded that even a low level of adulteration of ghee with animal body fats (5%) and vegetable oils/fats including coconut oil (10%) could be detected using this method

Herrera and Hartel (2000) reported that the microstructure of a fat crystal network is strictly dependent on the crystallization mechanisms, cooling rate, crystallization temperature and presence of emulsified fat, addition of vegetable oil and/or other minor components and agitation forces. In general, microstructure is affected by the solid-to-liquid ratio. However, at same level of SFC the microstructure can vary substantially based on the size of the crystals and crystal clusters and on the amount and type of bonds between them.

Narine and Marangoni (2002) reported that the purpose of cooling of liquid fat is to impart the required crystalline structure to the fat, as many important functional properties of fat-containing products (spreadability, and smoothness or graininess) are dictated by the characteristics of crystal network formed in the lipid phase.

Martini *et al*. (2002) studied the effect of cooling rate on crystallization behavior of milk fat fraction/sunflower oil blends The effect of cooling rate (slow: 0.1°C/min; fast: 5.5°C/min) on the crystallization kinetics of blends of a high melting milk fat fraction and sunflower oil (SFO) was investigated by pulsed NMR and DSC. For slow cooling rate, the majority of crystallization had already occurred by the time the set crystallization temperature had been reached. For fast cooling rate, crystallization started after the samples reached the selected crystallization temperature, and the solid fat content curves were hyperbolic. DSC scans showed that at slow cooling rates, molecular organization took place as the sample was being cooled to crystallization temperature and there was fractionation of solid solutions. For fast cooling rates, more compound crystal formation occurred and no fractionation was observed in many cases.

Campos *et al*. (2002) studied the effects of cooling rate on the macroscopic properties of a fat crystal network by crystallizing anhydrous milk fat (AMF) and lard either rapidly (5°C/min, Newtonian cooling) or slowly (0.1°C/min, stepwise cooling). AMF crystallized rapidly was harder than AMF crystallized slowly and had a higher SFC. Moreover, its solid state was in a more metastable polymorphic form. Upon slow crystallization, AMF had a lower SFC and its solid state was in a more stable polymorphic form. The microstructure was also different between the two treatments. When crystallized rapidly, crystallites were numerous and small, while a smaller number of larger crystallites were observed when crystallized slowly. Similar results were observed for lard crystallized under the same conditions.

Bhaskar *et al.* (2003) showed that if cooling is rapid the crystals will be many in number and small in size resulting in an increased surface area due to which more liquid fat will be absorbed. If the cooling is gradual the crystals will be larger and few.

Wiking *et al*. (2009) showed that the processing conditions such as cooling rate, agitation rate and crystallization temperature influence the milk fat crystallization who postulated that procoming condition affect the formation, morphology and size distribution of crystals, and thus the microstructure and texture of butter and butter blends. In general, at slow cooling rates, large crystals with a broad size distribution are formed, whereas many small and unstable crystals are present at fast cooling rates.

3. MATERIAL AND METHODS

The investigation entitled "Comparative study of low-fat spread preparation from cow and buffalo milk ghee" was carried out in the Division of Animal Husbandry and Dairy Science, College of Agriculture, Kolhapur, during the year 2015-2017. The materials used and methodology adopted to achieve the objective of the present investigation are reported in this chapter

3.1 MATERIALS

3.1.1 Ghee

Cow milk ghee and buffalo milk ghee was obtained from the local market of Kolhapur city.

3.1.2 Skim milk powder

Spray-dryed skim milk powder (SMP) was obtained from Kolhapur district milk producer union limited (Gokul).

3.1.3 Additives:

3.1.3.1 Stabilizer:

Carrageenan – Type II iota-carrageenan (Himedia) was used

3.1.3.2 Plasticizer:

Glycerol was used as plasticizer, obtained from Qualigens Fine Chemical, Mumbai**.**

3.1.3.3 Emulsifier:

Polyxyethylene sorbitan monooleate (Tween-80) (S.D.Fine-chem.ltd) was used.

3.1.3.4 Salts:

Iodized salt (Tata) was obtained from the local market of Kolhapur city.

3.1.3.5 Coloring Materials: Annatto butter color was used in experiment was obtained from local market of Kolhapur city.

3.1.3.6 Acidulates:

Citric acid (Qualigens Fine Chemical, Mumbai) was used.

3.1.4 Equipments:

3.1.4.1 Planetary mixer:

(SPAR Make, Taiwan) Planetary mixer was used for blending two phases. (Plate no.1)

3.1.4.2 Glassware:

All glassware of Borosil make was used for analytical work. It was Thoroughly cleaned and sterilized properly before use.

3.1.4.3 Weighing balance:

Electronic balance model M-3000, capacity 3000 g was used for weighing during the course of investigation.

3.1.5 Chemicals:

All the chemicals was used for the analytical work were of Analytical Reagent (AR) or Guaranteed Reagent (GR) grade which was manufactured by Merk, India Ltd/Glaxo India Ltd.

3.2 METHODOLOGY:

3.2.1 Effect of the rapid and slow pre-cooling on sensory characteristics of cow and buffalo milk ghee:

Initially both the ghee was melted at 70^{o}C followed by rapid and slow pre-cooling to 20°C in controlled condition and then cooling to 5^{o}C by quiescent storage under refrigeration. Then ghee was evaluated at 30°C for sensory characteristic viz. texture, consistency and color. The treatment detail as follows

CG_1: Cow milk ghee melted at 70^{o}C and rapidly pre-cooled to 20^{o}C

CG_2: Cow milk ghee melted at 70^{o}C and slowly pre-cooled to 20^{o}C

BG_1: Buffalo milk ghee melted at 70^{o}C and rapidly pre-cooled to 20^{o}C

BG_2: Buffalo milk ghee melted at 70°C and slowly pre-cooled to 20°C

3.2.2 Preparation of low-fat spread using cow and buffalo milk ghee.

Low-fat spread from cow and buffalo milk ghee was prepared as per protocol developed by Patange (2006) in planetary Mixer (SPAR Make, Taiwan). The procedure involves separate preparation and tempering of fat and serum phases before blending and emulsifying them. Ghee was heated up to 50°C, and then added with the emulsifier. It was then heated (in a water-bath) to 70°C before being rapidly cooled to 20°C (rate of cooling, 12°C/min) with continuous agitation in a chilled water-bath (2.5°C ± 1°C) and subsequently to 5°C by quiescent holding in a refrigerator for an overnight period. The cooled fat phase was then tempered to the blending temperature of 25^0C ± 1°C by holding in water bath for 6 h before use.

Skim milk powder as a source of MSNF was dispersed in water together with soluble ingredients followed by mixing with an electric blender, preheating (55°C), filtration (2- fold muslin cloth), pasteurization (72°C for 15 - 20 sec), cooling in an ice water-bath to 20°C and holding overnight in a refrigerator. Finally, when required this aqueous phase was acidulated using a dilute citric acid to the desired pH and warmed it to blending temperature.

The tempered fat phase was transferred to the bowl of planetary mixer and creaming will carried out using the flat beater attachment of the mixer for 30 sec at ‘medium’ speed. The serum phase was added in three equal installments. Blending was carried out after each addition of the serum phase using medium speed for 30 sec. The spreads was packed in 75 gm in plastic cups and closed with lids before being transferred to refrigerator (5°C). The treatment were as fallows

CGS- Low-fat spread using cow milk ghee

BGS-Low-fat spread using buffalo milk ghee

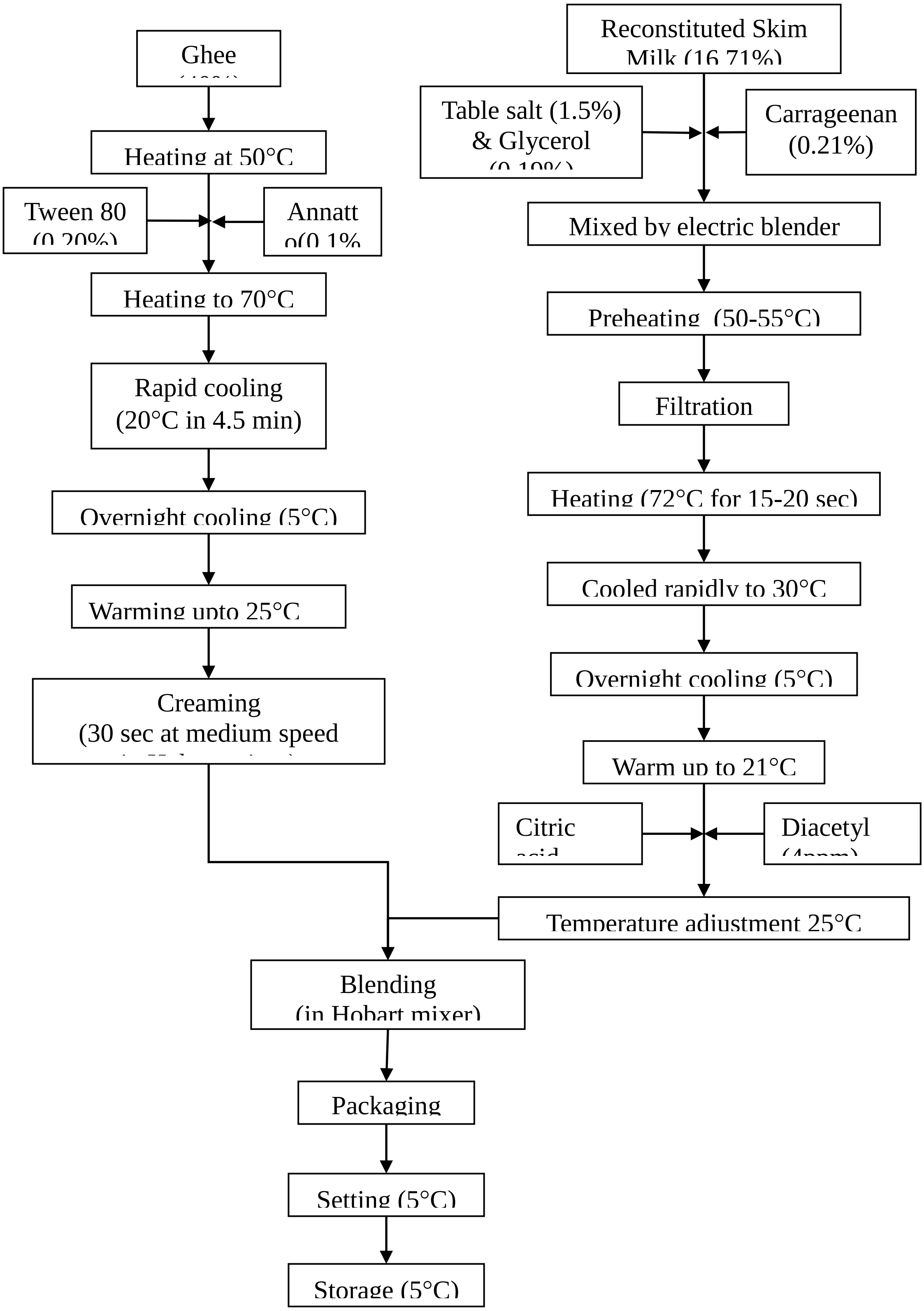

Fig. Flow diagram of manufacture of Low Fat Spread using Ghee

3.2.3 Effect of colour and flavour on qualities of LFS

To know the effect of colour and flavour, annatto colour and diacetyl flavour was added (@ 0.1% of ghee) and 4 ppm respectively in both the type of spread and then evaluated for sensory qualities.

3.2.4 Effect of exposure time on sensory properties of low fat spread prepared from cow and buffalo milk ghee.

This study was purposefully conducted with aim to know the effect of exposure time on sensory attributes of LFS. Then both prepared LFS were removed from refrigerator and immediately evaluated (CS_1 and BS_1). Its quality then evaluated after 10, 20 and 30 min intervals. The spread were kept in incubator maintained at 35±1°C. The treatments were as follows

CS_1: Cow ghee LFS removed immediately from Refrigeration.

BS_1: Buffalo ghee LFS removed immediately from Refrigeration

CS_2: Cow ghee LFS exposed for 10 min

BS_2: Buffalo ghee LFS exposed for 10 min

CS_3: Cow ghee LFS exposed for 20 min

BS_3: Buffalo ghee LFS exposed for 20 min

CS_4: Cow ghee LFS exposed for 30 min

BS_4: Buffalo ghee LFS exposed for 30 min

3.3 ANALYTICAL METHODS:

Ghee, skim milk powder as well as finished products were analyzed for their physico-chemical properties as given below.

3.3.1 Ghee:

3.3.1.1 Moisture:

The moisture content of ghee was determined as per the method given in IS: 3508-1966

3.3.1.2 Sleep point (melting point):

It was determined using capillary glass tube method (IS: 3508-1966).

3.3.1.3 Iodine value: The iodine value of ghee was determined as per the method given in ISO 3961:1996**.**

3.3.2 Skim Milk Powder

3.3.2.1 Moisture:

The moisture content in SMP was determined As per BIS: part XI (1981).

3.3.2.2 Fat content:

It was determined by gravimetric method As per BIS: part XI (1981).

3.3.2.3 Solubility index:

It was determined by following the procedure given in BIS: part XI (1981).

3.3.3 Spread:

3.3.3.1 Total Solid content:

The TS content was determined by gravimetric method described in the laboratory manual (1959) in which 2 g sample was dried to a constant weight in the hot air oven at 101±1^{o}C.

3.3.3.2 Fat content:

The fat content of the spread was determined By Mojonnier method (Laboratory Manual, 1959). About 5 gm of sample has been taken with 5 ml of distilled water, followed by addition of 2 ml concentrated ammonium solution and 9 ml of distilled water has been added, followed by gently shaking of the sample on mechanical shaker. Cooling and addition of the 10 ml ethyl alcohol (95%) has been followed and this mixture was added in the Mojonnieer tube and followed by shaking for 2 min, addition of 25 ml of petroleum ether and 25 ml diethyl ether and mixed thoroughly, upper layer was taken in aluminum dish and procedure was repeated with 15 ml of diethyl ether and petroleum ether each. Aluminum dish was allowed to 98^{o}C at oven and then cooled and weighed.

Fat % = wt. of fat extracted /wt. of sample × 100

3.3.3.3 Crude protein content:

Total crude protein content of spread was determined By Kjeldhal's method given by Manefee and Overman (1940) as described below:

About 0.2 to 0.3 g of sample was weighted in a 100 ml of digestion tube to which 1 g digestion mixture (1:4:: $CuSO_4$: K_2SO_4) and 10 ml of concentrated sulphuric acid was added. The tube content were digested in a kjeldahls digester till a clear bluish green digest was obtained. The flask was allowed to cool and then volume made up to 100 ml in a volumetric flask, from which 10 ml was taken in to a distillation flask. The content of the flask was neutralized by adding 15 ml of 50 Per cent Sodium Hydroxide solution. Steam distillation was carried out for 15 to 20 min. to collect distillate in a beaker containing 10 ml of saturated boric acid solution added with 3-4 drops of mixed indicator (mixture of equal volumes of 0.1 per cent methylene red and 0.1 per cent methylene blue in 98 % alcohol) after collection of 70-80 ml of distillate it was titrated with N/50 sulphuric acid to a pink end point. A blank was run by replacing the sample with distilled water and using the same procedure. The total protein content was calculated by using following formula:

Total protein (per cent) = (A-B) ×14 ×6.38/50×W

Where,

A= ml of 0.05 N H_2SO_4used in titration of the samples

B= ml of 0.05 N H_2SO_4used in titration of blank,

W= Weight of sample in grams

3.3.3.4 Total ash content:

The Total ash content was determined as per the AOAC (1980) method

3.3.3.5 Free fatty acid content:

The free fatty acid content of the spread was estimated by the method of Deeth *et al.* (1975) modified appropriately. 2 gram of sample was taken into 60 ml glass stoppered test tube to which 10 ml of extracting mixture containing isopropanol, petroleum ether and 4 N H_2SO_4 (40:10:1) was added and mixed thoroughly. This was followed by the addition of 6ml petroleum ether and 4 ml distilled water. The test tube was stoppered and tempered to 40C for 10 minutes. The content where vigorously shaken for 20 seconds. The 2 layers were allowed to separate and the upper layer (5 ml) was withdrawn and transferred to 50ml conical flask. After addition of 2 drops of methanolic phenolphthalein indicator the solution was titrated with 0.02N methanolic KOH. A blank in which sample was replaced with distilled water were used to obtain the background titration. The free fatty acid content (eqiv/g) of the sample was obtained from the following formula.

$$FFA(equiv/g)=T.N/P.W\times 10^3$$

Where,

T= Net titration volume

N= Normality of the methanolic KOH.

P= Proportion of upper layer titrated (volume of aliquot withdrawn/total volume of the upper layer)

W= Weight of the sample

3.3.3.6 pH:

The pH of the spread was measured by pH meter (Lab India Instruments Pvt. Ltd., Mumbai), pH of the spread sample (at 5^{o}C) was read directly inserting the electrode in the spread.

3.3.3.7 Oiling off and wheying off:

The procedure for the estimation of oiling off or free oil in butter described by deMan and Wood (1958) was used with certain modification for the spread as suggested by Prajapati (1988).

The spread sample was filled in a 200 ml of polypropylene tub and the held overnight at 5 ^{0}C. A plug of sample was removed from the tubwith cylinder sampling device made of brass, similarto a large cork borer (diameter 1.5 cm). Part of the plug was forced out with a plunger and a slice obtained by trimming off flush the end of the sampler using a knife. Successive slices, 3 mm thick each were then cut off. A set of five whatman paper filter- paper (dried in air oven at 100±20 ^{0}C for 1 h and cooled) was weighted and the 5 samples of slices placed at its center. Which was then weighed, held for 48 h at 20 ±2 ^{0}C and then transferred to refrigerator for 30 minutes. The spread sticking to the paper was scraped off and the filter-paper weighed to get the weight lost from the sample. Thereafter, the weighed filter papers were dried in an oven at 100±2 ^{0}C for 3 h, cooled in a desiccators and weighed again to determine the weight of absorbed oil. The amount of oil absorbed after deducting the total soluble solids in product was taken as absorbed after deducting the total soluble solids in the product was taken as oiling off from spread at 20 ^{0}C and the moisture absorbed as wheying off. **3.4 Sensory Evaluation**

3.4.1 Ghee:

The effect of rapid and slow pre-cooling on color, texture and consistency of ghee was carried out by a panel of 5 judges from Division of AHDS and Horticulture, A.C., Kolhapur. The linear scale was employed for this purpose (Annexure-I).

3.4.2 Spread:

The acceptability of spread was assessed by the panel of judges. The color and appearance, spreadability, body and texture, flavor and overall acceptability will assess by using 9-point rating scale (Annexure-II) as per (Amerine *et al.*, 1965). Spreadability of product was assessed by spreading piece of a bread at uniform temperature 5±1^{o}C.

3.4.3 Statistical Analysis:

Data generated during the course of investigation were analyzed using completely randomized design (CRD) technique according to Snedecor and Cochran (1994).

4. RESULTS AND DISCUSSION

Butter and other milk fat base product are economically important to Indian dairy industry. Butter is mostly used as a table spread due to their unique taste, textural characteristics and nutritional value. However the consumer demand has increased towards for Low-Fat Spread (LFS), due to its higher nutritional and physical advantages. Number of fat sources have been used in the preparation of LFS. Ghee also can be used as a source of fat in the preparation of the LFS. Indian dairy industry is well known for the production of ghee i.e. cow ghee and buffalo ghee are the major type of ghee. Considering the demand of LFS and use of ghee for preparation of LFS, the attempt was made to the comparative study of cow milk ghee LFS and buffalo milk ghee LFS. The results obtained during present investigation are presented and discussed under the following headings.

4.1. Physicochemical properties of cow and buffalo milk ghee.

4.2 The effect of method of rapid and slow precooling on sensory properties of cow and buffalo milk ghee.

4.3 Chemical analysis of skim milk powder used for experiment.

4.4 Effect of type of ghee on sensory attributes of LFS.

4.5 Effect of type of ghee on physico-chemical properties of LFS.

4.6 Effect of annatto colour on colour and appearance (score) of LFS.

4.7 Effect of diacytyl flavour on flavour (score) of LFS.

4.8 Effect of exposure time on sensory attributes of LFS Prepared from cow milk ghee and buffalo milk ghee at 35±1°C.

4.1. Physico-chemical properties of cow and buffalo milk ghee

Table 4.1 Physico-chemical properties of cow and buffalo ghee used in experiment

Properties	Types of ghee	Values*	SEm	CD	CV
Fat (%)	Cow ghee	99.50±0.3	0.03	NS	NS
	Buffalo ghee	99.50±0.4			
Iodine value (mg/ gm)	Cow ghee	31.90^{a}±0.7	0.7	0.15	0.31
	Buffalo ghee	33.10^{b}±0.7			
FFA (%)	Cow ghee	2.40^{a}±0.06	0.06	0.14	3.78
	Buffalo ghee	2.80^{b}±0.07			
Slip point (^{o}C)	Cow ghee	34.4^{a}±0.31	0.37	1.33	2.45
	Buffalo ghee	38.3^{b}±0.44			

***** mean ± SE of five replications within column followed by same letter are non-significantly different at p< 0.05. NS= Non-Significant.

From the above table 4.1 it was observed that the average fat content of cow ghee and buffalo ghee was 99.5 per cent. Ghee contains 99.5 per cent of fat and 0.5 per cent unsaponifiable matter was reported by Goyal, (2002). Further iodine value of cow and buffalo milk ghee was 31.9 and 33.1 mg/gm respectively. The lower iodine value for cow ghee might be presence of saturated fatty acid and absence of polyunsaturated fatty acid Mehta (2013).

Free fatty acid content in cow ghee was 2.4 percent whereas 2.8 percent in buffalo ghee (Table 4.1). Free fatty acids are undesirable in the milk fat products it could be due to the shorter-chain homologous are primarily responsible for the rancid flavour typified by butyric acid as reported by Borgstrom and Jonson (1986) and Munro *et al*., (1992). Yadav and Shrinivasan (1992) reported that the buffalo ghee appears to lack the FFA C_6C_{10} and C_{15} while cow ghee exhibited unique presence of $C_{18:2}$. They had not identified short chain fatty FFA C_2 to C_4 in cow or buffalo ghee. FFA content of cow ghee was 2.4 per cent and FFA content of buffalo milk ghee was 2.8 per cent.

From the Table 4.1 it was also observed that cow milk ghee has (34.4^{0}C) lower slip point than that of buffalo ghee (38.3^{0}C). Lakshminarayan and Rama Murthy (1985) reported an average melting point of cow ghee is 34.2^{0}C and melting point of buffalo ghee is 35.8^{0}C. Which was support the present findings. According to Changade *et al*, (2006), melting point of ghee is influenced by the kind of milk, rate of cooling, extent of crystallisation and entropy and enthalpy changes.

4.2 Effect of method of rapid and slow pre-cooling on sensory properties of cow and buffalo milk ghee.

The data pertaining to the effect of method of rapid and slow pre-cooling on sensory properties of cow and buffalo milk ghee are depicted in Table 4.2 and fig. 4.1

Table 4.2 .Effect of method of rapid and slow pre-cooling on sensory properties (score)* of cow and buffalo milk ghee

Type of ghee and method of pre-cooling	Color (score)*	Texture (score)*	Consistency (score)*
CG_1	67.31^c±0.26	5.28^a±0.12	85.12^c±0.80
CG_2	73.92^d±0.16	50.12^c±0.10	67.75^a±0.32
BG_1	22.44^a±0.70	11.88^b±0.11	94.16^d±0.71
BG_2	30.36^b±0.54	72.60^d±0.73	71.45^b±0.10
SEm	0.05	0.04	0.14
CD (5%)	0.15	0.13	0.26

***** mean ± SE of five replications within column followed by same letter are non-significantly different at $p< 0.05$.

Rate of fat cooling is known to influence its crystallisation behaviour which would affect the sensory properties of fat and the product preared therefrom. Therefore in the present study cow ghee and buffalo ghee were separately heated to 70^{0}C and followed by rapidly pre-cooled to 20^{0}C. Treatments for rapidly pre-cooled cow ghee and buffalo ghee were coded as CG_1 and BG_1 respectively. Simultaneously from the same lot both the ghee were taken in another pots and heated to 70^{0}C and allow to slow pre-cool to 20^{0}C in controlled condition. Treatments for slowly pre-cooled cow ghee and buffalo ghee were coded as CG_2 and BG_2 respectively. All the samples were transferred into refrigeration for quiescent storage at 5^0C for overnight period of time and on next day evaluated for sensory analysis and data were presented in above table 4.2.

It is reveal from the Table 4.2 that, rapidly pre-cooled cow ghee was light yellow colour (score 67.32) whereas, the slowly pre-cooled cow ghee recorded yellow colour (score73.92). It indicate that when cow ghee pre-cooled slowly had shifted towards pronounced yellow colour compare to pre-cooled rapidly. The colour of fat always appear deeper in eyes when melted than when in solid form as reported by Frankel *et al.,*(1958). Similarly buffalo ghee when pre-cooled rapidly had white appearance (score 22.44) as compared to slowly pre-cooled buffalo milk ghee which had creamish white appearance (score 30.36). It was observed that buffalo ghee pre-cooled rapidly had appearance more white than pre-cooled slowly. Bright yellow colour in cow ghee caused by the presence of carotene pigment which is associated with cow ghee and buffalo ghee lacks in carotene resulted in whitish with slightly greenish tinge due to presence bilirubin and biliverdin which gives it greenish tint as reported by Achaya (1997).

The texture score of cow ghee and buffalo ghee where significantly ($P<0.05$) affected by the method of pre-cooling. Rapidly pre-cooled cow ghee had very smooth and pasty texture with (score 5.28) than buffalo pre-cooled ghee (score 11.88). Further the texture characteristic of both the slow pre-cooled ghee also shown variation. The buffalo ghee had shown large grains (score 72.6) compared to cow ghee, which shown fine to medium grain (score 50.12). The present finding are well supported by the reports of Ahmed *et al*. (2008) who reported that the fat globule of buffalo milk ghee is course and bigger than cow milk ghee. Elzein *et al*. (2006) also reported much average globule size of buffalo milk fat (8.7µm) as compare to 3.8 µm for cow milk. Higher percentage of large fat globules has been observed only in buffalo milk.

The consistency score recorded for CG_1, CG_2, BG_1 and BG_2 ghee presented in Table 4.2 which was also significantly ($P<0.05$) affected by the type of ghee and method of pre-cooling. It was observed that the consistency score appear to be a solid hard (score 94.16) for rapidly pre-cooled buffalo ghee than rapidly pre-cooled cow ghee (score 85.12). It clearly indicated that slowly pre-cooled cow ghee has less solidity (score 67.77) than slowly pre-cooled buffalo ghee (score 71.45). The smaller number of large crystals forming under slowly pre-cooled conditions would have smaller surface area for the liquid fat to adhere, thus resulting in higher ratio of liquid fat to the surface area at the crystal interfaces. Similar findings were reported by Bhaskar *et al*. (2003).

4.3 Chemical analysis of skim milk powder used for experiment

Skim Milk Powder (SMP) is the product resulting from the partial removal of fat and water from pasteurized milk.

Table 4.3 Chemical analysis of skim milk powder used for experiment

Replications	Moisture (%)	Fat (%)	Solubility index (ml)
I	4.00	0.70	0.80
II	3.90	0.60	0.70
III	3.80	0.70	0.80
Mean	3.90	0.60	0.76
SE	±0.6	±0.3	±0.3

Table 4.3 reveals that the average moisture content of skim milk powder used for the investigation was 3.90 per cent, whereas average fat percentage was 0.65 per cent and solubility index was 0.76 ml. Codex Alimentarius Standard, (1999) also informed that skim milk powder should contain <1.5 per cent fat, <5 per cent moisture and <1 ml solubility index.

4.4 Effect of type of ghee on sensory attributes of LFS

It is said that the sensory qualities of the food is mostly important in determining its acceptance. Ideally, LFS should possess glistening light yellow color and easy to spread on the bread i.e. neither to hard nor too soft. It should be smooth, non-sticky and free from visible moisture and have butter like pleasant flavor. The LFS was prepared from cow and buffalo ghee separately (vide section 3.2.2) and evaluated for sensory attributes. The data for sensory attributes and ANOVA are presented in table 4.4 to 4.13 and fig. 4.2.

4.4.1 Effect of type of ghee on colour and appearance (score)* of LFS

The data pertaining to effect of fat on colour and appearance (score) of LFS are depicted in Table 4.4

Table 4.4 Effect of type of ghee on colour and appearance (score)* of LFS

Types of spread	Replications						SE m	CD	CV
	I	II	III	IV	V	Mean score*			
C.G S.	8.00	7.5	7.75	7.75	7.5	7.70^{b}±0.10	0.09	0.34	2.60
B.G S.	7.00	7.25	7.25	7.15	7.00	7.13^{a}±0.08			

***** mean ± SE within column followed by same letter are non- significantly different at p< 0.05.

Table 4.5 ANOVA for effect type of ghee on colour and appearance (score)

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	table f (0.05)	**intrprete 0.05**
Replications	5	replication ss	4.00	0.09	0.02	0.60	6.388	NS
grand total	74.15	treatment ss	1.00	0.81	0.81	21.81	7.709	*
Mean	7.42	error ss	4.00	0.15	0.03			
correction factor	549.8	**total**	9.00	1.05				

It can be observed from the Table 4.4 the significant (P<0.05) difference was observed in the score of colour and appearance of cow ghee LFS and buffalo ghee LFS. The cow ghee LFS was significantly (P<0.05) higher score (score 7.70) than buffalo ghee LFS (score 7.13). It was recorded that cow ghee based LFS had more score due to light yellow to pale yellow colour and appearance. Naturally butter spread has occurrence of pale yellow colour is desired (Patnge, 2006). Further, cow milk ghee contains higher amount of carotene pigment which is lack in the buffalo milk ghee (Achaya, 1997). Colour units of the cow ghee (15.90) were about five times higher than those from buffalo ghee (3.56) as reported by Changade *et al*. (2006).

4.4.2 Effect of type of ghee on body and texture (score)* of LFS

The data pertaining to effect of source of fat on body and texture (score) of LFS are depicted in Table 4.6

Table 4.6 Effect of type of ghee on body and texture (score)* of LFS

Types of spread	Replications						SEm	CD	CV
	I	II	III	IV	V	Mean score*			
C.G S.	8.00	7.50	8.25	8.00	7.75	7.90^{b}±0.11	0.09	0.35	2.65
B.G S.	7.25	7.25	7.25	7.50	7.25	7.35^{a}±0.08			

***** mean ± SE within column followed by same letter are non- significantly different at p< 0.05.

Table 4.7 ANOVA for effect of type of ghee on body and texture (score) of LFS

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	table f (0.05)	**intrpret e 0.05**
Replications	5	replication ss	4.00	0.21	0.05	1.31	6.388	NS
grand total	76.00	treatment ss	1.00	0.90	0.90	22.15	7.709	*
Mean	7.60	error ss	4.00	0.16	0.041			
correction factor	577.60	**total**	9.00	1.27				

From table 4.6 it is observed that the LFS prepared from cow ghee had significantly ($P<0.05$) more score (score 7.90) than buffalo ghee LFS (score 7.35). As mentioned above quality of ideal LFS should not have too hard and too soft body and texture. The texture of the buffalo milk fat is distinctly harder than the cow milk fat. This is due to of large amount of long chain saturated fatty acids (16:0 and 18:0) as compared to cow milk fat. Buffalo milk ghee contains high amount of triglycerides (8.7%) than cow milk fat (4.9%). Due to this difference the triglycerides crystallize much earlier in buffalo milk fat than cow milk fat and at a given temperature the amount of crystallized fat is much higher buffalo milk fat than cow milk fat as reported by Bector (2002).

4.4.3 Effect of type of ghee on spreadability (score)* of LFS

Spreadability is a rheological phenomenon determined by the chemical composition, solid fat content (SFC), and functionality of the fat crystal network as reported by Tang and Marangoni (2007).The data pertaining to effect of fat on spreadability (score) of LFS are depicted in Table 4.8

Table 4.8 Effect of type of ghee on spreadability (score)* of LFS

Types of spread	Replications						SE m	CD	CV
	I	II	II	IV	V	Mean score*			
C.G S.	8.00	8.00	7.75	8.00	8.15	7.98^{b} ±0.08	0.06	0.22	1.60
B.G S.	7.35	7.45	7.55	7.65	7.75	7.55^{a}±0.04			

***** mean ± SE within column followed by same letter are non- significantly different at $p< 0.05$.

The data presented in the Table 4.8 shown that the spredability score of LFS prepared from cow and buffalo ghee were significantly ($P< 0.05$) affected by the type of ghee used in the production of LFS. In table 4.8 the spreadability score of cow ghee LFS (7.98 score) is significantly higher than the buffalo ghee LFS (7.55 score). The less score of buffalo LFS is due to the hard body and texture of buffalo ghee. It was observed that at 5^{o}C temperature LFS prepared from buffalo ghee is harder than the LFS

Prepared from cow ghee. It was due to the cow milk ghee contains short chain fatty acid (4:0 to 12:0) and the unsaturated fatty acids which contribute softness to of the fat whereas long chain fatty acid contribute to its hardness and long chain acids are more in buffalo milk ghee. Due to this reason buffalo milk fat is harder than the cow milk fat as reported by Bector (2002). As the buffalo ghee is harder than the cow milk ghee therefore the LFS prepared from buffalo ghee is harder than the LFS prepared from cow milk ghee.

Table 4.9 ANOVA for effect of type of ghee on spreadability (score) of LFS

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	table f (0.05)	**intrprete 0.05**
Replications	5	replication ss	4.00	0.12	0.03	1.98	6.388	NS
grand total	77.65	treatment ss	1.00	0.46	0.46	30.07	7.709	*
Mean	7.77	error ss	4.00	0.06	0.015			
correction factor	602.95	**total**	9.00	0.65				

4.4.4 Effect of type of ghee on flavour (score)*of LFS

Flavor is the most important attribute of the spread like any other food product. It is one attribute that brings the consumer the product again and again. The data pertaining to effect of source of fat on flavour (score) of LFS are depicted in Table 4.10

Table 4.10 Effect of type of ghee on flavour (score)* of LFS

Types of spread	Replications						SEm	CD	CV
	I	II	II	IV	V	Mean score*			
C.G S.	6.70	6.75	6.50	6.75	6.80	6.70[a]±0.05	0.06	0.22	1.81
B.G S.	`7.00	7.15	7.25	7.10	7.25	7.18[b]±0.07			

***** mean ± SE within column followed by same letter are non- significantly different at p< 0.05.

Table 4.11 ANOVA for effect of type of ghee on Flavour (score) of LFS

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	table f (0.05)	**intrprete 0.05**
Replications	5	replication ss	4.00	0.04	0.01	0.60	6.388	NS
grand total	69.25	treatment ss	1.00	0.51	0.51	32.40	7.709	*
Mean	6.93	error ss	4.00	0.06	0.016			
correction factor	479.56	**total**	9.00	0.61				

From the Table 4.10 that the flavor score of LFS prepared from buffalo milk ghee was significantly (P<0.05) higher (score 7.18) as compared to LFS prepared from the cow milk ghee (score 6.70). Cow ghee LFS have more pleasant flavor than buffalo ghee Wadhwa and Jain (1984). In present investigation it was not reflected in LFS because of the expectation of the flavor from LFS is essential to have a slight butter like flavor rather than ghee flavor. Here it was also recorded that as in case of spread made from cow ghee had more pronounced ghee flavor resulted in decreased in flavour score and vice versa for buffalo ghee spread.

4.4.5 Effect of type of ghee on overall acceptability (score)*of LFS

The data pertaining to effect of source of fat on the overall acceptability (score) of LFS are depicted in Table 4.12 and its ANOVA of in Table 4.13

Table 4.12 Effect of type of ghee on overall acceptability (score)* of LFS

Types of spread	Replications						SEm	CD	CV
	I	II	II	IV	V	Mean score*			
C.G S.	8.00	7.75	7.75	7.85	8.15	7.95^{b}±0.06	0.05	0.21	1.61
B.G S.	7.50	7.25	7.00	7.15	7.23	7.23^{a}±0.04			

***** mean ± SE within column followed by same letter are non- significantly different at p< 0.05.

Table 4.13 ANOVA for effect of type of ghee on overall acceptability (score) of LFS

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	Table f (0.05)	**intrprete 0.05**
Replications	5	replication ss	4.00	0.19	0.05	3.29	6.388	NS
grand total	75.65	treatment ss	1.00	1.12	1.12	76.08	7.709	*
Mean	7.57	error ss	4.00	0.06	0.015			
correction factor	572.29	**total**	9.00	1.38				

From the Table 4.12 it was observed that overall acceptability score of LFS prepared from cow and buffalo milk ghee was significantly ($P<0.05$) affected. The overall acceptability score of LFS prepared from cow ghee (7.90 score) was more than LFS prepared from buffalo milk ghee (7.23 score). It was due to the cow ghee LFS scored higher in the colour and appearance score, body and texture score and spreadability score therefore overall acceptability score is also higher in the case of cow ghee LFS. Patange *et al.* (2015) observed that overall acceptability score of LFS 7.90 which was higher than present findings. It was might be due to not addition of annatto butter colour and diacetyl flavour in experimental LFS.

4.5 Effect of type of ghee on physico-chemical properties of LFS

4.5.1 Chemical analysis of low-fat spread

The data pertaining to chemical analysis of LFS are depicted in Table 4.14 and fig. 4.3.

The data from table 4.14 reveals that the chemical composition of low-fat spread prepared from cow milk ghee and buffalo milk ghee. After chemical analysis of both the samples, it was found that fat percentage of cow and buffalo ghee spread was 40.30 and 40.25 per cent**,** while crude protein content was 6.42 and 6.48 per cent for cow and buffalo spread respectively

Table 4.14 chemical analysis of low-fat spread

Type of spread	Fat (%)	Crude protein content (%)	Carbohydrate (%)	Ash (%)	T.S. (%)	pH	FFA (%)
CGS	40.30 ±0.05	6.42 ±0.01	8.20[a]±0.03	2.71 ±0.04	59.28 ±0.09	5.7 ±0.001	0.278[a]±0.004
BGS	40.25 ±0.10	6.48 ±0.04	8.16[b]±0.04	2.74 ±0.09	59.31 ±0.07	5.7 ±0.001	0.352[b]±0.003
CD	NS	NS	NS	NS	NS	NS	0.01

***** mean ± SE within column followed by same letter are non- significantly different at $p< 0.05$.

The carbohydrate content was 8.20 and 8.16 per cent for cow and buffalo based LFS respectively. It also observed that the ash percentage for LFS was 2.71 and 2.74 per cent for buffalo ghee LFS. The total solid, pH and FFA for LFS was 59.28 per cent, 5.7 and 0.278 per cent respectively whereas buffalo ghee LFS 59.31 percent, 5.7 and 0.352±0.003 percent respectively.

4.5.2 Effect of type of ghee on oiling off (%) of LFS

It is a melting property and emulsion stability of LFS in terms of oil expelled from the product. Frede and Buchheim (1994) defined that the percentage (weight %) of oil a butter sample releases under specified condition.

Table 4.15 Effect of type of ghee on oiling off (%) of LFS

Types of spread	Replications						SEm	CD	CV
	I	II	II	IV	V	Mean %			
C.G S.	3.92	3.96	4.2	3.98	4.14	4.04[b]±0.07	0.05	0.21	1.61
B.G S.	3.68	3.7	3.72	3.78	3.64	3.70[a]±0.03			

***** mean ± SE within column followed by same letter are non- significantly different at $p< 0.05$.

Table 4.16 ANOVA for effect of type of ghee on oiling off (%) of LFS

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	table f (0.05)	**intrpret e 0.05**
Replications	5	replication ss	4.00	0.03	0.01	0.74	6.388	NS
grand total	38.72	treatment ss	1.00	0.28	0.28	27.83	7.709	*
Mean	3.87	error ss	4.00	0.04	0.01			
correction factor	149.92	**total**	9.00	0.35				

As shown in the Table 4.15 and fig. 4.4 the oiling off percentage was significantly (P<0.0) affected by the type of ghee used for LFS. The LFS prepared from the cow ghee has more oiling off (4.4 per cent) than that of LFS prepared from the buffalo milk ghee (3.70 per cent). It might be due to cow ghee has higher liquid fraction (83 %) than that of buffalo ghee (62%) as reported by Armugham and Narayan (1979). Earlier workers reported that oiling off of spread ranging between 4.3 to 5.8 per cent (Bullock, 1966), 3.1 to 5.8 per cent (Prajapati *et al*. 1991) and 21.43 to 42.03 percent (Reddy *et al.*, 2000).

4.5.3 Effect of type of ghee on wheying off (%) of LFS

It is the property of the product indicating the amount of water expelled from the product upon gently pressing it at specific temperature. The data pertaining to Effect of fat on wheying off (%) of LFS are depicted in Table 4.17 and fig. 4.2.

Table 4.17 Effect of type of ghee on wheying off (%) of LFS

Types of spread	Replications						SEm	CD	CV
	I	II	II	IV	V	Mean %			
C.G S.	7.78	7.72	7.6	7.7	7.55	7.67^{b}±0.06	0.05	0.19	1.51
B.G S.	7.25	6.81	6.75	6.98	6.92	6.94^{a}±0.04			

***** mean ± SE within column followed by same letter are non- significantly different at p< 0.05.

Table 4.18 ANOVA for effect of type of ghee on wheying off (%) of LFS

Treatments	2	**source**	**df**	**ss**	**ms**	**cal-f**	table f (0.05)	**intrprete 0.05**
Replications	5	replication ss	4.00	0.14	0.03	2.84	6.388	NS
grand total	73.06	treatment ss	1.00	1.32	1.32	109.41	7.709	*
Mean	7.31	error ss	4.00	0.05	0.012			
correction factor	533.78	**total**	9.00	1.51				

From the Table 4.17 it was observed that wheying off percent was significantly (P<0.0) affected by fat. Wheying off percentage of the LFS prepared from cow milk ghee (7.67%) had more than LFS prepared from the buffalo milk ghee (6.94%). It was also observed that cow ghee low-fat spread had less firmness than the buffalo ghee low-fat spread. Similar observation were found by Dalaly *et al.*, (1968) who reported that the body firmness is generally inversely related to tendency of wheying off. Due to these reasons, cow ghee LFS had more wheying of percentage.

4.6 Effect of additon annatto colour on colour and appearance (score)* of LFS

The data pertaining to effect of annatto color on color and appearance (score) of LFS are depicted in Table 4.19 and fig. 4.5

Table 4.19 Effect of addition of annatto colour on colour and appearance (score) of LFS

Sample	Treatment	Mean score*	SEm	CD	CV
Without addition of annatto colour	Cow ghee spread	7.70^{b}±0.05	0.06	0.20	1.85
	Buffalo ghee spread	7.13^{a}±0.07			
With addition of annatto colour	Cow ghee spread	8.50^{d}±0.01			
	Buffalo ghee spread	8.10^{c}±0.03			

***** mean ± SE of five replications within column followed by same letter are non-significantly different at p< 0.05.

During the preparation of LFS from cow ghee and buffalo ghee spread, it was observed that the LFS prepared with addition of the Annatto colour had obtained more colour and appearance score than LFS prepared without addition of Annatto colour in both the cow and buffalo ghee spread. Annatto butter colour has the yellow colour. The present finding are accordance to research of Patange (2006) who used 0.1 percent butter annatto colour and improvement in color and appearance score of LFS. Among the several oil and water-soluble colorants, a mixture of oil soluble butter annatto colour and β- carotene (10mg/kg) imparted an acceptable yellow shade in o/w spread (Patel and Gupta, 1993). Due to addition of the annatto cow and buffalo ghee LFS imparted the attractive yellow colour to the both the cow and buffalo ghee spread. Therefore, the LFS prepared with addition of the annatto butter colour (0.1%) has obtained more score than of LFS prepared without addition of the annatto colour.

4.7 Effect of addition of diacetyl flavour on flavour (score)* of LFS

The data pertaining to effect of addition of diacytyl on flavour (score) of LFS are depicted in Table 4.20 and fig. 4.6

Table 4.20 Effect of diacetyl flavour on flavour (score)* of LFS

<table>
<tr><th>Sample</th><th>Treatment</th><th>Mean score*</th><th>SEm</th><th>CD</th><th>CV</th></tr>
<tr><td rowspan="2">Without addition of diacetyl flavour</td><td>Cow ghee spread</td><td>6.70^{a}±0.02</td><td rowspan="4">0.02</td><td rowspan="4">0.06</td><td rowspan="4">0.63</td></tr>
<tr><td>Buffalo ghee spread</td><td>7.18^{b}±0.01</td></tr>
<tr><td rowspan="2">With addition of diacetyl flavour</td><td>Cow ghee spread</td><td>7.30^{c}±0.03</td></tr>
<tr><td>Buffalo ghee spread</td><td>8.15^{d}±0.01</td></tr>
</table>

***** mean ± SE of five replications within column followed by same letter are non-significantly different at $p < 0.05$.

With texture or mouthfeel, flavour is the most important aspect of food. As low fat dairy spread is a blend of different dairy and nondairy ingredient, it may or may not have the desired flavour. Several attempts has been made to stimulate butter like flavour or cheese like flavour in spread. Numerous study have been conducted on the use of diacetyl flavour as a flaouring agent and concluded that diacetyl alone imparts definite culture flavour. Table 4.20 reveals that the LFS prepared with addition of diacetyl flavour had significantly obtained more flavour score than the LFS prepared without addition of diacetyl flavour in both the cow and buffalo ghee spread. Mostly judges accepted the spread with addition of diacetyl flavour than LFS without addition of diacetyl flavour. From the above statement, it was concluded that diacetyl flavour incorporated in to spread had more acceptability. Similar result was confirmed by Patange (2006). He optimized different level of diacetyl (0,2,4,10,15 ppm) were assessed for its effectiveness in imparting desired butter like flavour to the spread but he was found highest average score for sample containing 4 ppm diacetyl.

4.8 Effect of exposure time on sensory attributes (score)*of LFS Prepared from cow milk ghee and buffalo milk ghee at 35±1°C

4.8.1 Effect of exposure time on color and appearance (score)

From the Table 4.21 and fig. 4.7 it was seen that the score for color and appearance was range from 6.55 to 7.70. The maximum score was recorded for CS_1 followed by CS_2 (score 7.45). The color and appearance score was significantly decline for both the spread with increasing exposal time. When time for exposing the LFS more than 20 min the appearance of the product was observed to be dull similarly the colour of the product was not accepted by the panel of the judges.

Table 4.21 Effect of exposure time on sensory properties (score)* of LFS Prepared from cow milk ghee and buffalo milk ghee

Treatment	Colour and appearance	Body and texture	Spreadability	Flavour	Overall acceptability
CS_1	$7.70^{f}\pm0.05$	$7.90^{g}\pm0.07$	$7.98^{g}\pm0.02$	$6.70^{c}\pm0.09$	$7.95^{h}\pm0.05$
BS_1	$7.13^{d}\pm0.01$	$7.35^{e}\pm0.06$	$7.55^{d}\pm0.03$	$7.18^{f}\pm0.04$	$7.23^{e}\pm0.03$
CS_2	$7.45^{e}\pm0.02$	$7.79^{f}\pm0.06$	$8.10^{h}\pm0.07$	$6.76^{d}\pm0.02$	$7.80^{g}\pm0.09$
BS_2	$7.00^{c}\pm0.11$	$7.10^{d}\pm0.11$	$7.65^{e}\pm0.10$	$7.74^{g}\pm0.10$	$7.00^{d}\pm0.10$
CS_3	$7.00^{c}\pm0.08$	$7.10^{d}\pm0.04$	$7.92^{f}\pm0.03$	$6.11^{b}\pm0.08$	$7.60^{f}\pm0.08$
BS_3	$6.90^{b}\pm0.11$	$6.70^{c}\pm0.07$	$7.50^{c}\pm0.14$	$6.85^{e}\pm0.01$	$6.25^{b}\pm0.02$
CS_4	$6.55^{a}\pm0.04$	$5.25^{a}\pm0.01$	$6.15^{a}\pm0.07$	$6.00^{a}\pm0.11$	$6.55^{c}\pm0.03$
BS_4	$6.75^{a}\pm0.02$	$6.25^{b}\pm0.09$	$7.25^{b}\pm0.07$	$6.66^{d}\pm0.9$	$6.00^{a}\pm0.01$
CD	0.21	0.19	0.22	0.22	0.18
SEm	0.07	0.07	0.08	0.08	0.06
CV	2.17	2.10	2.33	2.56	1.94

***** mean ± SE of five replications within column followed by same letter are non-significantly different at $p< 0.05$.

4.8.2 Effect of exposure time on body and texture (score)

The data pertaining to effect of exposure time on body and texture (score) for LFS Prepared from cow milk ghee and buffalo milk ghee are depicted in Table 4.21 and fig. 4.8. Considering the exposure time of a cow ghee LFS it was observed that the score for body and texture of CS_1 (score 7.90) and CS_2 (score 7.79) were at par with each other. Body and texture scores were significantly ($p< 0.05$) decreased from the CS_1 to CS_4. It might be due to cow ghee contains more short chain of saturated fatty acid as compare to buffalo ghee. Short chain saturated fatty acid are responsible for smoothness in the product as reported by Bector (2002). In LFS prepared from buffalo ghee, the body and texture score was 7.30 at BS_1 which was decreased to 6.25 at BS_4. Body and texture score in buffalo ghee spread is less at BS_1 (score 7.35) and BS_2 (score 7.10) treatment as compared to cow ghee spread at CS_1 (7.90) and CS_2 (score 7.79). But it is also observed that body and texture score in cow milk ghee spread from the CS_3 treatment rapidly decreased as compared to buffalo milk ghee spread it might be due to cow ghee had less melting point and harder structure than the buffalo milk ghee as reported Lakshminarayan and Rama Murthy (1985).

4.8.3 Effect of exposure time on spreadability (score)

The data pertaining to effect of exposure time on spreadability (score) for LFS prepared from cow ghee and buffalo ghee are depicted in Table 4.21 and fig. 4.9. The spreadability scores of LFS prepared from cow ghee at treatment CS_1, CS_2, CS_3 and CS_4 were 7.98, 8.10, 7.92 and 6.15 respectively whereas the spreadability score for buffalo LFS were BS_1, BS_2, BS_3 and BS_4 were 7.55, 7.65, 7.50 and 7.25. It was observed that spreadability score was increased from CS_1 to CS_2 and BS_1 to BS_2 however, the increased in score was statistically at par with each other. As the time of exposure increased up to 10 min the cow ghee LFS shows a best results for spreadability. After the CS_2 treatment as the exposure time increased, the LFS was shown slight sticky appearance and resulted in significant decrease in spreadability score. As the body and texture score decreased spreadability of the cow ghee spread was also decreased. The reason behind this cow ghee had lower melting point (32.20^{0}C) than buffalo ghee (33.64^{0}C), higher viscosity values was recorded with cow ghee (33.893) than buffalo ghee as reported by Changade *et al*., (2006). Average liquid fraction was also more in the cow milk ghee (83%) than buffalo milk ghee (62%) as reported by Frankel *et al.* (1958).

4.8.4 Effect of exposure time on flavour (score)

The data pertaining to effect of time exposure on flavour (score) for LFS Prepared from cow milk ghee and buffalo milk ghee of LFS are depicted in Table 4.21 and fig. 4.10. It was observed that flavour score of cow ghee LFS was increased up to CS_2 (6.76). In CS_2 treatment flavour was more pleasant than CS_1 (6.62). It might be due to sample removed from refrigeration condition, flavoring compounds were not released in required amount due to low temperature but in CS_2 treatment the flavoring compounds may release in enough concentration and gives typical flavour so the score of CS_2 is more than CS_1. It was also observed that the flavour score was significantly ($P<0.05$) decreased from CS_3 (6.11) to CS_4 (6.00). In treatment CS_3 and CS_4, as the exposure time increased, the temperature of the cow ghee spread was increased simultaneously, further due to increases in temperature the intensity of flavour were decreased and thus flavour score was significantly decreased from CS_3 to CS_4. Similar results was found in case of buffalo ghee LFS.

4.8.5 Effect of exposure time on overall acceptability (score)

The data pertaining to effect of exposure time on overall acceptability (score) for expose of LFS prepared from cow milk ghee are depicted in Table 4.21 and fig. 4.11. It was observed that the overall acceptability score was significantly affected by the type of ghee and exposed time too. The overall acceptability score for CS_1, BS_1, CS_2, BS_2, CS_3, BS_3, CS_4 and BS_4 were 7.95, 7.23, 7.80, 7.00, 7.70, 6.25, 6.55 and 6.00 respectively. The maximum score for the overall acceptability was recorded to the spread of CS_1 (score 7.95) however it was at par with CS_2 indicating that for 10 min period of time the overall acceptability of cow spread was not affected. When the time passes the overall acceptability score was below 7 in case of spread made from buffalo ghee. The LFS was also shown decreased in overall acceptability score from 7.25 to 6.00. By considering all the sensory attributes it was recorded that spread could be consume up to 10 min of expose period after removing from the refrigerator.

5. Summary and Conclusions

5.1 Summary

Spreads are the products harmonizing with the idea of healthy nutrition. At the same time they have good taste and flavor as well as very good spreadability at refrigerator temperature and retain its stand up property even at high ambient temperature. Considerable efforts have been made in India for development of fat spreads of dairy and non-dairy type using a variety of ingredients viz, butter, butter oil, cream, paneer, channa, cheese, vegetable fat and ghee. The exploitation of ghee in the manufacture of low fat spread is the dire need of today's dairy industry due to its easy availability and better shelf life at ambient temperature. Indian dairy industry marketed ghee either as cow ghee or buffalo ghee or only as ghee. Both the ghee has differed in respect of color, texture and flavor because of differentiate in fatty acid profile. Buffalo milk fat is distinctly harder than cow milk fat. Considering the difference in behavior in properties of cow and buffalo milk ghee and importance of ghee in manufacture of low fat spread it was planned to study the 'Comparative study of low-fat spread preparation from cow and buffalo milk.

Initially cow and buffalo milk ghee was melt at 70^{o}C followed by rapid and slow pre-cooling to 20°C in controlled condition and then cooling to 5^{o}C by quiescent storage under refrigeration. This ghee was evaluated at 30°C for sensory characteristic viz. texture, consistency and color. The treatment were Cow milk ghee melted at 70^{o}C and rapidly pre-cooled to 20^{o}C (CG_1); Cow milk ghee melted at 70^{o}C and slowly pre-cooled to 20^{o}C (CG_2); Buffalo ghee milk melted at 70^{o}C and rapidly pre-cooled to 20^{o}C (BG_1) and Buffalo ghee milk melted at 70^{o}C and slowly pre-cooled to 20^{o}C(BG_2). These samples were evaluated for sensory study. LFS from cow and buffalo milk ghee was prepared by preparing aqueous phase and fat phase separately and then intermingling in planetary Mixer. The spreads was packed in 75gm in plastic cups and closed with lids before being transferred to refrigerator (5^{o}C). The treatment were as LFS using cow milk ghee (CGS) and LFS using buffalo milk ghee (BGS). Further the effect of colour and flavour on qualities of LFS was studied. To know the effect of colour and flavour, annatto colour and diacetyl flavour was added (@0.1% of ghee) and 4 ppm respectively in both the type of spread and evaluated for sensory qualities. Effect of exposure time on sensory properties of low fat spread prepared from cow and buffalo milk ghee was also studied. Generally LFS is expected to serve immediately after remove from refrigerator at 5^{0}C, however unfortunately it may expose to environment temperature for certain period of time before consumption. The prepared both the LFS were removed from refrigerator and immediately evaluated (CS_1 and BS_1). The quality of spread then evaluated after 10 (CS_2, BS_2) 20 (CS_3, BS_3) and 30 (CS4, BS_4) min. During this period the spread were kept in incubator maintained at 35±1^{0}C.

5.1.1 Physico-chemical properties of cow and buffalo milk ghee

The cow and buffalo milk ghee used for preparation of low fat spread in present study contain on an average 99.5 per cent fat, 31.9 and 33.1 mg/gm iodine value for cow and buffalo milk ghee respectively, 2.4 and 2.8 per cent of free fatty acid content in cow and buffalo ghee and 34.4^{0}C and 38.3^{0}C slip point of cow and buffalo milk ghee, respectively.

5.1.2 Effect of method of rapid and slow pre-cooling on sensory properties of cow and buffalo milk ghee.

Rapidly pre-cooled cow ghee was light yellow colour (score 67.32) whereas buffalo ghee when rapidly pre-cooled had white appearance (22.44) as compared to slowly pre-cooled buffalo milk ghee which had creamish white appearance (score 30.36). The texture score of cow and buffalo ghee were significantly affected by the method of pre-cooling. Rapidly pre-cooled cow ghee had very smooth and pasty texture with (score 5.28) than rapidly pre-cooled buffalo ghee (score 11.88). The consistency score appear to be solid hard (score 94.16) for rapidly pre-cooled buffalo ghee than rapidly pre-cooled cow ghee (score 85.12).

5.1.3 Chemical analysis of skim milk powder used for experiment

Skim milk powder used for the investigation had 3.90 per cent of moisture, whereas average fat percentage was 0.65 and solubility index was 0.76ml.

5.1.4 Effect of type of ghee on sensory attributes of LFS

The cow ghee LFS scored higher for colour and appearance (score 7.70), body and texture (score 7.90) and spreadability (score 7.98) than that of buffalo ghee LFS. Buffalo ghee LFS had more flavour score ()than cow ghee LFS. The overall acceptability score (7.95) was also significantly higher in the case of cow ghee LFS as compared to buffalo ghee LFS (7.25).

5.1.5 Effect of type of ghee on physico-chemical properties of LFS

5.1.5.1 Chemical analysis of low-fat spread

In present study it was found that fat percentage of cow and buffalo ghee spread was 40.30 and 40.25 per cent**,** while crude protein content was 6.42 and 6.48 for cow and buffalo ghee spread respectively. The carbohydrate content was 8.20 and 8.16 per cent for cow and buffalo based LFS respectively. It was also observed that the ash percentage for cow was 2.71 and 2.74 for buffalo ghee LFS. The total solid content, pH and FFA for cow was 59.28 per cent, 5.7 and 0.278 per cent whereas for buffalo ghee LFS it was found 59.31 per cent, 5.7 and 0.352 percent respectively.

5.1.5.2 Effect of type of ghee on physical properties of LFS

The LFS prepared from the cow ghee has more oiling off (4.4 per cent) than that of LFS prepared from the buffalo milk ghee (3.70 per cent).

May be because of higher liquid fraction in cow ghee than buffalo ghee. Wheying off percentage of the LFS prepared from cow milk ghee (7.67%) was more than LFS prepared from the buffalo milk ghee (6.94%). It might be due to less firmness of the cow ghee spread.

5.1.6 Effect of addition annatto colour on colour and appearance (score) of LFS

LFS prepared with addition of the annatto butter colour had obtained more colour and appearance score than the LFS prepared without addition of annatto colour in the case of both the cow and buffalo ghee spread**.**

5.1.7 Effect of addition of diacytyl flavour on flavour (score) of LFS

LFS prepared with addition of the diacetyl flavour had obtained more flavour score than the LFS prepared without addition of diacetyl flavour in the case of both the cow and buffalo ghee spread. The flavour score recorded for without addition of diacetyl flavour was 6.70 and 7.18 for cow and buffalo ghee LFS respectively whereas 7.30 and 8.15 for diacetyl flavour added cow and buffalo ghee LFS respectively.

5.1.8 Effect of exposure time on sensory properties (score) of LFS Prepared from cow milk ghee and buffalo milk ghee at 35±1^{0}C

The maximum score for the colour and appearance, body and texture and overall acceptability was recorded to cow milk ghee spread evaluated immediately after removed from refrigeration (CS_1) however it was at par with CS_2 indicating that cow ghee LFS maintain its spread characteristic up to 10 min period of time**.** In the case of LFS prepared from buffalo milk ghee also found similar results for controlled sample. Whereas spredability and flavour score were found higher in CS_2 and $BS_{2.}$

5.2 Conclusion

1 Slowly pre-cooled cow ghee had intense yellow colour than rapidly pre-cooled ghee, whereas slowly pre-cooled buufalo ghee has creamish white colour whereas rapidly pre-cooled cow ghee has white colour. Rapidly pre-cooled cow ghee had very smooth and pasty texture with (score 5.28) than rapidly pre-cooled buffalo ghee (score 11.88). The consistency score appear to be solid hard (score 94.16) for rapidly pre-cooled buffalo ghee than rapidly pre-cooled cow ghee (score 85.12).

2 The respective sensory scores for cow ghee and buffalo ghee LFS were, 7.70 and 7.13 for colour and appearance, 7.90 and 7.35 for body and texture, 7.98 and 7.55 for spreadability, 6.70 and 7.18 for flavour and 7.95 and 7.23 for overall acceptability.

3 There was non-significant difference in fat, crude protein, carbohydrates, ash and TS percentage in both cow and buffalo ghee LFS and free fatty acid content was more in buffalo ghee LFS than cow ghee LFS.

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ANNEXTURE-I

1 Colour (20-21°C)	White	Creamish white	Light yellow	yellow	pronounced yellow
2 Texture (20-21°C)	Smooth/ pasty	Fine grain	Medium grain	large grain	Extra-large grain
3 Consistency (20-21°C)	Essentially Fluid	Liquid	Optimally solid- liquid	Too large liquid	Hard/ solid

ANNEXTURE-II

Evaluation of low-fat spread on 9 point Hedonic scale

(Evaluation card)

1) Trial no:

2) Name of the evaluator: 3) Date of evaluation:

Sample no.	Sensory Characteristics				
	Colour and appearance	Body and texture	spreadability	Flavour	Overall acceptability
1					
2					
3					

Maximum score 9.0 for each characteristics

Sr. no.	Remark	Score
1	Like extremely	9
2	Like very much	8
3	Like moderately	7
4	Like slightly	6
5	Neither like or dislike	5
6	Dislike slightly	4
7	Dislike moderately	3
8	Dislike very much	2
9	Dislike extremely	1

Note: Score of 5.5 and above indicates acceptability within the score of 1 to 9.

Remarks:

1.

2.

Signature of the judge

8. VITA

Mr. Dhanake Vrishasen Vinayak

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

In

DAIRY SCIENCE

Thesis title	:	Comparative study of low-fat spread preparation from cow and buffalo milk ghee
Major field	:	Dairy Science
Biographical Information	:	Born at Vairag, Taluka-Barshi, District-Solapur (M.S) on 29thJune, 1993. Son of Shri. Vinayak Dhanake and Sou. Shashikala Dhanake.
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	:	Passed HSC, Vidyamandir Jr. College of Art and Science, Vairag Taluka Barshi, District-Solapur (M.S) in 2011.
	:	Received B.Sc. (Agri.) degree from. College of Agriculture, Kolhapur, Dist-Kolhapur (M.S) in 2015 with second class.
Achievements	:	1. NCC "**C**" Certificate with **"AAA**" grade, also **Gold** medallist and **Rank** holder of NCC. 2. University Youth festival participation. 3. College **Kabbadi** player. 4. Two times College wrestling **Gold** medal in 97 kgs and below wt. category. 5. College weightlifting **Gold** medallist. 6. Secretary of Agricos Career Development Forum, A.C. Kolhapur.

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E-mail	**:**	rajdhanake1993@gmail.com

Fig. 4.1 Effect of method of rapid and slow pre-cooling on sensory properties (score) of cow and buffalo milk ghee

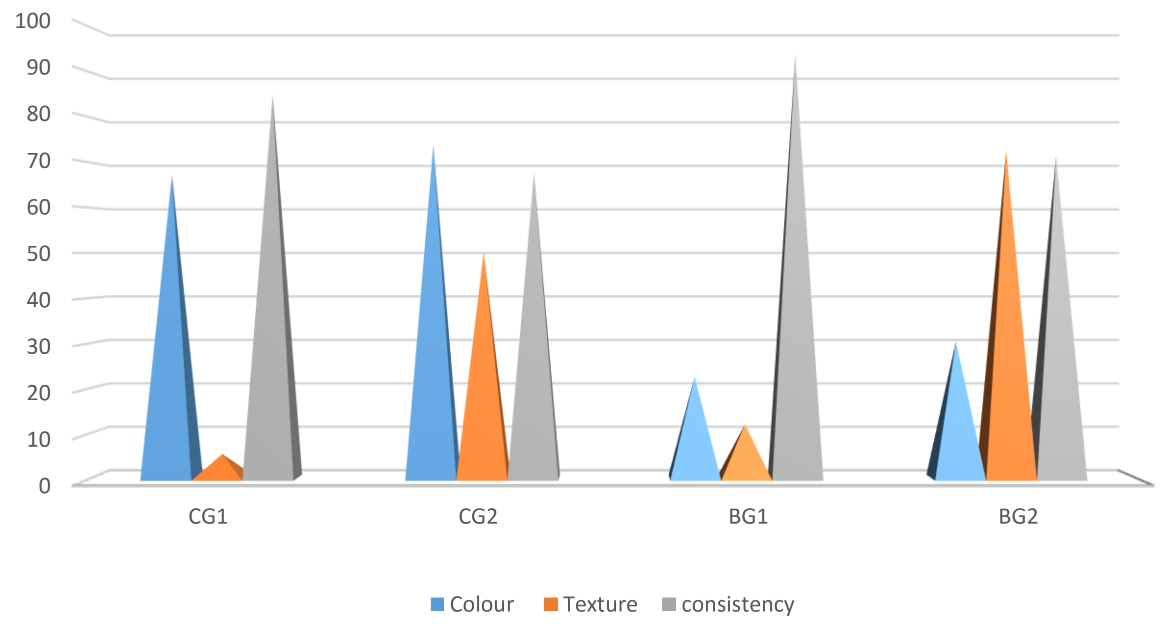

Fig. 4.1 Effect of method of rapid and slow pre-cooling on sensory properties (score) of cow and buffalo milk ghee

Fig 4.2 Effect of type of ghee on sensory attributes (score) of low-fat spread

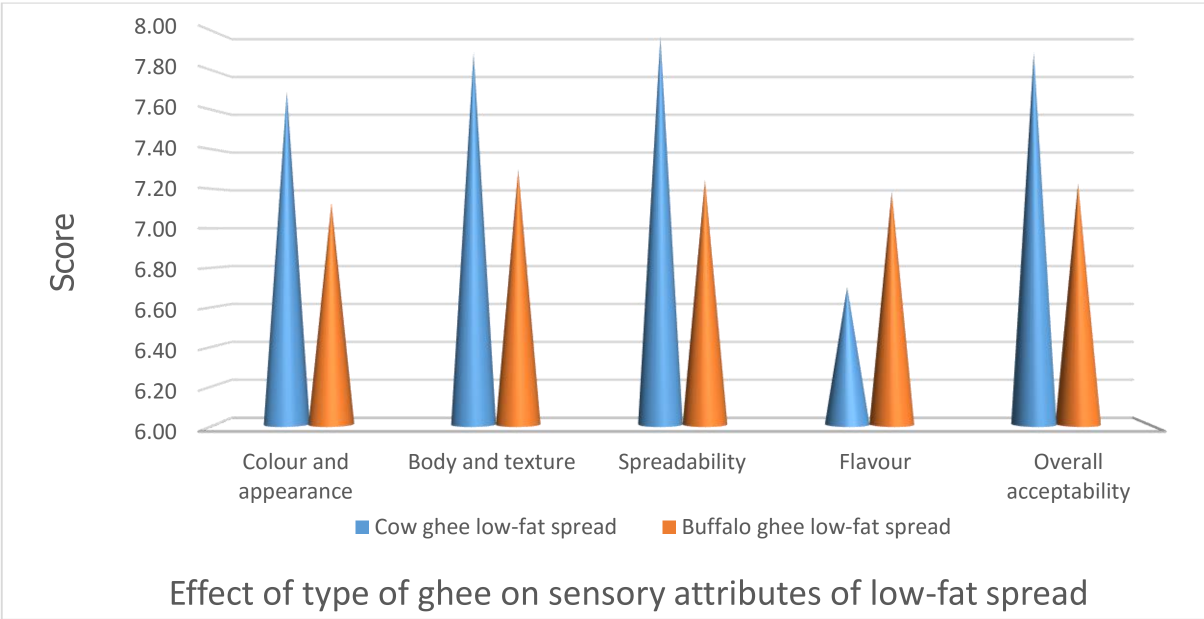

Fig 4.3 Effect of type of ghee on Chemical analysis of low-fat spread

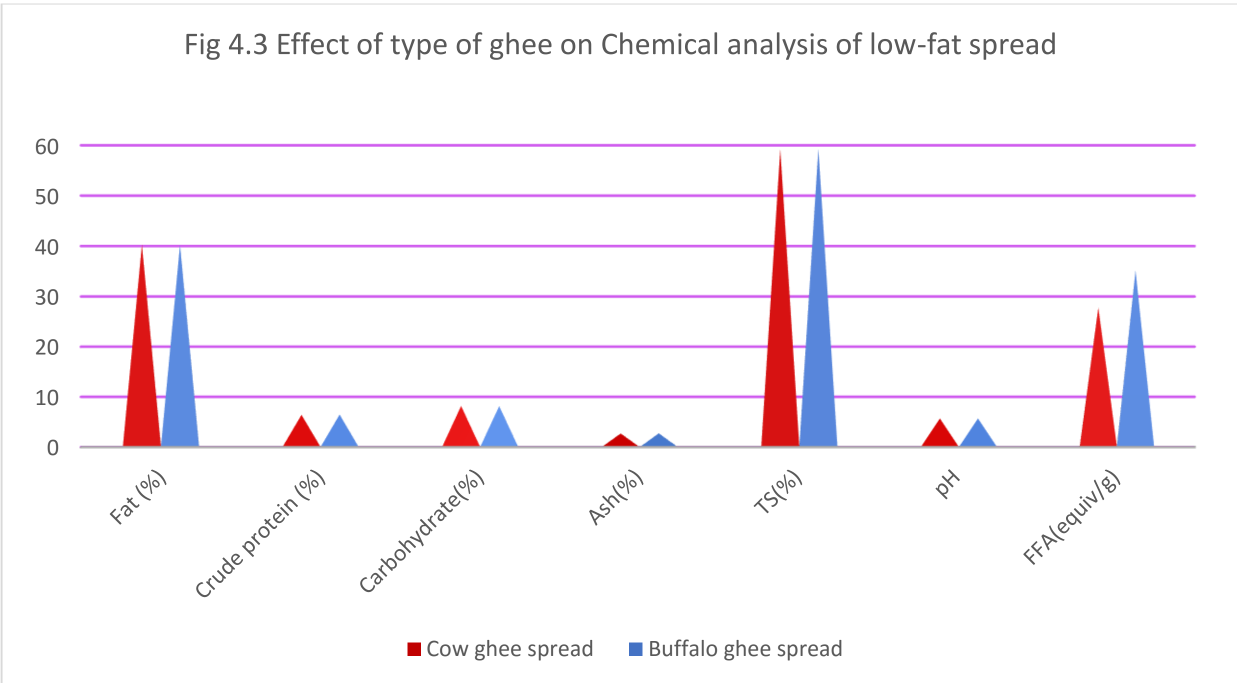

Fig 4.4 Effect of type of ghee on Oiling off and wheying off (%) of low-fat spread

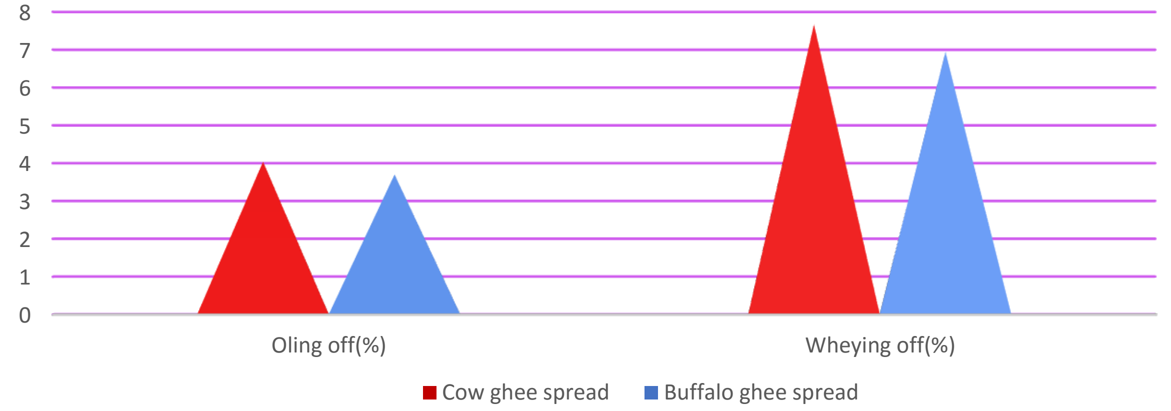

Fig 4.4 Effect of type of ghee on Oiling off and wheying off (%) of low-fat spread

Fig 4.5 Effect of addition of annatto colour on colour and appearance (score) of low -fat spread

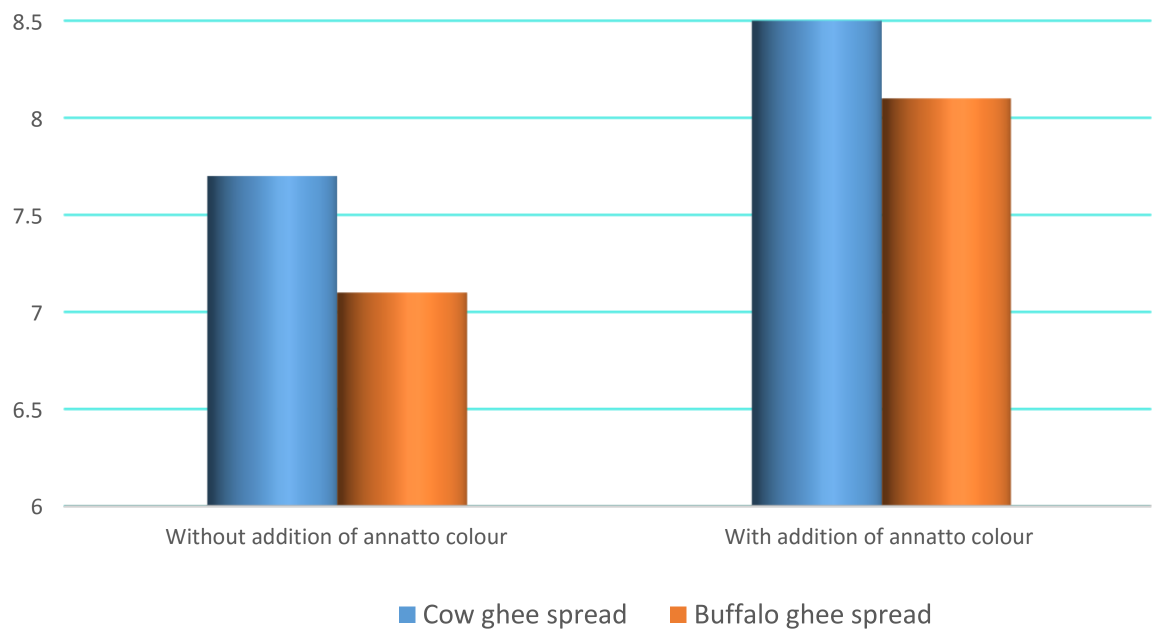

Fig 4.5 Effect of addition of annatto colour on colour and appearance (score) of Low -fat spread

Fig 4.6 Effect of diacetyl flavour on flavour (score) of low-fat spread

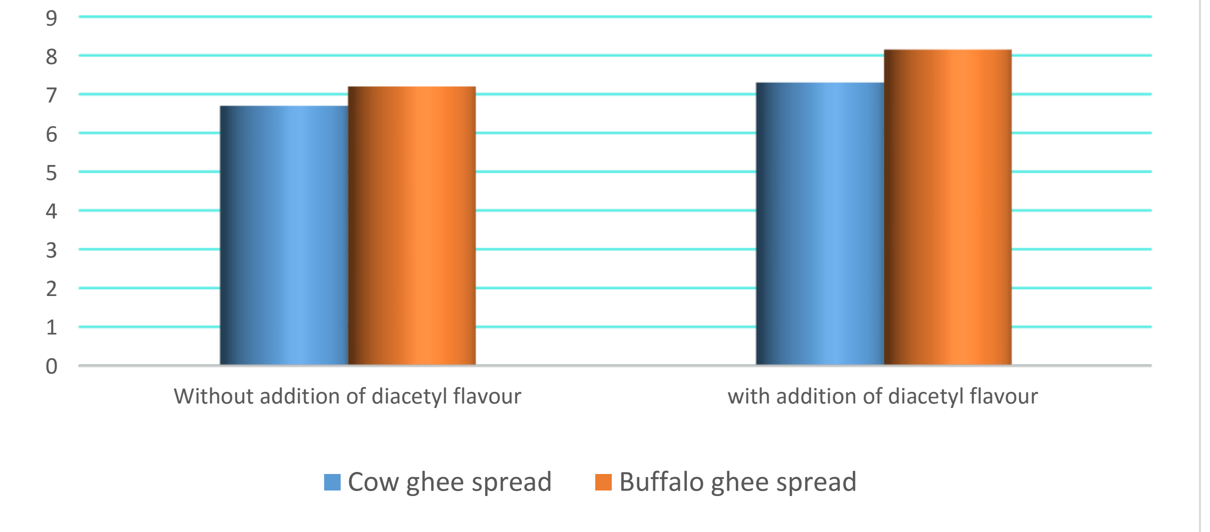

Fig 4.6 Effect of diacetyl flavour on flavour (score) of low-fat spread

Fig 4.7 Effect of exposure time on colour and appearance (score) of low fat spread

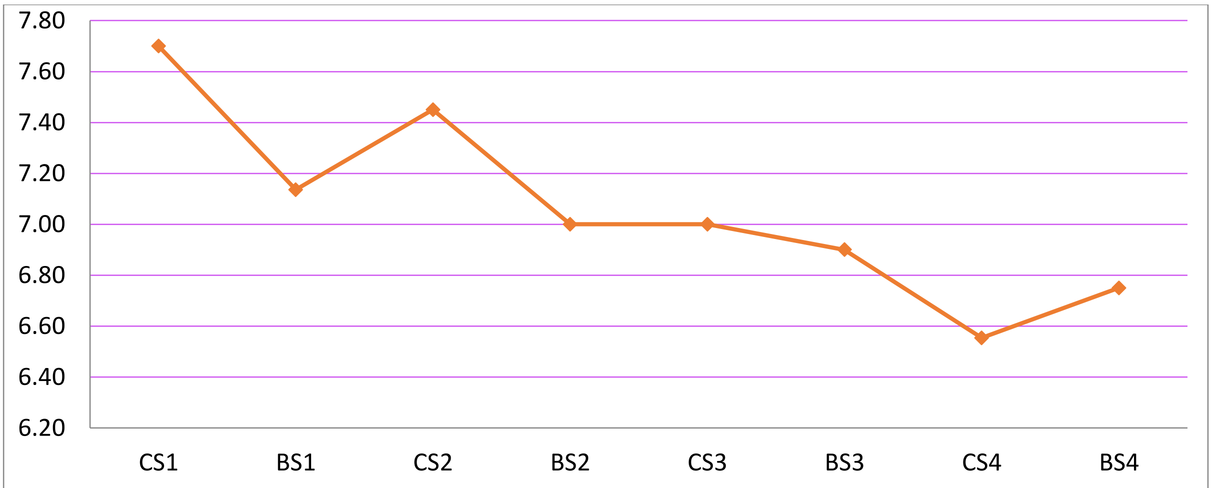

Fig 4.7 Effect of exposure time on colour and appearance (score) of low fat spread

Fig 4.8 Effect of exposure time on Body and texture (score) of low fat spread

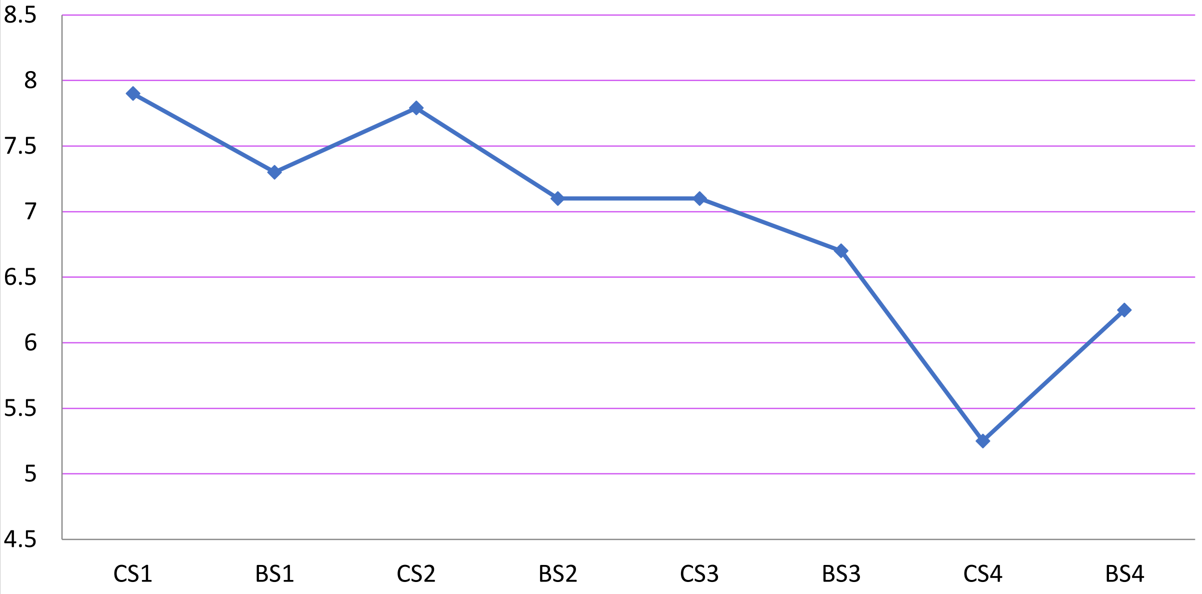

Fig 4.8 Effect of exposure time on Body and texture (score) of low fat spread

Fig 4.9 Effect of exposure time on Spreadability (score) of low fat spread

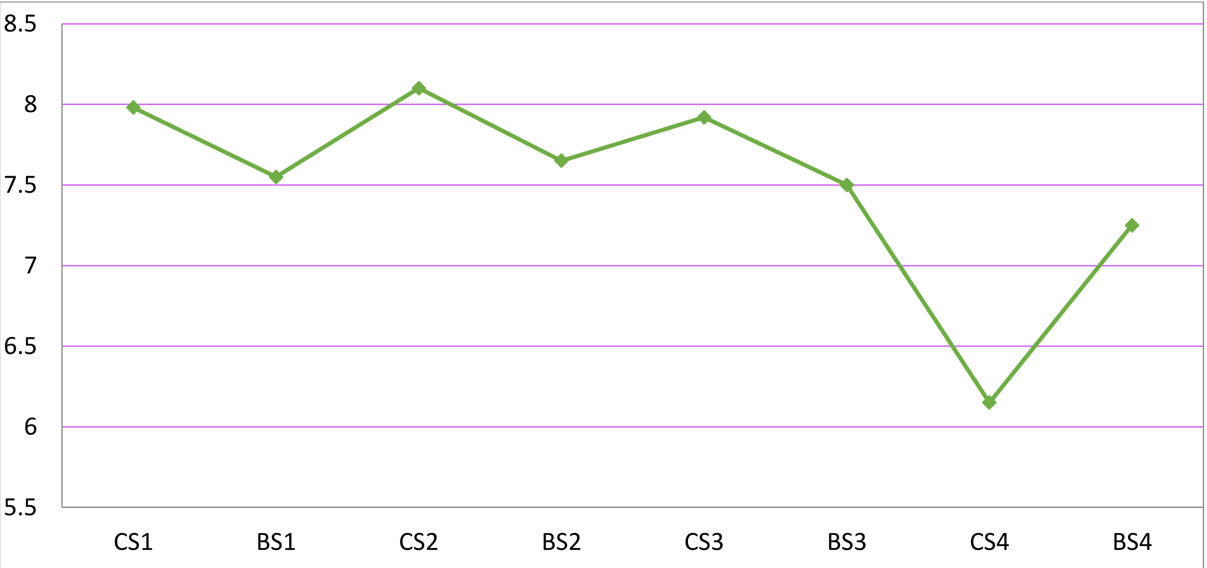

Fig 4.9 Effect of exposure time on Spreadability (score) of low fat spread

Fig 4.10 Effect of exposure time on Flavour (score) of low fat spread

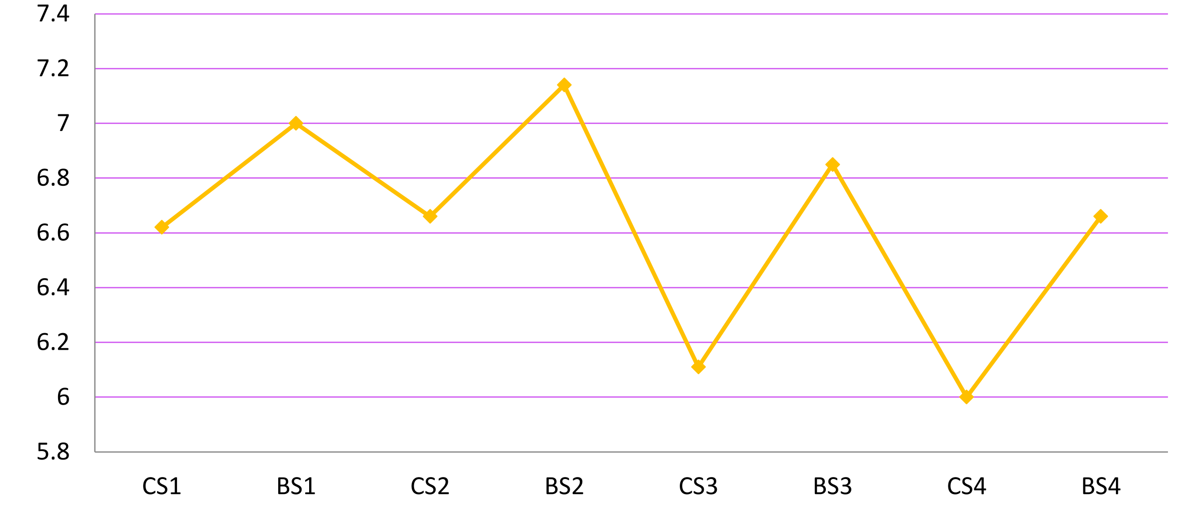

Fig 4.10 Effect of exposure time on Flavour (score) of low fat spread

Fig 4.11 Effect of exposure time on Overall acceptability (score) of low fat spread

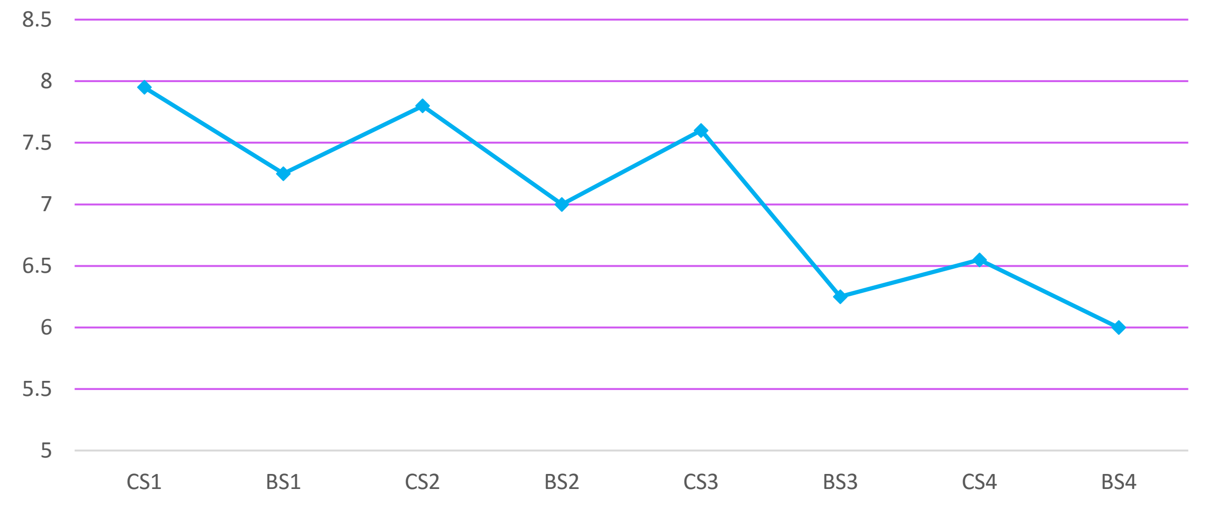

Fig 4.11 Effect of exposure time on overall acceptability (score) of low fat spread

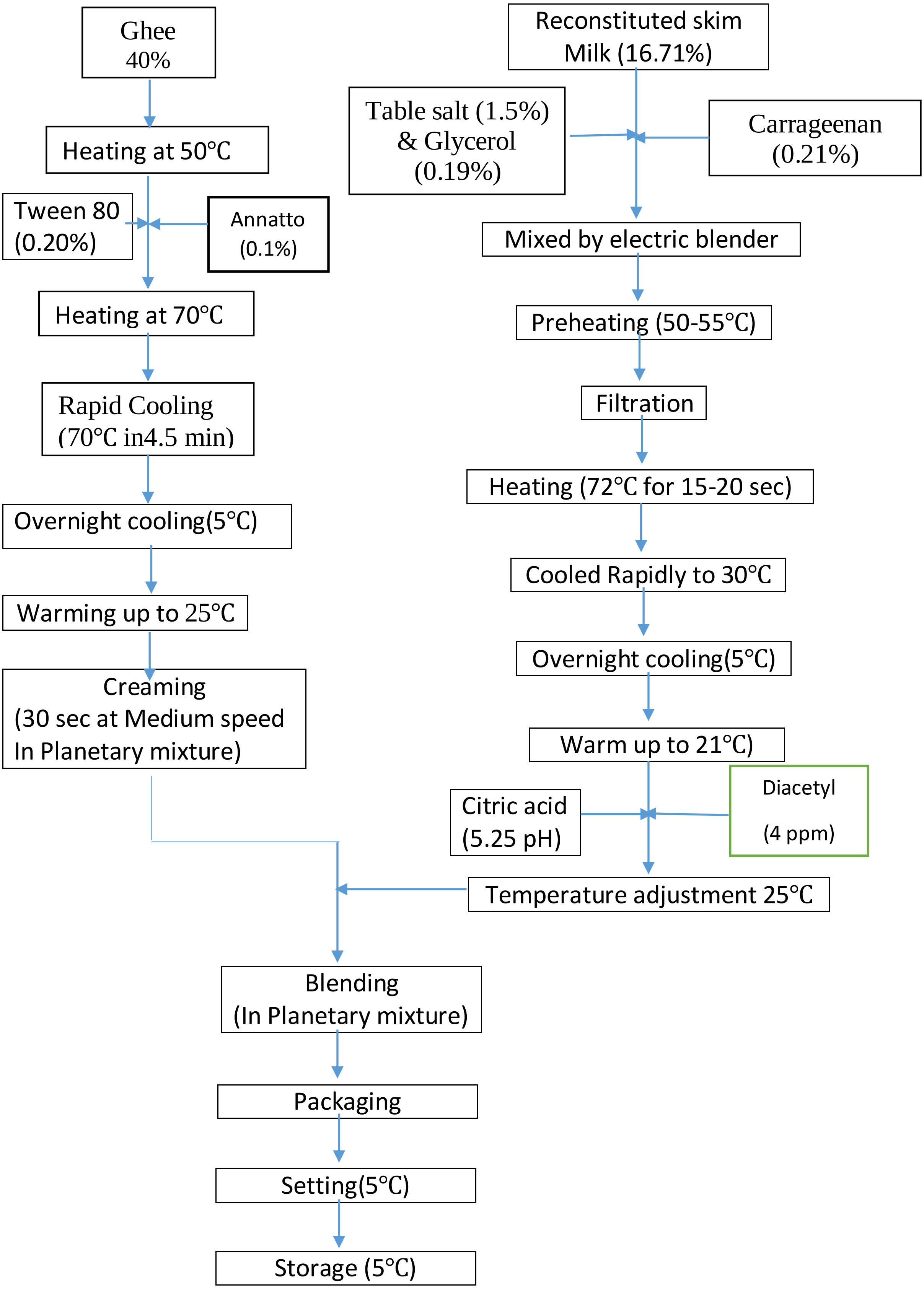

Fig. Flow diagram of manufacture of LFS using cow and buffalo Ghee with addition of of annatto colour and diacetyl flavour

Plate No. 1 Planetary mixture

Plate No. 2 Cow ghee LFS

Plate No. 3 Buffalo ghee LFS