A STUDY ON PHYSICO-CHEMICAL QUALITIES OF MARKET GHEE

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

MASTER OF TECHNOLOGY
IN
DAIRYING
(DAIRY CHEMISTRY)

BY

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KARNAL – 132001 (HARYANA), INDIA
2010

Regn. No. 2020805

Dedicated

To

My Guide

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MASTER OF TECHNOLOGY
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(DAIRY CHEMISTRY)

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ACKNOWLEDGEMENT

I convey my profound regards and feeling of gratitude to Dr. Darshan Lal for his expert guidance, constructive criticism and pertinent suggestions in planning and execution of this investigation.

I express my sincere thanks to members of Advisory Committee, Dr. Raman Seth, Principal Scientist, DC Division; Dr. Vivek Sharma, Senior Scientist, DC Division; Dr. Sumit Arora, Senior Scientist, DC Division; Dr. S.K. Atreja, Principal Scientist, ABC Division; Dr. A.A. Patel, Principal Scientist & Head, DT Division, and also to Dr. R.R.B Singh Principal Scientist, DT Division, for their valuable suggestions, encouragement and endless co-operation from time to time.

My sincere thanks are due to Dr. A.K Shrivastva, Director and vice-chancellor, NDRI, Karnal; Dr. G.S. Sharma, Ex-Head and Dr. B.K Wadhwa, Principal scientist & Head, DC Division, for providing all the necessary facilities for conducting this study.

My deep sense of gratitude and warm regards to Dr. Moti Ram , Mr. S.L. Ingle, Mr. Jagat singh, Mrs. Krishna and Mr. Kulvinder Singh for their sincere in-time help, affections and moral support during the course of this study.

I am deeply indebted to all the Scientists, Technical Officers and Staff of the Dairy Chemistry Division, for their necessary help as and when required.

I express heartfelt thanks to my seniors Ramesh sir and Prerana mem for not only their help and company, but also for all the refreshing moments we shared together.

Financial support received in the form of Institutional Fellowship from National Dairy Research Institute, Karnal, is duly acknowledged.

All are not mentioned but no one is forgotten.

A.S. Hariyani

Date: June. 14th, 2010

(ASHVINKUMAR S HARIYANI)

ABSTRACT

Ghee, most widely consumed Indian dairy product, is prepared from cow or buffalo milk or combination thereof. In India, the importance of ghee cannot be exaggerated. Consumers love its distinct flavor. In order to ensure a genuine product to the consumer, the Government of India has prescribed the compositional standards for ghee, under PFA act and Agmark rules. However, unfortunately, the producers or the middle-men involved in the ghee trade, in their greed to have more money, tend to adulterate ghee with cheaper oils and fats like vegetable oils, animal body fats, hydrogenated fats, and sometimes even the nonedible mineral oils, especially during lean season. In recent years, the problem of adulteration has assumed a very serious dimension. Several reports have appeared in the newspapers indicating rampant malpractices of ghee adulteration in different parts of the country. Therefore, the present project was undertaken to carry out a survey of collecting ghee samples from different parts of the country in different seasons and analyze them for physico-chemical parameters.

The present study revealed that the RM value, Polenske value, Saponification value, Iodine value and BR reading of ghee samples of organized dairies collected from different zones (North, South, East and West) in different seasons (Rainy, Winter and Summer) varied (with average) from 6.40 to 34.00 (24.98), 0.5 to 2.5 (1.39), 202.65 to 234.90 (222.88), 26.85 to 76.14 (39.29) and 40.50 to 51.85 (42.47), respectively. The corresponding values of ghee samples collected from unorganized dairies of Karnal city in different seasons (Rainy, Winter and Summer) were from 16.61 to 33.77 (29.57), 0.6 to 1.7 (1.28), 209.00 to 243.90 (228.85), 22.72 to 47.64 (33.02) and 38.80 to 44.60 (41.55), respectively. Similar values for pure ghee of cows and buffaloes of institute herd, collected simultaneously along with market ghee samples, were from 28.05 to 33.88 (30.86), 1.1 to 2.0 (1.61), 223.85 to 236.50 (229.72), 30.75 to 37.65 (33.43) and 40.60 to 42.50 (41.46), respectively.

It may be concluded from the present study that out of the total (66) market ghee samples (organized and unorganized), 24% samples failed to meet the requirements in terms of either RM value or BR reading or both as specified under PFA rules. Such cases were less in Summer and more in Rainy and Winter seasons. Similarly, these types of cases were more in Western region and Eastern region as against none in the Northern and southern region. Frequency of similar cases was more in unorganized dairy ghee samples of Karnal city than organized dairy ghee samples of all the four regions of the country investigated in the present study. However, in view of the small number of samples involved in the study, no serious consequences should be drawn at the country level from the present investigation.

सारांश

भारत देश में घी सबसे ज्यादा खाद्य पदार्थ में उपयोग किया जाता है। घी गाय या भैंस के दूध में से या तो दोनों के मिश्रण दूध से बनाया जाता है। ग्राहक घी का स्वाद अधिक पसन्द करते हैं। ग्राहकों को शुद्ध घी मिले, इसके लिए भारत सरकार ने घी का बंधारण पी.एफ.ए. अधिनियम और एगमार्क के तहत, निर्धारित किया है। दुर्भाग्य से, निर्माता या बीच के लोग जो घी के व्यापार में शामिल हैं, ज्यादा पैसा कमाने के लालच में घी में सस्ता तेल डालतें हैं, जैसे की वनस्पति तेल, वनस्पति घी, पशु चर्बी और अखाद्य मिनरल ऑयल भी डालतें हैं। अखबारों में भी समाचार आते हैं कि देश के अलग-अलग क्षेत्रों में घी में अनियत्रिंत रूप में मिलावट किया जाता है। इस बात को ध्यान में रखते हुए, इस परियोजना को शुरू किया गया, जिसमें देश के विभिन्न भागों से घी को संग्रह किया गया और उसका भौतिक-रसायनिक विश्लेष्ण किया गया।

वर्तमान अध्ययन से पता चला कि देश के अलग-अलग क्षेत्रों से (उतर, दक्षिण, पूर्व और पश्चिम), अलग-अलग ऋतु में (वर्षा, सर्दी और गर्मी) एकत्र डेयरी के घी के नमूने का आर.एम मूल्य, पोलेनस्के मूल्य, सेपोनिफीकेशन मूल्य, आयोडीन मूल्य एवम् बी. आर रीडिंग (औसत के साथ) 6.40 से 34.00 (29.48), 0.50 से 2.50 (1.39), 202.65 से 234.90 (222.88), 26.85 से 76.14 (39.29) और 40.50 से 51.85 (42.47) क्रमशः रिपोर्ट की गई हैं। इसके अतिरिक्त करनाल शहर की प्राईवेट डेयरी घी के नमूने के लिए अलग-अलग ऋतु में ऊपर दिये गये मान, क्रमशः 16.61 से 33.77 (29.57), 0.60 से 1.70 (1.28), 209.00 से 243.90 (228.85), 22.72 से 47.64 (33.02) और 38.80 से 44.60 (41.55) पाये गए हैं। संस्थान की गाय और भैंस के शुद्ध घी के लिए, अलग-अलग ऋतु में यह मान 28.05 से 33.88 (30.86), 1.10 से 2.00 (1.61), 223.85 से 236.50 (229.72), 30.75 से 37.65 (33.43) और 40. 60 से 42.50 (41.46) क्रमशः पाये गए हैं।

इस परियोजना से पता चलता है कि 66 घी के नमूने (एकत्र डेयरी और प्राईवेट डेयरी को मिलाकर) में से 24 प्रतिशत नमूने पी.एफ.ए. के हिसाब से आर.एम मूल्य या बी.आर रीडिंग या तो दोनों मूल्यों पर निष्फल हुए हैं। ऐसे मामले गर्मी में कम, बरसात और सर्दी में ज्यादा पाये गए हैं। उसी तरह ऐसे मामले देश के पश्चिम एवम पूर्व क्षेत्रों में ज्यादा पाए गए है। ऐसे मामले कि आवृति करनाल शहर की प्राईवेट डेयरी में ज्यादा और देश के अन्य क्षेत्रों की एकत्र डेयरी में कम पाया गया है। इस अभ्यास में कम घी के नमूने शमिल थे, इसलिए इस परियोजना को ध्यान में रखते हुए देश के स्तर पर कोई गंभीर परिणाम निकालना नहीं चाहिए।

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LIST OF ABBREVIATIONS

AGMARK	Agriculture Marketing
ANOVA	Analysis of Variance
AR	Analytical Reagent
AST	Apparent Solidification Time
BIS	Bureau of Indian Standards
BR	Butyro-Refractometer
BV	Bomer Value
CLT	Complete Liquefaction Time
CTD	Critical Temperature of Dissolution
DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
GBF	Goat body fat
GLC	Gas Liquid Chromatography
IDF	International Dairy Federation
IR	Infra Red
LR	Laboratory Reagent
L_{18}	Liquid fraction obtained at 18 ^{o}C
Min	Minute
^{o}C	Degree Centigrade
PFA	Prevention of Food Adulteration
PV	Polenske value
R_f	Resolving factor
RBO	Rice bran oil
RI	Refractive Index
RM	Reichert- Meissl
Rs	Rupees
S_{18}	Solid fraction obtained at 18^{o}C
S_{20}	Solid fraction obtained at 20^{o}C
S.E.	Standard Error

TLC	Thin Layer Chromatography
USM	Unsaponifiable Matter
UV	Ultraviolet
W.N	Whatman No

CHAPTER - 1

Introduction

1.0 INTRODUCTION

Ghee is by far the most important product widely consumed in the Indian sub-continent since time immemorial. It is prepared from cow or buffalo milk or combination thereof. It is known in different countries with different names such as ‘maslee’ or ‘samn’ or ‘samna’ in Egypt and Israel, ‘Roghan’ in Iran, and ‘Dahin hurr’ in Iraq. Butter oil, popular in western countries, is slightly different from ghee as the former has bland flavor. The origin of ghee making lies far beyond recorded history. The word ghee itself stems from old Sanskrit word ‘ghrit’ which means bright or to make bright. When sprinkled on fire, butter fat enhances its brightness. Before advent of modern techniques, especially of refrigeration, for furthering the sweet life of milk, the latter had either to be quickly consumed, or some means had to be found for its conversion into a more stable product, such as, ghee which satisfies this requirement. The absence of any but traces of moisture, lactose, and protein practically eliminates microbiological activity and extends the shelf life of ghee.

India is the largest producer of milk in the world, producing 108 million tones according to the latest estimates for 2009-10 (http://www.highbeam.com/doc/1G1-188920729.html). Ghee production forms the largest segment of the milk consumption and utilization pattern in India. Rising at annual growth rate of 5 %, ghee production has been estimated in 2009 to exceed 1.9 million tones, valued at Rs 475 billion, which has been estimated to be about 33% of total milk production and 58.9% of the total milk used for manufacture of dairy products. Over 10% of this production comes from organized sector, half of which is contributed by the co-operative sector. About 60% ghee is packed in bulk and 40% in consumer packs. Ghee production level varies from region to region, 57% in northern region, 9.5% in eastern, 23.5% in

western and 10% in southern region (Aneja *et al*., 2002). The average per capita consumption of ghee is less than 1.0 Kg per annum for the whole country (De, 1994). Ghee is traditionally marketed in mandis (market) where it is brought from village collection centers. Major ghee mandis have been established at Hathras and Khurja in Uttar Pradesh; Porbandar in Gujarat; Gantur in Andhra Pradesh; Erode in Tamilnadu; and Jodhpur in Rajasthan (Aneja *et al*., 2002). The production of ghee is higher in winter and lower in summer, corresponding to the months of higher and lower milk production. Ghee has been a regular export item from India since 1930s. Presently ghee is exported to Nepal, Bhutan, Bangladesh, the middle-east countries, and modest quantity to North-America (Aneja *et al*., 2002).

In India, ghee is mainly produced for food uses and is virtually consumed in every household because of its typical pleasant flavor and better palatability. About 2% only goes for ceremonial purpose. The vegetarian habit of great many Indian precluded from their diet hard animal fats like tallow or lard commonly used in the western countries. Sweets and meals, cooked in ghee enjoy special status, which are recognized for their distinguished flavor attributes derived from ghee. In its table use, ghee is served in hot melted form and used for garnishing the rice or spreading on chapattis. It is also used for shallow frying and deep frying of traditional Indian foods. It is also burnt in the Hindu religious ritual of Aarti. It is used in marriages and funerals, and for bathing murtis during worship. During prayers to Bhagavan Shiv on Maha Shivaratri, ghee is mixed along with four other substances: sugar, milk, dahi, and honey, which is called the Panchamrut. Also, it is used generously in Yagna as it is considered as food for Devtas.

Ghee is produced by heat desiccation of makkhan, butter or cream at 105-110^{o}C. Heat-induced changes in milk proteins/lactose during the clarification process imparts distinctive, pleasant cooked flavor to ghee (Aneja *et al*., 2002).

In general, ghee is prepared by four methods, namely, desi, creamery butter, direct cream and pre-stratification methods. The high heat applied to butter or cream removes moisture. Both are usually clarified at 110-120 ^{o}C. However, in southern India clarification is done at 120-140 ^{o}C, which gives mildly to highly cooked flavor to ghee (Ganguli and Jain, 1972).

As a human food, ghee has been considered immensely superior to other fats mainly because of the presence of characteristic short chain fatty acids, which are responsible for its better digestibility and anti-cancer property. Ghee performs a major and essential function as carrier of four fat-soluble vitamins viz., A, D, E, K and essential fatty-acids such as linolenic acid and arachidonic acid. Ghee is believed to be a coolant, capable of increasing mental power and physical appearance, and curative of ulcers and eye-diseases (Rangappa and Achaya, 1974).

In order to ensure a genuine product to the consumer, The Government of India has prescribed the compositional standards for ghee, under PFA act (2009) and Agmark rules (1981). However, unfortunately, the producers are the middle-men involved in the ghee trade, in their greed to have more money, tend to adulterate ghee with cheaper oils & fats like vegetable oils, animal body fats, hydrogenated fats, and sometimes even the nonedible mineral oils, especially during lean season. In recent years, the problem of adulteration has assumed a very serious dimension. Several reports have appeared in the newspapers indicating that rampant malpractices of ghee adulteration are going on particularly in the central & northern parts of the country. By adding animal body fats in ghee, the sacred holy food, the unscrupulous ghee traders are not only robbing the people of their money, but also playing with the religious sentiments, especially of the vegetarian section of the society, besides adversely affecting their health. It is not known as to what extent these types of malpractices of adulteration are prevailing in the ghee trade in our country and what quality of

ghee is available to the consumers. Therefore, the present project was undertaken to carry out a survey of collecting ghee samples from different parts of the country in different seasons and analyze them for physico-chemical parameters as laid down under legal and quality standards (Reichert-Meissl value, Polenske value, BR reading, Free Fatty Acids, Baudouin test, etc.) and also analyze them for quality tests like Crystallization time test, Apparent solidification time test (AST), Complete liquefaction time test (CLT), besides looking for thin layer chromatographic(TLC) profile of the unsaponifiable matter isolated from the ghee samples with a view to ascertain the authenticity of milk-fat.

Keeping the above things in mind, a systematic study was planned to be undertaken with the following objectives:

1) To study the physico-chemical attributes of market ghee samples collected from different regions of the country in different seasons.
2) To compare the purity of market ghee samples with laboratory made pure ghee.

CHAPTER - 2

Review of Literature

2.0 REVIEW OF LITERATURE

Lipids form one of the most important constituents of milk and milk products. Major part of milk lipids consists of triglycerides (generally called fats). Minor components of milk lipids include partial glycerides (mono- and di-glycerides), phospholipids, fat soluble vitamins, cholesterol, squalene, waxes, etc. In India, milk fat is mostly consumed in the form of ghee (clarified butterfat). However, due to its short supply, particularly in the lean (summer) season and comparatively more demand, expensiveness (costing 3 to 4 times as much as edible vegetable oils) and variable chemical composition, ghee is prone to adulteration by the unscrupulous traders in the market. The commonly used adulterants are vegetable oils and fats, animal body fats, mineral oils, starchy material, etc. Extensive survey of the literature reveals that several methods have been developed to detect the adulteration in ghee. These methods were mostly based on chemical parameters like fatty acid composition and the physico-chemical constants.

This review delineates the current status of our knowledge with regard to the present methods of manufacturing ghee and also the various methods commonly used for the detection of adulteration of milk fat with foreign fats. The literature is reviewed under the following four major heads:

1) Methods of ghee manufacture
2) Regional preference for ghee flavor
3) Methods to detect adulteration of ghee/milk fat
4) Market survey on ghee

2.1 METHODS OF GHEE MANUFACTURE

In general, ghee is prepared by following four methods namely Desi method, Creamery butter method, Direct cream method, Pre-stratification method (Ganguli and Jain, 1972),

2.1.1 Desi method

This method consists of churning cultured whole milk (dahi) with an indigenous corrugated wooden beater, separating the butter and clarifying it into ghee by direct open pan heating. Earthenware vessel was used earlier to boil milk and ferment it with a typical culture to convert it to dahi which in turn churned to separate the butter (Ganguli and Jain, 1972). The lot of makkhan (butter), fresh or accumulated over few days, is usually taken in a suitable open mud-pot or metallic vessel, heated and stirred over low fire to remove moisture. When practically all the moisture has been removed such a stage judged by experience. On cooling, when the residue has settled down, the clear fat is decanted into suitable container (De, 1994).

2.1.2 Creamery butter method

This is the standard method adopted in almost all organized dairies in India (De, 1994). The butter is heated in an improved ghee boiler, which consists of a stainless steel jacketed pan provided with stirrer, steam control valve, pressure and temperature indicator, etc., The solid mass of butter is heated under low steam pressure to melt the butter in completely liquid form. Later, the steam pressure in the jacket is raised so that liquid mass starts boiling with removal of moisture from the pan-contents at temperature above 90 ^{o}C. The final temperature of heating or clarification usually ranges from 110 to 120 ^{o}C, depending upon the region like in southern India ghee is clarified at 120 to 140 ^{o}C (Ganguli and Jain, 1972). The end point of clarification is indicated by the appearance of effervescence, which is much finer together with a browning of curd particle (Rangappa and Achaya, 1971). After cooling pan-contents to 70 ^{o}C, ghee is passed through seven pad ghee press filter, which consists alternatively of perforated stainless steel and nylon filter, followed by clarifier to remove fine ghee residue. After that ghee is cooled to 40 to 45 ^{o}C and packed in different sizes by Prepac machine.

2.1.3 Direct cream method

In this method, the cream is usually obtained by normal separation of milk at 40 to 45 ^{o}C. This cream is heated in the same ghee boiler described for the creamery-butter method. The procedure for heating and moisture removal, final temperature of clarification, removal of ghee residue and packaging also remain the same as for creamery-butter method. One of the important drawbacks of this method is a lower percentage recovery of ghee as compared to creamery-butter method (Rangappa and Achaya, 1971). This is due to much more solid-not-fat (SNF) content of cream (4.5 to 5.5%) than desi or white butter (1.0 to 1.5%). This higher SNF content in cream contribute to a large ghee-residue, which in turn cause greater fat loss in the same (De, 1996). This problem could be resolved by 'cream washing' process and this washed cream contain 1.0 to 2.0 % SNF content (De, 1996). It has been reported that direct cream method is most economical for preparing ghee and the product has better keeping quality (Ganguli and Jain, 1972).

2.1.4 Pre-stratification method

The principle of preparation of ghee by this method is that when butter is left undisturbed at temperature of 80 to 85 ^{o}C for 15 to 30 minutes, it separates into three distinct layers due to density differences, viz., a top layer of floating denatured particle of curd, a middle layer of fat, and a bottom layer of buttermilk. The bottom layer of buttermilk, containing 60 to 70% SNF and over 80% of moisture originally present in the butter, is discarded and the temperature of remaining two layer (denatured protein and oily portion) is raised to the usual clarification temperature of 110 to 120 ^{o}C (Aneja *et al*., 2002). This method is mostly adopted by organized dairies of country because of its major advantages over other method such as economy in fuel consumption, ghee with low acidity and comparatively longer shelf life (Ganguli and Jain, 1972).

2.2 REGIONAL PREFERENCE FOR GHEE FLAVOR

In India, The preference for flavor of ghee is distinctly different from region to region (Aneja *et al.,* 2002).

2.2.1 NORTHERN REGION

In this region of country, peoples preferred slight acidic to mildly curdy flavor of ghee with fine to medium size grain.

2.2.2 SOUTHERN REGION

In this region of country, peoples preferred mildly to highly cooked and aromatic flavor with medium size grain in Tamil Nadu, and coarse grain in Andhra Pradesh and Karnataka. The ghee from Tamil Nadu is characterized by added flavor from certain herbs and slight butyric flavor. This characteristic imparts pleasant flavor to rice and sambhar when garnished with ghee.

2.2.3 WESTERN REGION

In this region of country, people preferred mildly to strongly curdy flavor with coarse grains of 0.3 to 0.6 mm size.

2.2.4 EASTERN REGION

In this region of country, people preferred slightly to definitely cooked flavor with medium size grain.

2.3 METHODS TO DETECT ADULTERATION OF GHEE/MILK FAT

Various methods of detection of adulteration of ghee are covered under the following four major heads:

1) Method based on physical properties
2) Method based on chemical properties
3) Method based on tracer component of fats and oils
4) Miscellaneous method

2.3.1 METHODS BASED ON PHYSICAL PROPERTIES

Physical properties of oils and fats are important criteria for judging their quality and have also been used to determine their purity. Several methods, which were used to check the purity of ghee on the basis of physical properties, are as follows:

2.3.1.1 MELTING POINT

Melting point (slip point) of various oils and fats covers a wide range and this property has been employed for checking the adulteration of milk fat. Body fats (36-51.3°C) and vanaspati (37.8-38°C) have slightly higher melting point (Winton and Winton, 1999) while vegetable oils (20-30°C) have slightly lower melting point than milk fat (28-41°C) as reviewed by Arun *et al.* (2002). Although the average melting point of vanaspati ghee is reported to fall between 31 and 37°C (Schwitzer, 1956; Sharma and Singhal, 1995), Bolton (1999) reported that the melting point of vanaspati (hydrogenated oils) varies between 30 to 65°C depending upon the extent of hydrogenation. More the hydrogenation, higher is the melting point. Singhal (1973) reported that buffalo milk fat (33.4-34.2°C) has slightly higher melting point than cow milk fat (30.6-31.2°C) and ghee from cotton tract area showed considerably higher melting point (43.0-44.0°C) which resembles with that of animal body fats (43.9-51.0°C). Among the animal body fats, buffalo body fat showed the highest melting point (50.5-51.0°C), while pig body fat showed the lowest (43.9-44.6°C). Addition of animal body fats (buffalo, goat, pig and sheep) at 5 to 20 percent level increased the melting point of pure buffalo or cow ghee. Buffalo body fat caused the largest increase in melting point of ghee (5.7°C increase at 20% level), while pig body fat caused the least increase (2.2°C at 20% level). The study, however, concluded that adulteration up to 20 percent level does not make significant change in the melting point of ghee and, therefore, the method is not found to be useful for the detection of adulteration. Sharma and Singhal (1995) also confirmed these observations using body fats (buffalo, goat and pig) and vanaspati at 5 to 20 percent level of adulteration (irrespective of their mode of addition, either directly to ghee or through milk)

and noted that the increase in melting point was more with body fats than vanaspati.

2.3.1.2 APPARENT SOLIDIFICATION TIME (AST) TEST

The AST test was developed by Kumar *et al.,*(2009) to detect adulteration of milk fat with vegetable oils and animal body fats. The apparent solidification time is determined by taking three grams of melted fat sample in test tube (10.0 X 1.0 internal diameter) to apparently become solidified at 18°C.

Studies conducted by Kumar *et al.,*(2009) on the solidification behavior of various oils and fats including milk fat in terms of AST at the selected temperature (18°C) have revealed that the average AST values for buffalo and cow milk fat were 2 min 40 sec and 3 min 10 sec, respectively. The average AST values of pig body fat, goat body fat and vanaspati were 1 min 30 sec, 40 sec and 1 min 50 sec, respectively. On the other hand, all the vegetable oils studied remained liquid for an indefinite period. Addition of vegetable oils caused an increase in the AST values of buffalo and cow pure milk fat, whereas the addition of body fats and vanaspati (hydrogenated vegetable oils) resulted in the decrease in the AST values of buffalo and cow pure milk fat, depending on the amount of adulterant oils and fats added. Taking into account the overall range of AST values at 18°C pertaining to fresh, stored and seasonal samples of both buffalo and cow pure milk fat as the criteria for the detection of adulteration, it was found that the technique could detect the addition of individual vegetable oils at all the levels in case of cow milk fat but not in buffalo milk fat. Adulteration of buffalo milk fat with vanaspati (hydrogenated vegetable fat) was detectable at levels ≥10%, but not in cow milk fat. Addition of goat body fat to both cow and buffalo milk fat was detectable at levels ≥ 10%, whereas pig body fat was detectable only in buffalo milk fat at levels ≥ 10%.

2.3.1.3 COMPLETE LIQUIFICATION TIME (CLT) TEST

This test was developed by Amit Kumar (2008) to detect adulteration of milk fat with foreign fats and oils. The complete liquefaction time (CLT) of the

fat samples was recorded by observing the time taken by the solidified fat samples to get melted completely at a 44 ^{o}C.

At 44^{o}C, the CLT values of pure cow ghee samples ranged from 2 min 12 sec to 3 min 15 sec with the mean of 2 min 52 sec, while that of pure buffalo ghee ranged from 2 min 35 sec to 3 min 15 sec with the mean of 2 min 57 sec. There were large variation between CLT values of ghee samples collected over a period of whole year both for cows and buffaloes. Further, it was observed that both (cow and buffalo) type of ghee samples showed higher CLT values in the summer months (May to September) and lower CLT values in the winter months (November to March).

Addition of vegetable oils (palm, rice bran and soybean) caused a decrease, whereas addition of body fats (buffalo, goat and pig) resulted in an increase in the CLT values of cow and buffalo pure ghee at 44°C. This decrease or increase observed in CLT values caused by the addition of adulterant oils/ fats to ghee depended upon the amount of adulterants added. Higher the quantity of adulterant added, greater was the effect. However, in case of ghee samples containing body fats, samples with pig body fat showed slightly lower increase in CLT values than the samples containing buffalo and goat body fats.

Taking into account the overall range of CLT values at 44^{o}C, i.e. from 2 min 12 sec to 3 min 15 sec for pure ghee (cow and buffalo) as the criteria for the detection of adulteration, a perusal of the results on CLT values of adulterated ghee samples revealed that addition of vegetable oils individually at the levels of 5, 10 and 15 percent to either cow ghee or buffalo ghee was not detectable. However, among animal body fats, buffalo and goat body fats in either of the ghee were detectable at 10% level while pig body fat was detected only at higher (15%) level of adulteration.

2.3.1.4 CRYSTALLIZATION TIME TEST

Detection of added animal body fats in milk fats has always been a challenging task for dairy professionals. In the past, some physical methods, which were based on the partial solidification behaviour of milk fat such as opacity test (Singhal, 1980), crystallization test (Panda and Bindal, 1998 b) and apparent solidification time (AST) test (Arun Kumar, 2003), were developed and applied to detect the added animal body fats in milk fats (ghee). However, each of these methods has its own limitations. Among these tests, crystallization test was considered to be an excellent method to detect animal body fats. The Crystallization time test is defined as time taken for the onset of crystallization of milk fat at 17 ^{o}C when dissolved in a solvent mixture consisting of Acetone and Benzene (3.5:1.0).

Amit Kumar (2008) applied this test on samples of both cow and buffalo pure ghee pertaining to whole of the year collected on bimonthly basis, as well as the ghee samples added with adulterants (animal body fats and vegetable oils) individually at 5, 10 and 15% levels. Crystallization time for the pure cow ghee and pure buffalo ghee samples ranged from 6 min 50 sec to 16 min 20 sec and from 6 min 30 sec to 12 min 30 sec with the mean of 11 min 4 sec and 8 min 42 sec, respectively. The crystallization time of ghee samples increased when the samples were adulterated with vegetable oils (palm, rice bran and soybean) while it was found to decrease for ghee samples adulterated with animal (buffalo and goat) body fats. The extent of increase and decrease was dependent up on the level of adulteration with vegetable oils and animal body fats, respectively. Higher the level of adulterant oils/fats, greater was the effect. However, ghee samples adulterated with pig body fat, initially showed a decrease in crystallization time at 5% level of adulteration, which subsequently showed an increase as the level of adulteration increased. At 15% level of pig body fat adulteration, the values of crystallization time became closer to that of corresponding average values for pure ghee samples. The same trend was observed for both type of ghee (cow and buffalo).

The crystallization time was highest in the month of May while it was lowest in July. Generally, crystallization time is expected to be higher if the fat has more unsaturated fatty acid, i.e. crystallization time will be increased with the increase in BR reading and iodine value of a fat.

Amit Kumar (2008) concluded that the crystallization time test is a useful tool to detect addition of animal body fats particularly, buffalo and goat body fat to milk fat at 5% level. However, pig body fat could not be detected by this test when added to milk fat at any of the levels studied.

2.3.1.5 SOLIDIFICATION POINT

Solidifying point is defined as the temperature at which fat shows first sign of appearance of solid phase on cooling. Solidification temperature of milk fat depends very much on the procedure employed for cooling (Webb *et al.*, 1987). Rahn and Sharp (1928) reported solidification point of 19.7 and 23.6°C for samples of the same milk fat cooled by immersion at 14 and 20°C, respectively. The solidifying point of buffalo ghee (16.0-28.0°C) is reported (Rangappa and Achaya, 1974) to be slightly higher than cow ghee (15.0-23.5°C). The solidifying points for beef tallow and lard vary between 32 to 37°C and 25 to 30°C, respectively. Whereas, most of the vegetable oils, such as, sunflower oil, maize oil, soya oil, groundnut oil, cottonseed oil have very low solidification point in the range of –16 to 5°C, with the exception of plam oil (22-40°C), palm kernel oil (24-26.5°C) and coconut oil (22-23.5°C) whose values are close to that of animal fats reported above.

2.3.1.6 TITRE VALUE

The titre of ghee represents the highest temperature reached when the liberated water insoluble fatty acids are crystallized under arbitrarily controlled conditions. The titre is generally taken to represent the solidification point of the fatty acids, although they actually solidify over a range of temperature. For its determination, ghee (oils and fats) is saponified with glycerol-potassium hydroxide solution. The resulting soap is decomposed with sulphuric acid and

the liberated water-insoluble fatty acids are separated, washed free from mineral acid and dried. Titre is then determined on these fatty acids (BIS, 1981). Titre value of butterfat lies between 33 to 38°C (Hamilton and Rossell, 1986). Animal body fats such as beef tallow, lard and mutton tallow generally have a higher range (32-48°C). On the other hand, vegetable oils such as sunflower oil, safflower oil, soybean oil, groundnut oil, plam kernel oil, etc. generally have lower titre values (15-32°C), except cottonseed oil (30-37°C) and palm oil (40-47°C). Doctor *et al.,* (1940) reported the titre value of ghee as 33.5°C, whereas a survey conducted by Directorate of Marketing and Inspection, Govt. of India reported the titre value of ghee samples from cotton tract areas varying from 40.4 to 44.6°C (Singhal, 1973). At one time, the titre value was used for judging the quality of ghee produced in cotton tract areas, but later dispensed with as it was considered to be of not much use. Moreover, its use is limited due to the introduction of more modern instrumental methods of analysis.

2.3.1.7 DENSITY AND SPECIFIC GRAVITY

Density or specific gravity of the fatty oils have long been used in connection with the analysis and identification of oils. The Density of fats in their liquid form are commonly expressed as specific gravity rather than in terms of absolute density. The specific gravity of oils and fats depends upon their chemical composition and the temperature (Karleskind, 1996) at which density or specific gravity is measured.

Specific gravity values for cow ghee and buffalo ghee at 30°C are reported to be 0.9358 to 0.9443 and 0.9340 to 0.9444, respectively (Rangappa and Achaya, 1974). Walstra and Jenness (1984) reported that the liquid milk fat at 20°C in general, has a density of about 0.915 g/ml. Most of the edible vegetable oils and fats have a density in the range of 0.910 to 0.927 at 20°C. Animal body fats have slightly lower density values ranging from 0.894 to 0.906 at 20°C (Martin, 1979). With a view to detect adulteration, Singhal (1973) studied the density and specific gravity of ghee (including

cotton tract) and animal body fats (buffalo, goat, pig and sheep) at 40°C. Based on the narrow differences in the values for different type of oils and fats, it was concluded that detection of adulteration may not be possible using this property.

2.3.1.8 BÖMER VALUE (BV)

Bömer value is defined as the sum of the melting point of saturated triglycerides (isolated by diethyl ether method) and twice the difference between this melting point and that of the fatty acids obtained after the saponification of these triglycerides. This test, originally developed by Bomer in 1913 for the detection of lard in tallow, depends upon the triglyceride structure of the fat (Roos, 1963). The Bömer value of both cow and buffalo ghee ranges from 63 to 64, whereas those of animal body fats, e.g., goat, sheep and buffalo ranges from 68 to 69 and that of pig body fat from 75 to 76. Singhal (1980, 1987) reported that the Bömer value of ghee increased on adulteration with body fats even in the presence of vegetable oils, but not when vegetable oils alone were added. The method could be used as a confirmatory test for the detection of pig body fat in ghee. However, genuine cotton tract ghee, which behaved similar to adulterated ghee samples, could not be sorted out by this test and hence may be mistaken as adulterated ghee. Sharma and Singhal (1996) also successfully applied this test for the detection of body fats (buffalo, goat and pig) and vanaspati added to buffalo ghee at 20 percent level, irrespective of mode of adulteration either directly to ghee or through milk.

2.3.1.9 REFRACTIVE INDEX AND BUTYRO REFRACTOMETER READING

This property which concerns the degree of bending of light waves passing through a liquid or transparent solid is a characteristic for the particular liquid or solid. For oils and fats, it increases with the unsaturation and decreases with rise in temperature. In the case of milk fat, the constant may be determined readily with an Abbe refractometer at 40°C. The instrument is calibrated in butyro-refractometer (BR) readings instead of

absolute refractive indices. The refractive index of milk fat generally, ranges between 1.4538 and 1.4578 (Jenness and Patton, 1969; Walstra and Jenness, 1984). For animal fats at 40°C, it lies in the range of 1.4570 and 1.4630. For most of the vegetable oils, it ranges from 1.4600 to 1.4750 but for some vegetable oils like lauric fats (coconut and palm kernel oils) and palm oil, it lies in the range of 1.4480 to 1.4580 (Hamilton and Rossell, 1986; Karleskind, 1996).

Refractive index and BR readings are inter-convertible (BIS, 1981; Rangappa and Achaya, 1974; Bolton, 1999; Winton and Winton, 1999). The values for B.R. readings of milk fat (40-45) and vegetable oils and fats (above 50) are so wide apart (Singhal, 1980; Gunstone *et al*., 1994) that this property could be safely employed as an index for milk fat adulteration with vegetable oils and fats, except coconut oil (38-39) and palm oil (39-40). Feeding of cottonseed oil raises the BR reading by 5 units in case of ghee (Rangappa and Achaya, 1974). Normally, BR reading or refractive index of oils and fats increases with the increase in unsaturation and also chain length of fatty acids. The BR readings of animal body fats are in the range of 44 to 51 (Singhal, 1980). Adulteration of milk fat with animal body fats (Singhal, 1973; Sharma and Singhal, 1995) and vanaspati (Sharma and Singhal, 1995) at a level of 5 to 20 percent increased its BR readings. Recently, some workers (Arora *et al*., 1996; Lal *et al*., 1998) have developed a simple platform test for the detection of vegetable oil (refined mustard oil) added to milk at a level higher than 10 percent of the original fat on the basis of increase in BR reading of the fat. Arun Kumar (2003) reported that using general limit of BR reading as 40-43, adulteration of vegetable oil up to 5% in cow ghee and 15% in buffalo ghee can be detected. Amit Kumar (2008) concluded from his study that adulteration of milk fat with vegetable oils (except palm oil) added individually at 10 percent level in cow ghee and 15 percent level in buffalo ghee, using the general limit of BR reading as 40 to 43 for Haryana state(PFA, 2009), could be detected. However, adulteration of cow and

buffalo pure ghee with animal body fats could not be detected at any of the levels studied.

2.3.1.10 OPACITY TEST

Singhal (1980) developed an opacity test to detect the adulteration of ghee with animal body fats, based on the time taken by the melted fat sample (5.0 g) in a test tube (8 cm × 1.5 cm) to become opaque (OD>0.5) at 23°C using 590 nm (yellow) filter and observed that the normal ghee took more than 35 minutes, whereas animal body fats (buffalo, goat and sheep) took only 10 to 20 seconds to become opaque. In pure state, both cow and buffalo ghee took almost similar time to become opaque, but adulterated cow ghee took more time than the adulterated buffalo ghee to show a noticeable opacity. The difference in opacity time between pure and adulterated ghee at 5, 10 and 20 percent level, respectively, was 7 to 8, 9 to 10 and 14 to 16 min in case of adulteration with pig body fat; 18 to 20, 26 to 29 and 33 to 34 min in case of adulteration with goat body fat; and 22 to 26, 31 to 32 and 35 to 36 min in case of adulteration with buffalo body fat. He concluded that the adulteration with buffalo, goat and sheep body fats at 5 percent level and above could be safely detected by opacity test. He further recommended that if the sample exhibits opacity within 20 min, it is suspected to be adulterated with animal body fats particularly; buffalo, goat or sheep body fats. However, the limitations of this test are that the detection of pig body fat up to 10 percent level is difficult and ghee from cotton tract area also cannot be distinguished. Test also fails to detect the body fats in ghee in the presence of vegetable oils (Singhal, 1987).

In a modified procedure, Panda and Bindal (1998a) also studied the opacity profile of pure ghee and adulterants and recorded the opacity time as the time required by a fat sample at 23°C to acquire the O.D. in the range of 0.14 to 0.16 and consequent transmittance of 68 to 72. They reported that the opacity time of pure ghee (14-15 min) was much higher than that of ghee adulterated with animal body fats (2-9 min at 10% level and 3-11 min at 5% level of adulteration) and much lower than that of ghee adulterated with

vegetable oils (21-25 min at 10% level and 19-21 min at 5% level of adulteration). They also successfully employed the opacity test to detect the presence of vegetable oils and animal body fats when added directly to milk. These workers also observed the limitations of their test that the cotton tract area ghee (opacity time; 11-12 min) resembled with ghee adulterated with animal body fat such as pig body fat at 5 percent level (10-11 min), as reported earlier by Singhal (1973).

2.3.1.11 CRITICAL TEMPERATURE OF DISSOLUTION (CTD)

Critical temperature of dissolution (temperature at which turbidity appears on gradual cooling of the fat dissolved in a warm solvent or solvent mixture) is a characteristic of a particular fat which depends upon the nature of the solvent, nature and amount of most insoluble glycerides (usually tri-saturated glycerides) present in a fat as well as the mutual solubilizing power exerted on these glycerides by the soluble glycerides (Rangappa and Achaya, 1974; Boghra *et al.*, 1981). Bhide and Kane (1952) observed the CTD values for ghee and vanaspati in the range of 39 to 45°C and 62 to 72°C, respectively, employing a 2:1 (v/v) mixture of 95 percent ethanol and iso-amyl alcohol, and reported that gross adulteration of ghee with vanaspati could easily be detected. Delforno (1964) reported the CTD values for butter as 50.5 to 57.5°C (average 54°C), for coconut oil as 28 to 41°C (average 33°C) and for other oils and fats as 67 to 82.5°C (average 71-78°C). Similarly, the presence of body fats in ghee was detected by employing either a single solvent such as absolute alcohol (Delforno, 1964) or a solvent mixture of 95 percent ethyl alcohol and iso-amyl alcohol in the ratio of 2:1 (Bhide and Kane, 1952). Likewise, CTD was used for the detection of adulteration of ghee with mineral oils by Kane and Ranadive (1951) using aniline as solvent. The CTD test seems to be simple, but its efficacy is greatly affected by the free acidity (FFA) and rancidity (peroxides). Arun Kumar (2003), using solvent mixture (ethyl alcohol and iso-amyl alcohol, 2:1), reported that adulteration of ghee up to 15% level with vegetable oils, vanaspati and body fats could not be detected by CTD value.

2.3.1.12 FRACTIONATION OF MILK FAT

Fractionation is a thermally controlled process (with or without solvent) in which the milk fat is subjected to a specific temperature/time profile to allow a portion of milk fat to crystallize. Fractionation of fat with or without the use of solvent under suitable conditions of time and temperature combinations, followed by examination of fractions thus obtained has been exploited by some workers as a tool to detect foreign fats in milk fat. Different solvents that have been used for fractionation purpose include ethyl alcohol, acetone, hexane, isopropylalcohol and 2-nitropropane. Bhalerao and Kummerow (1956 a & b) separated the fat into solid (30%) and liquid (70%) fractions after dissolving it in the hot absolute alcohol and maintaining the same at 20°C for 2 hours. The acetone soluble fraction was iodinated and subsequently subjected to refractive index measurement. Using this method, the presence of foreign fats at 10 percent level could be detected.

Arun Kumar (2003) employed dry fractionation approach and applied AST test to detect adulteration of ghee with vegetable oil and animal body fats. The average AST value of solid fraction (S_{20}), solid fraction (S_{18}), liquid fraction (L_{18}) of pure buffalo ghee were 1 min 58 sec, 2 min 47 sec and 3 min 10 sec, and those for cow ghee were 2 min 30 sec, 3 min 21 sec and 3 min 31 sec respectively. Taking overall range of AST values of the S_{20}, S_{18} and L_{18} fraction of both pure buffalo ghee and cow ghee at 18 ^{o}C, as the basis for the detection adulteration in milk fat, the study revealed that fractionation technique could extend further help in detecting adulteration of only cow ghee especially with the mixture of goat body fat with individual vegetable oils or vanaspati even at 5 percent level, which otherwise not possible in the unfractionated ghee samples, thereby helping in increasing the sensitivity of detection of adulteration in case of cow ghee only.

Amit Kumar(2008) employed solvent fractionation approach using acetone as a solvent to get different fractions. Three temperatures (16, 8 and 4^{o}C) were selected for successive fractionation. The solid fractions obtained at 16 and 8^{o}C were named as S_{16} and S_{8}, respectively, while solid and liquid

fractions obtained at 4^{o}C were named as S_4 and L_4, respectively. On fractionation, animal body fats got concentrated in the first fraction (S_{16}) whereas vegetable oils got concentrated in the last fraction (L_4). Hence, first fraction was analyzed for complete liquification time (CLT) test performed at 44 and 46^{o}C, and last fraction was analyzed for BR reading and iodine value. Results of CLT test at 44^{o}C done for first fraction (S_{16}) of cow and buffalo pure ghee showed that the temperature of 44^{o}C is not suitable for application of CLT test on first fractions because it has failed in case of pure cow ghee as fraction of pure cow ghee obtained in the months of summer season (May to September) did not melt completely and some opacity remained throughout in the body of the fat fraction. CLT of solid fractions (S_{16}) of pure cow ghee and pure buffalo ghee at 46^{o}C ranged from 4 min 5 sec to 9 min and from 5 min 10 sec to 7 min 15 sec, respectively. Taking into account the overall range of CLT at 46^{o}C i.e. from 4 min 5 sec to 9 min for both cow and buffalo pure ghee and also considering the melting behaviour of samples at this temperature, it was observed that buffalo body fat adulteration along with either of the vegetable oils could be detected even at 5% level in case of cow ghee, while it was detected only at 15% level in buffalo ghee. Goat body fat along with either of the vegetable oils was detected at 10% level of adulteration in cow ghee, while it was detected only at 15% level of adulteration in buffalo ghee. However, pig body fat was detected at 15% level of adulteration with either of the vegetable oils in both cow and buffalo ghee.

2.3.1.13 SPECTROSCOPIC METHODS

Spectroscopic methods using visible (400-800 mμ), ultraviolet (200-400 mμ) and infrared (2-15 μ) regions have been used by many workers for characterization of fats and oils.

2.3.1.13.1 Tests Based on Visible Spectroscopy

Jha (1981) applied this technique for the detection of Cheuri (*Madhuca butyracea*) fat in ghee, a common adulterant in Nepal. Pure ghee showed no absorption band in visible range (600-700 nm), whereas Cheuri fat showed an

absorption band with maxima between 640 and 680 nm. Even 5 percent Cheuri fat added to ghee could be detected in this range.

2.3.1.13.2 Tests Based on Ultraviolet (UV) Spectroscopy

Ultraviolet (UV) spectroscopy has been applied for characterizing the poly-unsaturated fatty acids (PUFA) content of various oils and fats including milk fat and also for the detection of butterfat adulteration with foreign fats. Sharma (1989) examined UV spectrum of unsaponifiable matter extracted from ghee and animal body fats between 200 to 320 nm and observed first absorption maxima between 215 to 220 nm for both fats. Whereas, ghee sample showed a second maxima at 270 nm, which was shifted to 280 nm in case of animal body fats. However, on this basis, adulterated ghee could not be differentiated from pure ghee (Sharma, 1989; Arun Kumar, 2003).

2.3.1.13.3 Tests Based on Infra-Red (IR) Spectroscopy

The infra-red (IR) absorption has been extensively used in the analysis of lipids especially for cis- and trans- isomers. Unsaturated fatty acids of natural vegetable oils and fats are in cis- configuration and are isolated (non-conjugated). Partial hydrogenation or oxidation may result into formation of trans-isomers. Animal and marine fats may also contain small amounts of natural trans-isomers (Akoh and Min, 1998; Kirk and Sawyer, 1999). Bovine milk fat contains a low level (5%) of trans fatty acids in comparison with hydrogenated vegetable oils, in which the value may be as high as 50 percent due to non-stereospecific hydrogenation (Fox and McSweeney, 1998). For demonstrating the presence of hydrogenated fats in milk fat, some workers (Bartlett and Chapman, 1961; Firestone and Villadelmar, 1961) applied IR spectrophotometry and observed that the absorption maxima at 10.36 µ gets increased by the addition of hydrogenated fats containing iso-oleic acids (trans-octadecenoic acids).

Konevets *et al.,* (1987) studied the cis-trans configurations of individual fats (milk fat, animal body fat, vegetable fat and hydrogenated fat) and their mixtures using IR spectroscopy, and reported that the additions up to 10

percent of animal, vegetable and hydrogenated fats to milk fat could be detected. Sato *et al.,* (1990) used near IR spectroscopic method for the detection of as little as 3 percent foreign fat in milk fat. Sharma (1989) scanned the IR spectra of cow ghee, buffalo ghee, animal body fats (Buffalo, goat, sheep and pig) and ghee adulterated with body fats in the 4,000 to 600 cm^{-1} region and observed distinct differences between body fats and ghee in the region of 1300 to 1180 cm^{-1} and 1120 to 1100 cm^{-1}, respectively. Body fats showed the presence of 5 to 6 bands, while ghee showed only two bands. Ghee samples adulterated with body fats also showed 3 to 6 extra bands. Unsaponifiable matter extracted from the ghee, body fats and adulterated ghee samples also exhibited the similar pattern of bands as reported above for the whole fat. The above author concluded that the differences in IR spectrum of ghee and body fats could be used to detect ghee adulteration with body fats at 10 percent level. Recently, Arun Kumar (2003) reported that on the basis of increased level of trans isomers in ghee, as low as 5% of vanaspati added to ghee could be detected.

2.3.1.14 DILATATION BEHAVIOUR

This property is based on the thermal expansion behaviour of milk fat. Using this property, Kalsi (1984) reported different solid and liquid fractions of milk fat from different species in the temperature range of 10 to 80°C and observed that solid and liquid fractions in equal proportions are obtained at 33, 30 and 24.5°C in case of buffalo, cow and goat milk fats, respectively. Arun Kumar (2003) applied this property for detection of adulteration in ghee. He studied the proportion of solid and liquid fractions of pure and adulterated ghee at 5, 10 and 15% level and observed that the ratio of solid to liquid fraction for pure cow and buffalo ghee was 2.37 and 3.10 respectively. On the basis of solid/liquid ratio, it was found that vegetable oils could be detected in cow ghee while body fats and vanaspati could be detected in buffalo ghee even at 5% level.

2.3.1.15 MICROSCOPIC EXAMINATION OF FAT

Microscopic examination of the sterol crystals (Den Herder, 1955; IDF, 1965; BIS, 1981) has also been employed in the detection of adulteration of milk fat with the foreign fats especially vegetable fats. If the sterol crystals only show the form of a parallelogram with an obtuse angle of 100°, which is characteristic for cholesterol, the fat sample is considered to be free from vegetable fat. However, if the sterol crystals show the elongated hexagonal form with an apical angle of 108°, which is characteristic for phytosterols, or if some of the sterol crystals have a re-entry angle (Swallow's tail), which is characteristic for mixtures of cholesterol and phytosterols, the fat sample is considered to contain vegetable fat. Using this parameter, Arun Kumar (2003) reported that adulteration of ghee samples with 15% groundnut oil could be confirmed.

2.3.1.16 DIFFERENTIAL THERMAL ANALYSIS (DTA) AND DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DTA and DSC are both closely related thermo-analytical techniques which measure the physical properties such as phase transition and specific heats of foods as a function of temperature. DTA measures the difference in temperature (Δt) between a sample and an inert reference material as a function of temperature. In DSC thermograms, the area delineated by the output curve is directly proportional to the total amount of energy transferred in or out of the sample.

DTA and DSC have been used by different investigators for the detection of foreign fats in milk fat. Antila *et al.,* (1965) detected 5 percent coconut fat, cocoa fat and hardened vegetable fat in butterfat by DSC based on the differences in the shape of melting curves. However, tallow, lard and vegetable oil added at 5 percent level in butterfat could not be detected by this method. Roos and Tuinstra (1969) using DTA showed that addition of 5 to 10 percent of beef tallow in butterfat changed the solidification curve as solidification started earlier and showed two distinct minima in the curve. Lambelet *et al.,* (1980) detected goat body fat (more than 10%) in ghee by

DTA technique on the basis of differences in melting diagram and crystallization patterns of goat body fat and ghee. Using DSC, detection of foreign fats like pig and buffalo body fats (Lambelet and Ganguly, 1983) beef suet (Amelotti *et al.*, 1983) and chicken fat (Coni *et al.*, 1994) in milk fat was reported. The method, however, failed to detect coconut oil, cotton tract ghee and other animal body fats.

2.3.2 METHODS BASED ON CHEMICAL PROPERTIES

Certain well-known physical and chemical constants have been derived for the purpose of characterization of oils and fats. Among those constants three determinations, the Reichert-Meissl value, the Polenske value and the Iodine value, measure certain specific constituents of milk fats while other two, the Saponification value and Butyro-refractometer reading, give an overall average nature of the constituent fatty acids present (Rangappa and Achaya, 1974). These physico-chemical constants are described briefly in the following sections:

2.3.2.1 Reichert-Meissl (RM) value

RM value is the number of milliliter of 0.1 N alkali solution required to neutralize the steam volatile, water soluble fatty acids distilled from 5 g of fat under specified conditions. This constant for milk fat is quite significant since it is primarily a measure of butyric ($C_{4:0}$) and caproic ($C_{6:0}$) acid. RM value for milk fat ranges from 17 to 35, which is well above the value (generally 1) for all other fats and oils except coconut oil and palm kernel oil for which the value ranges between 4 to 8 (Singhal, 1973 & 1980). Feeding of cottonseed to milch animals lowers the RM value of ghee by 5 to 6 units (Rangappa and Achaya, 1974). The study conducted by Amit Kumar (2008) revealed that adulteration of pure cow ghee with animal body fats (buffalo body fat, goat body fat and goat body fat) and all vegetable oils (palm oil, rice bran oil and soybean oil) individually at 10 percent level, whereas in case of pure buffalo ghee at 15 percent level of adulteration could be detected using RM value as a base.

2.3.2.2 Polenske value

Polenske value denotes the number of millilitre of 0.1 N alkali solution required to neutralize the steam volatile and water insoluble fatty acids distilled from 5 g of fat under specified conditions. This value is substantially a measure of caprylic ($C_{8:0}$) and capric ($C_{10:0}$) acid. The Polenske value for milk fat ranges from 1.2 to 2.4. This value for other oils and fats (Singhal, 1980; Winton and Winton, 1999) is also low (less than 1) except the coconut oil (15-20) and palm kernel oil (6-12). Feeding of cottonseeds to milch animals reduces the Polenske value of ghee by 0.3 to 0.7 units (Rangappa and Achaya, 1974). Amit Kumar (2008) revealed that adulteration of cow and buffalo pure ghee with any of the adulterants either animal body fats or vegetable oils could not be detected at any of the levels studied, except adulteration of buffalo ghee with the palm and soybean oils which could be detected at 15 percent level.

2.3.2.3 Iodine value

Number of grams of iodine absorbed by 100 g of fat under specified conditions represents the iodine value. This constant is a measure of unsaturated linkages present in a fat. The iodine value for milk fat ranges from 26 to 35, which is low in comparison to most of the other fats and oils (Singhal, 1973). Animal body fats show slightly higher iodine value ranging from 36 to 49. Whereas, for vegetable oils, the value is very high (74-145) except coconut oil (6-10) and palm kernel oil (10-18). For hydrogenated fats, it lies in the range of 70 to 79. Feeding of cottonseed raises the iodine value of ghee up to 10 units (Rangappa and Achaya, 1974). Amit Kumar (2008) reported that iodine value can be a useful parameter in detecting adulteration of cow ghee as well as buffalo ghee with various vegetable oils (except palm oil) at the level of more than 10 percent.

2.3.2.4 Saponification value

Saponification value which denotes the number of milligrams of KOH required to saponify one gram of fat gives an indication of average molecular weight of fatty acids present. For milk fat, animal body fats, vegetable oils and hydrogenated fats, the value ranges from 210 to 233, 192 to 203, 170 to 197 and 197 to 199, respectively. Coconut oil and palm kernel oil show higher saponification value ranging from 243 to 262 (Jenness and Patton, 1969; Singhal, 1980). Feeding of cottonseeds to milch animals lowers this value by 7 units (Rangappa and Achaya, 1974).

Amit Kumar (2008) concluded from his study that based on saponification value, adulteration of pure cow ghee with either animal body fats or vegetable oils at 10 percent levels of adulteration could be detected except buffalo body fat which could be detected even at 5 percent level of adulteration. Whereas, in case of buffalo ghee, only 15 percent level of adulteration either with animal body fats (except pig body fat) or with vegetable oils (except palm oil) could be detected. However, when the data on cow and buffalo ghee was pooled, the study revealed that adulterants (body fats and vegetable oils) studied could be detected at 10 and 15 percent level of adulteration, except palm oil and pig body fats which could be detected only at 15 percent level of adulteration.

2.3.2.5. Tests Based on Gas Liquid Chromatography (GLC) of FAs

The technique of GLC, which utilizes retention time, a characteristic of a particular component under specified conditions, gives fatty acid profile of oils and fats obtained following the conversion of the triglycerides into more volatile methyl esters of their component fatty acids. Milk fat derived from ruminant animals, contains an exceptional number and variety of fatty acids from 4:0 to 26:0 (saturated) and from 10:1 to 22:5 (unsaturated). Body fats like tallow and lard contain mostly palmitic (16:0), stearic (18:0) and oleic acid (18:1), while vegetable oils consist mainly of palmitic, stearic, oleic and linoleic (18:2) acids. Coconut oil is the best known exception, containing lauric (14:0) and myristic (16:0) acids in very large amount (Rangappa and Achaya, 1974).

The GLC technique was employed by several workers for the detection of milk fat adulteration through determining the ratios of different fatty acids. Some workers (Wolff, 1960; Francesco and Avancini, 1961; Boniforti, 1962) employed GLC technique and reported that the milk fat sample with a ratio of $C_{12:0}$ / $C_{10:0}$ fatty acids >1.6 or $C_{4:0}$/$C_{6:0}$+$C_{8:0}$ fatty acids >1.8 was considered to be adulterated with margarine, coconut oil or tallow or pig body fat trans-esterified with butyric acid, while other workers (Provvedi and Cialella, 1961) suggested ratios of other fatty acids such as $C_{4:0}$ / $C_{12:0}$ > 3, $C_{18:1}$ / $C_{18:0}$ > 2 and $C_{12:0}$ / $C_{10:0}$ >1 for the detection of vegetable oils in butterfat. Similarly, many other workers used the different fatty acids ratios for checking the adulteration of milk fat with vegetable oils, margarine, beef tallow, lard, goat body fat, substituted fats, synthetic fats, etc. (Toppino *et al*., 1982; Ulberth, 1994; Sharma and Singhal, 1996; Panda and Bindal, 1997; Arun Kumar, 2003). Farag *et al*., (1983) determined the fatty acid profile of 3 fractions separated by fractional crystallization from cow and buffalo ghee adulterated with lard and margarine at various levels and reported that the amounts of 16:0, 18:0 and 18:1 acids were significantly changed with different adulteration levels and can be used as a marker to detect the admixture.

2.3.2.6 TESTS BASED ON THE NATURE AND CONTENT OF UNSAPONIFIABLE CONSTITUENTS

The unsaponifiable matter (USM) which can be obtained from oils and fats after saponification with alkali and subsequent extraction by a suitable organic solvent constitutes less than 2 percent by weight of fat. It is a repository of so many valuable constituents, like sterols (cholesterol and phytosterols), fat-soluble vitamin (A, D, E and K), hydrocarbons such as squalene, pigments, etc. Mineral oil, if added to oils and fats, will appear in USM (Kirk and Sawyer, 1999). Milk fat contains USM in the range of 0.30 to 0.45 percent by weight (Jenness and Patton, 1969) chiefly consisting of cholesterol (0.25 to 0.40% by weight of fat). Vegetable oils and animal body fats like lard and tallow have USM in the range of 0 to 2 and 0 to 1.0 percent, respectively (Hamilton and Rossell, 1986).

Sterols and tocopherols are the two most important constituents of USM, which have been used to detect the vegetable fats in milk fat by using various techniques like GLC, TLC, paper chromatography, etc.

2.3.2.6.1 TESTS BASED ON STEROLS

Sterols which represent maximum share of the USM range from 0.24 to 0.5 percent in butterfat, 0.03 to 0.14 percent in body fats and 0.03 to 0.5 percent in vegetable oils (Arun *et al*., 2002). Cholesterol is the characteristic sterol of animal fats, while sterols from vegetable sources consist of a mixture collectively called as phytosterols and include ß-sitosterol, stigmasterol, campesterol, brassicasterol, etc. Low concentration of cholesterol is also reported in the sterol fractions of vegetable oils and fats (Kirk and Sawyer, 1999). In addition to cholesterol, milk fat contains traces of lanosterol, dihydrolanosterol and ß-sitosterol (Webb *et al*., 1987). Vegetable fats contain the sterols mainly in the ester form, while animal body fats contain mostly the free form (Rangappa and Achaya, 1974). Milk fat contains cholesterol in free and ester form in the ratio of 10:1 (Fox, 1995).

The sterols can help to distinguish between fats of animal and vegetable origin, since the melting point of cholesterol acetate (112.76-116.40°C) is substantially lower than that of the acetates of any of the phytosterols (126-137°C). Adulteration of milk fat with vegetable oils is confirmed when melting point of sterol acetate fraction is more than 117°C (IDF, 1965; Rangappa and Achaya, 1974; BIS, 1981).

A circular paper chromatographic method based on the difference in the behaviour of USM isolated from fats in ghee using a solvent mixture of methyl alcohol: petroleum ether: water (80:10:10; v/v) was developed by Ramachandra and Dastur (1959) who reported that the spot of USM of ghee moved as a whole along with the solvent front, while that of ghee adulterated with animal body fats at 5 percent level or vanaspati at 10 percent level did not move at all when observed under UV light or when exposed to iodine vapours.

IDF (1966) recommended a TLC method for the detection of vegetable fats in milk fat based on the appearance of a small band of ß-sitosterol acetate in addition to the major band of cholesterol acetate using reversed phase system consisting of undecane / acetic acid-acetonitrile saturated with undecane. Ramamurthy *et al*., (1967) using thin layers of $CaCO_3$ and soluble starch (10 g + 4 g) impregnated with liquid paraffin and a solvent system consisting of methanol:acetic acid:water (20:5:1; v/v) as a developer reported that the presence of cottonseed oil, groundnut oil, sesame oil and hydrogenated fats at 10 to 13 percent level and coconut oil at 25 percent level in ghee could be detected on the basis of R_f values of 0.53 and 0.44 for cholesterol and phytosterols, respectively. Sharma (1989) carried out TLC of USM of ghee and animal body fats using hexane : ether : glacial acetic acid : ethyl alcohol (25:20:5:1, v/v) as the solvent system and reported that ghee samples adulterated with 10 percent body fats resulted in the appearance of an extra spot due to dihydrocholesterol present in body fats.

Using GLC technique, ß-sitosterol has been shown to be an index of vegetable fat addition (Colombini *et al*., 1978; Homberg and Bielefeld, 1979), however, by this method, addition of body fats cannot be detected as body fats also have cholesterol.

2.3.2.6.2 TESTS BASED ON TOCOPHEROL

Tocopherol, the important constituents of unsaponifiable matter of natural oils and fats which range from 0.002 to 0.005 percent in butterfat, 0.05 to 0.168 percent in vegetable oils except coconut oil which contains only 0.0083 percent and 0.0005 to 0.0029 percent in body fats (Bailey, 1951 and Arun *et al.*, 2002). Thus, tocopherol content of butterfat is low as compared to most vegetable oils and fats. Therefore, addition of vegetable fats to butter will result in a significant increase in tocopherol content of adulterated butterfat. Accordingly, some workers (Mahon and Chapman, 1954; Nazir and Magar, 1959; Markuze-Zofia, 1962; Keeney *et al*., 1971) have reported that vegetable fats and oils added to ghee could be detected on the basis of tocopherol content. However, body fats and coconut oil added to milk fat could not be

detected. Amit Kumar (2008) reported that HPLC analysis of tocopherol isomers showed that all the samples of cow and buffalo pure ghee contain all the three tocopherols studied (α, γ and δ) in appreciable proportion, and hence, analysis of tocopherol isomers did not help in the detection of vegetable oils adulteration in milk fat.

2.3.2.6.3 TESTS BASED ON GAS LIQUID CHROMATOGRAPHY OF TRIGLYCERIDES

Butterfat is composed predominately of triglycerides with 26 to 52 carbon number, while animal depot fats and common vegetable oils have 50 to 54 carbon number. Coconut and palm kernel oils contain short and medium chain length triglycerides with 30 to 52 carbon number, a range almost similar to butterfat (Parodi, 1969; Rangappa and Achaya, 1974)

Using GLC, Kuksis and McCarthy (1964) detected the presence of vegetable fat and lard in butterfat at 5 to 10 percent level based on the increase in the content of high molecular weight triglycerides, $C_{52:0}$ and $C_{54:0}$ peaks, respectively. Guyot (1978) using GLC of commercial butter found that triglycerides were in the order of $C_{36:0} > C_{38:0} > C_{40:0} > C_{42:0} > C_{44:0} > C_{50:0} > C_{52:0}$. Beef tallow and lard triglycerides ranged mainly from $C_{44:0}$ to $C_{54:0}$, with $C_{52:0}$ as main triglyceride. He found the ratio of $C_{52:0}/C_{50:0}$ was less than 1 in pure butter and between 2 & 3-4, in case of beef tallow & lard, respectively. He concluded that the $C_{52:0}/C_{50:0}$ ratio together with $C_{52:0}/C_{38:0}$ ratio gave a valuable indication of the possible addition of tallow or lard to butter. Marjanovic *et al*., (1984) reported that adulteration of the milk fat with margarine at 5 to 10% level could be detected on the basis that margarine had more triglycerides with 48 to 54 acyl carbon atoms than milk fat.

Some workers (Precht, 1990 & 1992; Lipp, 1996 a & b) compared the triacylglycerol composition of different fats as analyzed by GLC and designed a multiple linear regression equation by which foreign fats could be detected with substantially improved sensitivity. However, the method was suitable only when a single foreign fat was added to milk fat. Currently, European Union (EU) applies the method of Precht (1992) for triglyceride analysis as an official

method for evaluating the milk fat purity. Povolo *et al*., (1999) applied the above said official method of EU coupled with the determination of 3,5-cholestadiene content (Mariani *et al.,* 1994) and reported that the detection of beef tallow up to 0.5 to 1.0 percent using 3,5-cholestadiene analysis and up to 2 percent using multivariate statistical techniques could be done.

2.3.3 METHODS BASED ON TRACER COMPONENTS OF FATS AND OILS

Tracer components can be defined as those compounds which are present in adulterant oils and fats, either naturally or by addition, but absent in pure ghee. Addition of some tracer component in the likely adulterant of ghee has been suggested as a rapid and reliable tool to identify them in milk fat. A tracer can be a latent colour which is not detectable visually, but get identified by its colour reaction with certain chemicals or a colouring matter (natural or synthetic) which may impart direct colouration distinct from that of the natural colour of butterfat.

Among tracers, in India, sesame oil is added (5% by weight) to vanaspati according to food laws (PFA, 2009) for its detection in ghee by Baudouin test. The method is based on the development of a permanent crimson colour due to the reaction between furfural and sesamol formed by the hydrolysis of sesamolin (present in sesame oil) in the presence of concentrated HCl. Use of hydrofuramide (Kapur *et al*., 1960) or P-hydroxy benzaldehyde (Sharma, 1989) has also been suggested in place of furfural for this test. Another tracer is tannins which are assumed to be naturally present as impurities in palm oil. Ghee samples adulterated with palm oil give purssian blue colour with potassium ferricyanide and ferric chloride reagent and based on this reaction, Bector and Sharma (2002) have reported that palm oil added to ghee can be detected at the level of 5 percent. However, limitation of this method is that the ghee samples having BHA as antioxidant also give positive test.

Gamma oryzanol, a natural tracer, having antioxidant and cholesterol lowering properties, is found to be present in the rice bran oil. It was revealed to be a mixture of phytosteryl ferulates comprising cycloartenyl ferulate, 24-

methylenecycloartenyl ferulate, and campesteryl ferulate as major components (Xu and Godber, 1999; Chen and Bergman, 2005; Iqbal *et al.*, 2005). Crude rice bran oil contains ≤2 percent (v/v) oryzanol (Norton, 1995). This compound has been indicated as a marker of rice bran oil in other edible oils (Singhal *et al.*, 1997). Apart from rice bran oil, gamma oryzanol is also present as natural component of corn and barley oils (http://www.cncahealth.com/health-notes.htm?ContentID=2850009). On the basis of presence of gamma oryzanol, some methods have been developed recently to detect it in other edible oils. Nasirullah *et al.,* (1992) reported a thin layer chromatographic method while Shukla *et al.,* (2004, 2005) reported a colorimetric method for detection of rice bran oil in other vegetable oils. Amit Kumar employed colorimetric method (2008) and TLC method (2009) for detection of rice bran oil in ghee, based on the gamma oryzanol, could detect rice bran oil even at 2% and 5% level in ghee, respectively.

2.3.4 MISCELLANEOUS METHODS

In this section, methods such as to detect mineral oil and cotton seed oil, and methods based on enzymatic hydrolysis, etc., are described in brief.

2.3.4.1 TEST FOR MINERAL OIL

Adulteration of common edible oils and fats including milk fat with cheaper mineral oils, such as paraffin oil, heavy and light fuel oil, petroleum jelly, etc., has become a widespread phenomenon because of the price difference. Unlike oils and fats, mineral oils are not saponifiable by alkali. This characteristic behaviour of mineral oils has been used as the basis for their detection in edible oils and fats. Using Holde's test, the presence of as little as 0.3 percent of mineral oil in a fat can be detected by saponifying 10 drops of test sample (1 ml) with 5 ml of 0.5 N ethanolic potassium hydroxide solution and adding 5 ml of water to the hot soap solution and noting the appearance of turbidity (Winton and Winton, 1999). Arun *et al.* (2005b) have also reported

the detection of liquid paraffin added to ghee at the rate of 0.5 percent and above using Holde's test as described by Winton and Winton (1999).

2.3.4.2 TESTS BASED ON THE CONTENT OF SPECIFIC FATTY ACIDS

Certain specific fatty acids such as butyric acid, erucic acid, iso-valeric acid, iso-oleic acid, cyclopropenoic acids, etc. which are either characteristic or absent in milk fat or present in less quantity as compared to adulterant fats have been used as an index for the detection of foreign fats in milk fat. Unusual fatty acids like iso-oleic acid, iso-valeric acid, erucic acid, cyclopropenoic acid, etc. are altogether absent in milk fat, but are found in vegetable fats.

Butyric acid is found only in milk fat, but not in adulterant fats. Some workers (Harper and Armstrong, 1954; Eckizen and Deki, 1976) reported that any decrease in butyric acid content of milk fat below 9.6 mole percent would indicate its adulteration with foreign fat. The presence of iso-valeric acid in dolphin oil has been used as the basis for its detection in milk fat by several workers (Tappi and Menziani, 1952; Antoniani and Cerutti, 1954; Parrozzano and Mancinelli, 1954; D'Arrigo, 1955; Bottini and Campanello, 1955; Priori, 1955) using ascending paper chromatography.

2.3.4.2.1 TEST FOR COTTONSEED OIL

Fatty acids containing cyclopropene ring, viz., malvalic ($C_{18:1}$) and sterculic ($C_{19:1}$) acids which are altogether absent in milk fat, but are characteristic of cottonseed oil (Bailey *et al*., 1966; Pandey and Suri, 1982) have been used as a tool by few workers (Shenstone and Vickery, 1961; Christie, 1970) for the detection of cottonseed oil in milk fat and also to distinguish cotton tract ghee from normal ghee using Halphen test or methylene blue reduction test.

2.3.4.2.1.1 HALPHEN TEST

This test is based on the development of a crimson colour due to the reaction between cyclopropenoic acids (constituents of cottonseed oil) and Halphen reagents (1% sulphur solution in CS_2 and equal volume of iso-amyl

alcohol) after incubation for an hour in a boiling bath of saturated sodium chloride solution. This test finds its application for differentiating the cotton tract ghee from normal ghee (Singhal, 1980) as well as for the detection of cottonseed oil in milk fat.

2.3.4.2.1.2 METHYLENE BLUE REDUCTION TEST

Singhal (1980) developed a methylene blue reduction test for the identification of cotton tract ghee and reported that the colour of methylene blue dissolved in chloroform: methanol (1:1) was decolourised by cotton tract ghee or ghee added with cottonseed oil due to the presence of cyclopropenoic acids, while normal pure ghee did not reduce the colour of methylene blue dye.

2.3.4.2.1.3 HYDROXAMIC ACID TEST

It is a colorimetric test which is used to distinguish between butterfat and other vegetable and animal fats (Nelson, 1954) based on the fact that fats derived from milk (cow, goat, sheep, etc.) will form water soluble hydroxamic acid–iron complexes. These complexes appear as a pink to purple colour in water layer. The hydroxamic acid-iron complexes formed from fatty acid esters in vegetable fats except coconut oil and of animal fats are insoluble in water and do not contribute a distinctive pink to purple colour to the water layer. Shipe (1955a & b) modified the above test by separating the water soluble and water insoluble hydroxamates. The soluble fraction containing the complexes of butyric, caproic, caprylic and capric acids is extracted with a butanol-ethanol mixture which removes the caprylic and capric acids and leaves the other two in the water. The relative proportion of these two pairs of acids estimated by comparing the colour intensity in the aqueous phase before and after extraction could be used to distinguish butterfat from other fats.

2.3.4.3 RAPID COLOR BASED VEGETABLE OILS DETECTION TEST

The Bieber's test, hitherto employed for the detection of almond oil adulteration with Kernel oil, was suitably modified by Sharma *et al.* (2005) to detect the adulteration of ghee with vegetable oils. Their results showed the presence of orange brown colour in case of refined vegetable oils and fats, whereas in case of pure ghee samples no colour was observed. By this method, adulteration of ghee with different vegetable oils to the tune of 5-7% could be detected.

2.3.4.4 TESTS BASED ON ENZYMATIC HYDROLYSIS

Lipases (triacylglycerol acyl hydrolases) are the enzymes that catalyze the reversible hydrolysis of triacyl glycerols under natural conditions. Pancreatic lipase which specifically hydrolyzes the primary hydroxyl positions of glycerides can catalyze the complete breakdown of triacylglycerols to free fatty acids and glycerol (Akoh and Min, 1998) involving at least one isomerization step, in addition to the three hydrolytic steps (Coleman, 1963), as follows:

Triglyceride $\rightarrow$ 1,2 Diglyceride $\rightarrow$ 2 Monoglyceride $\leftrightarrows$ 1 Monoglyceride $\rightarrow$ Glycerol

Pancreatic lipase digests some classes of milk triglycerides more rapidly than others (de Man, 1961; Weihe, 1961; Raghuveer and Hammond, 1967; Soliman and Younes, 1986) due to difference in their glyceride structure especially with regard to fatty acids distribution. Further, triglycerides containing unsaturated fatty acids in 1 and 3 positions are hydrolyzed faster as compared to those having saturated fatty acids in these positions (Coleman, 1963). Fats such as buffalo milk fat with higher melting triglycerides and long chain saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) were hydrolyzed slowly by pancreatic lipase as compared to cow milk fat (Ramamurthy and Narayanan, 1974). Also, triglycerides with low melting points greatly contribute to the faster rate of hydrolysis (Lakshminarayana and Ramamurthy, 1986).

It is expected that other fats such as body fats and vegetable fats which have the preponderance of long chain fatty acids will also have different rates of hydrolysis vis-à-vis milk fat. Information on this aspect is obscure. Moreover, it is also not known whether the rate of hydrolysis of fat by lipase can be used for detecting milk fat adulteration.

2.4 MARKET SURVEY ON GHEE:

Recently, Benerjee *et al*. (2002) have reviewed the present status of ghee in India and reported a wide variations in the moisture, fat, curd, free fatty acids (FFA) contents and peroxide value of the ghee samples collected from different markets of India. The moisture content in market ghee varied from 0.02 to 0.79%, fat from 93.5 to 99.9%, curd from 0.51 to 6.5%, free fatty acid from 0.01 to 2.95% (as oleic acid) and peroxide value from 0.2 to 6.0 m.eq O_2 /kg fat. Adulteration of ghee with vanaspati was detected by various workers (Sharma and Zariwala, 1978; Ghatak and Bandyopadhyay, 1989 and Benerjee, 2002) but none of the workers found presence of animal body fats in market ghee samples.

Physico-chemical constants of market ghee have been studied by many workers and they have found wide variation in RM value, Polenske value, Saponification value, Iodine value and BR reading. The RM value of ghee samples collected from different markets varied from 1.0 to 38.50, Polenske value from 0.2 to 3.2, Saponification value from 174.70 to 246.83, Iodine value from 20.30 to 50.70 and BR reading varied from 40.1 to 49.10 (Benerjee *et al*., 2002).

Amit Kumar (2008) collected eleven ghee samples of known brand from different region of country, and analyzed for RM value, Polenske value, BR reading at 40^{o}C, Iodine value, Crystallization time test, and CLT test at 44^{o}C. These samples were also subjected to solvent fractionation, and first solid fractionation (S_{16}) obtained was analyzed for CLT test at 46^{o}C, while liquid fraction (L_4) obtained on fractionation was analyzed for BR reading at 40^{o}C and Iodine value. On the basis of results obtained he concluded that out

of eleven ghee samples collected from the market, nine samples are of pure ghee while two samples (18%) are possibly adulterated with animal body fats.

The above review reveals that ghee is obtained by heat clarification of milk fat and there is no similar product in the country. There are different flavor preferences for ghee in different region of the country. In order to ensure a genuine product to the consumer, the Government of India has prescribed the compositional standards for ghee, under PFA rules (2009) and AGMARK rules (1981). However, unfortunately, the producers or the middle-men involved in the ghee trade, in their greed to have more money, tend to adulterate ghee with cheaper oils and fats like vegetable oils, animal body fats, hydrogenated fats, and sometimes even the inedible mineral oils, especially during lean season. The detection of foreign fats in milk fat is a very complex phenomenon. Although several methods based on the physico-chemical characteristics of oils and fats have been developed to detect the various types of adulterant fats such as animal body fats and vegetable oils in milk fat, but most of the methods are quite tedious, time consuming and have one or the other limitation. Literature also reveals that pig body fat (lard) among the animal body fats, and coconut and palm oils among the vegetable oils, pose lot of difficulties owing to their resemblance with milk fat in many respects. The detection methods available till date are mainly based on the physico-chemical constants, fatty acid profile, sterol analysis, partial solidification behaviour, etc. Market survey on ghee revealed that there was wide variation found in the physico-chemical qualities of market ghee samples from different region of country. In the past, not much research work has been done to know the physico-chemical qualities of ghee from different regions of the country in different seasons, so a systematic work has been planned to study the physico-chemical attributes of market ghee samples collected from different regions of the country in different seasons with a view to find variations in physico-chemical constants of ghee and also to reveal milk fat adulteration with foreign fats.

CHAPTER - 3

Materials and Methods

3.0 MATERIALS AND METHODS

This chapter deals with the materials and methodologies used in the present study on physico-chemical qualities of market ghee.

3.1 CHEMICALS AND REAGENTS

3.1.1 Chemicals:

3.1.1.1 Glacial acetic acid, Oxalic acid, Potassium hydroxide pellets, Sodium hydroxide pellets, Sodium thiosulphate, Hydrochloric acid and Sulphuric acid (AR grade, Qualigens fine chemicals, Mumbai, India), Glycerol (AR grade, Ranbaxy Laboratories Ltd., Punjab, India), Iodine monochloride (Central Drug House Pvt. Ltd., New Delhi, India), Phenolphthalein, Potassium dichromate (LR grade, Glaxo Laboratories Ltd., Mumbai, India), Potassium iodide (AR grade, Sisco Research Laboratories Pvt. Ltd, Mumbai, India), Sodium sulphate anhydrous (LR grade, NICE, Kochi, India).

3.1.1.2 Solvents: Acetone, Benzene (GR grade, Loba chemie, Mumbai, India), Carbon tetrachloride, Diethyl ether, Ethanol absolute, Hexane (s.d. Fine-chem Ltd., Mumbai, India), Ethyl acetate (LR grade, Qualigens fine chemicals, Mumbai, India), Methanol (AR grade, Sisco Research Laboratories Pvt. Ltd, Mumbai, India),Toluene (LR grade, Thomas baker chemicals ltd., Mumbai, India).

3.1.1.3 TLC plates (Silica Gel 60 TLC, coated with silica gel): MERCK specialities private Ltd, Mumbai, India.

3.1.1.4 Cholesterol, Ergosterol, Stigmasterol, Cholesterol acetate: Sigma-Aldrich Chemie, USA.

3.1.2 Reagents:

3.1.2.1 Alcoholic potassium hydroxide (0.5N): About 35 to 40 g of potassium hydroxide pellets were dissolved in about 20 ml distilled water and then volume was made to one litre with ethyl alcohol. The strength of the solution was adjusted to 0.5 N with the help of 0.5 N standard oxalic acid solution.

3.1.2.2 Dilute sulphuric acid: Approximately 25 ml of concentrated sulphuric acid was diluted to 1000 ml with distilled water and its strength was adjusted until 40 ml of diluted sulphuric acid neutralized 2 ml of the 50% (w/w) sodium hydroxide solution.

3.1.2.3 Ethyl alcohol (neutralized): Ethyl alcohol was neutralized using 0.1N sodium hydroxide solution in presence of phenolphthalein indicator before its use.

3.1.2.4 Furfural solution: Two percent solution of furfural, distilled not earlier than 24 hours prior to the test, in rectified spirit.

3.1.2.5 Hydrochloric acid solution (0.5N): About 42 ml of concentrated hydrochloric acid was added slowly into 500 ml distilled water from the side of container and then the volume was made up to 1000 ml in a volumetric flask. The strength of the solution was adjusted to 0.5 N with the help of 0.5 N standard potassium hydroxide solutions.

3.1.2.6 Hydrochloric acid solution (25%): Exactly 25 ml of concentrated hydrochloric acid was added slowly into volumetric flask containing distilled water from the side of container and then the volume was made up to 100 ml.

3.1.2.7 Oxalic acid solution (0.1N): Exactly 0.63 g of oxalic acid was dissolved in distilled water and volume was made to 100 ml in a volumetric flask.

3.1.2.8 Oxalic acid solution (0.5N): Exactly 3.15 g of oxalic acid was dissolved in distilled water and volume was made to 100 ml in a volumetric flask.

3.1.2.9 Phenolphthalein solution: One gram of phenolphthalein was dissolved in 5 ml of distilled water and then volume was made to 100 ml with absolute ethyl alcohol.

3.1.2.10 Potassium dichromate solution: Five gram of potassium dichromate was dissolved in distilled water and then volume was made to 1000 ml.

3.1.2.11 Potassium iodide solution (10%): Prepared freshly by dissolving 10 g of potassium iodate in 100 ml of distilled water.

3.1.2.12 Sodium hydroxide solution (0.1 N): About 4.2 g of sodium hydroxide was dissolved in distilled water and volume was made up to 1000 ml in a volumetric flask. The strength of the solution was adjusted to 0.1 N with the help of 0.1 N standard oxalic acid solution.

3.1.2.13 Sodium hydroxide solution (10%): About 10 g of sodium hydroxide pellets were dissolved in distilled water and then volumes was made to 100 ml.

3.1.2.14 Sodium hydroxide solution (50%; w/w): Exactly 50 g of sodium hydroxide pellets were dissolved in equal weight of distilled water and kept in a bottle protected from CO_2.

3.1.2.15 Sodium thiosulphate solution (0.1 N): Approximately 24.8 g of sodium thosulphate crystals were dissolved in CO_2 free water, and titrated with potassium dichromate solution as described in SP: 18(Part XI)-1981.

3.1.2.16 Starch solution (indicator): Triturate 0.5 gram of potato starch was dissolved in 3 ml cold distilled water, which was poured into 100 ml of boiling water and boiled for 3 min. After cooling, the supernatant was decanted off.

3.1.2.17 Wijs' reagent: Five milliliter of iodine monochloride was dissolved in about 900 ml of glacial acetic acid and mixed vigorously. To 5.0 ml of the diluted iodine monochloride solution, 10 ml of potassium iodide solution was added and titrated with 0.1 N standard sodium thiosulphate solution, using

starch solution as an indicator. The volume of the solution was adjusted till strength of the diluted solution was 0.2 N.

3.1.2.18 Potassium hydroxide (50%, W/V): Exactly 125 g of Potassium hydroxide was dissolved in distilled water and volume was made to 250 ml in a volumetric flask.

3.2 EQUIPMENTS

3.2.1 Boiling water-bath: The Laboratory Glassware Co., Ambala Cantt., India.

3.2.2 Butyro refrectometer: Naveen Scientific Industries, Ambala, India.

3.2.3 Cream separator: Kamdhenu, KD-60E, Benny Impex, New Delhi, India.

3.2.4 Digital temperature control oven: Narang Scientific Works Pvt. Limited, New Delhi, India.

3.2.5 Electric heater: Vikrant, Jain Enterprises, India.

3.2.6 Electronic balance: Sartorius, England and Mettler AT-200, Switzerland.

3.2.7 Filter paper: Whatman No. 4 of 12.0 cm diameter, England

3.2.8 Refrigerated water-bath: J Lab Tech, Daihan Labtech Co. Ltd., Korea.

3.3 COLLECTION AND PREPARATION OF SAMPLES

3.3.1 Collection of Milk and Preparation of Ghee

Cow and buffalo milk used for the preparation of respective ghee samples were collected in three different seasons (rainy, winter and summer, respectively in August, January and May) from the Experimental Dairy of the Institute. Cow milk was a bulk milk consisting of mixture of the milk obtained from the herd of Karan Swiss, Karan Fries, Sahiwal and Tharparkar breeds. Buffalo milk used was also bulk milk obtained from Murrah breed only.

Samples of cow/buffalo ghee were prepared by creamery butter method (De, 1994). Soon after the collection of milk samples, these were warmed to 40°C and separated into cream using mechanical cream

separator. The cream was pasteurized at 77°C for 5 minutes, cooled to room temperature and then kept in a refrigerator (5 to 10°C) for few hours (3 to 5 hours) for aging. Butter was prepared under standard conditions (9°C in summer and 13°C in winter) by churning the cream using mixture. The butter was then heated on direct flame in a stainless steel vessel and clarified into ghee with continuous stirring at temperature of 120°C/flash. Ghee was then filtered through 6-8 fold muslin cloths followed by Whatman No.4 filter paper, filled in glass bottles, cooled to room temperature and kept in a refrigerator at a temperature of 5 to 10°C till further analysis. Three ghee samples each of cow and buffalo in each of three different seasons were used as control samples for comparison of market ghee samples.

The flow chart for the preparation of control ghee samples was as follows:

Collection of cow/buffalo milk

↓

Cream separation (at 40^{o}C)

↓

Churning to butter

↓

Clarification at 120^{o}C/ flash

↓

Filtration (Muslin cloth and W.N. 4 filter paper)

↓

Ghee

3.3.2 Collection of Market ghee samples

Market ghee samples of organized dairies were collected from different regions (North, South, East and West) of the country in three different seasons (rainy, winter and summer, respectively in August, January and May). Three samples were collected from each region making it to a total of 12 samples in each season, thereby totaling to 36 samples in three seasons. Ten samples of unorganized dairies of Karnal city were also collected in each season and analyzed simultaneously along with the samples of organized dairies, thereby making a total of 30 samples in this case. After collection, all

samples were kept in a refrigerator (5-10^{o}C) till they were analyzed for their physico-chemical qualities and other tests to be analyzed.

3.3.3 Preparation of Adulterated Ghee Samples (positive control samples)

For the preparation of adulterated positive control ghee samples, pure ghee samples prepared from Institute herd milk were heated to 60-70°C for 10 min before mixing. The adulterants like vanaspati, mineral oil (liquid paraffin), rice bran oil and sunflower oil were added to ghee at 15% level individually, to serve as a positive control for Baudounin test, Mineral oil test, Rice bran oil test and rapid vegetable oil detection test, respectively.

3.4 METHODS OF ANALYSIS

In this section, various methods followed to study the physico-chemical qualities of various market ghee samples are described briefly.

3.4.1 PHYSICO-CHEMICAL CONSTANTS

The physico-chemical constants such as Reichert-Meissl (RM) value, Polenske value (PV), Saponification value, Iodine Value and Butyro-refractometer reading of the control ghee samples and market ghee samples were determined by the methods as described in SP: 18 (Part XI) – 1981 (BIS, 1981). The details of the procedures for these physico-chemical constants are as follows:

3.4.1.1 Determination of Reichert-Meissl (RM value) and Polenske Value

Accurately 5.0 g of sample were weighed in a Polenske flask and then saponified with 20.0 g of glycerol and 2.0 ml of 50% (w/w) sodium hydroxide solution on a direct flame. Then 93 ml of freshly boiled distilled water were added followed by 50 ml of dilute sulphuric acid. The flask was immediately connected with the distillation apparatus and 110 ml of the distillate was collected within 21 minutes. The flask was replaced with 25 ml cylinder and

the flame was removed. The distillate was cooled in a water bath maintained at 15^{o}C for 10 min. Then it was filtered through a dry 9 cm Whatman No.4 filter paper and 100 ml of the filtered distillate were titrated against 0.1 N sodium hydroxide solution using phenolphthalein as an indicator. Similarly, a blank test was also done by using all reagents in similar fashion except fat sample. From this, the RM value was calculated as follows:

$$\text{Reichert-Meissl Value} = 1.10\ (T_1\text{-}T_2).$$

Where;

T_1 = Volume (ml) of 0.1 N NaOH solution used for sample titration

T_2 = Volume (ml) of 0.1 N NaOH solution used for blank titration

For Polenske value (PV), the condenser, 25 ml cylinder, 110 ml flask and the filter paper were washed with three successive washings of 15 ml portions of cold water followed by neutralized alcohol. The washings with neutralized alcohol were collected and then titrated against 0.1 N sodium hydroxide solution using phenolphthalein as an indicator. Similarly, a blank was also done. From this, the Polenske value was calculated as follows:

$$\text{Polenske Value} = T_3\text{-}T_4$$

Where;

T_3 = Volume (ml) of 0.1 N NaOH solution used for sample titration

T_4 = Volume (ml) of 0.1 N NaOH solution used for blank titration

3.4.1.2 Determination of Saponification Value

Accurately 2.0 g of sample were weighed into a round flat bottom flask and to this 25 ml of 0.5 N alcoholic potassium hydroxide solutions were added. Then the contents were refluxed in a water bath for about one hour. At the end of saponification, the contents were titrated against 0.5 N hydrochloric acid when it was hot, using phenolphthalein as an indicator.

A blank test was carried out using the same quantity of potassium hydroxide under similar conditions. From this, the saponification value was calculated as follows:

$$\text{Saponification Value} = 28.05\ (T_2\text{-}T_1)/W$$

Where;

T_2 = Volume (ml) of 0.5 N hydrochloric acid required for the blank

T_1 = Volume (ml) of 0.5 N hydrochloric acid required for the sample, and

W = Weight (g) of the sample taken for the test

3.4.1.3 Determination of Iodine Value

Iodine value of control ghee samples and market ghee samples were determined by the Wij's method as described in SP: 18 (Part XI) – 1981.

Accurately 0.4 g of sample was weighed in a clean and dry iodine flask and was dissolved in 15 ml of carbon tetrachloride. Then 25 ml of the Wij's reagent were added and the flask was stoppered. The contents were then mixed and kept in dark for one hour. After one hour, 20 ml of 10 percent potassium iodide solution and about 150 ml of distilled water were added to the iodine flask and mixed. The contents were titrated against 0.1 N sodium thiosulphate solution using starch solution as an indicator. A blank test was also carried out using the same quantities of the reagents. From this, the iodine value was calculated as follows:

$$\text{Iodine Value} = 12.69\ (B - S)\ N / W$$

Where;

B = Volume (ml) of standard sodium thiosulphate solution required for the blank

S = Volume (ml) of standard sodium thiosulphate solution required for the sample

N = Normality of the standard sodium thiosulphate solution, and

W = Weight (g) of the sample taken for the test

3.4.1.4 Determination of Butyro-Refractometer (BR) Reading at 40°C

Butyro-Refractometer (BR) reading control of ghee samples and market ghee samples were determined by the method as described in SP: 18 (Part XI) – 1981.

Before determining the BR reading of a sample, the temperature of the refractometer was adjusted to 40.0 ± 0.1°C using circulatory water bath and the prisms were cleaned and dried completely. The refractometer was calibrated with the standard provided by the company before taking the reading of the different samples. A drop of the molten fat sample was placed on the lower prism of the refractometer and the prisms were closed and held for 2 minutes. After adjusting the instrument and light to get the most distinct reading possible and bringing the temperature to 40°C, the BR reading of the fat was recorded.

3.4.2 MISCELLANEOUS METHODS

3.4.2.1 Baudouin test for detection of vanaspati

The test as described in SP: 18 (Part XI) – 1981 was performed as follows:

In a dry test tube, 5.0 ml of melted ghee sample was taken, to which 5.0 ml of concentrated hydrochloric acid was added followed by 0.4 ml of 2% furfural solution and shaken vigorously for two minutes. The mixture was allowed to separate. Positive test is indicated by development of permanent pink/red color in the acid layer. Confirmation is done by adding 5.0 ml of distilled water and shaking again. If the color in acid layer persists, vanaspati is present in ghee. If the color disappears it is absent in ghee.

3.4.2.2 Turbidity test for detection of mineral oil

Detection of mineral oil in ghee samples was carried out by Holde's test (Winton and Winton, 1999) as described by Arun *et al.,* (2005). The method, in brief, was as follows:

One ml of melted ghee sample was taken in a test tube (15 cm X 15 mm O.D). To this, 5.0 ml of 0.5N alcoholic KOH solution was added. Then it was subjected to heating on direct flame using wire gauge for five minutes or until complete saponification took place. After this, about 5.0 ml of cold water was added to it and observed for turbidity. Appearance of turbidity marked the presence of mineral oil. But no turbidity indicated the absence of mineral oil.

3.4.2.3 Colorimetric method for detection of rice bran oil

Colorimetric procedure developed by Shukla *et al.* (2004) for the detection of rice bran oil in other vegetable oils on the basis of gamma oryzanol was essentially adopted in the present study for its detection in ghee, as modified by Amit Kumar *et al.,* (2009). The test was performed as follows:

In a dry test tube, 1 ml of the melted fat/oil sample was taken, to which 1.5 ml of hexane was added to dissolve it. Then 0.5 ml of dilute (25%) hydrochloric acid and 0.5 ml of 5% sodium nitrite solution were added to the same test tube and mixed, followed by the addition of 1 ml of 10% sodium hydroxide solution. An orange-red color appeared in the presence of gamma oryzanol after addition of sodium hydroxide, which became the basis for detection of rice bran oil in ghee.

3.4.2.4 Rapid color based test for detection of vegetable oils

This test was done according to the method of Sharma *et al.* (2007) as follows:

One ml of clear molten ghee was dissolved with 1.5 ml of hexane in test tube. To this one ml of color developing reagent (distilled water, sulphuric acid and nitric acid in the ratio of 20:6:14, respectively) added, shaken vigorously and kept undisturbed till it is separated into two layers. The appearance of a distinct orange tinge in the upper layer indicates the presence of vegetable oils/fats including vanaspati.

3.4.2.5 Crystallization time test

Crystallization test was done according to the method of Panda and Bindal (1998b) as follows:

0.8 ml of clear melted fat sample was transferred separately to the glass tube (specifications: length- 10 ± 0.1 cm, internal diameter- 1.0 ± 0.02 cm, external diameter- 1.2 ± 0.02 cm) with the help of 1 ml pipette. To this, 2.5 ml of the solvent mixture (acetone: benzene:: 3.5:1) was added. The contents in the glass tube were mixed thoroughly and placed in a water bath maintained at 20^{o}C/5 min for temperature equilibration. Thereafter, the tube was placed in a water bath essentially maintained at 17±0.2^{o}C till the onset of crystallization of fat in the tube and the time of onset of crystallization was noted down.

3.4.2.6 Complete Liquefaction Time (CLT) Test

The complete liquefaction time (CLT) of the fat samples was recorded by observing the time taken by the solidified fat samples to get melted completely at 44 ^{o}C as described by Kumar (2008). The method, in brief, was as follows:

Three gram of the completely melted fat sample was taken into a test tube (12 x 100 mm) and kept in an oven maintained at 60°C for a period of 5 minutes. Thereafter, the test tube, containing fat sample, was kept in a refrigerator (6-8^{o}C) for 45 min for solidification of the melted fat sample. After that the solidified sample was subjected to liquefaction process at 44^{o}C for complete melting of the sample. The time for the sample to liquefy completely was recorded as CLT using stop watch.

3.4.2.7 Apparent Solidification Time (AST) Test

The AST of the fat samples was recorded by studying the time taken by the melted fat samples to become apparently solidified at 18±0.2 ^{o}C as described Kumar *et al*.,(2009). The method, in brief, was as follows:

In this test, 3 g of the completely melted fat samples were placed in test tubes (10 x 1.0 cm ID) and maintained at 60°C for a period of 5 min. The test tubes were then kept in a refrigerated water bath maintained at 18 ± 0.2. ^{o}C The test tubes were observed constantly until the apparent solidification of fat samples took place, which was confirmed by non-movement of fat samples on tilting the test tube. At this stage, when the fat sample was apparently solidified, the time taken for the same was recorded as AST using a stop watch.

3.4.3 Thin layer chromatography of unsaponifiable matter

In order to check the presence of vegetable oils in market ghee samples, technique of thin layer chromatography (TLC) was applied using the method of Sebastian and Rao (1974) with following modification:

1. TLC plates (Silica Gel 60 TLC, coated with silica gel): MERCK specialities private Ltd, Mumbai, India.
2. Instead of whole fats, unsaponifiable matter dissolved in chloroform was used as spotting material.

The method, in brief, was as follows:

3.4.3.1 Extraction of unsaponifiable matter from the samples

Accurately, 5.0 g of ghee samples were weighed and saponified by refluxing with 50 ml of ethyl alcohol and 7 ml of 50 percent (w/v) potassium hydroxide solution for 30 minutes. After cooling, 150 ml of distilled water were added and the contents were transferred to a separating funnel. The unsaponifiable matter was extracted three times with 50 ml of peroxide-free diethyl ether each time. All the three ether extract were combined together and washed with water to make it alkali free. The alkali-free ether extract was dried over sodium sulphate (anhydrous) and finally the ether was evaporated on water bath under reduced pressure.

3.4.3.2 Application of unsaponifiable matter on TLC plates and its development

By means of a micropipette, 10 µl of unsaponifiable matter extracted from the samples of control ghee (cow and buffalo) and market ghee samples, after dissolving in chloroform, were spotted on the TLC plates at a distance of about 2 cm from the bottom.

These plates were introduced into chromatographic vessel having solvent mixture consisting of cyclohexane:ethyl acetate:water in the proportion of 600:200:1 by volume, as developing solution. The plates were developed according to ascending chromatographic technique for about five hours. The development was discontinued when the solvent (developer) front had travelled over height of about 17 cm, as measured from the solvent front level in the vessel. The developed plates were dried in the air for 30 minutes to remove excess of the solvent followed by drying in an oven maintained at 100^{o}C for 3 minutes. The plates were then taken out and allowed to cool at room temperature and sprayed uniformly with 25 percent phosphomolybdic acid in ethanol (98%, v/v). The plates were kept back in drying oven till distinct bluish bands were developed which took 3 to 5 minutes. Simultaneously, reference solution consisting of pure cholesterol ester, cholesterol, and phytosterols (ergosterol and stigmasterol) were also run under similar conditions.

3.5 STATISTICAL ANALYSIS

Data reported were expressed as mean values with standard errors. In experiments, wherever required, two-way analysis of variance (ANOVA) with a subsequent least significant difference (LSD) test was applied for multiple sample comparison to test for any significant differences ($P<0.05$) in the mean values of all the groups as described by Snedecor and Cochran (1994), using the statistical program of Microsoft® Excel Version 5.0 (Microsoft Corporation, Redmond, WA, U.S.A.).

CHAPTER - 4

Results and Discussion

4.0 RESULTS AND DISCUSSION

A study on the physico-chemical qualities of market ghee sold in four different regions (North, South, East and West) of India in three different seasons (rainy, winter and summer) to know the regional and seasonal variations in the physico-chemical composition of market ghee samples, was undertaken. Beside this, the study was also aimed to compare the purity of market ghee samples with laboratory made pure ghee samples with a view to detect adulteration (if any) with foreign oils and fats like animal body fats, vegetable oils, vanaspati and mineral oil.

For this purpose, 36 branded market ghee samples of organized dairies were collected from four different regions in three different seasons. Three samples were collected from each region making it to a total of 12 samples in each season, thereby totaling to 36 samples in three seasons. Beside this, ten unbranded samples of ghee of unorganized dairies of Karnal city were also collected in each season and analyzed simultaneously along with the samples of organized dairies, thereby making a total of 30 samples in this case. Therefore, a total of 66 samples of market ghee were analyzed in the present study.

For the purpose of comparison of market ghee samples, pure ghee samples from the milk of institute herd of cows and buffaloes were also separately prepared in the laboratory in three different seasons (rainy, winter and summer) simultaneously by creamery butter method. Three samples each of cow ghee and buffalo ghee in each of three different seasons were used as control samples, thereby making a total of 18 control ghee samples.

Adulterated positive control ghee samples were also prepared in the laboratory by adulterating pure ghee with adulterants like vanaspati, mineral oil (liquid paraffin), rice bran oil and sunflower oil, to serve as a positive control for Baudouin test, mineral oil test, rice bran oil test and rapid vegetable oil detection test, respectively.

The market ghee samples collected from organized as well as unorganized dairies along with pure control ghee samples were analyzed for physico-chemical constants, such as:

I. Reichert-Meissl (RM) value

II. Polenske value

III. Saponification value

IV. Iodine value

V. Butyro-refractometer (BR) reading at 40^{o}C

Besides physico-chemical constants, ghee samples were also analyzed for the following parameters, with a view to ascertain the purity of market ghee samples:

1. Apparent solidification time (AST) test at 18^{o}C
2. Complete liquefaction time (CLT) test at 44 ^{o}C
3. Crystallization time test at 17 ^{o}C
4. Baudouin test for detection of vanaspati in ghee samples
5. Rapid color based test for detection of vegetable oils
6. Detection of rice bran oil on the basis of tracer component (gamma oryzanol)
7. Turbidity test for detection of mineral oil
8. Thin layer chromatography of unsaponifiable matter isolated from ghee samples

The results obtained in the present study on the above said parameters are given in the Tables 4.1 to 4.23 and plates 4.1 to 4.9.

4.1. Physico-chemical constants of pure ghee (control) and market ghee (organized and unorganized) samples in different seasons for different regions.

Ghee, like other fats and oils, is characterized by certain physico-chemical properties (RM value, Polenske value, saponification value, iodine value, BR reading and free fatty acids) which have been found to be the basis for the fixation of certain physico-chemical constants for defining the chemical quality of the product. These constants serve as an indication of the types of component fatty acids present in fats. They also enable the detection of fat adulteration qualitatively and, in some instances quantitatively. These physico-chemical properties, however, show some natural variation depending upon factors like method of manufacture, age and condition of the sample, species, breed, individuality of animal, stage of lactation, number of lactation (age of animal), season of the year, region of the country, feed of the animal etc (Doctor *et al.,* 1940; De, 1994; Banerjee *et al*., 2002).

Prevention of Food Adulteration rules, 1955 (PFA, 2009) have prescribed the requirements for two physico-chemical constants Butyro-refractometer (BR) reading at 40^{o}C and Reichert-Meissl (RM) value, besides free fatty acids (as oleic acid) and moisture percent for defining the quality of ghee. Whereas, AGMARK standards (AGMARK, 1981) specify the physico-chemical constants i.e. BR reading, RM value and Polenske value, besides free fatty acids (as oleic acid) and moisture for the same purpose. These PFA and AGMARK standards of ghee are presented in Appendix-I and II respectively. The iodine value, saponification value and polenske value for milk fat range between 26 to 35, 210 to 233 and 1.2 to 2.4, respectively (Jenness & Patton, 1969).

The result obtained in the present study on Physico-chemical constants such as Reichert-Meissl value, Polenske value, saponification value, iodine value and Butyro-refractometer reading (at 40^{o}C) of cow and buffalo pure ghee, and ghee from organized dairies for different regions and unorganized dairies of Karnal city in different seasons are described in Tables 4.1 to 4.9, The overall range of physico-chemical constants of pure ghee of cow and

buffalo and that of organized dairy and unorganized dairy ghee samples are described in Tables 4.10 to 4.12 and the analysis of variance of physico-chemical constants of pure ghee and market ghee are presented in Tables 4.13 to 4.14.

4.1.1 Physico-chemical constants of pure ghee (control) and market ghee (organized and unorganized) samples in Rainy season (August) for different regions.

Tables 4.1 to 4.3 show the results on physico-chemical constants such as Reichert-Meissl value, Polenske value, Saponification value, Iodine value and Butyro-refractometer reading (at 40^{o}C) of cow and buffalo pure ghee, and ghee from organized and unorganized dairies in rainy season.

Table 4.1 Physico-chemical constants of cow and buffalo pure ghee in Rainy season (August).

Pure ghee	Reichert-Meissl value	Polenske Value	Saponification value	Iodine Value	BR Reading at 40^{o}C
Cow ghee	28.71±0.41	1.90±0.05	224.49±0.40	34.75±0.64	42.23±0.14
Buffalo ghee	33.37±0.36	1.43±0.08	231.29±0.55	32.02±0.63	41.57±0.18

Data represent mean ± S.E. of three determination

In rainy (August) season, the range (with average) of RM value, Polenske value, Saponification value , Iodine value and BR reading at 40 ^{o}C for pure ghee of institute herd of cows were 28.05 to 29.48 (28.71), 1.8 to 2.0 (1.9), 223.85 to 225.23 (224.49), 33.49 to 35.63 (34.75) and 42.0 to 42.50 (42.23) respectively. The corresponding values for pure ghee of institute herd of buffaloes were 32.67 to 33.88 (33.37), 1.3 to 1.6 (1.43), 230.26 to 232.14 (231.29), 30.75 to 32.80 (32.02) and 41.20 to 41.80 (41.57) respectively.

These results for rainy season samples of laboratory made pure ghee have indicated that the average RM value and Saponification value were higher in buffalo ghee than in cow ghee. On the other hand, the average polenske value, iodine value and BR reading were lower in buffalo ghee than in cow ghee.

Table 4.2 Physico-chemical constants of market ghee from organized dairies in rainy season (August) for different regions.

Ghee from four regions of country	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40°C
N-1	32.45	1.8	229.98	35.59	40.85
N-2	31.13	1.5	221.08	36.09	40.50
N-3	31.13	1.5	227.47	35.46	41.30
Mean±S.E.	31.57±0.44	1.6±0.10	226.17±2.64	35.68±0.20	40.88±0.23
S-1	27.60	1.4	222.25	44.70	41.60
S-2	27.17	2.3	229.85	42.64	41.15
S-3	27.94	1.4	225.20	44.16	40.60
Mean±S.E.	27.57±0.22	1.7±0.3	225.77±2.21	43.84±0.61	41.12±0.22
W-1	8.69	1.5	214.15	65.95	45.60
W-2	10.10	0.8	203.25	58.12	45.95
W-3	6.40	2.1	223.56	47.26	41.50
Mean±S.E.	8.40±1.07	1.40±0.37	213.65±5.86	57.11±5.41	44.35±1.42
E-1	32.01	1.8	213.39	33.32	40.50
E-2	24.31	1.5	220.49	39.10	41.50
E-3	18.92	1.3	222.47	44.47	42.80
Mean±S.E.	25.08±3.79	1.50±0.14	218.78±2.75	38.96±3.21	41.60±0.66

N- sample from north region, S- sample from south region, W- sample from west region,E- sample from east region

Table 4.2 depicts the physico-chemical constants of market ghee from organized dairies in rainy season (August) for different regions of the country. The range (with average) of RM value, Polenske value, saponification value, iodine value and BR reading at 40 ^{o}C for ghee samples of northern region were respectively 31.13 to 32.45 (31.57), 1.5 to 1.8 (1.6), 221.08 to 229.98 (226.17), 35.46 to 36.09 (35.68) and 40.50 to 41.30 (40.88). For southern region, these values were respectively 27.17 to 27.94 (27.57), 1.4 to 2.3 (1.7), 222.25 to 229.85 (225.77), 42.64 to 44.70 (43.84) and 40.60 to 41.60 (41.12).

The similar values for ghee samples of western region were respectively 6.4 to 10.10 (8.40), 0.8 to 2.1 (1.4), 203.25 to 223.56 (213.65), 47.26 to 65.95 (57.11) and 41.50 to 45.95 (44.35), and for eastern region were respectively 18.92 to 32.01 (25.08), 1.3 to 1.8 (1.5), 213.39 to 222.47 (218.78), 33.32 to 44.47 (38.96) and 40.50 to 42.80 (41.60).

The above part of the study of market ghee samples from organized dairies in rainy season for different regions (Table 4.2) revealed that the RM values of all the northern and southern ghee samples studied were within the prescribed PFA standards. However, the RM values of all the three ghee samples of western region were extremely low and did not meet the prescribed specification. For eastern region, one out of three ghee samples showed lower RM value and also failed to meet the legal specification. On the other hand, BR reading of all the ghee samples taken from different regions of the country were within the limits except two samples of western region which showed slightly higher value than the PFA limits. Polenske values of all the ghee samples of different regions were within the general range of 1.2 to 2.4, except for one sample of western region for which the value was 0.8, being slightly lower. Similarly saponification values of all the market ghee sample of rainy season of all the regions were within the general range of 210 to 233, except for one sample of western region for which the value was 203.25, being slightly lower. Iodine values of ghee samples of northern region were somewhat close to the general reported range of 26 to 35. However, for other three regions, the values were by and large higher than the general values

especially of western regions in which one sample showed iodine value of as high as 65.95.

Table 4.3 Physico-chemical constants of market ghee from unorganized dairies in rainy season (August) of Karnal city.

Ghee from unorganized dairies of Karnal city	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40^{o}C
NOD-1	16.61	1.3	213.85	47.64	44.60
NOD-2	31.79	1.2	224.68	31.42	41.15
NOD-3	31.79	1.6	225.50	32.98	40.20
NOD-4	29.70	1.3	224.96	39.19	41.60
NOD-5	31.79	1.6	227.14	36.75	41.30
NOD-6	30.05	1.4	226.61	35.94	41.45
NOD-7	33.77	1.6	224.68	27.11	40.25
NOD-8	32.23	1.4	233.29	33.17	40.60
NOD-9	28.42	1.4	227.65	34.05	41.15
NOD-10	29.48	1.1	223.85	30.00	38.80
Mean±S.E.	29.56±1.52	1.39±0.05	225.22±1.52	34.82±1.79	41.11±0.46

NOD- Non-organized dairy/Unorganized dairy

The results of physico-chemical constants of market ghee samples from unorganized dairies of Karnal city in rainy season (August) are presented in Table 4.3. The RM value, Polenske value, saponification value, iodine value

and BR reading at 40 ^{o}C varied from 16.61 to 33.77 (29.56), 1.1 to 1.6 (1.39), 213.85 to 233.29 (225.22), 27.11 to 47.64 (34.82) and 38.80 to 44.60 (41.11), respectively.

The analysis of market ghee samples of unorganized dairies of Karnal city collected in rainy season (August) revealed that out of ten samples one sample showed considerably lower RM value and slightly higher BR reading, while another one sample showed BR reading less than the PFA limits. For Polenske value, one out of ten samples showed lower value of 1.1, while rest of the sample were in the general range of 1.2 to 2.4. However, saponification values of all the samples were within the general range of 210 to 233. On the other hand, Iodine values of four out of ten samples were on higher side of general range of 26 to 35, and one sample among them even showed as high as 47.64.

4.1.2 Physico-chemical constants of pure ghee (control) and market ghee (organized and unorganized) samples in winter season (January) for different regions.

The results on physico-chemical constants such as Reichert-Meissl value, Polenske value, saponification value, iodine value and BR reading reading (at 40^{o}C) of cow and buffalo pure ghee, and ghee from organized and unorganized dairies in winter season are described in Tables 4.4 to 4.6.

Table 4.4 Physico-chemical constants of cow and buffalo pure ghee in winter season (January).

Pure ghee	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40^{o}C
Cow ghee	28.60±0.25	1.40±0.18	226.38±1.63	35.48±1.20	41.50±0.49
Buffalo ghee	31.83±0.42	1.30±0.15	231.16±0.72	33.49±0.43	41.13±0.16

Data represent mean ± S.E. of three determination

In winter (January) season, the range (with average) of RM value, Polenske value, saponification value, iodine value and BR reading at 40 ^{o}C for pure ghee of NDRI herd of cows were respectively from 28.16 to 29.04 (28.60), 1.2 to 1.7 (1.4), 224.40 to 229.62 (226.38), 33.50 to 37.65 (35.48)

and 40.60 to 42.30 (41.50). The corresponding values for pure ghee of institute herd of buffaloes were respectively 31.02 to 32.45 (31.83), 1.1 to 1.5 (1.30), 230.05 to 232.52 (231.16), 32.85 to 34.32 (33.49) and 40.80 to 41.35 (41.13).

As observed for rainy season, the results for laboratory made pure ghee samples of winter season have also indicated that the average RM value and saponification value were higher in buffalo ghee than in cow ghee. On the other hand, the average Polenske value, iodine value and BR reading were lower in buffalo ghee than in cow ghee.

The physico-chemical constants of market ghee from organized dairies in winter season (January) for different regions of the country are presented in Table 4.5. The range (with average) of RM value, Polenske value, Saponification value, Iodine value and BR reading at 40 ^{o}C for ghee samples of northern region were respectively 26.62 to 30.58 (28.42), 0.7 to 1.7 (1.2), 230.26 to 234.67 (232.29), 32.35 to 35.80 (34.38) and 41.20 to 41.70 (41.40). For southern region, these values were respectively 27.72 to 29.37 (27.90), 0.6 to 1.2 (0.97), 218.32 to 234.90 (227.09), 26.85 to 31.28 (28.89) and 41.35 to 41.75 (41.50).

The similar values for ghee samples of western region were respectively 8.5 to 21.89 (13.20), 0.6 to 2.4 (1.67), 202.65 to 230.11 (211.83), 31.87 to 76.14 (52.29) and 41.05 to 51.85 (46.20), and for eastern region were respectively 13.75 to 29.80 (22.11), 0.5 to 1.7 (1.13), 212.79 to 232.92 (223.46), 28.76 to 50.52 (38.43) and 40.50 to 46.05 (42.93)

Table 4.5 Physico-chemical constants of market ghee from organized dairies in winter season (January) for different regions.

Ghee from four regions of country	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40^{o}C
N-1	30.58	1.2	234.67	32.35	41.20
N-2	28.05	0.7	230.26	35.80	41.70
N-3	26.62	1.7	231.95	35.00	41.30
Mean±S.E.	28.42±1.15	1.20±0.28	232.29±1.28	34.38±1.04	41.40±0.15
S-1	27.72	1.1	228.06	26.85	41.40
S-2	29.37	1.2	218.32	28.55	41.35
S-3	26.62	0.6	234.90	31.28	41.75
Mean±S.E.	27.90±0.79	0.97±0.18	227.09±4.81	28.89±1.29	41.50±0.12
W-1	21.89	2.4	230.11	31.87	41.05
W-2	9.20	0.6	202.65	76.14	51.85
W-3	8.50	2.0	202.72	48.85	45.70
Mean±S.E.	13.20±4.35	1.67±0.54	211.83±9.14	52.29±12.84	46.20±3.12
E-1	29.80	1.2	232.92	28.76	40.50
E-2	22.77	1.7	224.67	36.00	42.25
E-3	13.75	0.5	212.79	50.52	46.05
Mean±S.E.	22.11±4.64	1.13±0.34	223.46±5.84	38.43±6.39	42.93±1.63

N- sample from north region, S- sample from south region, W- sample from west region,E-sample from east region

From the above part of the study of market ghee samples from organized dairies in winter season for different regions (Table 4.5) it can be seen that the RM values of all the northern and southern ghee samples

studied were within the PFA standards prescribed for cotton tract and non cotton tract areas. However, the RM values of two out of three ghee samples of western region were extremely low and did not meet the prescribed specification given under the PFA rules. For eastern region, one out of three ghee samples showed lower RM value and also failed to meet the legal specification. On the other hand, BR reading of all the ghee samples taken from different regions of the country were within the limits except two samples of western region and one sample of eastern region which showed the values above the PFA limits. For Polenske values, one out of three ghee samples of each region showed value lower than the general range of 1.2 to 2.4. Saponification values of all the market ghee samples of winter season of all the regions were close to the general range of 210 to 233, except for two samples of western region for which the values were slightly lower. Iodine values of ghee samples of northern and southern regions were within the general reported range of 26 to 35. However, two samples of western region and one sample of eastern region were much higher than the general range. One of the sample of western region even showed very high value of 76.14.

Table 4.6 shows the result on physico-chemical constants of market ghee samples from unorganized dairies of Karnal city in winter season (January). The RM value, Polenske value, Saponification value, Iodine value and BR reading at 40 ^{o}C varied from 27.61 to 31.13 (29.41), 0.6 to 1.7 (1.19), 209.00 to 243.90 (230.19), 22.72 to 36.22 (30.24) and 38.80 to 42.90 (41.05), respectively.

The analysis of market ghee samples of unorganized dairies of Karnal city collected in winter season (January) revealed that out of ten samples, two samples showed RM value lower and two other samples showed BR reading less than the PFA limits. For Polenske value, three out of ten samples showed lower values. For saponification value and iodine value, one out of ten samples fell below the general range of 210 to 233 and 26 to 35 respectively.

Table 4.6 Physico-chemical constants of market ghee from unorganized dairies in winter season (January) of Karnal city.

Ghee from unorganized dairies of Karnal city	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40^{o}C
NOD-1	29.37	1.4	231.77	31.05	40.80
NOD-2	30.90	0.7	232.60	30.50	38.80
NOD-3	28.82	1.7	236.92	22.72	41.15
NOD-4	28.93	0.9	229.50	30.82	42.05
NOD-5	27.65	1.3	243.90	36.22	42.90
NOD-6	27.61	0.6	238.28	30.33	41.75
NOD-7	31.13	1.5	221.60	28.15	40.35
NOD-8	29.37	1.2	209.00	33.18	42.15
NOD-9	30.67	1.2	230.85	31.10	40.85
NOD-10	29.60	1.4	227.50	28.33	39.70
Mean±S.E.	29.41±0.39	1.19±0.11	230.19±3.05	30.24±1.10	41.05±0.38

NOD- Non-organized dairy/Unorganized dairy

4.1.3 Physico-chemical constants of pure ghee (control) and market ghee (organized and unorganized) samples in summer season (May) for different regions.

Tables 4.7 to 4.9 depicts the physico-chemical constants such as Reichert-Meissl value, Polenske value, saponification value, iodine value and BR reading (at 40^{o}C) of cow and buffalo pure ghee, and ghee from organized and unorganized dairies in summer season.

Table 4.7 Physico-chemical constants of cow and buffalo pure ghee in Summer season (May).

Pure ghee	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40°C
Cow ghee	29.70±0.33	1.80±0.05	230.84±0.40	32.79±0.58	41.27±0.40
Buffalo ghee	32.96±0.36	1.70±0.15	234.20±1.15	32.09±0.37	41.07±0.24

Data represent mean ± S.E. of three determination

In summer (May) season, the range (with average) of RM value, Polenske value, Saponification value, Iodine value and BR reading at 40 ^{o}C for pure ghee of institute herd of cows were respectively from 29.26 to 30.36 (29.70), 1.7 to 1.9 (1.8), 230.36 to 231.65 (230.84), 31.70 to 33.70 (32.79) and 40.70 to 42.05 (41.27). The corresponding values for pure ghee of institute herd of buffaloes were respectively 32.78 to 33.66 (32.96), 1.5 to 2.0 (1.70), 232.82 to 236.50 (234.20), 31.51 to 32.78 (32.09) and 40.60 to 41.40 (41.07).

As observed for rainy and winter seasons, the results on laboratory made pure ghee samples of summer season have also indicated that the average RM value and Saponification value were higher in buffalo ghee than in cow ghee. On the other hand, the vice-versa was true for other physico-chemical constants studied.

Table 4.8 shows the physico-chemical constants of market ghee from organized dairies in summer season (May) for different regions of the country. The range (with average) of RM value, Polenske value, saponification value, iodine value and BR reading at 40 ^{o}C for ghee samples of northern region were respectively 30.80 to 32.45 (31.86), 1.2 to 1.6 (1.40), 224.40 to 231.89 (227.26), 33.09 to 37.98 (35.96) and 41.60 to 43.25 (42.23). For southern region, these values were respectively 27.72 to 32.56 (30.25), 1.1 to 1.5 (1.30), 215.87 to 225.50 (221.47), 32.87 to 33.50 (33.11) and 41.85 to 42.40 (42.15).

Table 4.8 Physico-chemical constants of market ghee from organized dairies in summer season (May) for different regions.

Ghee from four regions of country	Reichert-Meissl value	Polenske Value	Saponification value	Iodine Value	BR Reading at 40°C
N-1	32.34	1.40	224.40	36.81	41.85
N-2	30.80	1.20	231.89	37.98	43.25
N-3	32.45	1.60	225.50	33.09	41.60
Mean±S.E.	31.86±0.53	1.40±0.11	227.26±2.33	35.96±1.47	42.23±0.51
S-1	30.47	1.10	225.50	32.97	42.40
S-2	27.72	1.30	215.87	32.87	42.20
S-3	32.56	1.50	223.03	33.50	41.85
Mean±S.E.	30.25±0.51	1.30±0.11	221.47±2.88	33.11±0.19	42.15±0.16
W-1	29.92	1.80	228.86	32.48	41.05
W-2	34.00	1.70	226.07	30.77	41.40
W-3	32.56	1.50	223.86	36.83	41.60
Mean±S.E.	32.16±4.92	1.67±0.28	226.26±4.86	33.36±6.32	41.35±2.01
E-1	24.09	1.20	223.97	33.50	42.50
E-2	22.22	1.30	224.62	34.45	42.25
E-3	17.60	0.90	213.07	50.55	47.25
Mean±S.E.	21.30±1.92	1.13±0.12	220.55±3.74	39.50±5.53	44.00±1.62

N- sample from north region, S- sample from south region, W- sample from west region,E-sample from east region

The similar values for ghee samples of western region were respectively 29.92 to 34.00 (32.16), 1.5 to 1.8 (1.67), 223.86 to 228.86 (226.26), 32.48 to 36.83 (33.36) and 41.05 to 41.60 (41.35), and for eastern region were respectively 17.60 to 24.09 (21.30), 0.9 to 1.3 (1.13), 213.07 to 223.97 (220.55), 33.50 to 50.55 (39.50) and 42.25 to 47.25 (44.00)

It can be seen from the above part of the study of market ghee samples from organized dairies in summer season for different regions (Table 4.8) that the RM values of ghee samples of all the region studied were within the prescribed PFA standards, except for the eastern region for which one out of three ghee samples showed lower RM value and also failed to meet the legal specification. Likewise, BR reading of all the ghee samples taken from different regions of the country were within the limits, except one sample of eastern region which showed higher value than the PFA limits. Polenske values of all the ghee samples of different regions were within the general range of 1.2 to 2.4, except for one sample each of southern and eastern region which were slightly lower than the general limits. Saponification values of all the market ghee sample of summer season of all the regions were within the general range of 210 to 233. Iodine values of ghee samples of all the regions were almost close to the general reported range of 26 to 35, except for one sample of eastern region which was very high (50.55).

The results of physico-chemical constants of market ghee samples from unorganized dairies of Karnal city in summer season (May) are presented in Table 4.9. The RM value, Polenske value, saponification value, iodine value and BR reading at 40 ^{o}C varied from 21.23 to 32.22 (29.74), 1.0 to 1.7 (1.27), 221.07 to 242.00 (231.16), 27.69 to 39.08 (34.01) and 41.70 to 44.05 (42.51), respectively.

The analysis of market ghee samples of unorganized dairies of Karnal city collected in summer (May) season revealed that out of ten samples one sample showed RM value lower and BR reading higher than the PFA limits. For Polenske value, four out of ten samples showed slightly lower values than the general range of 1.2 to 2.4. For saponification value and iodine value,

most of the samples were close to the general range of 210 to 233 and 26 to 35, respectively.

Table 4.9 Physico-chemical constants of market ghee from unorganized dairies in summer season (May) of Karnal city.

Ghee from unorganized dairies of Karnal city	Reichert-Meissl Value	Polenske Value	Saponification Value	Iodine Value	BR Reading at 40^{o}C
NOD-1	29.48	1.20	221.07	38.31	43.20
NOD-2	30.03	1.00	223.31	39.08	42.85
NOD-3	31.24	1.50	223.84	32.00	41.70
NOD-4	28.05	1.70	233.75	35.89	43.25
NOD-5	21.23	1.60	235.62	27.69	41.20
NOD-6	30.25	1.10	226.00	31.56	42.25
NOD-7	31.57	1.30	236.71	34.40	42.45
NOD-8	31.57	1.20	231.65	35.29	44.05
NOD-9	32.22	1.00	242.00	32.18	41.95
NOD-10	31.79	1.10	237.66	33.70	42.20
Mean±S.E.	29.74±1.02	1.27±0.07	231.16±2.26	34.01±1.06	42.51±0.26

NOD- Non-organized dairy/Unorganized dairy

4.1.4 Physico-chemical constants of pure ghee (cows and buffaloes pooled together) and market ghee of organized dairies (all regions pooled together) and unorganized dairies of Karnal city in different seasons.

The overall range along with mean value of physico-chemical constants such as Reichert-Meissl value, Polenske value, saponification value, iodine value and BR reading (at 40^{o}C) of pure ghee (cows and buffaloes pooled together), and ghee from organized (all regions pooled

together) and unorganized dairies of Karnal city in three seasons (rainy, winter and summer) is described in Tables 4.10 to 4.12.

Table 4.10 Physico-chemical constants of pure ghee (cows and buffaloes pooled together) in three different seasons (rainy, winter and summer)*.

Season	RM value	Poleske value	Saponification value	Iodine value	BR reading
Rainy	28.05-33.88	1.3-2.0	223.85-232.11	30.75-35.63	41.20-42.50
Mean±S.E.	31.03±1.07	1.66±0.11	227.89±1.55	33.38±0.73	41.90±0.18
Winter	28.16-32.45	1.1-1.8	224.40-232.52	32.85-37.65	40.60-42.30
Mean±S.E.	30.21±0.75	1.41±0.10	228.77±1.33	34.48±0.72	41.31±0.24
Summer	29.26-33.66	1.5-2.0	230.36-236.50	31.51-33.70	40.60-42.05
Mean±S.E.	31.33±0.76	1.75±0.07	232.51±0.93	32.54±0.34	41.16±0.21
Overall range**	28.05-33.88	1.1-2.0	223.85-236.50	30.75-37.65	40.60-42.50
Mean±S.E.**	30.86±0.48	1.61±0.06	229.72±0.85	33.43±0.39	41.46±0.14

*Data represents range and mean±S.E. of six determinations
** Data represents range and mean±S.E. of 18 determinations

The present study revealed that the overall range with average of RM value, Polenske value, Saponification value, Iodine value and BR reading of pure ghee (cows and buffaloes pooled together), were from 28.05 to 33.88 (30.86), 1.1 to 2.0 (1.61), 223.85 to 236.50 (229.72), 30.75 to 37.65 (33.43) and 40.60 to 42.50 (41.46), respectively. From the Table 4.10, it can be observed that average RM value, Polenske value and Saponification value were slightly higher while Iodine value and BR reading were slightly lower in summer season than in rainy and winter seasons. However, the analysis of variance of the data on physico-chemical constants of pure cow ghee (Table 4.13) and buffalo ghee (Table 4.14) revealed that there were no significant seasonal variations in all the physico-chemical constants (RM value,

Polenske value, Saponification value, Iodine value and BR reading). The results obtained in the present study on the RM value, Polenske value, Saponification value, Iodine value and BR reading of pure cow and buffalo ghee are in general agreement with those reported by earlier workers (Singhal, 1973; Rangappa & Achaya, 1974; Lal & Narayanan, 1984; Sharma and Singhal, 1995; Kumar, 2008).

Table 4.11 Physico-chemical constants of ghee samples of organized dairies (all regions pooled together) in three different seasons (Rainy, Winter and Summer)*.

Season	RM value	Poleske value	Saponification value	Iodine value	BR reading
Rainy	6.40 - 32.45	0.8 – 2.3	203.25 – 229.98	33.32-65.94	40.50-45.95
Mean±S.E.	23.15±2.79	1.57±0.11	221.09±2.21	43.89±2.80	41.98±0.54
Winter	8.50-30.58	0.5-2.4	202.65-234.90	26.85-76.14	40.5-51.85
Mean±S.E.	8.50±2.31	1.24±0.17	223.66±3.40	34.49±4.04	43.00±0.95
Summer	17.60-34.00	0.9-1.8	213.07-231.89	30.77-50.5	41.05-1.62
Mean±S.E.	28.89±1.45	1.37±0.07	223.88±1.46	35.48±1.49	42.43±0.46
Overall range**	6.40-34.00	0.5-2.5	202.65-234.90	26.85-76.14	40.50-51.85
Mean±S.E.**	24.98±1.34	1.39±0.07	222.88±1.41	39.29±1.76	42.47±0.39

*Data represents range and mean±S.E. of 12 determinations
** Data represents range and mean±S.E. of 36 determinations

Table 4.11 depicts that the RM value, Polenske value, Saponification value, Iodine value and BR reading of ghee samples of organized dairies (all regions pooled together in different seasons (Rainy, Winter and Summer) varied (with average) from 6.40 to 34.00 (24.98), 0.5 to 2.5 (1.39), 202.65 to 234.90 (222.88), 26.85 to 76.14 (39.29) and 40.50 to 51.85 (42.47), respectively.

Table 4.12 Physico-chemical constants of unorganized dairies ghee samples of Karnal city in three different seasons (Rainy, Winter and Summer)*.

Season	RM value	Poleske value	Saponification value	Iodine value	BR reading
Rainy	16.61-33.77	1.1-1.6	213.85-233.29	27.11-47.64	38.80-44.60
Mean±S.E.	29.53±1.52	1.39±0.05	225.22±1.52	34.82±1.79	41.11±0.46
Winter	27.61-31.13	0.6-1.7	209.00-243.90	22.72-36.22	38.80-42.90
Mean±S.E.	29.40±0.39	1.19±0.11	230.19±3.05	30.24±1.10	41.05±0.38
Summer	21.23-32.22	1.0-1.7	221.07-242.00	27.69-39.08	41.20-44.05
Mean±S.E.	29.74±1.02	1.27±0.07	231.16±2.26	34.01±1.06	42.51±0.26
Overall range**	16.61-33.77	0.6-1.7	209.00-243.90	22.72-47.64	38.80-44.60
Mean±S.E.**	29.57±0.60	1.28±0.04	228.85±1.40	33.02±0.84	41.55±0.24

*Data represents range and mean±S.E. of 10 determinations
**Data represents range and mean±S.E. of 30 determinations

Table 4.12 shows that RM value, Polenske value, Saponification value, Iodine value and BR reading of ghee samples of ghee samles collected from unorganized dairies of Karnal city in different seasons (Rainy, Winter and Summer) varied (with average) from 16.61 to 33.77 (29.57), 0.6 to 1.7 (1.28), 209.00 to 243.90 (228.85), 22.72 to 47.64 (33.02) and 38.80 to 44.60 (41.55), respectively.

Table 4.13. ANOVA for physico-chemical constants of pure cow ghee in different seasons

Source of variation	RM value			Polenske value			Saponification value			Iodine value			BR reading at 40 °C		
	DF	M.S.S	F	DF	M.S.S	F	DF	M.S.S	F	DF	M.S.S	F	DF	M.S.S	F
season	2	1.101	3.137 NS	2	0.181	4.405 NS	2	31.8296	10.625 NS	2	5.8012	2.632 NS	2	0.763	1.783 NS
Error	6			6			6			6			6		

NS- Non significant

Table 4.14 ANOVA for physico-chemical constants of pure buffalo ghee in different seasons

Source of variation	RM value			Polenske value			Saponification value			Iodine value			BR reading at 40 ^{o}C		
	DF	M.S.S	F	DF	M.S.S	F	DF	M.S.S	F	DF	M.S.S	F	DF	M.S.S	F
season	2	1.910	4.329 NS	2	0.081	1.489 NS	2	8.8434	4.081 NS	2	2.072	2.817 NS	2	0.221	1.829 NS
Error	6			6			6			6			6		

NS- Non significant

4.2 Apparent solidification time (AST), Complete liquefaction time (CLT) and Crystallization time tests of pure ghee (control) and market ghee (Organized and unorganized dairies) samples in different season for different region.

Milk fat melts over a wide range of temperature. It is antipicited to be liquid above 40 ^{o}C and completely solidified below -40 ^{o}C (Fox, 1995). At ambient temperature milk fat is partially solidified. Based on solidification and liquefaction behavior of milk fat, some tests such as Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test have been developed by various workers (Kumar, 2008; Kumar *et al,* 2009) and have been employed as one of the criteria for establishing the purity of milk fat, so as to detect the adulteration in milk fat with other cheaper oils and fats. The Apparent solidification time (AST) test of the sample is defined as the time taken by the melted fat sample to get solidified apparently at 18 ^{o}C. Pure ghee sample of both cow and buffalo show AST in the range of 2 min-30 sec to 3 min-26 sec. Any deviation from these values gives an indication of adulteration of milk fat (Kumar *et al,* 2009). The Complete liquefaction time (CLT) test of the fat sample is defined as time taken by solidified fat sample to get melted completely at 44 ^{o}C. Pure ghee sample of cow and buffalo show CLT in the range of 2 min-12 sec to 3 min-15 sec. Any deviation from these values gives an indication of adulteration of milk fat (Kumar, 2008). The Crystallization time test is defined as time taken for the onset of crystallization of milk fat at 17 ^{o}C when dissolved in a solvent solvent mixture consisting of Acetone and Benzene (3.5:1.0). Pure ghee sample of cow and buffalo show crystallization time in the range of 6 min-30 sec to 16 min-20 sec. Any deviation from these values gives an indication of adulteration of milk fat (Kumar, 2008).

Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of cow and buffalo pure ghee and ghee from organized and unorganized dairies are described in Tables 4.15 to 4.23

4.2.1 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of pure ghee and market ghee (organized and unorganized) samples in rainy season (August) for different regions.

Tables 4.15 to 4.17 depicts the Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of cow and buffalo pure ghee and ghee from organized and unorganized dairies.

Table4.15 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of pure cow and buffalo ghee in rainy season (August).

Pure ghee	Apparent solidification time (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time at 17 ^{o}C (Min-Sec)
Cow ghee	3-10±0-04	2-47±0-07	8-00±1-00
Buffalo ghee	2-39±0-04	2-53±0-06	7-28±1-12

Data represent mean ± S.E. of three determination

In rainy (August) season (Table 4.15), the range (with average) of AST value, CLT value, Crystallization time value for pure ghee of institute herd of cows were respectively from 3-05 to 3-15 (3-10) min-sec, 2-35 to 2-58 (2-57) min-sec,and 6-00 to 9-00 (08-00) min-sec. The corresponding values for pure ghee of institute herd of buffaloes were respectively 2-30 to 2-48 (2-39) min-sec, 2-41 to 2-54 (2-53) min-sec, and 5-06 to 9-05 (7-28) min-sec.

The results on AST, CLT and Crystallization time observed in the present study in rainy season are broadly in agreement with the general values of AST (2 min-30 sec to 3 min-26 sec), CLT (2 min-12 sec to 3 min-15 sec) and Crystallization time (6 min-30 sec to 16 min-20 sec) reported for cow and buffalo ghee by earlier workers (Kumar, 2008; Kumar *et al*, 2009).

Table 4.16 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of ghee from organized dairies in rainy season (August).

Ghee from different regions of country	Apparent solidification time (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time at 17 ^{o}C (Min-Sec)
N-1	2-30	3-45	4-20
N-2	2-41	3-50	4-55
N-3	2-07	4-35	4-25
S-1	2-39	2-25	7-10
S-2	4-20	2-35	10-20
S-3	2-45	2-40	7-30
W-1	> 5-0	1-15	>20-00
W-2	3-35	3-18	6-20
W-3	>5-0	2-55	6-30
E-1	2-20	3-10	6-25
E-2	3-10	2-34	20-00
E-3	2-07	3-25	4-30

N- sample from north region, S- sample from south region, W- sample from west region,E- sample from east region

It can be seen from Table 4.16 that the AST values of market ghee samples from organized dairies in rainy season for all the four regions varied from 2-07 min-sec to more than 5-00 min-sec. Out of twelve samples, only five samples (42% cases) were within the normal AST range (2 -30 to 3 -26 min-sec) reported for cow and buffalo ghee. Two samples of western region even showed very high AST time of more than five minutes. These two samples had shown very low RM values as reported earlier in section 4.1 (Table 4.2) for the sample of western region. Even third sample of the same

region which had shown low RM value also exhibited the AST value outside the normal range. Similarly, one sample of eastern region which had failed for RM value also exhibited the abnormal AST value of 2-07 min-sec which is below the normal range. It was observed in general that samples which had abnormal AST values also had abnormal physico-chemical constants in most of the cases.

The CLT values of market ghee samples of rainy season from organized dairies of all the regions varied from 1-15 to 4-35 min-sec. Out of the twelve samples, only 50% samples were within the normal CLT range (2 -12 to 3 -15 min-sec).

The crystallization time of market ghee samples of organized dairies in rainy season from different regions varied from 4-20 to more than 20-00 min-sec. Out of twelve samples, only four samples (33% cases) were within the normal reported range 6 -30 sec to 16 -20 min-sec. One sample of western region which had earlier shown low RM value, even showed crystallization time of more than 20 minutes.

In general, it was observed that such samples which had abnormal CLT and abnormal crystallization time, coinciding with abnormal AST value, had also shown abnormal physico-chemical constants.

The AST, CLT and Crystallization time of market ghee samples of unorganized dairies of Karnal city in rainy season (Table 4.17) varied from 1-26 to 3-35 min-sec, 2-30 to 5-20 min-sec and 1-00 to 20-00 min-sec, respectively. Out of ten samples, only five samples (50% cases) showed AST in normal range (2 -30 to 3 -26 min-sec), only four samples (40% cases) showed CLT in normal range (2 -12 to 3 -15 min-sec) and only one sample(10% cases) showed normal crystallization time (6-30 to 16-20 min-sec). It was observed that only one sample which had normal AST, CLT and crystallization time values, also had normal physico-chemical constants. Rest all other nine samples did not match their corresponding physico-chemical constants.

Table 4.17 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of ghee from unorganized dairies (Karnal city) in rainy season (August).

Ghee from unorganized dairies of Karnal city	Apparent solidification time (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time test at 17 ^{o}C (Min-Sec)
NOD-1	1-26	5-20	1-00
NOD-2	2-50	3-15	8-33
NOD-3	2-10	3-20	1-00
NOD-4	3-35	3-40	20-00
NOD-5	2-30	3-30	3-30
NOD-6	2-40	3-15	1-20
NOD-7	3-00	3-30	4-35
NOD-8	2-40	3-15	4-20
NOD-9	2-04	3-40	2-10
NOD-10	2-15	2-30	20-00

NOD- Non-organized dairies/Unorganized dairies

4.2.2 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of pure ghee and market ghee (organized and unorganized) samples in Winter season (January) for different regions.

The results on Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of cow and buffalo pure ghee and ghee from organized and unorganized dairies are described in Tables 4.18 to 4.20.

Table 4.18 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of pure cow and buffalo ghee in winter season (January).

Pure ghee	Apparent solidification time (AST) at 18 °C (Min-sec)	Complete liquefaction time (CLT) at 44 °C (Min-sec)	Crystallization time at 17 °C (Min-sec)
Cow ghee	3-06±0-04	2-17±0-01	12-25±1-12
Buffalo ghee	2-34±0-05	2-37±0-02	8-38±0-03

Data represent mean ± S.E. of three determination

In winter (January) season (Table 4.18), the range (with average) of AST value, CLT value, and Crystallization time value for pure ghee of institute herd of cows were respectively from 2-58 to 3-13 (3-06) min-sec, 2-15 to 2-20 (2-17) min-sec, 10-40 to 11-50 (12-25) min-sec. The corresponding values for pure ghee of institute herd of buffaloes were respectively 2-25 to 2-43 (2-34) min-sec, 2-35 to 2-39 (2-37) min-sec, and 4-15 to 9-20 (8-38) min-sec.

The results on AST, CLT and Crystallization time observed in the present study in winter season for laboratory made pure cow and buffalo ghee samples are broadly in agreement with the general values of AST (2 min-30 sec to 3 min-26 sec), CLT (2 min-12 sec to 3 min-15 sec) and Crystallization time (6 min-30 sec to 16 min-20 sec) reported for cow and buffalo ghee by earlier workers (Kumar, 2008; Kumar *et al*, 2009).

Table 4.19 shows that the AST values of market ghee samples from organized dairies in winter season for all the four regions varied from 1-35 min-sec to more than 5-00 min-sec. Out of twelve samples, only three samples (25% cases) were within the normal AST range (2 -30 to 3 -26 min-sec) reported for cow and buffalo ghee. One sample of western region even showed very high AST time of more than five minutes while another one sample of same region showed appreciably low AST value 1-35 min-sec. These two samples had shown very low RM values as reported earlier in section 4.1 (Table 4.5) for the samples of western region. Similarly, one

Table 4.19 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of ghee from organized dairies in Winter season (January).

Ghee from different regions of country	Apparent solidification time test (AST) at 18 °C (Min-sec)	Complete liquefaction time test (CLT) at 44 °C (Min-sec)	Crystallization time test at 17 °C (Min-sec)
N-1	2-40	2-18	13-28
N-2	2-07	2-40	11-35
N-3	2-13	2-30	15-30
S-1	2-40	2-55	7-25
S-2	2-19	2-48	8-10
S-3	2-21	2-40	12-42
W-1	2-20	2-05	4-10
W-2	1-35	> 5 mins	1-00
W-3	> 5	2-12	1-45
E-1	2-24	3-00	5-20
E-2	3-12	2-50	5-45
E-3	2-11	2-15	5-10

N- sample from north region, S- sample from south region, W- sample from west region,E- sample from east region

sample of eastern region which had failed for RM value also exhibited the abnormal AST value 2-11 min-sec which is below the normal range. It was observed in general that samples which had abnormal AST values also had abnormal physico-chemical constants in most of the cases. Almost similar type of observation have been made earlier for rainy season for the sample of these regions.

The CLT values of market ghee samples of winter season from organized dairies of all the regions varied from 2-04 to more than 5 min. Out

of the twelve samples, nearly 80% samples were within the normal range (2-12 to 3 -15 min-sec).

The crystallization time of market ghee samples of organized dairies in winter season from different regions varied from 1-00 to more than 20-00 min-sec. Out of twelve samples, only four samples (33% cases) were within the normal reported range (6-30 sec to 16 -20 min-sec). Two samples of western region which had earlier shown low RM value of 9.20 and 8.50 exhibited crystallization time of 1-00 and 1-45 min-sec, respectively.

In general, here also it was observed that such samples which had abnormal CLT and abnormal crystallization time, coinciding with abnormal AST value, also showed abnormal physico-chemical constants.

Table 4.20 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of ghee from unorganized dairies(Karnal city) in Winter season (January).

Ghee from unorganized dairies of Karnal city	Apparent solidification time (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time at 17 ^{o}C (Min-Sec)
NOD-1	2-30	3-30	6-20
NOD-2	2-10	4-20	4-10
NOD-3	2-18	3-35	6-15
NOD-4	2-10	3-15	6-10
NOD-5	1-25	4-05	1-00
NOD-6	1-50	3-20	5-40
NOD-7	2-30	2-55	4-35
NOD-8	2-15	3-15	4-45
NOD-9	2-10	3-00	6-30
NOD-10	2-25	2-40	6-35

NOD- Non-organized dairies/Unorganized dairies

The AST, CLT and Crystallization time of market ghee samples of unorganized dairies of Karnal city in winter season (Table 4.20) varied from 1-25 to 3-30 min-sec, 2-40 to 4-20 min-sec, and 1-00 to 6-35 min-sec, respectively. Out of ten samples, only two samples (20% cases) showed AST in normal range (2 -30 to 3 -26 min-sec), only five samples (50%) showed CLT in normal range (2 -12 to 3 -15 min-sec) and only two samples (20%) showed normal crystallization time (6-30 to 16-20 min-sec).

4.2.3 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of pure ghee and market ghee (organized and unorganized) samples in Summer season (May) for different regions.

Tables 4.21 to 4.23 show the Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time tests of cow and buffalo pure ghee and ghee from organized and unorganized dairies.

Table 4.21 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of pure cow and buffalo ghee in summer season (May).

Pure ghee	Apparent solidification time (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time at 17 ^{o}C (Min-Sec)
Cow ghee	3-28±0-40	3-02±0-41	15-43±0-47
Buffalo ghee	2-40±0-03	3-12±0-04	11-20±0-37

Data represent mean ± S.E. of three determination

In rainy (August) season (Table 4.21), the range (with average) of AST value, CLT value, Crystallization time value for pure ghee of institute herd of cows were respectively from 3-20 to 3-35 (3-28), 2-56 to 3-10 (3-02), and 14-35 to 17-14 (15-43). The corresponding values for pure ghee of institute herd of buffaloes were respectively 2-36 to 2-45 (2-40), 3-06 to 3-20 (3-12), and 10-18 to 12-28 (11-20).

The result on AST, CLT and Crystallization time for laboratory made pure cow and buffalo ghee samples of summer season observed in the

present study are in broad agreement with the general value of AST (2 min-30 sec to 3 min-26 sec), CLT (2 min-12 sec to 3 min-15 sec) and Crystallization time (6 min-30 sec to 16 min-20 sec) reported for cow and buffalo ghee by earlier workers (Kumar, 2008; Kumar *et al*, 2009).

Table 4.22 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of ghee from organized dairies in summer season (May).

Ghee from different regions of country	Apparent solidification time test (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time test (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time test at 17 ^{o}C (Min-Sec)
N-1	2-32	2-20	15-29
N-2	2-35	2-55	14-50
N-3	2-40	3-12	9-13
S-1	2-29	3-10	13-13
S-2	2-36	3-14	8-38
S-3	2-40	2-58	8-40
W-1	3-23	2-50	15-26
W-2	2-45	2-34	16-18
W-3	2-33	2-40	15-22
E-1	2-45	2-16	15-10
E-2	2-33	2-34	13-39
E-3	2-50	3-11	12-48

N- sample from north region, S- sample from south region, W- sample from west region,E- sample from east region

From Table 4.22 it can be seen that the AST values of market ghee samples from organized dairies in summer season for all the four regions varied from 2-29 min-sec to more than 2-50 min-sec. Out of twelve samples, nearly 92% samples were within the normal AST range (2 -30 to 3 -26 min-sec) reported for cow and buffalo ghee. It was observed in general that

samples which had normal AST values also had normal physico-chemical constants in most of the cases.

The CLT values of market ghee samples of summer season from organized dairies of all the regions varied from 2-16 to 3-14 min-sec. All the twelve samples were within the normal CLT range of 2 -12 sec to 3 -15 min-sec.

The crystallization time of market ghee samples of organized dairies in summer season from different regions varied from 8-38 to 16-18 min-sec. Like CLT, all the twelve samples were within the normal reported range of 6 -30 sec to 16 -20 min-sec.

In general, it was observed that most of the samples which had normal CLT and normal crystallization time, coinciding with normal AST value, also showed normal physico-chemical constants.

The AST, CLT and Crystallization time of market ghee samples of unorganized dairies of Karnal city in summer season (Table 4.23) varied from 2-05 to more than 5-00 min-sec, 1-27 to 2-58 min-sec, and 7-50 to more than 20-00 min-sec, respectively. Out of ten samples, two samples (20%) showed AST in normal range (2 -30 to 3 -26 min-sec), five samples (50%) showed CLT in normal range (2 -12 sec to 3 -15 min-sec) and three samples (30%) showed normal crystallization time (6-30 to 16-20 min-sec).

Table 4.23 Apparent solidification time (AST) test, Complete liquefaction time (CLT) test and Crystallization time test of ghee from unorganized dairies in summer season (May) of Karnal city.

Ghee from unorganized dairies of Karnal city	Apparent solidification time (AST) at 18 ^{o}C (Min-Sec)	Complete liquefaction time (CLT) at 44 ^{o}C (Min-Sec)	Crystallization time at 17 ^{o}C (Min-Sec)
NOD-1	2-18	2-50	14-00
NOD-2	2-27	2-46	7-50
NOD-3	2-05	2-58	11-45
NOD-4	3-20	2-05	>20
NOD-5	4-45	2-08	>20
NOD-6	2-25	2-30	>20
NOD-7	>5	2-00	>20
NOD-8	2-10	2-17	>20
NOD-9	>5	1-27	>20
NOD-10	3-05	1-37	>20

NOD: Non-organized dairies/Unorganized dairies

4.3 Color and turbidity based tests for detection of adulteration in ghee

This section delineates the Baudouin test for detection of vanaspati, rapid color based test for detection of vegetable oil, detection of rice bran oil on the basis of tracer component (γ-oryzanol) and turbidity test for detection of mineral oil. The results based on the above parameters for the ghee samples organized and unorganized dairies are discussed below.

It was observed that Baudouin test (Plate-1), rice bran oil test (Plate-2) and mineral oil test (Plate-3) were negative in all the ghee samples of organized dairies from different regions as well as unorganized dairies of Karnal city for all the seasons studied. However, rapid vegetable oil detection test (Plate-4 & 5) was positive for six samples of organized dairies and five

samples of unorganized dairies in rainy season (August). Similarly, four samples of organized dairies and five samples of unorganized dairies showed positive test for vegetable oil in winter (January). Likewise, in summer also three samples of organized dairies and five samples of unorganized dairies showed positive test for vegetable oil.

4.4 Thin layer chromatography of unsaponifiable matter isolated from ghee samples

In the past, a few successful attempts have been made to detect the adulteration of milk fat on the basis of minor components such as unsaponifiable constituents (Sharma, 1989). The level of unsaponifiable matter (USM), being present in the range of 0.0 to 2.0% in vegetable oils, 0.0 to 1.0% in animal body fats (Hamilton and Rossel, 1986) and 0.3 to 0.45% in milk fat (Jenness and Patton) cannot form the basis of adulteration detection. However the presence of minor constituents in the USM of vegetable oils and fats and their absence in that of milk fat, which can be detected by some chromatographic technique, may be the right choice to check such adulteration.

Thin Layer Chromatography (TLC) of USM extracted from pure ghee (cow and buffalo), market ghee samples (from organized and unorganized dairies), vegetable oil (sunflower oil), and body fat (pig body fat) was carried out and the typical chromatograms depicting the separation of unsaponifiable constituent (mainly sterol) of some samples along with standards are shown in Plates 6 to 9.

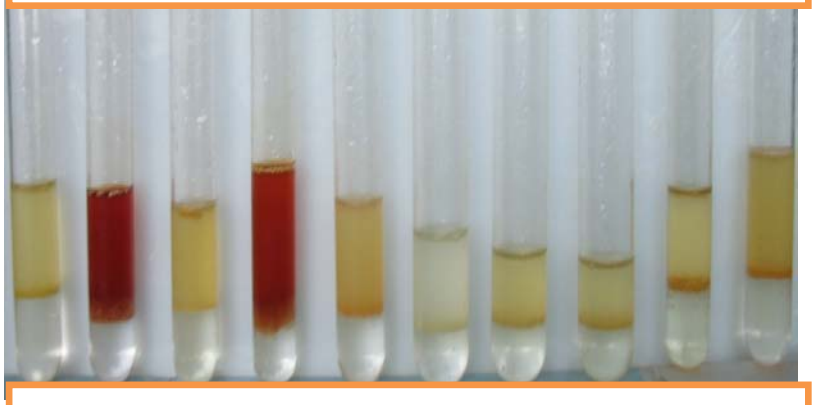
A comparison of the bands of the standard cholesterol, stigmasterol, ergosterol, pure cow ghee and buffalo ghee, vegetable oil, body fats and market ghee samples revealed that all the samples showed prominent band with similar distance moved from the spotting line as that of standard cholesterol (Plate-7 & 8). Similarly, stigmasterol and ergosterol showed very little difference with cholesterol in respect of their distance covered from the spotting line. It was observed that pure vegetable oil showed more number of bands than pure cow and buffalo ghee, and animal body fat (Plate-8). It was also observed that some market ghee samples also showed the appearance

Plate-1 Baudouin test for detection of vanaspati in ghee

1 2 3 4 5 6 7 8 9 10

1-Pure cow ghee (-ve control),2- Cow ghee (+ve control),3- Buffalo ghee (-ve control),4- Buffalo ghee (+ve control), 5,6,7, 8, 9 and 10 are Market ghee samples.

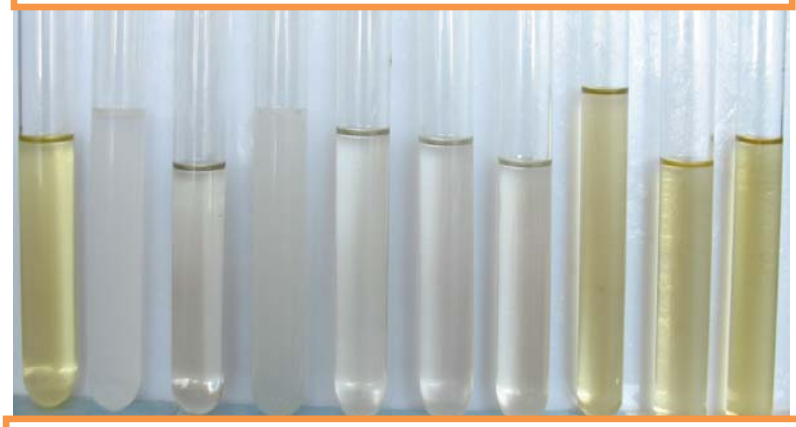
Plate-2 Colorimetric rice bran oil test



1 2 3 4 5 6 7 8 9 10

1-Pure cow ghee (-ve control),2- Cow ghee (+ve control),3- Buffalo ghee (-ve control),4- Buffalo ghee (+ve control), 5,6,7, 8, 9 and 10 are Market ghee samples.

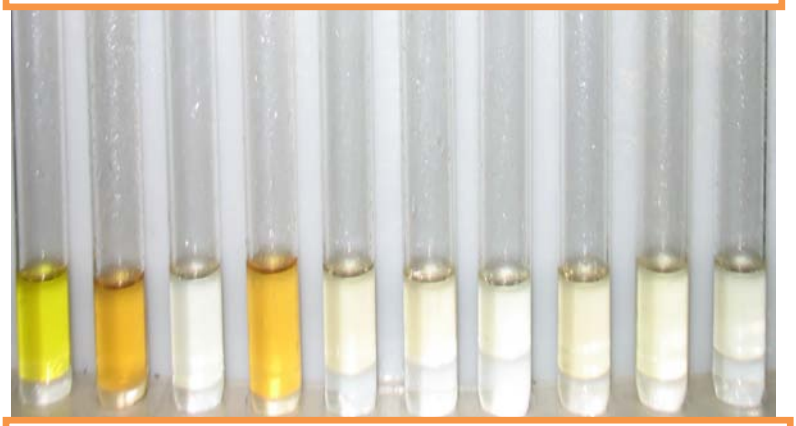
Plate-3 Mineral oil test



1 2 3 4 5 6 7 8 9 10

1-Pure cow ghee (-ve control),2- Cow ghee (+ve control),3- Buffalo ghee (-ve control),4- Buffalo ghee (+ve control), 5,6,7, 8, 9 and 10 are Market ghee samples.

Plate-4 Vegetable oil detection test



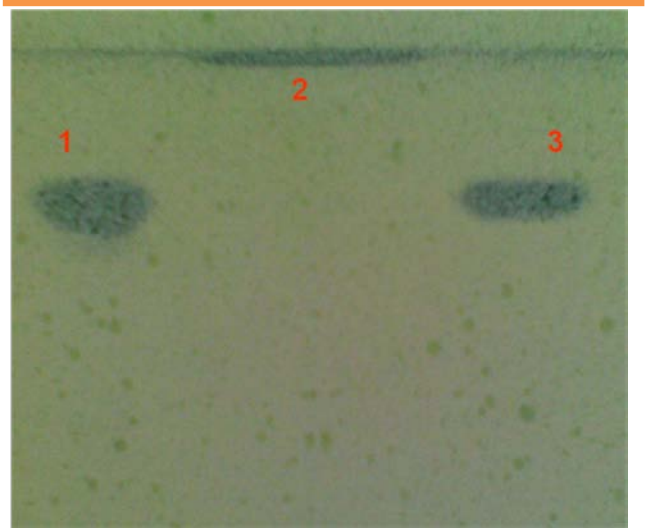
1 2 3 4 5 6 7 8 9 10

1-Pure cow ghee (-ve control),2- Cow ghee (+ve control),3- Buffalo ghee (-ve control),4- Buffalo ghee (+ve control), 5,6,7, 8, 9 and 10 are Market ghee samples.

Plate-5 Vegetable oil detection test

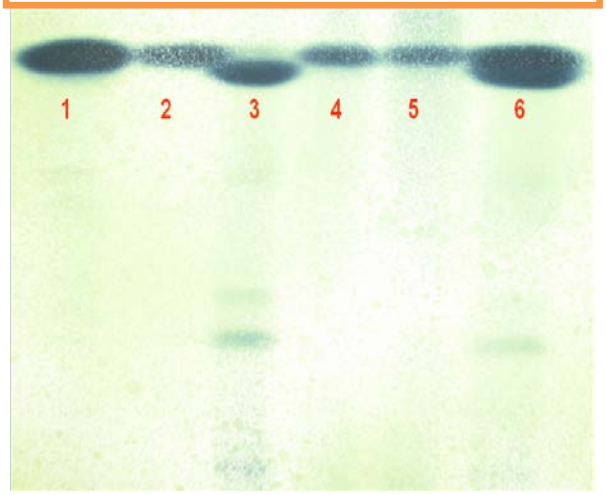
Market ghee samples: 1,2,3,4 shows –ve test
Market ghee samples: 5, 6,7,8,9 and 10 show +ve test

Plate- 6 TLC profile of standard substances



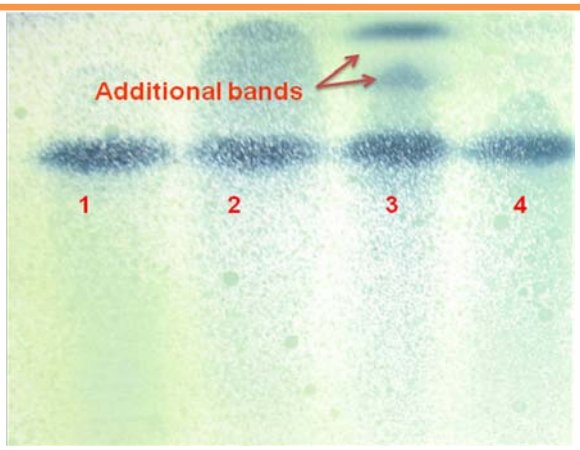
1–Cholesterol,2-Choleserolester, 3-stigmasterol

Plate- 7 TLC profile of standard substances and USM of pure ghee



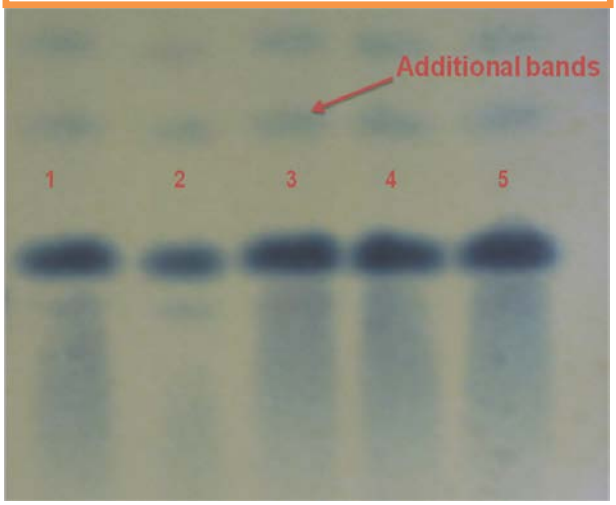
1 – Cholesterol, 2- Stigmasterol, 3- Ergosterol, 4- USM of pure cow ghee, 5-USM of pure buffalo ghee, 6-mixture

Plate- 8 TLC profile of USM of pure ghee, vegetable oil and animal body fat.



1-pure cow ghee, 2- pure buffalo ghee, 3- vegetable oil, 4- animal body fat

Plate- 9 TLC profile of USM of market ghee



1, 2, 3, 4, 5 → market ghee samples show the additional bands

additional bands as shown by the pure vegetable oil (Plate-9). Those samples which have shown high iodine value and BR reading and also positive vegetable oil detection test were found to have additional bands in the TLC profile in most of the cases.

The cholesterol ester showed a separate band which moves with solvent front at a distance higher than that of standard cholesterol (as shown in Plate-6). Although milk fat contain 10 to 15% of the cholesterol as cholesterol ester, but ghee sample have not shown a separate band of cholesterol ester (as shown in Plate-7 & 8), because during saponification of the sample, the latter get converted into its alcoholic form i.e. cholesterol. Recently, Kumar *et al*, (2005) has reported that the additional bands on the thin layer chromatogram only in vegetable oils and their absence in milk fat may form the basis for detection of vegetable oil added to ghee, which corroborates our observation on the additional bands appearing in case of pure vegetable oil (Plate-8) and in some of the market ghee samples showing high Iodine value and positive vegetable oil test (Plate-9).

4.5 GENERAL DISCUSSION

In the present study, it was observed that for the laboratory made pure ghee samples of all the seasons (rainy, winter and summer), the average RM value and saponification value were higher in buffalo ghee than in cow ghee. Whereas, the average Polenske value, iodine value and BR reading were lower in buffalo ghee than in cow ghee.

Higher RM value and saponification value while lower Polenske value, Iodine value and BR reading in laboratory made pure buffalo ghee than laboratory made pure cow ghee, as observed in the present study, may be attributed to the species characteristics, since the study was carried out under identical conditions of feeding and management. Similar kind of relationship in physico-chemical constants of pure cow ghee as well as buffalo ghee have also been reported by earlier workers (Rangappa and

Achaya, 1974; Bector and Narayanan, 1974; Lal and Narayanan, 1984; Kumar, 2008) which corroborate our observations.

The study on physico-chemical constants of ghee samples of organized dairies of all the four regions has revealed that out of 36 branded market ghee samples, 8 samples (22%) in total (comprising four in rainy season, three in winter season, and one in summer season) failed to meet the legal requirements in terms of either RM value or BR reading or both. Similarly, out of 30 ghee samples of unorganized dairies of Karnal city, 8 samples (27%) in total (comprising two in rainy, four in winter, and two in summer) also failed to meet the legal specification in respect of either RM value or BR reading or both. The study also revealed that such cases were less in summer and more in rainy and winter season. This was probably because of the less demand in summer as compared to rainy and winter seasons. It was further observed that similar cases which failed to meet the PFA specifications were more in western region (5 out of 9 samples, 56%) and eastern region (3 out of 9 samples, 33% cases) as against none in the northern and southern region. The frequency of such cases was found to be 27% in unorganized dairies ghee samples of Karnal city as compared to 22% in organized dairies ghee samples of all the four regions (north, south, east and west) in three seasons (rainy, winter and summer) studied.

The deviation of some market ghee samples from the PFA standards in terms of either RM value or BR reading or both may be possibly because of the presence of foreign fats like vegetable oils and animal body fats. Some market ghee samples have shown low RM value as compared to that prescribed in PFA standards. RM value for milk fat ranges from 17 to 35, which is well above the value (generally 1) for all other fats and oils except coconut oil and palm kernel oil for which the value ranges between 4 to 8 (Singhal, 1973 & 1980). Therefore, this parameter has been oftenly employed as a criterion to differentiate milk fat from other oils and fats, and also to establish the purity of milk fat. RM value, which primarily measures steam volatile and water soluble fatty acids such as butyric ($C_{4:0}$) and caproic

($C_{6:0}$) acids, is a significant physico-chemical constant, as these short chain fatty acids are found only in milk fat and not in animal body fats and vegetable oils.

Some market ghee samples have shown high BR reading at 40 ^{o}C as compared to that prescribed in PFA standards. Under the PFA specifications, BR reading of pure ghee varies from 40.0 to 43.0 for most of the areas, whereas the values may lie between 41.5 and 45.0 for some notified cotton tract areas (Appendix-l). Literature shows that the values for BR reading for animal body fats (44 to 51) and vegetable oils (above 50) are higher than milk fats (Kumar *et al,* 2002). The higher BR reading observed in case of some market ghee samples may be again because of the possibility of presence of animal body fats and vegetable oils.

As reported above for RM and BR reading, some market ghee samples also showed deviations in Polenske value, saponification value and iodine value from their normal range of values for pure milk fat. Lower Polenske and saponification values and higher Iodine value observed in case of some market ghee samples may be again possibly because of the presence of some vegetable oils. The Polenske value, saponification value and Iodine value of milk fat are reported to fall in the general range of 1.2 – 2.4, 210 – 233 and 26 – 35, respectively. Polenske value for other oils and fats(singhal 1980; Winton and winton, 1999; Kumar *et al*., 2002) is also low (less than 1) except the coconut oil (15 – 20) and palm kernel oil (6 - 12). Saponification value for animal body fats, vegetable oils and hydrogenated fats ranges from 192-203, 170-197 and 197-199, respectively. Coconut oil and palm kernel oil show higher saponification value ranging between 243-263 (Jenness and Patton, 1969; Singhal, 1990; Kumar *et al.,* 2002). Animal body fats show slightly higher Iodine value ranging from 36-49. Whereas, for vegetable oils, the value is very high (74-145) except coconut oil (6-10) and palm kernel oil (10-18). For hydrogenated fats, it lies in the range of 70-79. Low Iodine value observed in very few market samples may be probably

because of the addition of coconut or palm oil, as both these oils have very low Iodine value.

Physico-chemical properties of market ghee of organized and unorganized dairies in Uttar Pradesh, Bombay, Bihar, Orissa, Calcutta and Kalyani have been studied by several workers (Singh and Singh, 1960; Sharma and Zariwala, 1978; Anon, 1983; Ghatak and Bandyopadhayay, 1989; Banerjee *et al.,* 2002) who have reported wide variations in RM value, Polenske value, saponification value, iodine value and BR reading. The RM value of ghee samples collected from different markets varied from 1.00 to 38.50, Polenske value varied from 0.2 to 3.2, saponification value varied from 174.70 to 246.83, Iodine value varied from 20.30 to 50.70 and BR reading varied from 40.10 to 49.10. The results obtained in the present study on market ghee samples of organized dairies from different region of the country and unorganized dairies of Karnal city are in general agreement with the above reports.

As observed for physico-chemical constants, deviations were also observed in case of some market ghee samples for other physico-chemical parameters like AST, CLT and crystallization time tests. The AST, CLT and crystallization time of milk fat are reported to fall in the general range of 2-30 to 3-26 min-sec, 2-12 to 3-15 min-sec and 6-30 to 16-20 min-sec, respectively. AST value for body fats (pig body fat and goat body fat) and hydrogenated vegetable oil is reported to vary between 0-40 to 1-50 min-sec, where as vegetable oils have no AST value. Recently, Kumar *et al* (2009) have reported that addition of body fats to milk fat can decrease the AST value of milk fat, whereas addition of vegetable oils can increase the AST value of milk fat. Similarly, Kumar (2008) has reported that the addition of vegetable oils (palm oil, rice bran and soyabean) can cause a decrease in CLT value of milk fat, whereas addition of body fats (buffalo, goat and pig) can result in an increase in CLT value of milk fat. Kumar (2008) also reported that the crystallization time of ghee samples increase when sample are adulterated with vegetable oil (palm oil, rice bran and soyabean) while it

decreases on adulteration with animal body fats (buffalo and goat). It is obvious from the above parameters that addition of mixture of body fats and vegetable oils can have a neutralizing effect on these physico-chemical parameters. However, partitioning of the sample into solid and liquid fractions on the basis of crystallization property, thereby enriching the solid fraction with the body fat and hydrogenated fats, and liquid fraction with vegetable oils, can be a useful proposition in this regard (Kumar, 2003; Kumar, 2008).

From the study, it is apparent that there are few samples of both organized dairies (all regions) as well as unorganized dairies (Karnal city) which have shown positive rapid vegetable oil detection test and also shown additional bands on the TLC chromatogram, indicating the presence of vegetable oil, but their Iodine value, BR reading and other physico-chemical constants were normal. This may be due to the possibility of adulteration with some vegetable oils like palm oil, coconut oil etc. which have BR reading and saponification value close to that of milk fats, and RM value and Iodine value less than that of milk fats. It is also possible that addition of these foreign fats to milk fats might be at lower level so that it escapes the legal network of parameters. Moreover, the wide variations in physico-chemical constants of the milk fat of two species (cow and buffalo) makes it vulnerable to adulteration by accommodating the small level of addition of such vegetable oils which can camouflage due to their resemblance with milk fat in some of the physico-chemical properties.

CHAPTER - 5

Summary and Conclusions

5.0 SUMMARY AND CONCLUSION

A study on the physico-chemical qualities of market ghee sold in the organized dairies of India and unorganized dairies of Karnal city was carried out. For this, 36 branded samples collected from organized dairies from different regions (North, South, East and West) in different season (rainy, winter and summer) and 30 samples collected from unorganized dairies of Karnal city along with laboratory made 18 samples of pure cow and buffalo ghee were analyzed for physico-chemical constants (RM value, Polenske value, Saponification value, Iodine value and BR reading at 40 ^{o}C) and other physico-chemical parameters like Apparent solidification time test at 18 ^{o}C, Complete liquefaction time test at 44 ^{o}C, Crystallization time test at 17 ^{o}C, Baudouin test for vanaspati, rapid vegetable oil detection test, Colorimetric test for rice bran oil, Turbidity test for mineral oil and TLC profile of unsaponifiable constituent.

The present study revealed that the RM value, Polenske value, Saponification value, Iodine value and BR reading of ghee samples of organized dairies collected from different zones (north, south, east and west) in different seasons (rainy, winter and summer) varied (with average) from 6.40 to 34.00 (24.98), 0.5 to 2.5 (1.39), 202.65 to 234.90 (222.88), 26.85 to 76.14 (39.29) and 40.50 to 51.85 (42.47), respectively. The corresponding values of ghee samples collected from unorganized dairies of Karnal city in different seasons (rainy, winter and summer) varied (with average) from 16.61 to 33.77 (29.57), 0.6 to 1.7 (1.28), 209.00 to 243.90 (228.85), 22.72 to 47.64 (33.02) and 38.80 to 44.60 (41.55), respectively. Similar values for pure cow and buffalo ghee (pooled together) collected simultaneously along with market ghee samples varied (with average) from 28.05 to 33.88 (30.86), 1.1 to 2.0 (1.61), 223.85 to 236.50 (229.72), 30.75 to 37.65 (33.43) and 40.60 to 42.50 (41.46), respectively. For pure ghee, RM value and Saponification value were higher while Polenske value, Iodine value and BR reading were lower in buffalo ghee than cow ghee.

Out of 36 branded market ghee samples of organized dairies from all the regions, 8 samples (22%) in total (comprising four in rainy season, three in winter season, and one in summer season) failed to meet the legal requirements in terms of either RM value or BR reading or both. Similarly, out of 30 ghee samples of unorganized dairies of Karnal city, 8 samples (27%) in total (comprising two in rainy, four in winter, and two in summer) also failed to meet the legal specification in respect of either RM value or BR reading or both. The study also revealed that such cases were less in summer and more in rainy and winter season, probably because of the less demand of ghee in summer as compared to rainy and winter seasons.

It was further observed that similar cases which failed to meet the requirements of PFA standards were more in western region (5 out of 9 samples, 56%) and eastern region (3 out of 9 samples, 33% cases) as against none in the northern and southern region. Further, it was observed that frequency of occurring of these types of cases was found to be 27% in ghee samples of unorganized dairies of Karnal city as compared to 22% in ghee samples of organized dairies of all the four regions (North, South, East and West) in all the three seasons studied.

From the study, it was observed that for Polenske value, 8 out of 36 ghee samples (comprising one in rainy, five in winter and two in summer, total of 22% cases) of organized dairies of all the four regions and 8 out of 30 ghee samples (comprising one in rainy, three in winter and four in summer, total of 27% cases) of unorganized dairies of Karnal city were not within the normal range (1.2 to 2.4). For saponification value, 5 out of 36 ghee samples (comprising one in rainy, four in winter and none in summer, total of 14% cases) of organized (all four regions) and 9 out of 30 ghee samples (comprising none in rainy, four in winter and five in summer, total of 30% cases) of unorganized dairy (Karnal city) ghee samples were not within the normal range (210 to 233). For Iodine value, 20 out of 36 ghee samples (comprising eleven in rainy and five in winter and four in summer, total of 56% cases) of organized dairy (all four regions) and 9 out of 30 ghee samples (comprising four in rainy, two in winter and three in summer, total of 30%

cases) of unorganized dairy (Karnal city) ghee samples were not within the normal range (26 to 35).

The study revealed that using AST test, 17 out of 36 ghee samples (comprising seven in rainy, nine in winter and one in summer, total of 47% cases) of organized dairies of all the four regions and 21 out of 30 ghee samples (comprising five in rainy, eight in winter and eight in summer, total of 70 % cases) of unorganized dairies of Karnal city were not within the normal range (2 -30 to 3 -26 min-sec). For CLT, 16 out of 36 ghee samples (comprising six in rainy, ten in winter and none in summer, total of 44% cases) of organized (all four regions) and 16 out of 30 ghee samples (comprising six in rainy, five in winter and five in summer, total of 53 % cases) of unorganized dairies of Karnal city were not within the normal range (2 -12 sec to 3 -15 min-sec). For Crystallization time test, 14 out of 36 ghee samples (comprising eight in rainy, six in winter and none in summer, total of 39% cases) of organized dairy (all four regions) and 24 out of 30 samples (comprising nine in rainy, eight in winter and seven in summer, total of 80% cases) of unorganized dairies of Karnal city were not within the normal range (6-30 to 16-20 min-sec). In general, it was observed that those samples which had abnormal CLT and abnormal crystallization time, coinciding with abnormal AST value, had also shown abnormal physico-chemical constants.

Baudouin test, mineral oil test and γ-oryzanol test were negative in all the ghee samples. However, 13 out of 36 ghee samples of organized dairies of all the four regions and 19 out of 30 ghee samples of unorganized dairies of Karnal city showed positive vegetable oils detection test.

Few ghee samples of organized dairies (all four regions) and unorganized dairies have shown additional band on TLC chromatogram when compared with that of pure ghee indicating the presence of vegetable oil. But these samples showed normal Iodine value, BR reading and other physico-chemical constants. Possibly vegetable oils like palm oil and coconut oil might be added which have some physico-chemical properties close to that of milk fat. Hence, TLC is a useful tool for detecting such adulteration.

Form the study of market ghee samples of organized dairies from different regions of the country and unorganized dairy ghee samples of Karnal city, it may

be inferred that legal parameters like RM value and BR reading are not sufficient enough to check the purity of milk fat and there is a need to consider for inclusion of other physico-chemical parameters like Apparent Solidification Time (AST) test, Complete Liquefaction Time (CLT) test, Crystallization time test and TLC profile of unsaponifiable matter under the legal rules, so as to have wider sense on the status of milk fat purity, before declaring the sample as adulterated.

At the end, it may be concluded from the present study that out of the total (66) market ghee samples (organized and unorganized), 24% samples failed to meet the requirements in terms of either RM value or BR reading or both as specified under PFA rules. Such cases were less in summer and more in rainy and winter seasons. Similarly, such cases were more in western region and eastern region as against none in the northern and southern region. Frequency of such cases was more in unorganized dairy ghee samples of Karnal city than organized dairy ghee samples of all the four regions of the country investigated in the present study. However, at the end it may be suggested that since the number of samples analyzed in the study were small because of certain limitations, therefore no serious consequences at the country level should be drawn from the present investigation in terms of frequency of samples not meeting the legal requirements or other physico-chemical parameters with respect to any season or region of the country.

Bibliography

BIBLIOGRAPHY

Achaya, K.T. and Banerjee, B.N. 1946. The fatty acid and glyceride structures of Indian buffalo milk and depot fats, and some characteristics of Eastern animal fats. *Biochem. J.* **40**: 661-669.

AGMARK.1981. Ghee Grading and Marking Rules, 1938 (as amended). Government of India, Ministry of Food and Agriculture, Department of Agriculture, New Delhi.

Akoh, C.C. and Min, D.B. 1998. Food lipids. Chemistry, Nutrition and Biotechnology, Marcel Dekker, Inc.

Alam, T. 2005. Facts and figures of dairy industry. In G. Chakraborthy, Indian Dairy Industry. pp. 11-23. Published by capital offset, Navin Shahdara, Delhi.

Ali, M. and Tremazi, S.A. 1966. A study on the effect of adulteration on butterfat constants. *Pakistan J. Sci.*, **18:** 124-129.

Amelotti, G., Brianza, M. and Lodigiano, P. 1983. Application and limits of differential scanning calorimetry to the detection of beef suet in butter. *Rivista Italiana delle Sostanze Grasse*, **60**(9): 557-564. [Cited: *Dairy Sci. Abstr.* (1984), 46 (4): 2480].

Amit Kumar. 2008. Detection of Adulterants in ghee. *Ph.D. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Amit Kumar, Lal, D. Sharma, V., Raman Seth. 2009. A Simple Colorimetric test for detection of Rice bran oil in Ghee. *Indian J. Dairy Sci.*, **62**(1): 71-73.

Aneja, R.P., Mathur, B.N., Chandan, R.C., Banerjee, A.K. 2002. *Technology of Indian Milk Product.* A Dairy India Publication, New Delhi, India.pp.184-187.

Anon. 1983.ICAR Project report, Final report of the ICAR Scheme on Standardization of Industrial Practices for Manufacturing Ghee– Haringhata Center. .[Cited by Banerjee *et al.,* 2002]

Antila, V., Luoto, K. and Antila, M. 1965. Detection of adulteration of milk fat by means of DSC. *Meijeritied Ackakausk*, **25**: 37-42. [Cited: *Dairy Sci. Abstr.* (1966), **28**: 356].

Antoniani, C. and Cerutti, G. 1954. Detection of isovaleric acid in fats in the presence of butyric acid and caproic acids. *Ann. Sper. Agr.*, **8**(3): 801-807 [Cited: *Dairy Sci. Abstr.* (1955), **17**(2): 167].

AOCS. 1959. Poly-unsaturated acids. *Official and Tentative Methods of the American Oil Chemist's Society, Illinois.*

Arlene Karan . 1998. Separation of oryzanol from crude rice bran oil. *M. Sc. Thesis* submitted to chemical engineering university, Toronto. (Cf: www.collectionscanada.ca/obj/s4/f2/dsk1/tape10/PQDD_0010/MQ40908.pdf).

Arora, K.L., Lal, D., Seth, R. and Ram, J. 1996. Platform test for detection of refined mustard oil adulteration in milk: A short communication. *Indian J. Dairy Sci.* **49**(10): 721-723.

Arora, S. and Rai, T. 1997. Milk fat fractions- properties and applications. A Review. *J. Dairying, Foods & Home Sci*. **16**(3): 143-155.

Arul, J., Boudreau, A., Makhlouf, J., Tardif, R. and Greneir, B. 1988. Distribution of cholesterol in milk fat fractions. *Journal of Dairy Research.* 55: 361-371.

Arumughan,C. and Narayanan, K.M. 1979. Grain formation in ghee (butterfat) as related to structure of triglycerides. *Journal of Food Science and Technology.* **16**: 242-247.

Arun Kumar, Lal, D., Seth, R. and Sharma, R. 2002. Recent trends in detection of adulteration in milk fat- A Review. *Indian J. Dairy Sci*. **55**(6): 319-330.

Arun Kumar, Lal, D., Seth, R. and Sharma, V. 2005 a. Detection of groundnut oil and vanaspati in ghee by thin layer chromatography. *Indian J. Dairy Sci.*, **58**(4): 250-252.

Arun Kumar, Lal, D., Seth, R. and Sharma, V. 2005 b. Turbidity test for detection of liquid paraffin in ghee. *Indian J. Dairy Sci.*, **58**(4): 298.

Arun Kumar, Lal, D. Sharma, V., Raman Seth. 2005. Detection of Groundant oil and Vanaspati in Ghee by Thin Layer Chromatography. *Indian J. Dairy Sci.*, **62**:pp. 71-73.pp.250-252.

Arun Kumar, Lal, D., Seth, R. and Sharma, V. 2009. Apparent solidification time test for detection of foreign oils and fats adulterated in clarified milk fat, as affected by season and storage. *International Journal of Dairy Technology* ., **62**: 33-38.

Arun Kumar. 2003. Comparative studies on physico-chemical properties of various oils and fats with a view to detect adulteration in ghee. *Ph.D. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Bailey, A.E. 1951. Industrial oil and fat products. Interscience Publishers Inc., New York.

Bailey, A.V., Harris, J.H. and Skau, E.L. 1966. Cyclopropenoid fatty acid content and fatty acid composition of crude oils from twenty-five varieties of cottonseed. *J. Amer. Oil Chemists Soc.* **43:** 107-110.

Baker, B.C. 1970. The fractionation of butter fat and the properties of selected fractions. *XVIII Int. Dairy Congr*., IE: 241.

Banerjee, S.,Sarkar.K., Ghatak, P.K., Ray, P.R. and Bandyopadyay.2002. Present status of ghee – A review. Beverage and Food world: 27-29.

Banfi, S., Bergna, M., Povolo, M. and Contarini, G. 1999. Programmable temperature vaporizer (PTV) applied to the TG analysis of milk fat. *J. High Resolution Chromatography.* **22:** 93-96.

Bank, W., Clapperton, J.L. and Girdler, A.K. 1985. On the fractional melting of milk fat and the properties of the fractions. *J. Sci, Food Agric.* 36: 421-432.

Barron, L.J.R., Hierro, M.T.G. and Santa-Maria, G. 1990. HPLC and GLC analysis of triglyceride composition of bovine, ovine and caprine milk fat. *J. Dairy Res.*, **57**(4): 517-526.

Bartlett, J.C. and Chapman, D.G. 1961. Detection of hydrogenated fats in butterfat by measurement of cis-trans conjugated unsaturation. *J. Agric. Food Chem.* **9:** 50-53 [Cited: *Dairy Sci. Abstr.* (1961), **23:** 1505].

Bector, B.S. and Sharma, V. 2002. Physico chemical characteristics of ghee adulterated with palm oil and its detection. *NDRI Annual Report*, pp. 44-45.

Bector,B.S. and Narayanan, K.M.1974. Effect of thermal oxidation on the physico-chemical constants of ghee. *Indian J. Dairy Sci.,* **27**: 90-93.

Bhalerao, V.R. and Kummerow, F.A. 1954. A method for the detection of foreign fats in dairy products. *J. Dairy Sci.* **37**(2)**:** 156-161.

Bhalerao, V.R. and Kummerow, F.A. 1956a. Modification of the refractive index method for the detection of foreign fats in dairy products. *J. Dairy Sci.* **39**(7): 947-955.

Bhalerao, V.R. and Kummerow, F.A. 1956b. A summary of methods for the detection of foreign fats in dairy products. *J. Dairy Sci.* **39**(7): 956-964.

Bhide, P.T. and Kane, J.G. 1952. Critical temperatures of dissolution of ghee and hydrogenated oils (vanaspati). *Indian J. Dairy Sci.*, **5**(4): 183-187.

BIS. 1981. Handbook of Food analysis, SP- 18, *Part XI- Dairy Products*. Bureau of Indian Standards. Manak Bhavan, New Delhi.

Boghra, V.R., Singh, S. and Sharma, R.S. 1981. Present status of the tests used for the detection of adulterants in ghee. *Dairy Guide*, **81:** 21-31.

Bolton, R.E. 1999. Oils fats and fatty foods: Their practical examination. 1st edn. Biotech Books Delhi. pp-22-301.

Boniforti, Z. 1962. Gas chromatographic study of the fatty acids of butter made in Italy and other countries; application to adulteration of commercial butter. *Ann. Falsif. Expert. Chim.*, Paris, **55**: 255-263.

Bottini, E. and Campanello, F. 1955. Detection of hydrogenated dolphin oil in butter by means of paper chromatography. *Ann. Sper. Agr.* **9**(1): 11-20. [Cf: *Dairy Sci. Abstr.* (1955), **17:** 345].

Chakrabarty, M.M., Bandhyopadhyay, C. and Gayen, A.K. 1968. Detection of adulteration of fats by TLC of trisaturated glycerides: Detection of hydrogenated groundnut oil, tallow and *mohua* (Mowrdh) oil in butterfat (ghee). *J. Chromatography*, **36**: 84-90.

Chakrabarty, M.M., Bhattacharya, D. and Gayen, A.K. 1969. Detection of adulteration of butterfat (ghee) by random rearrangement reaction and TLC. *J. Chromatography*, **44**: 116-127.

Chakrabarty, M.M., Bhattacharya, D., Gayen, A.K. and Chakrabarty, M.K. 1980. Argentation silica gel thin layer chromatographic detection of adulteration of butterfat (ghee). *Science and Culture*, **46**(9): 336-337.

Chen, M. H. and Bergman, C.J. 2005. A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. *J. Food Composition and Analysis*. 18. 139-151.

Chen, P.I.C. and deMan, J.M. 1966. Composition of milk fat fractions obtained by fractional crystallization from acetone. *Journal of Dairy Science*. 49: 612-616.

Chourasia, L.O., Patil, M.N. and Jaiswal, P.K. 1994. Detection of kokum fat in ghee by thin layer chromatography. *Journal of the Oil Technologists' Association of India.* **26**(4): 115.

Christie, W.W. 1970. Cyclopropane and cyclopropene fatty acids. In: Topics in Lipid Chemistry. Vol. 1. Edited by F.D. Gunstone, Logos Press, London. pp. 1-49.

Coleman, M.H. 1963. The pancreatic hydrolysis of natural fats. 3. The influence of the extent of hydrolysis on monoglyceride composition. *J. Amer. Oil Chemists Soc.*, **40**: 568-571.

Collomb M., Spahni M., Bühler T. 1998. Analysis of triglycerides II. Validation of a multilinear regression method for the determination of foreign fats in milk fat. Trav. Chim. Aliment. Hyg. **89** 75-83.

Collomb, M. 1997. Is it possible to determine non milk fats in milk fat? *Schweizerische Milchzeitung.* **123** (47): 7. [Cited in Dairy Sci. Abstr. (1998) 60: 6934].

Colombini, M., Amelotti, G. and Vanoni, M.C. 1978. Adulteration of butter with beef suet and lard: II. GLC of sterols and UV spectrophotometric analysis. *Rivista Italiana delle Sostanze Grasse*. **55(11)**: 356-361.

Coni, E., Pasquale, M.D., Coppolelli, P. and Bocca, A. 1994. Detection of animal fats in butter by differential scanning calorimetry: A pilot study. *J. Amer. Oil Chemists Soc.* **71**(8): 807-810.

Contarini, G., Leardi, R., Pezzi, C. and Toppino, P.M. 1993. Evaluation of butter purity by analysis of triacylglycerols and fatty acids, *Rivista Italiana Delle Sostanze Grasse.* **70**(10): 491-499. [Cited in Dairy Sci. Abstr. (1994) 56: 7883].

D'Arrigo, G. 1955. Identification of traces of isovaleric acid in butter adulterated with hydrogenated dolphin oil. *Olii. Min.* **32**(1): 2-7. [*Cited: Dairy Sci. Abstr.*, (1955), **17**: 9687].

DasGupta, S. M. 1939. Influence of dietary carotene on the carotene and vitamin A contents and Reichert and iodine value of butterfat with special reference to seasonal variations. *Indian J. Vet. Sci.*, 9: 249-266.

De, Sukumar. 1996. Indian dairy products. In *Outlines of Dairy Technology*. Oxford University Press, New Delhi. pp. 382-466.

Deffense, E. 1993. Milk fat fractionation today: A review. *JAOCS*. 70 (2): 1193-1201.

Delforno, G. 1964. Detection of foreign fats in butter using the crismer test. *Latte*, **38**(11): 921-924.

DeMan, J.M. 1961. Physical properties of milk fat. I. Influence of chemical modification. *J. Dairy Res.* **28**: 81-86.

Den Herder, P.C. 1955. Detection of adulteration of butter with foreign fats by examination of the sterols. *Neth. Milk Dairy J.* **9**: 261-274.

DeRuig, W.G. 1968. The detection of foreign fat in milk: Infra-red spectroscopy of Dutch butterfat. *Neth. Milk Dairy J.* **22**(3): 104-113.

Doctor, N.S., Bajerjee, B.N. and Kothavalla, Z.R. 1940. Studies on some factors, affecting the physical and chemical constants of ghee. *Indian J. Vet. Sci.* **10**: 63.

Eckizen, A. and Deki, M. 1976. Reports of the Central Customs Lab., No.16: 67. Kobe Customs Lab., Ikertaku, Kobe-Shi, Japan.

Farag, R.S., Abo-Raya, S.H., Ahmed, F.A., Hewedi, F.M. and Khalifa, H.H. 1983. Fractional crystallization and gas chromatographic analysis of fatty acids as

a means of detecting butterfat adulteration. *J. Amer. Oil Chemists Soc.* **60**(9): 1665-1669.

Firestone, D. and Villadelmar, M.D.L. 1961. Determination of isolated trans unsaturation by infra-red spectrophotometry. *J. Ass. Of. Agri. Chem.* **44**(3): 459-464.

Fox, P. F. and. McSweeney, P. L. H. 1998. Milk lipids. In *Dairy Chemistry and Biochemistry*. Blackie Academic Professional/Chapman and Hall, New York. Pp. 67–149

Fox, P.F. 1995. Advanced Dairy Chemistry. 2. Lipids. 2nd Edn. Chapman & Hall., London, New York and Madras.

Francesco, F.D. and Avancini, D. 1961. The adulteration of butter with trans-esterified fats. *Latte*, **35**: 809-817.

Francesco, F.D., Avancini, D., Michelotti, P.G., Stefani, R. and Savoia, M. 1971. Unsaponifiable matter in butter. *Scienza Aliment.* **17**(2): 31-43. [Cited: *Dairy Sci. Abstr.* (1971), **33**: 4825].

Ganguli, N.C. and Jain, M.K. 1972. Ghee: its chemistry, processing and Technology. *J. Dairy Sci.* **56(1)**: 19-25.

Ghatak, P.K. and Bandyopadhyay, A.K. 1989. Indian Dairyman. **41**: 515.[Cited by Banerjee et al, 2002]

Gunstone, F.D., Harwood, J.L. and Padley, F.B. 1994. *The lipid handbook*, 2nd edn., Chapman & Hall, London.

Gupta, P.R. Dairy India 2007.Edition six.pp.32-63.

Gurr, M.I., Harwood, J.L. and Frayn, K.N. 2002. Lipid biochemistry, 5th Edn. Blackwell Science Ltd., UK.

Guyot, A.L. 1978. Detection of animal body fats in milk fat by triglyceride gas liquid chromatography. *20th Int. Dairy Congr., Vol.E*, pp. 400.

Hamilton, R.J. and Rossel, J.B. 1986. Analysis of oils and fats. Elsevier Applied Science Publishers, London.

Harper, W.J. and Armstrong, J.V. 1954. Measurement of butyric acid in fat with reference to the detection of substitute fats in dairy products. *J. Dairy Sci.* **37**: 481-487.

Hendrickx, H. and Huyghebaert, A. 1968. Detection of substitution fats containing intersterified fats, in factory butter by thin layer chromatography. *Meded. Rijksfac. Landb Wet. Gent*, **33(2):** 331-346. [Cited *Dairy Sci. Abstr*.(1969), **31**: 3148]

Hendrickx, H. and Huyghebaert, A. 1970. Butterfat and substitute fats - some new aspects. *The Milk Industry*, pp.18-23.

Homberg, E. and Bielefeld, B. 1979. Detection of vegetable fats in butterfat by gas chromatographic analysis of sterols. *Zeitschrift fur Lebensmittel-Untersuchung und Forschung*, **169**(6): 464-467. [Cited: *Dairy Sci. Abstr.* (1980), **40**: 3092].

http://www.cncahealth.com/health-notes.htm?ContentID=2850009(γ-Oryzanol)

http://www.mcxindia.com/products_ricebranref_oil.html, 2006

IDF. 1965. Detection of vegetable fat in milk fat by phytosteryl acetate test. *FIL-IDF*, **32.**

IDF. 1966. Detection of vegetable fat in milk fat by thin layer chromatography of steryl acetates. *FIL-IDF*, **38**.

India's milk production (http://www.highbeam.com/doc/1G1-188920729.html)

Iqbal, S.; Bhanger, M.I. and Anwar, F. 2005. Antioxidant properties of some commercially available varieties of rice bran in Pakistan. *Food Chemistry*. 93. 265-272.

Javeed, S. I., Rathi, S.D. and Ingle, V.M. 1986. Physico-chemical constants of ghee prepared from Deoni cow, crossbred cow and Surti buffalo milk. *Indian J. Dairy Sci*. 39: 491-492.

Jenness, R. and Patton, S. 1969. Milk Lipid. In *Principles of Dairy Chemistry*. John Wiley & Sons, Inc. pp. 30-72.

Jha, J.S. 1981. Spectrophotometeric studies of Cheuri (*Madhuca butyracea*) fat and ghee mixtures .*J. Amer. Oil Chem. Soc*. **58**: 843-845.

Kalsi, P.S. 1984. Dilatometeric study of solid/liquid fractions of milk fat from different species. *MSc. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal.

Kamm, W.; Dionisi, F.; Hischenhuber, C.; Schmarr, H.G. and Engel, K.H. 2002. Rapid detection of vegetable oils in milk fat by on-line LC-GC analysis of β-sitosterol as marker. European Journal of Lipid Science and Technology, 104 (11), 756 - 761.

Kane, J.G. and Ranadive, G.M. 1951. Aniline point of some fats. *Journal of Scientific and Industrial Research.* **10**B: 62-66.

Kapur, O.P., Srinivasan, M. and Subrahmanyan, V. 1960. A study in the preparation and properties of hydrofuramide. *Journal of Scientific and Industrial Research.* **19**(B): 509.

Karleskind, A. 1996. Oils and fats (Manual). A Comprehensive Treatise, Vol.2.

Katrak, B.N., Achaya, K.T. and Banerjee, B.N. 1946. The analytical constants of ghee. *Current Science*. 15: 107- 108.

Kayalegian, K.E., Hartel, R.W. and Lindsay, R.C. 1993. Applications of modified milk fat in food products. *J. Dairy Sci.* **76**: 1782-1796.

Keeney M., Bachman K.C., Tikriti H.H., King R.L. 1971. Rapid vitamin E method for detecting the adulteration of dairy products with non-coconut vegetable oils. *J. Dairy Sci.* **54**: 1702-1703.

Kirk, R.S. and Sawyer, R. 1999. Pearson's composition and analysis of foods. Addison-Wesley Longman, Inc.

Konevets, V.I., Roganova, Z.A. and Smolyanskii, A.L. 1987. Use of infra-red spectroscopy for analysing fats of different origin. *Izvestiya Vysshikh Uchebnykh Zavadenii, Pischevaya Tekhnologiya*, **1**: 64-67. [*Cited: Dairy Sci. Abstr.*, (1988), **50**: 1973].

Krienke, W.A. 1953. Fractionation by selective solidification as an aid in detecting butterfat adulteration. *J. Dairy Sci.*, **36**(6): 567.

Kuksis, A. and McCarthy, M.J. 1964. Triglyceride gas chromatography as a means of detecting butterfat adulteration. *Journal of the American Oil Chemists' Society.* **41**(1): 17-21.

Kumar, A., Lal, D., Seth, R. and Sharma, R. 2002. Recent trends in detection of adulteration in milk fat- A Review. *Indian J. Dairy Sci*. **55**(6): 319-330.

Kumar. 2003. Comparative studies on physico-chemical properties of various oils and fats with a view to detect adulteration in ghee. *Ph.D. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Kumar, A. 2008. Detection of Adulterants in ghee. *Ph.D. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Lakshminarayana, M. and Ramamurthy, M.K. 1986. Cow and buffalo milk fat fractions. Part III. Hydrolytic and autoxidative properties of milk fat fractions. *Indian J. Dairy Sci*. **39**(3): 251-255.

Lal, D. 1982. Effect of lactation number on the physico-chemical status of milk lipids. *Ph.D. Thesis* submitted to the Kurukshetra University, Kurukshetra

Lal, D. and Narayanan, K.M. 1984. Effect of lactation number on fatty acid and physico-chemical constants of milk fat. *Asian J. Dairy Res*., **2**(4): 191-195.

Lal, D., Seth, R., Arora, K.L. and Ram, J. 1998. Detection of vegetable oils in milk. *Indian Dairyman.* **50**(7): 17-18.

Lal, D., Seth, R, Sharma, R. and Arun Kumar. 2005. Approaches for detection of adulteration in milk fat- An overview. *Indian Dairyman.* **57**(10): 31-43.

Lambelet, P. and Ganguli, N.C. 1983. Detection of pig and buffalo body fat in cow and buffalo ghee by differential scanning calorimetry. *Journal of the American Oil Chemists' Society* **60**(5): 1005-1008.

Lambelet, P., Singhal, O.P. and Ganguli, N.C. 1980. Detection of goat body fat in ghee by differential thermal analysis. *Journal of the American Oil Chemists' Society.* **57**(4): 364-366.

Latif, A. and Mazloum, U. 1969a. Some chemical studies on animal and vegetable fats. Part 1: A trial to differentiate between cow and buffalo butterfat (Samn). *Indian J. Dairy Sci.* **22**: 162-167.

Latif, A. and Mazloum, U. 1969b. Some chemical studies on animal and vegetable fats. Part 2: Identification of animal and vegetable fats and oils. *Indian J. Dairy Sci.* **22**: 168-172.

Lechat, G., Varchon, P., Kuzdazal-Savoie, S., Longlois, D. and Kuzdazal, W. 1975. Fractionated crystallization of anhydrous milk fat. *Lait.* 55 (545/546): 293.

Lipp, M. 1996a. Comparison of PLS, PCR and MLR for the quantitative determination of foreign oils and fats in butter fats of several European countries by their triglyceride composition. *Zeitschrift fur Lebensmittel-Untersuchung und-Furschung*. 202: 193-198. [Cited: *Dairy Sci. Abstr.* (1996), **58**: 45469].

Lipp, M. 1996b. Determination of adulteration of butter fat by its triglyceride composition obtained by GC. A comparison of the suitability of PLS & neural network. *Food Chemistry.* 55: 389-395.

Luf, W., Stock, A. and Brandl, E. 1987. Detection of foreign fat in milk by triglyceride analysis. II. Detecting adulteration by evaluating triglyceride combinations. *Osterreichische Milchwirtschaft*, **42**: 29-35. [Cited: *Dairy Sci. Abstr.* (1988), **50**(4): 1959].

Magidman, P., Herb, S.F., Luddy, F.E. and Riemenschneider, R.W. 1963. Fatty acids of lard. 2. Quantitative estimation by silicic acid and gas liquid chromatography. *Journal of the American Oil Chemists' Society.* **40**(3): 86-88.

Mahon, J.H. and Chapman, R.A. 1954. Detection of adulteration of butter with vegetable oils by means of the tocopherol content. *Analytical Chemistry.* **26**(7): 1195-1198.

Mariani, C., Venturini, S., Fedeli, E. and Contarini, G. 1994. Detection of refined animal and vegetable fats in adulteration of pure milk fat. *J. Amer. Oil Chem. Soc*. **71**: 1381-1384.

Marjanovic, N., Jankouits, I., Turkulov, J., Karlouic, D., Caric, M., Milanovic, S. and Zagorak, M. 1984. Determination of the triglyceride composition of milk fat by gas chromatography. *Mijekarstuo*, **34**: 67-75. [Cited: *Dairy Sci. Abstr.*, **47**: 474].

Markuze-Zofia R. Z. H. 1962. Method for the estimation of tocopherol content of butter and its use for the detection of adulteration with vegetable fats. *Warsz.* **13**(3): 255-262. [Cited: *Dairy Sci. Abstr.* (1963), **24**: 3392].

Martin, P.G. 1979. Oils and fats. *Manuals of food quality control. 3. Commodities. Food & Agricultural Organization of the United Nations, Rome.* pp: 271-285.

Mathew, T.V. and Kamath, K.S. 1978. Detection of vegetable oils in ghee and vice-versa by reverse phase thin layer chromatography. *Res. & Ind.* **23**: 168-169.

Morris, R.J., Macphee, R.D. and Randall, E.L. 1952. Ultraviolet spectrophotometry in detection of food product substitutes. *Analytical Chem.* **24**(9): 1396-1399.

Mulder, H. and Walstra, P. 1974. The milk fat globule. Centre for Agricultural Publishing and Documentation Wageningen, The Neitherlands.

Murthy, G.K. 1955. Reichert-Meissl value: Its importance and limitation in the detection of foreign fats in butterfat. *J. Dairy Sci.* **38**: 317-318.

Nasirullah; Krishnamurthy, M. N. and Nagaraja, K. V. 1992. Methods for detection of rice-bran, mustard, karanja oils and rice-bran deoiled cake. *Fat Science Technology*. 94(12): 457 – 458.

Naviglio, D. and Raja, C. 2003. Quality control of butter by means of capillary column RTX 65-TG. *Industrie Alimentary*. **42**: 742-756. [Cited in Dairy Sci. Abstr. (2004) 66: 298].

Nazir, D.J. and Magar, N.G. 1959. Tocopherol content of Indian butter and its use in detecting adulteration of butterfat. *Indian J. Dairy Sci.* **12**(3/4): 125-132.

Nelson, W.L. 1954. A rapid test for distinguishing between butterfat and fats from plant and other animal sources. *Food Technology*. **8**: 385-386.

Norris, R., Gray, I.K., McDowell, A.K.R. and Dolby, R.M. 1971. The chemical composition and physical properties of fractions of milk fat obtained by a commercial fractionation process. *Journal of Dairy Research.* 38: 179-191.

Norton, R.A. 1995. Quantification of steryl ferulate and p-coumarate esters from corn and rice. *Lipids.* 30. 269-274.

Panda, D. and Bindal, M.P. 1997. Detection of adulteration in ghee with vegetable oils using GLC based on a marker fatty acid. *Indian J. Dairy Sci.* **50**(2): 129-135.

Panda, D. and Bindal, M.P. 1998a. Detection of adulteration in ghee with animal body fats and vegetable oils using opacity test. *J. Dairying, Foods & Home Sci.* **17**(1): 31-36.

Panda, D. and Bindal, M.P. 1998b. Detection of adulteration in ghee with animal body fat and vegetable oils using crystallization test. *Indian Dairyman*. **50**(9): 13-16.

Pandey, S.N. and Suri, L.K. 1982. Cyclopropenoid fatty acid content and iodine value of crude oils from Indian cottonseed. *J. Amer. Oil Chemists Soc.* **59**(2): 99-101.

Paradkar, M.M., Singhal, R.S. and Kulkarni, P.R. 2001. An approach to the detection of synthetic milk: 3. Detection of vegetable oil and sodium bicarbonate. *International Journal of Dairy Technology*. **54**(1): 34-35.

Parodi, P.W. 1969. Detection of a synthetic milk fat. *Aust. J. Dairy Technol.* **24**(4): 56-59.

Parodi, P.W. 1971. Detection of synthetic and adulterated butterfat. III. Triglyceride fatty acid analysis. *Aust. J. Dairy Technol.* **26**(4): 155-158.

Parodi, P.W. 1972. A simple TLC procedure for detecting interesterified fats. *Australian Journal of Dairy Technology.* **27**(2): 60-62.

Parodi, P.W. 1973. Detection of synthetic and adulterated butterfat. 4. GLC triglyceride analysis. *Australian Journal of Dairy Technology.* **28**(1): 38-42.

Parodi, P.W. and Dunstan, J.R. 1969. Detection of synthetic and adulterated butterfat: 2. Trisaturated triglycerides analysis. *Australian Journal of Dairy Technology.* **24**(4): 190-195.

Parrozzano, R. and Mancinelli, F. 1954. Detection of hydrogenated dolphin oils in butter using paper chromatography. *Boll. Lab. Chim. Prov.* **5**(2/3): 43-50. [Cited: *Dairy Sci. Abstr.* (1955), **17**(4): 343].

Patel, A.A. and Frede, E. 1991. Studies on thermal properties of cow and buffalo milk fats. *Lebensm.-Wiss.u.-Technol*., 24: 323-327.

Patel, M.D., Patel, B.M. and Dave, C.N. 1944. A comparative study of the quality of ghee from cow and buffalo under uniform dietary conditions including heavy feeding of cotton seed. *Indian J. Vet. Sci*., 14: 97-100.

PFA. 2009. Prevention of Food Adulteration Act, 1954 & rules, 1955 (As amended) Eastern Book Company, 1267, Kashmere Gate, Delhi.

Pinto, C.M., Contreras, O., Carrasco, R.E., Brito, C.C., Molinae, L.H. and Leon, Y.S. 2002. Determination of authenticity of pure milk fat. Linear discriminant analysis of triacylglycerides. *Agro sur*. **30**(1): 59-67. [Cited in Dairy Sci. Abstr. (2002) 64: 6578].

Povolo, M., Bonfitto, E., Contarini, G., Toppino, M. and Daghetta, A. 1999. Study on the performance of three different capillary gas chromatographic analysis in the evaluation of milk fat purity. *J. High Resolution Chromatography*, **22**(2): 97-102.

Precht, D. 1990. Quantification of animal body fats and vegetable fats in milk by triglyceride analysis. *22nd Int. Dairy Congr.* **1**: 234.

Precht, D. 1992. Detection of foreign fat in milk fat. 1. Quantitative detection of triacyl-glycerol formulae. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung*. **194**: 1-8.

Priori, O. 1955. Chromatographic detection of hydrogenated dolphin oils in market butters. *Boll. Laboratori Chim. Prov.* **6**(2): 45-46. [Cited: *Dairy Sci. Abstr.* (1955), **7**: 10].

Provvedi, F. and Cialella, G. 1961. Gas chromatographic analysis of some food fats. *Revista Italia Sostanze Grasse*. **38**(8): 361-366. [Cited: *Dairy Sci. Abstr.* (1962), **24**(5): 1452].

Raghuveer, K.G. and Hammond, E.G. 1967. Fatty acid composition and flavour of auto-oxidized milk fat. *J. Dairy Sci.* **50**(8): 1200-1205.

Rahn, O. and Sharp, P.F. 1928. Physik der. *Milchewirtschaft*, Paul Parey, Berlin.

Ramachandra, B.V. and Dastur, N.N. 1959. Application of paper chromatography to differentiate ghee from other fats. Part 1. Behaviour of unsaponifiable matter on chromatograms. *Indian J. Dairy Sci.* **12**(3/4): 139-148.

Ramachandra, B.V. and Dastur, N.N. 1960. Application of paper chromatography to differentiate ghee from other fats. II. Directly spotting fats on chromatogram. *Indian J. Dairy Sci.* **13**(1): 29-44.

Ramamurthy, M.K. and Narayanan, K.M. 1971. Fatty acid compositions of buffalo and cow milk fats by gas liquid chromatography (GLC). *Milchwissenschaft*, **26**(11): 693-696.

Ramamurthy, M.K. and Narayanan, K.M. 1972. Polyunsaturated fatty acids of buffalo and cow milk fat. *Milchwissenschaft*. **27**(11): 695-697.

Ramamurthy, M.K. and Narayanan, K.M. 1974. Hydrolytic and auto-oxidative properties of buffalo and cow milk fats as influenced by their glyceride structure. *Indian J. Dairy Sci.* **27**(4): 227-233.

Ramamurthy, M.K., Narayanan, K.M., Bhalerao, V.R. and Dastur, V.N. 1967. A TLC method for detection of adulteration of ghee with vegetable fats. *Indian J. Dairy Sci.*, **20**(1): 11-13.

Rangappa, K.S. and Achaya, K.T. 1974. Indian dairy products. Asia Publishing House, Bombay. pp. 255-326.

Rego, M.D.C. and Garcia,-Olmedo, R. 1963. Chromatographic analysis of butter, margarine and their mixtures. *An. Bromatol. Madr.* **15**(1): 53-79.

Rego, M.D.C., Carballido, A. and Villanua, L. 1964. Spectrophotometric analysis of butter margarine and their mixtures. *An. Bromat.*, **16**(4): 455-430. [Cited: *Dairy Sci. Abstr.* (1968), **28**(1): 332].

Renterghem, R.Van. 1997. The triglyceride composition of Belgian butter in view of EU controls on milk fat purity. *Milchwissenschaft.* **52**: 79-82.

Roos, J.B. 1963. Methods for the detection of foreign fat in milk fat. *Int. Dairy Fed. Ann. Bull.* **4**: 2-54.

Roos, J.B. and Tuinstra, L.G.M.Th. 1969. The detection of foreign fat in milk fat. 2. Differential thermal analysis of Dutch butterfat. *Neth. Milk Dairy J.* **23**(1): 37-45.

Sampath, S.R. and Anantakrishnan, C.P. 1956. Seasonal variations in the Reichert and Iodine values of milk fat. *Indian J. Dairy Sci*. 9. 135-141.

Sato, T., Kawano, S. and Iwamoto, M. 1990. Detection of foreign fat adulteration of milk fat by near infra-red spectroscopic method. *J. Dairy Sci.* **73**: 3408-3413.

Schwitzer, M.K. 1956. Margarine and other food fats. Leonardo Hill Books Ltd., London.

Sebastian, J. and Rao, M.B. 1974. Detection of the presence of vegetable oils and fats in ghee (butterfat). *Milchwissenschaft*, **29**(9): 533-534.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. 6th Edn., *Iowa State College*. Press, Ames, Iowa.

Sharma, R. 1992. Physico-chemical properties of ghee prepared from milk adulterated with vanaspati and body fats. *M.Sc. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Sharma, R. and Singhal, O.P. 1995.Physico-chemical constants of ghee prepared from milk adulterated with foreign fat. *Indian J. Dairy & Bio. Sci.*, **6**: 51-53.

Sharma, R. and Singhal, O.P. 1996. Fatty acid composition, bomer value and opacity profile of ghee prepared from milk adulterated with foreign fats. *Indian J. Dairy Sci.* **49**(1): 62-67.

Sharma, S.K. 1989. Studies on unsaponifiable matter of ghee (clarified butterfat) and animal body fats with a view to detect adulteration. *Ph.D. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Sharma, U.P. and Zariwala. 1978.J .Fd. Sci. Technol., **15:** 118.[Cited by Banerjee et al, 2002]

Sharma, V., Lal, D. and Sharma, R. 2005. Development of a rapid colorimetric test for detection of vegetable oil adulteration in ghee. *NDRI Annual Report*, pp. 26.

Sharma, V., Lal, D. and Sharma, R. 2007. Color Based Platform Test for the Detection of Vegetable oils/Fats in ghee . *Indian J. Dairy Sci.*, **60**(1): 16-18.

Shenstone, F.S. and Vickery, J.R. 1961. Occurrence of cyclopropene acids in some plants of the Order Malvales. *Nature*. **190**(1-3): 168-169.

Shipe, W.F. 1955a. Identification of fats by urea fractions. *J. Assoc. Off. Agric. Chem.* **38**: 156.

Shipe, W.F. 1955b. M53. A modified hydroxamate method for determining the short chain fatty acids in fats. *J. Dairy Sci.* **38**(6): 599.

Shukla, A.K.; Dixit, A. K. and Singh, R. P. 2005. Detection of adulteration in edible oils. *J. Oleo Science.* 54(6): 317-324.

Shukla, A.K.; Johar, S.S.; Dixit, A. K. and Singh, R. P. 2004 Identification of physically refined rice bran oil and its simple detection in other oils. *J. Oleo Science.* 53 (8) 413-415.

Silverberg, H.D. 1962. Mineral oil in foods. *J. Assoc. Off. Agric. Chem*. **45**(2): 241-243.

Singh, K.P. and Singh, S.N.1960 Indian J Dairy sci.**13:** 143-149.[Cited by Banerjee et al, 2002]

Singhal, O.P. 1973. Studies on ghee (clarified butterfat) and animal body fats with a view to detect adulteration. *Ph.D. Thesis* submitted to Panjab University, Chandigarh, India.

Singhal, O.P. 1980. Adulterants and methods for detection. *Indian Dairyman*. **32**: 771-774.

Singhal, O.P. 1987. Detection of adulteration in butterfat. *NDRI Annual Report*. pp. 64-65.

Singhal, O.P. Ganguly, N.C. and Dastur, N.N. 1973. Physico-chemical properties of different layers of ghee (clarified butterfat). *Milchwissenschaft*. **28**(8): 508-511.

Singhal, R.S., Kulkarni, P.R. and Rege, D.V. 1997. Edible oils and Fats. In Handbook of indices of food quality and authenticity. Woodhead Publishing Ltd. pp. 301-357.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. 6th Edn., *Iowa State College*. Press, Ames, Iowa.

Sofia, P.Z. 2005. Assessment of methods for detection of palm oil and/ or coconut oil in ghee. *M.Sc. Thesis* submitted to National Dairy Research Institute (Deemed University), Karnal, India.

Soliman, M.A. and Younes, N.A. 1986. Adulterated butterfat: Fatty acid composition of triglycerides and 2-monoglycerides. *J. Amer. Oil Chemists Soc.* **63**(2): 248-250.

Tappi, G. and Menziani, E. 1952. Detection of hydrogenated dolphin oil in butter. *Ann. Chim.* **42**: 446-462. [Cited: *Dairy Sci. Abstr.* (1952), **15**(9): 7439].

Tawde, S. and Magar, N.G. 1957. Fractionation of butterfat by urea complexes and its use in detecting adulteration. *Indian J. Dairy Sci.* **10**: 43-47.

Toppino, P.M., Contarini, G., Traversi, A.L., Amelotti, G. and Gargano, A. 1982. Gas chromatographic parameters of genuine butter. *Rivista Italiana delle Sostanze Grasse*, **59**(12): 592-610.

Ulberth, F. 1994. Detection of milk fat adulteration by linear discriminant analysis of fatty acids. *J. Assoc. Off. Agric. Chem.* **77**(5): 1326-1334.

Velu, T. 1971. The detection of adulteration of butterfat. *Madras Agric. J.* **58**: 901-902.

Venkatachalam, V. and Sundaram, S. 1957. Test for mineral oil in edible oils and ghee. *Current Science.* **26**(5): 157.

Walstra, P. and Jennes, R. 1984. Dairy chemistry and physics. John Wiley & Sons Inc. New York.

Webb, B.H., Johnson, A.H. and Alford, J.A. 1987. Fundamentals of dairy chemistry. CBS Publishers & Distributors, Delhi.

Weihe, H.D. 1961. Technical note: Interesterified butteroil. *J. Dairy Sci.* **44**: 944-947.

Winton, A.L. and Winton, K.B. 1999. Techniques of food analysis. Allied Scientific Publishers, Bikaner.

Wolff, J.P. 1960. Gas chromatography in the control of butter purity. *Ann. Falsif. Expert. Chim.* **53**: 318-325. [Cited: *Dairy Sci. Abstr.* (1961) 23: 272].

Xu, Z. 2005. Lipids. In Handbook of food analytical chemistry. John Wiley & Sons, Inc., Publication. pp. 479-490.

Xu, Z. and Godber, J.S. 1999. Purification and identification of components of gamma oryzanol in rice bran oil. *J. Agricultural and Food Chemistry*. 47. 2724-2728.

Yen, G.C. and Hwang, L.S. 1984. A simple method for the detection of rice bran oil in vegetable oils. *Shin P' in K'o Hsueh*. 11: 213-217.

Appendix I
Standards of Ghee under PFA Rules (2009)

Sr. No.	Name of the State & U.T.	BR Reading at 40°C	RM (Reichert Meissl) value (Min)	Percentage of	
				FFA (as Oleic acid) (Max)	Moisture (Max)
1.	Bihar, Chandigarh, Delhi, Punjab, Haryana (Areas other than cotton tract areas), West Bengal (Areas other than Bishnupur sub-division), Sikkim, Jharkhand.	40-43	28	3.0	0.5
2.	Manipur, Meghalaya, Mizoram, Arunachal Pradesh, Orissa, Uttaranchal, Nagaland, Tripura, Assam, Goa, Kerala, Himachal Pradesh, U.P., J & K, Rajasthan (Areas other than Jodhpur Divn), Haryana (Cotton tract areas), Lakshadweep, Maharashtra(Areas other than cotton tract areas).	40-43	26	3.0	0.5
3.	Karnataka (Belgaum district), Madhya Pradesh (Areas other than cotton tract areas), Pondicherry, Chhatisgarh.	40-44	26	3.0	0.5
4.	Andhra Pradesh, Daman & Diu, Dadar & Nagar Haveli, Karnataka (Areas other than Belgaum district)	40-43	24	3.0	0.5
5.	Andaman & Nicobar Island, Tamil Nadu.	41-44	24	3.0	0.5
6.	Gujarat (areas other than cotton tract).	40-43.5	24	3.0	0.5
7.	Gujarat (cotton tract areas), Madhya Pradesh (Cotton tract areas), Maharashtra (cotton tract areas), Rajasthan (Jodhpur sub division), West Bengal (Bishnupur sub division).	41.5-45	21	3.0	0.5

a) Baudouin test shall be negative

b) By cotton tract is meant the areas in the state where cotton seed is extensively fed to the cattle and so notified by the State Govt. concerned.

c) Usually such cotton tract areas ghee has low RM value and high BR reading compared to other areas

d) Ghee may contain BHA not more than 0.02% as antioxidant.

Appendix II
Standards of Ghee under AGMARK Rules (1981)

S.No	Characteristics	All India	Regional *	
			Winter (Sep.-Feb.)	Summer (Mar.-Aug.)
1	Baudoin test		Negative	
2	Phytosterol acetate test		Negative	
3	BR reading at 40°C	40.0 – 43.0	41.5 – 44.0	42.5 – 45.0
4	Reichert – Meissl value	Not less than 28.0	Not less than 23.0	Not less than 21.0**
5	Polenske value	1.0 – 2.0	0.5 - 1.2	0.5 – 1.0
6	Moisture (%)	Not more than 0.3		
7	Percentage of free fatty acids (as oleic acid)			
a	Special Grade (Agmark Red Label)	Not more than 1.4		
b	General Grade (Agmark Green Label)	Not more than 2.5		

*Normal physical and chemical constants of Ghee produced in recognized cotton tracts@ (of Saurashtra and Madhya Pradesh) to which grade designation marks may be applied.

@ By cotton tract is meant that area where cotton seed is extensively fed to the cattle.

**Ghee with Reichert Meissl value between 19 and 21 shall be graded only after a Phytosterol Acetate test has been performed and the result thereof found to be negative.

N.B. - Percentage of Free Fatty Acids (as Oleic acid) shall not exceed 3.0 for Standard Grade Ghee.