

**QUALITY OF MILK FAT IN MARKET SAMPLES OF BUTTER,
GHEE AND PANEER**



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)**

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

DAIRY CHEMISTRY

BY

RAKESH

B.Tech. (Dairy Technology)

**DAIRY CHEMISTRY DIVISION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)**

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APPROVED BY


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This is to certify that the thesis entitled “**Quality of milk fat in market samples of butter, ghee and paneer**” submitted by **Rakesh** in partial fulfillment of the requirement for the award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY CHEMISTRY** of the **ICAR-National Dairy Research Institute, (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

I wish him all success.

Dated: 27th / July / 2016

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*Dedicated
To My
Beloved Family
&
Respected Guide*

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ABBREVIATIONS

AGMARK	Agricultural Marking
AR	Analytical reagent
AST	Apparent Solidification Time
BV	Biological value
BIS	Bureau of Indian standard
BR	Butyro-Refractometer
CLT	Complete Liquification Time
CTD	Critical Temperature of Dissolution
CTT	Crystallization time test
DSC	Differential Scanning Calorimetry
EU	European union
FAAS	Flame atomic absorption spectrometry
FFA	Free fatty acid
FID	Flame ionization detector
FSSR	Food safety standard and regulation
GAIN	Global Agriculture Information Network
GFAAS	Graphite Flame atomic absorption spectrometry
GLC	Gas liquid chromatography
GR	Guaranteed reagent
GS ₃	Trisaturated glyceride
HPTLC	High performance thin layer chromatography
IDF	International Dairy Federation
IR	Infra red
ISI	Indian standards institute
LOD	Limit of detection
LOQ	Limit of quantification
mμ	Milli micron
MMT	Million Metric Ton
NCR	National capital region
nm	Nanometer

NMR	Nuclear magnetic resonance
°C	Degree centigrade
OD	Optical density
OS	Organized sector
%	Percent
PCA	Principal component analysis
PER	Protein efficiency ratio
PFA	Prevention of food adulteration act
PUFA	Poly unsaturated fatty acid
R _f	Retention factor
RM	Reichert- Meissl
RP-TLC	Reversed-phase thin layer chromatography
RP-HPLC	Reversed-phase high performance liquid chromatography
Sn	Stereospecific number
STGs	Saturated triglycerides
TAG	Triacylglycerol
TLC	Thin layer chromatography
TMS	Trimethyl silyl ether
UOS	Unorganized sector
USD	United state dollar
USM	Unsaponifiable matter
UV	Ultraviolet

ABSTRACT

In the present investigation, quality of milk fat in market samples of butter, ghee and paneer was studied. It was observed from the research that 2nd order UV derivative spectrophotometry did not give any conclusive evidence of detection of tallow adulteration in ghee. It was observed that physico-chemical constant like Polenske value was not helpful for the detection of adulteration of milk fat with animal body fat, vegetable oil and margarine. However, Reichert-Meissl value could detect animal body fat and soybean oil adulteration up to 10% and 5% level, respectively in cow ghee and 20 percent level in buffalo ghee. Butyro-refractometer (BR) reading at 40°C could detect adulteration of cow ghee with lard and soybean oil at 20% level but in case of buffalo ghee values were within the range given by FSSR (2011) and adulteration even to the tune of 20% could not be detected. A slight decrease was observed in RM value of milk fat extracted from paneer, the reason could be the incomplete extraction of water soluble fatty acid (C_{4:0} and C_{6:0}) in soxhlet extraction method due to the non-polar nature of chloroform. It was also concluded from the present study that RP-TLC protocol could detect the adulteration of ghee, paneer and butter with vegetable oil and margarine, respectively at 5% level of adulteration. It was observed in the present study of market ghee samples (organized and unorganized), 19.2 % samples failed to meet the legal requirements (FSSR, 2011) and 61.5 % samples found adulterated through RP-TLC and low-resolution GLC. Similarly 28.5% and 21.71% market samples of butter and paneer, respectively failed to meet the legal requirement. 42.81% and 39.13% market samples of butter and paneer, respectively were found adulterated through low-resolution GLC and RP-TLC. The TAG profiles of goat tallow, buffalo tallow, lard, margarine and soybean oil had higher relative concentration of C50, C52 and C54. The TAG profile of coconut oil had higher concentration of C34, C36 and C38. In soybean oil C54 and in lard C52 were found better marker to check the authenticity of milk fat. Adulteration of coconut oil alone and in conjunction with lard in buffalo ghee could be detected at 5% level by observing the increase in sum of TAG (C34+C36+C38). In case of all the adulterants other than coconut oil, sum of TAGs (C50+C52+C54) increased as the level of adulteration increased.

सारांश

वर्तमान अध्ययन में बाजार में बिकने वाले बटर, घी और पनीर के नमूनों की वसा की गुणवत्ता को जांचा गया। अध्ययन से ये पाया गया कि दूसरे आर्डर की डेरीवेटिव अल्ट्रावायलेट स्पेक्ट्रोस्कोपी दुध की वसा में पशुओं के शरीर की वसा को जांचने में कोई फलसवरूप नतीजा नहीं दे पाई। यह भी देखा गया की भौतिक एवं रासायनिक मापदंड जैसे की पोलंस्की संख्या दूध वसा में पशुओं की शरीर की वसा, वनस्पति तेल और मार्जरीन की मिलावट को जांचने में सक्क्षम नहीं थी। जबकि रेचरत मिसेल संख्या के दवारा पशुओं के शरीर की वसा १० प्रतिशत तक एवं सोयाबीन तेल की ५ प्रतिशत तक की मिलावट को गाय के घी में और पशुओं की शरीर की वसा एवं सोयाबीन तेल की की मिलावट भैंस के घी में २० प्रतिशत तक जाँच किया जा सकता है। बी.र. रीडिंग के दवारा गाय के घी में सूअर की चर्बी की वसा और सोयाबीन तेल की मिलावट को २० प्रतिशत के सत्तर तक जांचा जा सकता है। लेकिन भैंस के घी की मिलावट किसी भी प्रकार के मिलावटी तेल एवं वसा से २० प्रतिशत तक के सत्तर तक जांचा नहीं जा सकता है। वर्तमान अध्ययन से ये भी पाया गया कि जो शुद्ध वसा पनीर से निचोड़ी जाती है, उसकी रेचरत मिसल संख्या शुद्ध घी की तुलना में कम होती है, इसका कारण पानी में घुलनशील फैटी एसिड ($C_{4.0}$ और $C_{6.0}$) है जो क्लोरोफॉर्म के नॉन-पोलर स्वभाव के कारण सक्सहलेत विधि में पूरी तरह से निकल नहीं पाते है। वर्तमान अध्ययन से यह भी निष्कर्ष निकला कि आर.पी. टी. एल. सी क्रोमैटोग्राफी प्रोटोकॉल के दवारा घी एवं पनीर में सोयाबीन तेल की और बटर में मार्जरीन की मिलावट को ५ प्रतिशत तक जांचा जा सकता है। बाजार के संगठित और असंगठित क्षेत्रों के घी के नमूनों की जाँच करने पर पाया गया कि १९.२ प्रतिशत नमूनों में गुणवत्ता भौतिक एंड रासायनिक मापदंडों (फ.स.स.र, २०११) से कम पाई गई और ६१.५ प्रतिशत नमूने आर.पी. टी. एल. सी एवं लो रेसोलुशन गैस लिक्विड क्रोमैटोग्राफी दवारा मिलावटी पाए गये। उसी तरह पनीर के २८.५ प्रतिशत और बटर के २१.७१ प्रतिशत नमूने संगठित और असंगठित क्षेत्रों में भौतिक एंड रासायनिक मापदंडों पर गुणवत्ता में खरे नहीं उतरे। ४२.८१ प्रतिशत बटर के नमूने और ३९.१३ प्रतिशत पनीर के नमूने संगठित और असंगठित क्षेत्रों में आर.पी. टी. एल. सी एवं लो रेसोलुशन गैस लिक्विड क्रोमैटोग्राफी दवारा मिलावटी साभित हुए। ट्रिग्लिसरोल प्रोफाइल से पता चला था कि कार्बन संख्या सी५०, सी५२ और सी५४ की सांदरता पशुओं के शरीर की वसा (भेड़, सूअर, बकरी और भैंस), सोयाबीन तेल और मार्जरीन में ज्यादा होती है। कार्बन संख्या सी३४, सी३६ और सी३८ की सांदरता नारियल तेल की ट्रिग्लिसरोल प्रोफाइल में ज्यादा पाई गई। सोयाबीन के तेल में सी५४ और सूअर की शरीर की वसा में सी५२, दो ट्रिग्लिसरोल चिहक मिले जो दूध वसा की मिलावट जांचने में सहयोगी साभित हुए। ट्रिग्लिसरोल सी३४, सी३६ और सी३८ के योग के बढ़ने के अध्यन से अकेले नारियल के तेल की और नारियल के तेल को सूअर की शरीर की वसा के साथ मिला कर भैंस के घी में डालने पर ५ प्रतिशत सत्तर तक की मिलावट का पता लगा सकते है। नारियल तेल को छोड़ कर सभी प्रकार की मिलावट, जो अध्यन के इस्तमाल में लायी गई, इनसे ट्रिग्लिसरोल (सी५०+सी५२+सी५४) के योग में बढ़ोतरी देखी गई, जैसे-जैसे इनकी मिलावट की दर को दूध वसा में बढ़ाया।

CHAPTER -1

Introduction

INTRODUCTION

Milk fat is an important food component and plays a significant role in economics, nutrition, and physical and chemical properties of milk and milk derived products. The market price of milk fat is almost 3 times more than the price of edible vegetable oils/fats. It is also a good source of fat soluble vitamins and essential fatty acid. Due to increased demand, milk fat has been a target of the fraudulent practices, such as replacement with cheaper or more readily available non milk fat of plant and animal origin. Replacements of milk fat with fat from other source are not only an economic fraud, but now also pose risk to human health.

India is the largest producer of milk in the world, producing 154 million tones according to the latest estimates for 2015-16 (GAIN REPORT, 2015). Yet, per capita milk availability is around 322 g per day. Butter, ghee and paneer are the products which are prepared and sold by both organized and unorganized dairy sector. At the same time these products have large consumer base in India. Ghee production forms the largest segment of the milk consumption and utilization pattern in India. All India consumption patterns of ghee, butter and paneer are 27.5%, 6.5% and 2%, respectively (GAIN REPORT, 2014). Calendar year 2016 combined butter and ghee (clarified butter) production is estimated to rise 3% to 5.2 MMT on rising domestic demand due to population growth and demographic shifts (GAIN REPORT, 2015). 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). In the past, Rani (2013) and Aghav (2014) reported that 33.3% samples of ghee and 46.6% samples of paneer, respectively failed to meet the requirement in term of physico-chemical constants.

Ghee (from Sanskrit *ghṛita* meaning "sprinkled") is clarified butter and by far the most important product widely consumed in the Indian sub-continent since time immemorial. Its image as a natural product and its organoleptic attributes, nutritional value, and functional properties make it suitable for numerous food applications. Ghee is believed to be a coolant, capable of

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increasing mental power and physical appearance, curative of ulcers and eye-diseases (Rangappa and Achaya, 1974). Ghee is produced by heat desiccation of makkhan, butter or cream at 105-110°C. heat induced change in milk protein and lactose during clarification process imparts distinctive, pleasant cooked flavor to ghee (Aneja *et al.*, 2002). In order to ensure a genuine product to the consumer, the Government of India has prescribed the compositional standards for ghee, under FSSR (2011) and Agmark rules (1981).

Paneer is a coagulated milk product obtained by heating and acidulation, followed by filtration and pressing. Paneer contains entire milk casein, part of denaturated whey proteins, almost all fat, colloidal salts and soluble milk solids in proportion to moisture retained. It represents south Asian variety of soft cheese. It is non fermentative, non-rennet, non-melting and unripened type of cheese similar to white cheese (Chandan, 2007). According to the FSSR (2011), chhana or paneer means the product obtained from the cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 70.0% moisture and milk fat content shall not be less than 50.0% of the dry matter basis. Bureau of Indian standard (BIS, 1983) permits maximum of 60% moisture and minimum of 50% fat in dry matter for paneer.

About 5% of milk produced in India is converted into paneer. Paneer market size is around 15,500 tonnes at Rs. 1860 million (USD 46.5%).The unorganized sector, local sweet shops account for most of production. The market size of paneer is growing at 6 to 7 % per annum. Paneer is a rich source of animal protein available at a comparatively lower cost and form an important source of animal protein for vegetarians. Soni (1979) reported that paneer has very high nutritive value, protein efficiency ratio (PER) being 3.4 and biological value (BV) 85.86 for buffalo milk paneer as compared to cow milk paneer (PER 2.3, BV 81.88) and mixed milk paneer (PER 2.3, BV 80.38), respectively.

Butter may be defined as a fat concentrate, obtained by churning cream, gathering the fat into compact mass and then working it. As per FSSR (2011), Butter means the fatty product derived exclusively from milk of cow and/or buffalo or its products principally in the form of water-in-oil type of an emulsion. The product may be with or without added common salt and starter cultures of

harmless lactic acid and/or flavour producing bacteria. Table butter shall be obtained from pasteurized milk and/or other milk products which have undergone adequate heat treatment to ensure microbial safety. It shall be free from animal body fat, vegetable oil and fat, mineral oil and added flavour. It shall have pleasant taste and flavour free from off flavour and rancidity. It may contain food additives permitted in the Regulations. It must contain not less than 80% by weight of milk fat, not more than 1.5% by weight of curd and not more than 3% by weight of common salt. Butter adulteration and fraud and attempts at their control have a long history. Trade in butter was radically affected by the introduction of margarine in industry. Before the production of margarine, some cheaper substance like chalk, potato starch and color was used as adulterant in commercial butter. With arrival of margarine, a new phase arrived in counterfeiting of butter. Falsely labelled butter flooded the markets all over world.

Worldwide food safety and quality are considered significant issues that are directly related to health of consumers and ethics of the food industry. Consumer are increasingly looking for trusted trademark of food products, and expect the producer and seller to supply high quality products (Reggattieri *et al.*, 2007). However, in recent years, several reports have appeared in the newspapers indicating that rampant malpractices of milk fat adulteration are going on particularly in the central and northern parts of the country like “synthetic paneer” is sold in the market in bulk quantities. The problem of adulteration has assumed a very serious dimension. By adding concoction of vegetable oil/fat and 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. Such a situation has tarnished the image of dairy industry, not only in India, but abroad also. Unfortunately, unorganized sector involved in the milk fat trade, in their greed to have more money, tend to adulterate milk fat with cheaper oils and fats like vegetable oils, animal body fats, hydrogenated fats, and sometimes even the non-edible mineral oils, especially during lean season. It is not known as to what extent these types of malpractices of adulteration are prevailing in the trade of these products in our country and what quality of these product available to the consumers on local dairies and road side

Introduction

dhabas. Extensive survey of the literature revealed that in the past, several techniques have been employed to detect the adulterants in milk fat, yet it is realized that they have their own limitations in establishing the type and the level of the adulterants. Hence, the methodology available till date is not adequate in dealing with the hazard of adulteration of milk fat. No single test can detect all types of adulterant fats/oils. Therefore, oftenly more than one tests have to be employed to confirm the purity of the milk fat. Traditional methods like R.M. value, Polenske value and B.R. reading are not fool proof methods in establishing the genuineness of milk fat. In order to ensure a genuine product to the consumer, the Government of India has prescribed the compositional standards for milk fat, under FSSR (2011) and AGMARK rules (1981). However these standards are not comprehensive and can hardly establish the type and the level of added adulterants in the present scenario of rampant and science based adulteration practices. This may be because of wide variations in the physico-chemical makeup of milk fat owing to different factors like animal species, feeding practices and nutritional management etc. Therefore, there is a deficiency in the data pertaining to quality of milk fat in said products. Deficiency in quality data is due to lack of adequate methodology to check adulteration being done in precise and scientific manner. Animal body fat adulteration at low concentration is still undetectable. Recently, RP-TLC has been reported to detect vegetable oil in ghee. Similarly, GLC/derivative spectrophotometry based method has been reported to detect presence of tallow in milk fat. Therefore, the present study was proposed with the following objectives:

1. To standardize and optimize the derivative spectrophotometry method for detection of tallow in milk fat.
2. To evaluate the quality of milk fat in market samples of butter, ghee and paneer.

CHAPTER -2

Review of Literature

REVIEW OF LITERATURE

This review delineates the various tests commonly used for the detection of adulteration of milk fat with foreign fat and market survey of ghee, butter and paneer. The review covered under this chapter has been discussed in the following headings:-

- 2.1 Tests based on physico-chemical constants of milk fat
- 2.2 Latest methodology to check the adulteration of milk fat
- 2.3 Market survey of ghee and butter
- 2.4 Market survey of paneer

2.1 Tests based on physico-chemical constants of milk fat

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:

2.1.1 Reichert-Meissl value

RM value is the number of millilitre 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 and 1980). Feeding of cottonseed to milch animals lowers the RM value of ghee by 5 to 6 units (Rangappa and Achaya, 1974). Singhal (1973) in their study on the physico-chemical properties of different layers of ghee obtained after one month storage at 30 to 35°C reported that the liquid layer exhibited a higher RM value than solid layer. Kumar (2008) had

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reported that adulteration of pure cow ghee with all animal body fats and all vegetable oils 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. Patel (2011) concluded from research that RM value can detect more than 5% adulteration in cow ghee while 25% in case of buffalo ghee. However, in case of mix fat detection was not possible before 50% in buffalo ghee and not up to and beyond 75% in cow ghee.

Upadhyay (2014) reported that adulteration of groundnut oil when added singly in cow ghee could be detected as low as 10% level, however pooling of the respective data of cow and buffalo ghee revealed that detection of groundnut oil could be done at 15% and above level using R.M. value.

2.1.2 Polenske value

Polenske value denotes the number of millilitre of 0.1 N alkali solutions 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). Singhal (1973) reported that the liquid portion of ghee obtained on storage at 30 to 35°C for one month showed higher Polenske value than the solid portion.

Kumar (2008) had reported that on the basis of Polenske value, that adulteration of cow and buffalo pure ghee with any of the adulterants either animal body fats or vegetable oils could not be detected even at 20 percent level, except adulteration of buffalo ghee with the palm and soybean oils which could be detected at 15 percent level.

Patel (2011) reported that it was not possible to detect adulteration up to 5% in cow ghee with animal body fats and up to 20% in case of mix fat by using Polenske value. Mix fat was detectable in buffalo ghee at 15% level using Polenske value.

The study revealed that polenske value did not help in the detection of groundnut oil added to cow ghee and pooled data of cow and buffalo ghee at any of the levels (5, 10 and 15%) studied (Upadhyay, 2014).

2.1.3 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 interconvertible (ISI, 1966; Rangappa and Achaya, 1974; Richards Bolten, 1999; Winton and Winton, 1999). The general values for BR readings of milk fat (40-43) 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 indicator 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 by 5 units in case of ghee (Singhal, 1973; Rangappa and Achaya, 1974; Singhal, 1980). 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 reading. 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.

Bhalerao and Kummerow (1954a and 1956b) reported that the refractive index of whole fat cannot give reliable results about adulteration and suggested

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the use of alcohol fractionation (soluble and insoluble fractions) to increase the concentration of adulterants in one of these fractions, causing enough shift in the refractive index and thus making the detection of the adulterants easier. They further improved the degree of accuracy of detection by subjecting the above alcohol insoluble fraction to acetone fractionation, and subsequent iodination of the acetone soluble fraction before determining refractive index. 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. Kumar (2003) reported that using general limit of B.R. reading as 40-43, adulteration of vegetable oil up to 5% in cow ghee and 15% in buffalo ghee could be detected. Kumar (2008) reported that adulteration of rice bran and soybean oils added to cow milk fat individually could be detected at 10% level. Kumar (2013) reported a detection limit of 10% and 15% for the adulteration of soybean oil in cow and buffalo ghee, respectively. Kumar (2008) and Kumar (2013) noticed that the adulteration of ghee with animal body fat could not be detected by using B.R. reading up to the level of study i.e. 15%.

Upadhyay (2014) studied that adulteration of groundnut oil when added singly in cow ghee could be detected at as low as 10% using B.R. reading, while in buffalo ghee, detection of adulteration of groundnut oil was possible at the level 15% using B.R. reading. However, B.R. Reading was of no use in detection of goat body fat in all the ghee samples. The combination of groundnut oil and goat body fat added to cow ghee could be detected at the level of 20% and above using B.R. reading, while in case of buffalo ghee, the combination of adulterants were not detectable (Upadhyay, 2014).

2.1.4 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). Liquid fraction obtained as a result of ghee storage at 30 to 35°C for one month showed higher iodine value than solid fraction (Singhal, 1973). Upadhyay (2014) reported that adulteration of groundnut oil when added singly in cow ghee could be detected as low as 5% level, while in buffalo ghee, detection of adulteration of groundnut oil was possible at the level of 10% and above using iodine value.

2.1.5 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). Liquid portion of ghee separated on storage at 30 to 35°C for one month showed higher saponification value than solid portion (Singhal, 1973).

Several earlier workers (Achaya and Banerjee, 1946; Murthy, 1955; Ali and Tremazi, 1966; Velu, 1971) have reported that physico-chemical constants failed to detect the adulteration of milk fat with beef tallow, refined cottonseed oil, hydrogenated oils and even coconut oil separately or in mixture even up to 10 percent level. Singhal (1973, 1980) also employed the physico-chemical constants for detecting the animal body fats (buffalo, goat, sheep and pig) added to buffalo and cow ghee and reported that the values for Reichert-Meissl, Polenske, and BR indices remained within the legal limits for normal ghee, when adulterated with animal body fats at 20 percent level. However, when adulteration was done at 50 percent level, the values remained within the legal limits set for cotton tract ghee. Sharma and Singhal (1995) also confirmed the above findings using body fats (buffalo, goat and pig) and vanaspati ghee irrespective of mode of adulteration whether added directly to ghee or through milk. Kumar (2008) had reported that adulteration of pure cow ghee with either animal body fats or vegetable oils at 10 percent levels of adulteration could be

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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.

2.1.6 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 sesamol (present in sesame oil) in the presence of concentrated HCl. Use of hydrofurfamide (Kapur *et al.*, 1960) or p-hydroxy benzaldehyde (Sharma, 1989) has also been suggested in place of furfural for the above test.

2.2 Latest methodology to check the adulteration of milk fat

2.2.1 Based on recent techniques of spectroscopy

Ntakatsane *et al.*, 2013 reported the great potential of fluorescence spectroscopy to rapidly discriminate between authentic and adulterated milk fat, and also to classify the samples according to their source of fat. More importantly, fluorescence spectroscopy could detect up to 5% of vegetable oil in butterfat. The pure and adulterated samples were discriminated based on the total concentration of saturated fatty acids, and also on the three major fluorophores: tryptophan, tocopherols, and riboflavin.

Legg *et al.* (2011) reported the quantification of selected metals in various oils and fats (rice oil, canola oil, sunflower oil, corn oil, soy oil, olive oil, light margarine, regular margarine and butter) was carried out using flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS) after sample emulsification. FAAS was used to determine the Na, K, Ca, Mg, Zn and Fe levels in the samples, while GFAAS was used for quantifying Cr, Ni, As, Pb, Cd, Cu and Mn, as these elements appeared in the samples at much lower concentrations. Atomic absorption spectrometry proved to be a promising approach for the analysis of metals in emulsified edible oils and fats. Additionally, under appropriate emulsification conditions (formulation, stirring time and temperature), the emulsions were homogeneous, had excellent stability, and had appropriate viscosity. The proposed method has proved to be simple, sensitive, reproducible, and economical.

Jirankalgikar and De (2014) reported that the normal UV spectra of cow ghee and tallow did not reveal sufficient information that can be useful for detection of tallow adulteration in cow ghee. Hence, the second order derivative UV spectra of the samples were recorded. The two absorption peaks, one at about 238 nm (Peak 1) and the other around 297 nm (Peak 2) are seen in all the samples. The absorbance of the Peak 1 remains almost same in all the samples while in Peak 2, the absorbance is the highest in ghee and steadily decreases with the increasing concentration of tallow in ghee and is the lowest in tallow. Between these two peaks in each of the spectra, one remains unchanged while the absorbance of another peak changes with the changing proportion of tallow in ghee. It was felt that the ratio of absorbance of these two peaks may be useful in quantifying the tallow adulteration in ghee and the ratio of absorbance (Peak 2 absorbance/Peak 1 absorbance, i.e., B/A) was calculated. A graph was prepared by plotting B/A value against the concentration of tallow in ghee to see the linearity. The decrease in this ratio is quite linear ($R^2 = 0.989$), i.e., proportionate to the concentration of adulteration. Hence, it will be useful in detecting and quantifying adulteration of tallow in cow ghee.

Picariello *et al.* (2013) developed a robust and efficient method, based on high resolution ^{13}C NMR spectroscopy, for detecting the adulteration of butterfat with synthetic low and medium carbon number TAG (triacylglycerol) mixtures. In milk fat TAGs, butyric acid is esterified exclusively at the sn-3 position of the glycerol backbone. Thus, the method relies on ^{13}C NMR monitoring of the carbonyl resonance of butyric acid at the sn-2 glycerol position, which is diagnostic of synthetic or interesterified TAGs. The ^{13}C NMR based analysis overcomes the pitfalls of traditional pattern recognition methodologies for the assessment of butter authenticity, which can be ineffective when butter is adulterated with synthetic TAGs obtained from FA in proportions similar to those of natural milk fat. The method achieves reliable quantification up to 2.5% w/w of adulterating fats (LOQ) and can detect quantities as low as 1% w/w of synthetic TAGs blended with butter (LOD).

Nicolaou *et al.* (2010) reported that Fourier transform infrared spectroscopy and multivariate analysis could detect the adulteration of sheep

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and goat milk in cow milk and vice-versa and advocated the used of this technique for authenticating milk and milk product.

Uysal *et al.* (2013) studied on the determination of butter adulteration with margarine using Raman spectroscopy. The results revealed that a combination of Raman spectroscopy with chemometrics and artificial neural network (ANN) methods can be applied for testing butter adulteration. Fourier transform infrared spectroscopy combined with partial least square (PLS) model has reported successfully to be used in the quantitative analysis of lard in butterfat (Nurrulhidayah *et al.*, 2013) and analyzing the mixture of lard with other animal fats (Man and Mirghani, 2001).

2.2.2 Based on physico-chemical methods

Aktas and Kaya (2001) carried out a study in which differential scanning calorimetry (DSC) melting and crystallization curves of butterfat, beef body fat (BBF) and margarine were formed by cooling gradually from 70 to 40°C. Results obtained show that DSC technique could be used in order to determine the adulteration of butterfat. Upadhyay (2014) reported that goat body fat could be detected individually and in combination at a level of 5% in both cow ghee and buffalo ghee by DSC technique but it could not help in detection of lower level of groundnut oil in both cow and buffalo ghee.

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. Upadhyay (2014) concluded from her work that groundnut oil added individually in cow ghee could be detected only at a level of 15% using CLT and CTD test. However, in pooled data of samples of cow ghee and buffalo ghee, adulteration of groundnut oil added singly could not be detected using CLT test, while it could be detected at a level of 15% using CTD test.

Kumar (2003) reported that fractionation of milk fat into solid and liquid fraction did not offer any extra help using BR. reading. However, using apparent solidification time (AST) test, fractionation technique could extend help in the detection of adulteration of cow ghee especially with the mixtures of goat body fat with vegetable oils even at 5% level, which otherwise was not possible in the

un-fractionated ghee samples. According to Kumar (2008), solvent fractionation offered help in lowering the detection limit of adulteration in terms of iodine value of last liquid fraction. He also reported that complete liquification time (CLT) test can help considerably to detect the adulteration in ghee especially at 44°C without fractionation. However fractionation technique has offered further advantage in lowering the detection limits particularly when the CLT is done at 46°C which otherwise could not be possible without fractionation, when mixture (body fats and vegetable oils) is added. Zachariah (2005) reported that fractionation of cow ghee (at 25°C) followed by estimation of the yield of the liquid fraction was a simple method to detect adulteration of ghee with coconut oil or palm oil even at 5% level as the yield of the liquid fraction obtained at 25°C was higher for adulterated samples than for pure cow ghee. Gandhi *et al.* (2014) reported that RM value for pure cow ghee ranged from 28.60 to 30.36 with an average of 29.50, whereas that for pure buffalo ghee ranged from 31.46 to 34.98 with an average of 33.30. Palm olein and sheep body fat added individually could be detected only at 15 per cent levels in pooled cow and buffalo ghee samples based on RM value determination. Mixture of palm olein and sheep body fat was detectable at 6+14 (20) and 9+21 (30) per cent levels. However, after fractionation, even lower level of 3+7 (10) per cent which was not detectable before fractionation, also became detectable. Upadhyay *et al.* (2014) conducted a study for the development of protocol for the solvent fractionation of pure ghee (cow and buffalo), animal body fat (goat body fat) and vegetable oil (groundnut oil) using two different temperature-time combinations which can successively be used for enriching the first solid fraction with the animal body fat and the last liquid fraction with the vegetable oil (groundnut oil), while keeping most of the ghee concentrated into the middle fraction. The selected temperature-time combinations were 15°C for 15 minutes and 2°C for 2 hours respectively. The fractions obtained using selected temperature-time combination may further be used for the detection of adulteration of pure ghee with these cheaper fats and oils by conducting physico-chemical analysis on the fractions.

Patel (2011) reported that on the addition of body fats, AST of cow ghee decreased, but the decrease was not very prominent up to the addition of 5% of

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body fats, while in case of buffalo ghee, the level of detection for sheep, goat, buffalo and pig body fats were 10, 15, 10 and 15 percent levels and concluded that AST could detect the adulteration of ghee, when the level of adulteration varies from 5 to 15% depending upon the type of adulterant. However, in real situation, where origin of samples is not known it is difficult to ascertain the purity of milk fat.

Upadhyay (2014) concluded from the result on the unfractionated samples that by using AST test, adulteration of groundnut oil added individually could be detected in cow ghee at 5% and higher, while at a level of 10% and higher in buffalo ghee, and in pooled samples of cow and buffalo ghee.

2.2.3 Based on gas liquid chromatography of triacylglycerol

Differences in the chemical composition of triglycerides have also been used as a basis for the detection of adulteration of milk fat with foreign fats by some workers. Based on their fatty acid composition, the possible number of triglycerides in milk fat is calculated to be greater than 1,300 (Barron *et al.*, 1990). Butterfat is composed predominantly of triglycerides with 26 to 52 carbon number, while animal depot fats and common vegetable oils other than coconut and palm kernel oil has 50 to 54 carbon number. Coconut and palm kernel oil contain short and medium chain length triglyceride with 30 to 52 carbon number, a range almost similar to butterfat (Rangappa and Achaya, 1974). Glycerides of ghee are subject to less variation than the fatty acids. Glycerides having lower chain and unsaturated fatty acids are most soluble.

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. Parodi and Dunstan (1969) reported that the trisaturated glycerides (GS₃) analysis could be used for the detection of beef tallow in Australian butterfat at 5 to 10 percent level. Parodi (1971, 1973) carried out GLC analysis of butterfat adulterated at various levels with beef tallow and reported that out of the total samples analyzed, 59.8 percent at 10 percent level, 35.7 percent at 15 percent level, 9 percent at 20 percent level and 2.7 percent at 25 percent level of adulteration were not detectable based on the triglycerides and fatty acid ratios.

However, subsequently in 1973, the same author using the similar technique observed that adulteration of butterfat samples with beef tallow (20% level) and vegetable oils (5% level) was detectable.

Guyot (1969) 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. The $C_{52:0} / C_{50:0}$ ratio was found < 1 in pure butter and between 2 and 3-4, in case of beef tallow and lard, respectively. He concluded that the $C_{52:0} / C_{50:0}$ triglyceride ratio together with $C_{52:0} / C_{38:0}$ ratios give a valuable indication of the possible addition of tallow or lard to butter. Vanoni *et al.* (1978) used GLC for fatty acid analysis of triglycerides and 2 monoglycerides isolated from the liquid and solid fractions of fat crystallized from acetone at 0°C . The author reported that the ratio of $C_{16:0}/C_{18:2}$ fatty acid in 2 monoglycerides of the liquid fraction could detect beef suet in butter at 10% level. Marjanovic *et al.* (1984) reported that adulteration of the milk fat with margarine at 5 to 10 percent level could be detected on the basis that margarine had more triglycerides with 48 to 54 acyl carbon atoms than milk fat. Stock (1985) detected 5-10% of cattle tallow, vegetable oils in cow milk fat using Timm's (1985) method. Similarly, Luf *et al.* (1987) could also detect 5 to 10 percent of beef tallow and lard as well as vegetable oils /fats in milk fat based on $C_{52:0} / C_{40:0}$ and $C_{50:0}-C_{54:0} / C_{38:0}-C_{40:0}$ triglycerides, respectively. Precht (1990, 1992) compared the triacylglycerol composition of different fats as analyzed by GLC and designed multiple linear regression equations 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. Povolito *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.

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Several investigators (Contarini *et al.*, 1993; Renterghem, 1997; Collomb, 1997; Banfi *et al.*, 1999) carried out the triacylglycerol analysis by GLC and modified it to detect adulteration of milk fat.

Pinto *et al.* (2002) did linear discriminant analysis of triacylglycerides to determine the authenticity of pure milk fat. The objective of the study was to propose a methodology for the detection of adulteration of milk fat. Naviglio and Raja (2003) proposed a gas chromatographic analysis of butter using a capillary column having 65% phenyl methyl silicone as stationary phase. This method allows the detection of the presence of extraneous vegetable and animal fats in a simple, rapid and precise way, even if minimum quantities of these fats are added to the butter.

All the methods based on glyceride analysis suffer from major limitations that they require capillary columns with flow splitter which require skilled hand for operation and maintenance. At present our laboratories are rarely equipped with such sophisticated columns. Secondly, all these methods being based on increase or decrease of glyceride ratio escape detection in most of the cases (Parodi, 1973) and thus can't ensure a foolproof detection. The adulteration of bovine milk fat with foreign fat can be detected by the analysis of its triacylglycerol (TAG) composition in combination with TAG formulae to calculate S-values. With genuine milk fats these S-values must not exceed certain limits already laid down in German and European analytical standards since 1994. Consequently, the composition of genuine milk fat from different continents still fits the S-ranges formerly established for Europe, and the TAG method most likely can be applied globally. Limitations have to be considered with technologically processed milk fat or fat from low-fat milk products such as skim milk or buttermilk (Molkentin, 2007). Pure milk fat, together with mixtures of milk fat containing two levels of four foreign fats (coconut fat, sunflower oil, lard and beef tallow), were analyzed by both the conventional capillaryGC method and UFM-GC. Sheep, goat and buffalo milk fats were also analyzed. The reference material CRM-519 was used for calibration. Repeatability and reproducibility values were always below the limits reported in the Official method, demonstrating the suitability of UFM-GC for the evaluation of milk fat purity. The investigation on sheep, goat and buffalo milk fat confirmed that the S ranges,

calculated for cow milk fat, are not applicable to the evaluation of genuineness of non-bovine milk fats (Povolo *et al.*, 2008). Gutierrez *et al.* (2009) utilized gas liquid chromatography to determine triacylglycerol in milk and non-milk fat. The values of triacylglycerol were subjected to linear discriminant analysis to detect and quantify the non-milk fat in milk fat. The samples of raw milk fat were adulterated with non-milk fat (fish oil and corn oil) in proportions of 0, 5, 10 and 20 percent. The first function obtained from linear discriminant analysis allowed the correct classification of 94.4% of the samples with less than 10% level of adulteration.

Kala (2013) detected the adulteration of ghee and the detection level was found to be 2% in case of beef tallow and coconut oil addition. Vegetable fat and lard addition could be detected at 5% and 6.3%, respectively.

2.2.4 Based on chromatography of sterols

Sterols which represent maximum share of the USM are found to be ranged 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 (Bailey, 1951). Cholesterol is the characteristic sterol of animal fats, while sterols from plant sources are consisted of a mixture collectively called as phytosterols and include β -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 β -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). Animal sterols have 98 percent cholesterol, 1 percent dehydrocholesterol, and some ergosterol and possibly campesterol. The vegetable sterols have 57 to 96 percent sitosterol, 1 to 32 percent campesterol, 0 to 21 percent stigmasterol and 0 to 3 percent cholesterol (Guyot, 1969). Milk fat also contains cholesterol in free as well as ester form in 10:1 ratio (Fox, 1983).

The sterols can help to distinguish between fats of animal and vegetable origin, since the melting point of cholesterol acetate (114°C) is substantially lower than that of the acetates of any of the phytosterols (126-137°C). Sterol

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determination is the most effective and versatile measurement, because it provides information not only on the detection and extent of adulteration, but also on the possible identity of the adulterant (Sheppard *et al.*, 1985).

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). They 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. However, ghee from cottonseed fed animals behaved like adulterated ghee. Gallego (1969) applied the descending paper chromatography to the USM of butterfat and margarine mixture and observed that margarine at 10 percent level could be detected on the basis of presence of vegetable sterols (ergosterol, stigmasterol, sitosterol). Sharma (1989) applied paper chromatography to the USM of ghee, body fats and their mixture, and observed that ether soluble fraction of ghee adulterated with buffalo body fat at 10 percent level exhibited two spots. However, pig body fat in ghee could not be detected by this method.

International dairy federation (IDF), 1966 recommended a TLC method for the detection of vegetable fats in milk fat based on the appearance of a small band of β -sitosterol acetate in addition to the major band of cholesterol acetate using reverse 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 gm + 4 gm) impregnated with liquid paraffin and developing solvent consisting of methanol: acetic acid: water (20:5:1, v/v) 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 separation of cholesterol and phytosterols having R_f values of 0.53 and 0.44, respectively.

A simple TLC method was developed by Chourasia *et al.* (1994) by spotting USM on silica gel G coated plates using chloroform : acetone : acetic acid (90:10:1, v/v) as the developing solvent. Based on the appearance of a characteristic violet spot in adulterated ghee observed at R_f 0.55 to 0.60, Kokum

(*Garcinia indica*) fat in ghee up to a level of 5 percent could be detected. 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 pure body fats and ghee samples adulterated with 10 percent body fat resulted in the appearance of an extra spot due to dihydrocholesterol present in body fats. TLC of free and esterified sterol digitonides could detect adulteration of ghee with 10 percent body fats, while TLC of trimethyl silyl ethers (TMS) from USM failed to detect ghee adulteration. GLC of sterols has also been used in recent years for the detection of adulteration of milk fat with vegetable oils and fats. Among the various phytosterols, β -sitosterol has been very widely used as an index of adulteration (Parodi, 1967, 1969; Zurcher *et al.*, 1976; Colombini *et al.*, 1978; Homberg and Bielefeld, 1979). Samridhi (2012) detected coconut oil and designer oil at 4% level using RP-HPLC. A new method, involving the use of high-performance thin-layer chromatography, has been developed by De *et al.* (2013), which detects 10–20% (w/w) of adulterant tallow in cow ghee. The method consists of highperformance thin-layer chromatographic separation of both unsaponifiable and saponified portions using n-hexane–diethyl ether–glacial acetic acid (4:6:0.2, v/v) (for unsaponifiable portion) and n-hexane–diethyl ether–glacial acetic acid (6.5:3.5:0.2, v/v) (for saponifiable portion) as mobile phases. Beef tallow was mixed with cow ghee in various proportions (5 to 90%) to obtain admixtures of beef tallow with cow ghee. The analysis of the samples of cow ghee containing different proportions of beef tallow revealed that the addition of beef tallow to cow ghee affected the chromatographic profile; the effect increased with increasing proportions of beef tallow. Ghee adulterations with tallow at levels down to 20% are clearly seen visually in the chromatographic profile. Rani (2013) concluded that the separation of cholesterol and phytosterols could be achieved by using RP-HPLC over C₁₈ column at 205 nm. Analysis of USM of adulterated ghee samples revealed that the minimum level of detection in case of coconut oil was to the tune of 5%, designer vegetable oil 2%, refined soybean oil 1%, refined sunflower and groundnut oil 2%.

Kumar *et al.*, 2013 detected the adulteration of soybean oil in milk fat at 5% level by normal-phase thin layer chromatography. Upadhyay *et al.* (2014)

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reported that RP-TLC of reference standards, unsaponifiable matter of pure ghee (cow and buffalo), pure vegetable oils, and the ghee adulterated with these vegetable oils ($\geq 1\%$) was carried out on plates with different stationary phases, viz., RP-18, RP-8, and RP-2 plates using solvent system comprising of petroleum ether, acetonitrile, and methanol. The results revealed that adulteration of vegetable oils in ghee can easily be detected at a level of as low as 1 percent on RP-18 and RP-8 plates, while RP-2 plate did not offer any help in detection of adulteration. Using the reversed-phase thin layer chromatographic protocol standardized in the present study the adulteration of ghee with adulterant oils such as soybean oil, sunflower oil and groundnut oil could be detected up to 1% level while, designer oil up to 2 % level. Similarly, coconut oil addition in ghee could be detected up to 7.5 % level (Rani *et al.*, 2015).

2.3 Market survey of ghee and butter

Kumar (2008) collected eleven ghee samples of known brand from different regions of the country, and analyzed for RM value, Polenske value, and BR reading at 40 °C, iodine value, crystallization time test, and CLT test at 44°C. These samples were also subjected to solvent fractionation, and first solid fractionation (S_{16}) obtained was analyzed for CLT test at 46°C, while liquid fraction (L_4) obtained on fractionation was analyzed for BR reading at 40°C and iodine value. On the basis of results obtained, he concluded that out of eleven ghee samples collected from the markets, nine samples were of pure ghee while two samples (18%) were possibly adulterated with animal body fats. Kumar (2010) carried out a study on the physico-chemical qualities of market ghee sold in the organized dairies of India and unorganized dairies of Karnal. Out of 36 branded ghee samples of organized dairies from all the regions, 8 samples (22%) failed to meet legal requirements in term of either RM value or BR reading or both. Similarly, out of 30 ghee samples from unorganized dairies of Karnal city, 8 samples (27%) also failed to meet the legal specification in term of RM value and BR reading or both.

Rani (2013) reported that all the samples from organized sector met the legal standards for ghee, however among the unorganized sector ghee samples 33.33% were below standard. RP-HPLC and RP-TLC profile of the USM from

market ghee samples revealed that 80% of all the samples from organized sector and about 73% from unorganized sector were found to contain vegetable oil/fat.

Fifteen commercial and 22 control samples of ghee were analysed for triglyceride compositions by low-resolution short capillary column gas chromatography. A sample survey based on triglyceride profile and standardised (S) values calculation revealed up to 43% branded ghee samples and 87.5% samples of unbranded ghee did not conform to triglyceride profiles of control ghee (Kala, 2013). The fat content of ghee based sweets obtained to nearly 50% of the edible portion through gas liquid chromatography of triacylglycerol composition (Kala *et al.*, 2016).

Subrahmanyam (1981) collected 350 butter samples in Kerala (India), only 5 met all requirements of the Prevention of Food Adulteration Act.

Twenty samples each of khoa, burfi, rasogolla, ice cream, butter, ghee and infant milk foods were collected by Rao *et al.* (2004) from the distinctive marketing agencies in Hyderabad city and analysed for chemical quality and possible adulteration. All the samples from cooperative dairies and most of the samples from private dairies could meet the minimum legal standards, whilst samples from unorganised sectors (vendors, sweetmeat shops and small traders) could not meet the minimum legal requirements. Some samples of khoa, burfi and ice cream were found adulterated with starch, whilst vegetable oil was detected as adulterant in some market samples of burfi and ghee.

2.4 Market survey of paneer

Aghav (2014) conducted the analysis of market samples of paneer collected from local market (unorganized sector) of Karnal city showed that out of fifteen market samples seven samples (46.6%) had lower RM value compared with FSSAI and AGMARK standards, two samples do not lie within the normal range of Polenske value compared with AGMARK standards. Iodine value of six samples was higher than normal range of 26-35. BR reading of seven samples showed higher value compared with FSSAI and AGMARK standards, hence, these samples may be containing external fats. While saponification value and free fatty acid (% lactic acid) content were found to be normal in all fifteen paneer samples. Goyal *et al.* (2007) compared the physico-chemical properties and

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microbiological quality of paneer from different markets in Agra in Uttar Pradesh, India, with a control sample. It was shown that acidity (0.30-0.45%) and moisture content (49.23-51.20%) were lower in the control sample, while fat (18-23%), protein (17.45-19.23%), lactose (1.25%) and ash contents (1.60-2.9%) were highest in the control sample. The highest total viable bacterial count (TVC) was in paneer from Bijilighar (860-1420 thousand/g), while the highest coliform count was in paneer from Sadar (94-150/g). The lowest TVC and coliform count were in control paneer (758-100 thousand/g and 30-50/g, respectively). The highest yeast and mould counts were from paneer from Bhagwan Talkies (127-175/g), while the lowest was in control paneer (50-65/g). The higher microbial counts in market paneer indicate the unsanitary conditions during its preparation and sale. Das and Ghatak (1999) conducted a study to assess the quality of paneer available in sweet-shops in and around Calcutta. A total of 50 samples (45 market and 5 laboratory) of paneer were analysed for chemical and microbiological quality. Out of 45 market samples of paneer, 19 samples did not conform to the legal standard in respect of fat content. Rajorhia *et al.* (1984) reported that 18 paneer samples from Karnal and 7 from Delhi markets, and 15 samples manufactured under controlled conditions at the National Dairy Research Institute, resp., had the following mean chemical and microbiological composition: moisture, 51.9, 50 and 50.7%; fat, 26.6, 27.7 and 27.1%; protein, 17.7, 17.6 and 18%; lactose, 2.1, 2.0 and 2.3%; ash, 1.8, 2.2 and 1.9%; lactic acid, 0.76, 0.80 and 0.78%; total viable count, 761.4 million, 202.1 million and 3.5 million/g; coliform count, 6.08 million, 25.06 million and 2091/g; and yeast/mould count, 7918, 30 100 and 132/g. The poor microbiological quality of the market samples showed a need for improved hygiene standards during manufacture, and the importance of refrigerated transport and storage. Nayak and Bector (1998) collected 10 samples of paneer from Karnal and Delhi markets during March-April 1996. Contaminants in the form of formaldehyde, hydrogen peroxide, urea and alkali neutralizer were detected. About 70% of samples did not conform to Indian Standards in respect of fat content. Presence of a high content of C18:1, C20:0, C22:0 and C20:4 fatty acids and the absence of C4:0 suggested that some samples may have been adulterated with foreign fats.

Sharma and Lavania (1987) reported that 50 khoa samples (35 market and 15 laboratory) were analysed for chemical and microbiological quality. The presence of alum in market samples of khoa made them unsuitable for human consumption. The chemical quality was well within the legal standards but the microbiological quality was poor. Ghatak and Bandypadhyay (1989) collected 57 khoa samples from retail sweet dealers in Calcutta and reported that the samples were mainly of good texture and organoleptic quality (3 had a flat and 2 had rancid flavour). Chemical composition was as follows (mean values in parentheses): moisture 23.8-32.7% (26.3); fat, 17.6-27.3% (24.3); protein, 21.6-23.3% (22.8); lactose, 19.4-21.2% (20.8); ash, 3.57-3.85% (3.71); titratable acidity, 0.23-0.78% (0.58); and peroxide value 0.06-0.18 (0.13). Iodine test was positive for 5 samples, which indicated that they were contaminated with starch.

Zariwala *et al.* (1974) collected a total of 551 samples of khoa from retailers in Bombay city between 1966 and 1971 and reported average composition (plus or minus SD) of $28.13 \pm 2.39\%$ moisture, $27.14 \pm 3.12\%$ fat and $17.67 \pm 1.80\%$ lactose. 291 samples did not conform to the Indian Standards Institution specification for moisture (less than or equal to 28.0%), and 174 samples did not conform to the specification for fat % (more than or equal to 26%). Starch was not detected in any of the samples, but some had a poor flavour, which may have been due to the presence of stabilizers. The majority of samples were of good texture and organoleptic quality.

Recently, Kala (2012) analysed the fatty acid composition of Khoa a heat-desiccated milk product and compared with those obtained with cow milk, buffalo milk and toned milk. Fatty acid methyl esters were analysed by capillary gas chromatography (GC). Among the Khoa samples analysed, 57% showed fatty acid composition characteristic of milk fat. The ratio of major saturated fatty acids to unsaturated fatty acids (S/U) including 18:1 trans fatty acids was calculated for all the fats. The GC profiles of 43% Khoa samples showed the composition of fatty acids not conforming to milk fats. The Khoa samples adulterated with non-milk fats were further confirmed by cholesterol and triglyceride estimation.

It can be deduced from the above review that milk fat, which bears the highest price tag among all the edible fats, plays a significant role in the economics, nutrition, and physico-chemical properties of milk and milk products.

Review of Literature

In India, milk fat is mostly consumed in the form ghee and butter. Similarly texture of paneer is also very much dependent on fat content of the paneer. These products are also manufactured at large scale by the unorganized sector like local dairies and sold in open market as well as to the road side dhabas. Review suggests that work has been done on the quality of these products with reference to compositional aspects but no systematic work had been done to know the quality of milk fat in market samples of butter, ghee and paneer. It is not known to what extent these types of malpractices of adulteration are prevailing in the trade of these products in our country and what quality of fat is being used in these products available to the consumer on local dairies and road side dhabas. There is a lack of sufficient data and regular monitoring for the quality of butter, ghee and paneer sold in unorganized sector like road side dhaba and local dairies. Animal body fat adulteration at low concentration is still undetectable. Recently, RP-TLC has been reported to detect the presence of vegetable oil in ghee. Similarly, GLC based method has been reported to detect the presence of tallow in milk fat. However, the efficiency of these methods in fat isolated from different dairy products has not been tested. Therefore, a systematic research has been planned to assess the quality of milk fat in butter, ghee and paneer.

CHAPTER –3

Materials & Methods

MATERIALS AND METHODS

For present investigation on quality of milk fat in market samples of butter, ghee and paneer, the materials and methodologies employed are dealt in this chapter.

3.1 CHEMICALS AND REAGENTS

3.1.1 CHEMICALS

Glacial acetic acid, Potassium hydroxide pellets, Sodium hydroxide pellets, and Sulphuric acid (AR grade, Qualigens fine chemicals, Mumbai, India), Glycerol (AR grade, Ranbaxy Laboratories Ltd., Punjab, India), Hydrochloric acid (GR grade, Loba Chemie., Mumbai, India), Phenolphthalein (AR, s.d. fine-chem Ltd., Mumbai, India) , Phosphomolybdic acid (HPLC grade, Mumbai, India), calcium sulphate (ExcelaR, Qualigens Fine Chemicals), Cellulose powder (AR, s.d. Fine-chem Ltd., Mumbai, India).

3.1.1.1 SOLVENTS

Chloroform (HPLC, Thermofisherscientific Pvt. Ltd., Mumbai), Acetonitrile (HPLC, s.d. Fine-chem Ltd., Mumbai, India), Methanol (HPLC grade), Petroleum Ether (VETEC, sigma Aldrich chemical Pvt. Ltd., Bangalore), Hexane (HPLC, Loba Chemie., Mumbai, India), Ethanol absolute (Jiangsu Huxari International Trade Co. Ltd., China).

3.1.1.2 Reversed-phase TLC plates

TLC aluminium sheets silica gel 60 RP-18 F₂₅₄S TLC plates (Merck Specialities Pvt. Ltd. Mumbai).

3.1.2 Reagents

3.1.2.1 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.

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3.1.2.2 Ethyl alcohol (neutralized):

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

3.1.2.3 Phenolphthalein solution

One gram of phenolphthalein indicator was dissolved in ethyl alcohol and then volume was made to 100 ml with absolute ethyl alcohol.

3.1.2.4 Oxalic acid solution (0.1N):

Exactly 0.63 gm of oxalic acid was dissolved in distilled water and volume was made to 100 ml in a volumetric flask.

3.1.2.5 Sodium hydroxide solution (0.1 N)

About 4.0 gm 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 solutions.

3.1.2.6 Sodium hydroxide solution (50%; w/w)

Exactly 50 gm of sodium hydroxide pellets were dissolved in equal weight of distilled water and kept in a bottle protected from CO₂.

3.1.2.7 5% Methanolic potassium hydroxide solution

Exactly 5.0 gm of potassium hydroxide was dissolved in methanol and volume was made to 100 ml in volumetric flask.

3.2 EQUIPMENTS

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

3.2.2 Butyro- refractometer: (Naveen Scientific Industries, Ambala, India).

3.2.3 Centrifuge: (Remi Udyog, Mumbai, India).

3.2.4 Cream separator: (Kamdhenu, KD-60E, Benny Impex, New Delhi, India).

3.2.5 Digital temperature controller oven: (Narang Scientific Works Pvt. Limited, New Delhi, India).

- 3.2.6 Electric heater:** (Vikrant, Jain Enterprises, India).
- 3.2.7 Electronic balance:** (Precisa XB 220A, Switzerland).
- 3.2.8 Rotary vacuum evaporator:** (Metrex Scientific Instruments Pvt. Ltd. New Delhi, India).
- 3.2.9 Vortex mixer:** (Spinix, Tarsons Products Pvt. Ltd., Kolkata, India).
- 3.2.10 Desiccator:** (Tarsons products Pvt. Ltd., Kolkata, India).
- 3.2.11 water bath with thermostat:** (The laboratory Glassware Co., Ambala Cantt, India).
- 3.2.12 Gas chromatography:** GC-2010 plus, Shimadzu
- 3.2.13 Heating mantle:** ABC India, capacity 1000 ml.
- 3.2.14 UV-Visible spectrophotometer:** UV-2700, Shimadzu.
- 3.2.15 Filter paper:** Whatman no.1 and 4 of 12.0 cm diameter, (GE Healthcare UK Ltd., UK), Made in China.
- 3.2.16 Thimbles:** Whatman cellulose extraction thimbles (single thickness), 41×123 mm (internal diameter and external length), (GE Healthcare UK Ltd., UK).
- 3.3 Collection and Preparation of Adulterant Fats**

Three body fats (buffalo, sheep and pig), vegetable oil and margarine were used as the adulterant fats in the present study. Sheep body fat and pig body fat were prepared separately from their respective adipose tissues collected from the local slaughter house. The adipose tissues of buffalo body fat were collected from slaughter house located at New Delhi. These adipose tissues after collection were washed thoroughly under running water. After draining out the residual water, the adipose tissues were heated at 130 to 150°C (buffalo-150°C, Sheep-140°C and pig-130°C) over direct flame in a stainless steel vessel till a transparent liquefied animal body fat was obtained. The liquid fat thus obtained was filtered through 6-8 fold muslin cloth followed by Whatman No. 4 filter paper, filled in polyethylene bottle, cooled to room temperature and kept in a refrigerator (5-10°C) for their further use as the adulterant fats. For the

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preparation of adulterated milk fat samples, milk fat, vegetable oil and animal body fats were heated to 60-70°C for 10 min before mixing.

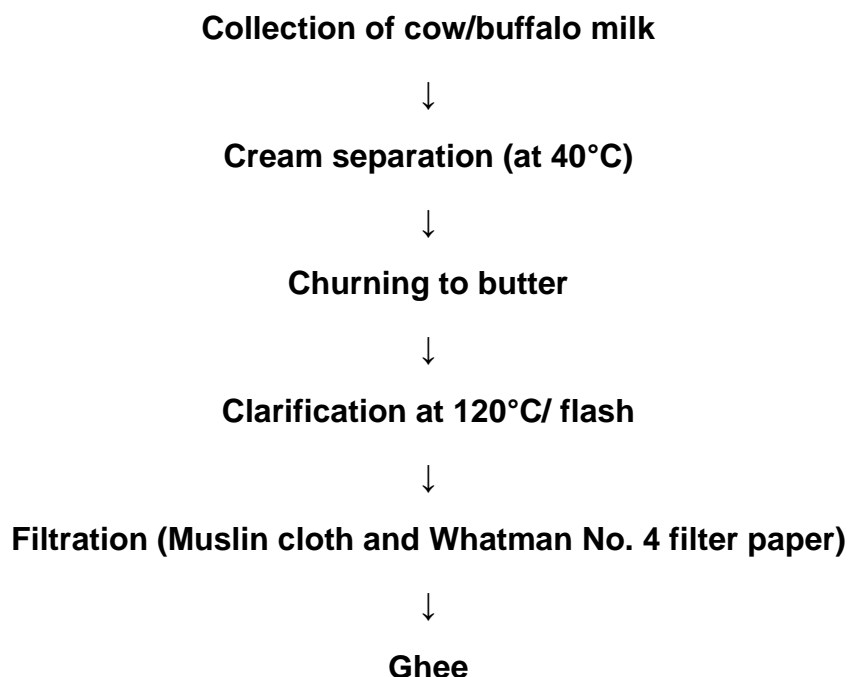
3.4 Collection and preparation of samples

Authentic pooled samples of raw cow and buffalo milk were separately collected fresh from the Experimental Dairy, Department Of Dairy Technology, N.D.R.I., Karnal and used in all the experiments.

3.4.1 Preparation of Ghee

Samples of cow/buffalo ghee were prepared by creamery butter method (De, 2010). 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. Butter was prepared under standard conditions (13°C in winter) by churning the cream using hand operated butter churn. 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 polyethylene bottles, cooled to room temperature and kept in a refrigerator at a temperature of 5 to 10°C till further analysis.

The flow chart for the preparation of cow/ buffalo ghee was as follows.



3.4.1.1 Preparation of adulterated ghee samples

For the preparation of adulterated ghee samples, ghee, soybean oil, coconut oil, lard, buffalo body fat and goat body fat were heated and maintained at 65-70°C for the 10 min before mixing. The adulterant fats/oils were added to ghee individually as well as in their combination.

Table 3.1 Adulterated samples used in the study

SI. No.	Samples	Adulterants	Levels (%)
1	Pure ghee (cow and buffalo)	-	-
2	Cow ghee	Soybean oil Lard Buffalo tallow Goat tallow	5, 10, 20 5,10, 20 5, 10, 20, 50 5, 10, 20
3	Buffalo ghee	Soybean oil Lard Buffalo tallow Goat tallow Coconut oil	5, 10, 20 5,10, 20 5, 10, 20, 50 5, 10, 20 5, 10, 20
4	Buffalo ghee	Coconut oil + lard	5+5, 10+10, 20+20

At individual levels, these adulterants were separately added to ghee at 5, 10 and 20 percent levels. In case of combined mixture, coconut oil (5, 10 and 20 percent) and lard (5, 10 and 20 percent) were added in ghee, representing a total adulteration of 10, 20 and 40 percent levels, respectively. After the addition of adulterant oil and fats to ghee, the samples were thoroughly mixed.

3.4.2 Preparation of paneer sample

Paneer samples were prepared by the method of Aneja *et al.* (2002). Standard milk (4.5% fat) for paneer making was first heated up to 90°C and cooled to 76°C, and then 1% citric acid solution was added in to milk by stirring with the help of flat steel ladle, at this point, clear whey separates out, allowing the curd to sink in bottom of stainless steel vat. After 10 minutes of coagulation,

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the whey was removed from curd. And the curd was pressed in paneer hoops at pressure of 2-3 kg/sq cm for 10-15 min. Then prepared paneer dipped in cooled water at 4°C for 1-2 hours.

3.4.2.1 Preparation of adulterated paneer samples

These samples were prepared in laboratory to establish whether the fat extracted using Soxhlet procedure could be used for the purpose of checking the adulteration by physico- Chemical as well as other studied methods.

3.4.2.1.1 Preparation of adulterant fat emulsion:

To get the adulterant fat, emulsion was prepared using milk fat and vegetable oils. Admixture of milk fat and vegetable fat was prepared by mixing vegetable oil to milk fat @ 5%, 10 and 20%. Liquid detergent (teepol) was added @ 0.5% of the fat to the admixture and hand mixed to get an emulsion.

Example: To prepare two liters of adulterated milk containing 5% fat the following procedure was adopted:

100 gm of adulterant fat (5% adulterated milk fat) was mixed well with 0.5 gm of liquid detergent at 60°C- 65°C and then about 1.9 liters of hot skimmed milk was added to this emulsion by continuous stirring. The resultant milk was having about 5% of fat and this milk was used for paneer preparation. Similarly other samples containing higher amount of vegetable oils were prepared.

3.4.2.1.2 Preparation of adulterant paneer:

For the preparation of adulterated paneer samples, the milk containing about 5% of the adulterant fat as stated in the above mentioned example, was used in place of pure milk and rest of the procedure remained the same as in case of control paneer preparation (Aneja *et al.*, 2002).

3.4.2.2 Extraction of fat from paneer samples

One hundred gram of Paneer sample was first mixed with almost equal weight (100 gm) of cellulose powder and added calcium sulphate @ 10% of the cellulose powder and mashed in pestle and mortar and then transferred in extraction thimbles. The thimble was then placed in soxhlet extractor which was connected to flask containing about 250 ml chloroform and attached with condenser of soxhlet assembly, having the provision of a heater. The heating at

a temperature of about 65-70°C was started. The extraction process was finished in about 3 hour. After that, the flask containing the extracted fat in the solvent was removed and connected to vacuum rotary evaporator to remove the solvent. The residual solvent was removed by nitrogen flushing. The extracted paneer fat free from solvent was used for the analysis.

3.4.3 Preparation of butter

Samples of butter were prepared by churning of unripened cream (De, 2010). 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 stored for ageing at 5-10°C. Butter was prepared under standard conditions (13°C in winter) by churning the cream using hand operated butter churn. After the churning butter was stored into deep freezer, until it was used.

3.4.3.1 Preparation of adulterated butter samples

For the preparation of adulterated butter samples margarine was used as adulterant fat. Margarine and butter were melted at 70°C and allowed to stand at 70°C for 30 min., after that molten samples were filtered separately through three fold of muslin cloth to get rid of larger curd particles and filtered material (filtrate) was used for the preparation of samples. The filtrate of pure butter was adulterated with filtrate of margarine @ 5, 10, 20 percent level followed by clarification at 120°C and then filtration through filter paper to get clear fat. The fat obtained so was stored in sample bottles and kept under refrigeration till analysis.

3.5 Collection of market samples of butter, ghee and paneer

The samples of butter, ghee and paneer manufactured and sold by local dairies, road side dhabas and organized sector of NCR were collected and analyzed for physico-chemical constant, RP-TLC, and TAGs profiling. 16 samples of each butter, ghee and paneer were collected from unorganized sector, and 5, 10 and 7 samples of butter, ghee and paneer respectively, were collected from organized sector. After collection all samples were kept in a refrigerator (5-10°C) till they were analyzed for their quality by the above said methods.

3.6 Methods of analysis

In this section, various methods followed to study the Physico-Chemical constants of milk fat samples are described briefly.

3.6.1 Physico-chemical constants of milk fat extracted from paneer, butter and ghee

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

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

Accurately 5.0 g of sample was 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°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. From this, the RM value was calculated as follows:

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

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:

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

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

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

Butyro-Refractometer (BR) reading of control ghee samples and adulterated ghee samples were determined by the method as described in BIS, (1981) SP: 18 (part XI). Before determining the BR reading of a sample, the temperature of the refractometer was adjusted to $40.0 \pm 0.1^\circ\text{C}$ 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 prism was 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.

3.6.1.3 Determination of Free Fatty Acids (FFA)

10 g of the sample was weighed in a 250 ml conical flask. In a second flask 50 ml of alcohol was brought to the boiling point and while still above 70°C it is neutralized in the presence of phenolphthalein (using 0.5 ml) with 0.1 N sodium hydroxide. The neutralized alcohol was poured on ghee in the flask and the contents of the flask were mixed. Flask content were boiled and while still hot, titrated with 0.1N sodium hydroxide, shaking vigorously during the titration. The end point of the titration was reached when the addition of a single drop produced a slight but definite colour change persisting for at least 15 seconds.

$$\text{FFA percent by weight (\% oleic acid)} = 2.82 T/W$$

Where;

T= amount of 0.1 N NaOH used during titration

W= weight of sample

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3.6.1.4 Baudouin test

The test as described in BIS, (1981) SP: 18 (part XI) was performed as follows:

In a dry test tube, 5.0 ml of melted ghee sample was taken, to which 5 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 adding 5 ml of distilled water and shaking again. If the colour in acid layer persists, vanaspati is present in ghee. If the colour disappear it is absent in ghee.

3.6.2 Reversed-phase thin layer chromatography (RP-TLC) of unsaponifiable matter

In order to check the presence of vegetable oils in milk fat samples, RP-TLC of unsaponifiable matter was performed. Unsaponifiable matter from fat samples was extracted as per the method standardized by Sharma *et al.* (2009) and applied on RP-TLC plates as per the method described by Rani *et al.* (2015).

3.6.3 Triacylglycerols (TAGs) profiling using Low- resolution GLC

Triacylglycerol profile of fat of control and market samples were analyzed by low- resolution chromatography using BP5, capillary column 2.5 m length (cut from 15 m X 0.25 mm X 0.25 μ m) in gas chromatograph (Shimadzu GC 2010 PLUS), equipped with flame ionization detector and temperature control module. A GLC programme standardized in the lab by Hazra (2016) which was essentially based on the method of Kala (2013) was used for the analysis of the samples.

Table 3.2 Details of temperature programmed

Ramp Rate	Temperature	Hold Time
	80°C	0.50 min
50°C/min	190°C	1min
6°C/min	335°C	5min

Injector Condition-

- 1) Temp of injector-350°C
- 2) Split ratio-1:50
- 3) Pressure-13.4Kpa
- 4) Total Flow 79ml/min (N₂)
- 5) Column Flow 1.49ml/min
- 6) Linear velocity-56.4cm/s
- 7) Purge flow3ml/min
- 8) Injection volume-1µl

Detector (FID) Condition-

- 1) FID temperature-370°C
- 2) Make up flow-30ml/min, H₂ flow -40ml/min, Air flow-400ml/min

GC solution software provided by the GLC Company was used to determine the percentage of different tri-glycerol present in different fat sample

3.6.3.1 Sample preparation

Molten and filtered fat sample was dissolved in Hexane in such proportion so that the concentration of the sample was 5 mg/ml. To get this concentration 25mg of molten fat sample was transferred to a 5ml volumetric flask and volume was made to 5ml with hexane. Contents of the flask were mixed gently for 1 min to have a uniform sample.

3.6.4 Derivative spectroscopy

The normal absorption spectra and 2nd order derivative absorption spectra of ghee and tallow were analyzed as per the method described by Jirankalgikar and De (2014).

One gram of pure ghee and tallow were taken separately, melted by warming, 10 ml chloroform was added, sonicated for 15 min to make it homogenous and were used as stock solution. Mixture of ghee and tallow in different proportions ranging from 9:1 to 1:9 were prepared by using the stock solutions of ghee and tallow. 10% v/v solution in chloroform of different samples,

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i.e. cow ghee, tallow as well as cow ghee containing different proportion of tallow, as prepared above, were used for the study.

3.7 STATISTICAL ANALYSIS

The data obtained in the present study was subjected to one-way analysis of variance (ANOVA) for the significant difference in the samples via Tukey's Multiple Comparison test performed at 95% confidence interval with GraphPad Prism software (version 5.01for windows), San Diego California, USA. Two-way analysis of variance (ANOVA) was applied for checking the effect of different level/adulterant on the studied parameter and comparison between means was made by critical difference (CD) value. The results were expressed as means \pm standard error of mean for physic-chemical analysis. Similarly, for GLC analysis, results were expressed as mean \pm standard deviation of mean.

CHAPTER -4

Results and Discussion

RESULTS AND DISCUSSION

In the present investigation, quality of milk fat in market samples of butter, ghee and paneer was evaluated. There were two objectives of the study. The first objective was aimed to standardize and optimize the derivative spectrophotometry method for detection of tallow in milk fat. The second objective was to evaluate the quality of milk fat in market samples of butter, ghee and paneer.

In order to meet the objective of the study, control samples of the three products were prepared in the laboratory, National Dairy Research Institute, Karnal. To study the quality of milk fat in market samples of paneer from both organized and unorganized sector, milk fat was extracted with the help of chloroform using soxhlet extraction method. Similarly, for analysis of quality of milk fat in market samples of butter, milk fat was extracted using the heat clarification method.

For market survey, 10 branded ghee samples, 7 branded paneer samples and 5 branded butter samples from organized dairies were collected from NCR. Beside this, 16 samples of each butter, ghee and paneer from unorganized dairies of NCR were also collected. Therefore, a total of 70 samples of three products were analyzed in the present study.

Milk fat extracted from market samples of the said product collected from organized as well as unorganized dairies along with control samples were analyzed for physico-chemical constants, such as:

- I. Reichert-Meissl value (RM-value)
- II. Polenske value (P-value)
- III. Butyro-refractometer reading at 40 (B.R.-reading)
- IV. Free fatty acid value (FFA %)

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

Results & Discussion

- I. Spectral analysis- i.e. Derivative spectroscopy to detect tallow in ghee
- II. Baudouin test for detection of vanaspati in ghee samples
- III. Reversed-phase thin layer chromatography of unsaponifiable matter of fat extracted from market samples to ascertain the presence of vegetable oil/fat in ghee.
- IV. Low-resolution gas liquid chromatography for triacylglycerol analysis

4.1. To standardize and optimize the derivative spectrophotometry method for detection of tallow in milk fat.

Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. Thus, the information content of a spectrum is presented in a potentially more useful form, offering a convenient solution to a number of analytical problems, such as resolution of multi-component systems, removal of sample turbidity, matrix background and enhancement of spectral details. Derivatisation of sets of digital data is well known method of separation useful signals from noised data (Talsky, 1994). Historically, the beginning of derivative spectrophotometry is dated on 1953 when the first analogue spectrophotometer was build by Singleton and Cooler (Talsky, 1994). But the fast development of this technique started in 70s of twentieth century, when new generation of spectrophotometers controlled by computers were constructed. An apogee of its popularity occurred in 80s of last century. Derivative spectrophotometry is now a reasonably priced standard feature of modern micro-computerized UV/Vis spectrophotometry (Ojeda and Rojas, 2004).

Derivative spectroscopic methods using ultra-violet (200 to 400 nm) region had been used for characterizing pure ghee and buffalo tallow (Jirankalgikar and De, 2014). In the present study, samples of pure ghee (buffalo and cow), buffalo tallow and ghee samples adulterated with buffalo tallow were scanned in the Ultraviolet (200 to 320 nm) regions.

Pure ghee (buffalo and cow), buffalo tallow and ghee samples adulterated with buffalo tallow (50+50) were dissolved in chloroform of different grade and scanned on double beam UV-2700 spectrophotometer, between 200 to 400 nm chromatogram showed first absorption maxima between 280 and 290 nm (Figures 4.1 to 4.2). Thus, it was concluded from the results that the normal spectra of pure ghee (cow and buffalo) and buffalo tallow behaved in almost similar fashion. Therefore, it can be inferred from the above findings that Ultra-violet (UV) spectroscopic examination of fats and oils did not give any indication for demonstrating the adulteration in ghee. Consequently, ghee samples adulterated with buffalo tallow, individually at 50% percent levels also did not show any difference from pure ghee in terms of absorption maxima (Fig. 4.2). The results obtained in the present investigation were in full agreement with those of Singhal (1973) and Sharma (1989), who also opined that UV spectroscopy did not offer any help in detecting adulteration in milk fat.

The normal UV spectra of pure ghee and tallow did not reveal sufficient information that can be useful for detection of tallow adulteration in cow and buffalo ghee. Hence, in the present study, second order derivative UV spectra of the samples were recorded as per the method described by Jirankalgikar and De (2014). They concluded from the research that the two absorption peaks, one at about 238 nm (Peak 1) and the other around 297 nm (Peak 2) were seen in cow ghee, tallow and the adulterated samples of cow ghee with tallow. The absorbance of the Peak 1 remained almost same in all the samples while in Peak 2, the absorbance was the highest in ghee and steadily decreases with the increasing concentration of tallow in ghee and was the lowest in tallow. Between these two peaks in each of the spectra, one remains unchanged while the absorbance of another peak changed with the changing proportion of tallow in ghee. It was felt that the ratio of absorbance of these two peaks may be useful in quantifying the tallow adulteration in ghee and the ratio of absorbance (Peak 2 absorbance/Peak 1 absorbance, i.e., B/A) was calculated. But, in the present study it was observed that the second order derivative UV spectra did not offer any help in detecting the adulteration in ghee because no characteristics differences were observed in the second order spectra of pure ghee (cow and buffalo) and tallow studied in the UV (200-400 nm) region, indicating that there

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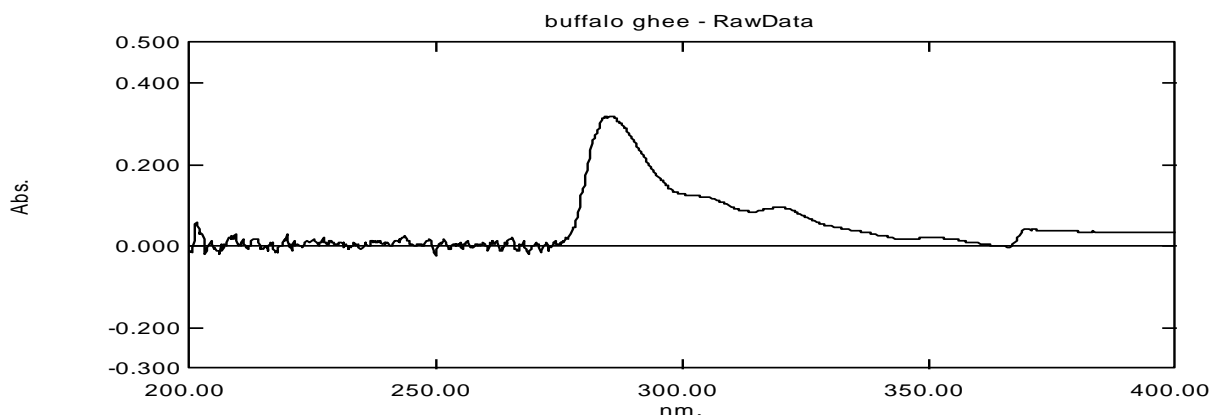
are common functional groups present in pure ghee and buffalo tallow. I even the effect of solvent change and concentration (Table 4.1 and 4.2) on second order UV spectra did not offer any help in getting the observation similar to Jirankalgikar and De (2014). Even, I did not observe characteristic peaks at 238 and 297 nm. In the present investigation a different kind of second order UV spectra as compared to Jirankalgikar and De (2014) was observed. Pure ghee, tallow and (50+50) adulterated ghee sample with buffalo tallow showed absorption maxima in second order UV spectra between 240 and 270 nm (Figure 4.3 to 4.4). Since the method could not give results as reported by earlier observer, and even in present study no variation in spectra of ghee and tallow was seen, so the working on its derivative spectroscopy was discontinued.

Table: 4.1 Stock 1% in chloroform

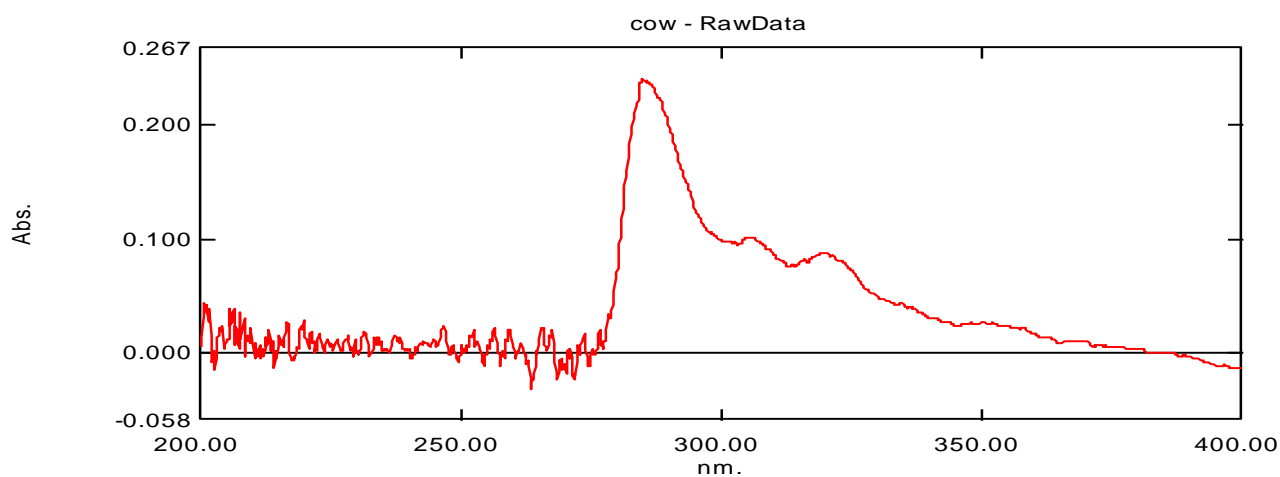
Samples	Peak 1 (λ , nm)	Peak 1 (absorbance, A)	Peak 2 (λ , nm)	Peak 2 (absorbance, B)	B/A
Cow ghee	238	-0.005	297	0.003	0.6
Buffalo ghee	238	-0.006	297	0.003	0.5
Tallow	238	-0.007	297	0	0
Cow ghee + tallow (50+50)	238	0.008	297	0.004	0.5
Buffalo ghee + tallow (50+50)	238	0.005	297	0.002	0.4

Table: 4.2 Stock 10% in chloroform

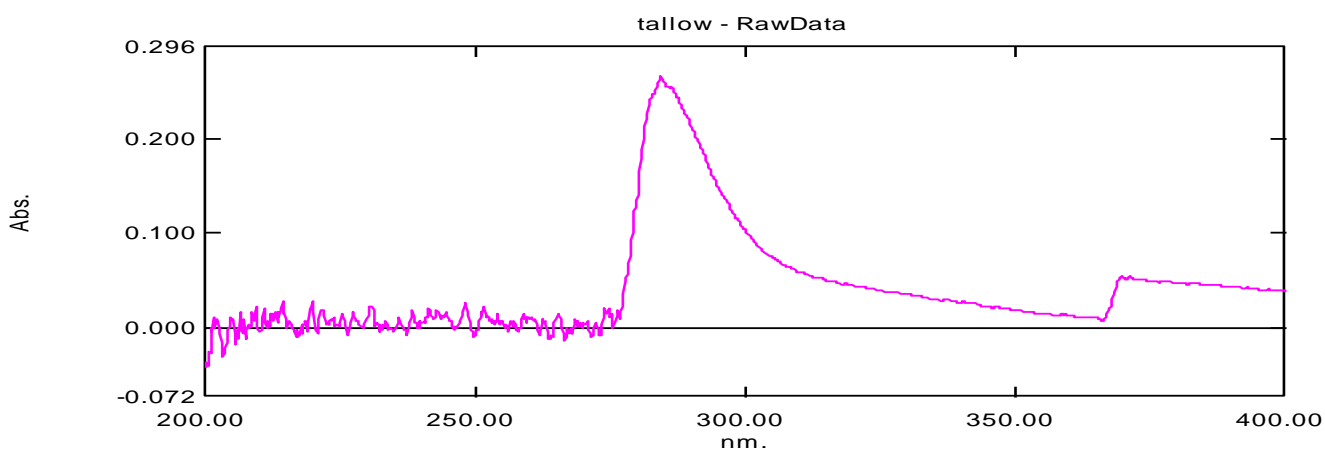
Samples	Peak 1 (λ , nm)	Peak 1 (absorbance, A)	Peak 2 (λ , nm)	Peak 2 (absorbance, B)	B/A
Cow ghee	238	0.035	297	0.001	0.028
Buffalo ghee	238	0.014	297	0.007	0.5
Tallow	238	0.063	297	0.004	0.06
Cow ghee + tallow (50+50)	238	0.035	297	0.002	0.05
Buffalo ghee + tallow (50+50)	238	0.026	297	0.006	0.23



A- Pure buffalo ghee



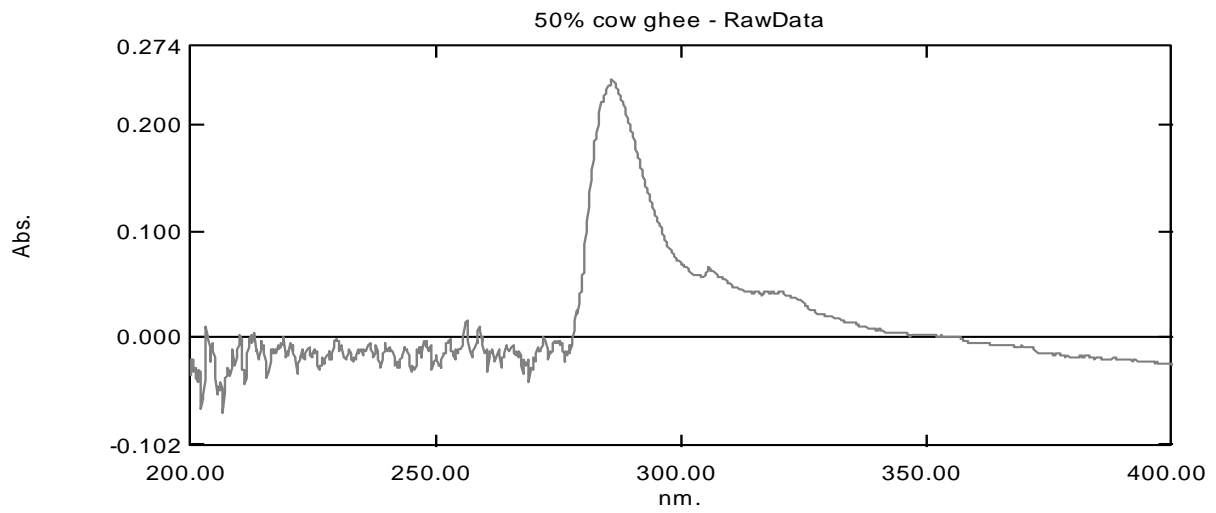
B-Pure cow ghee



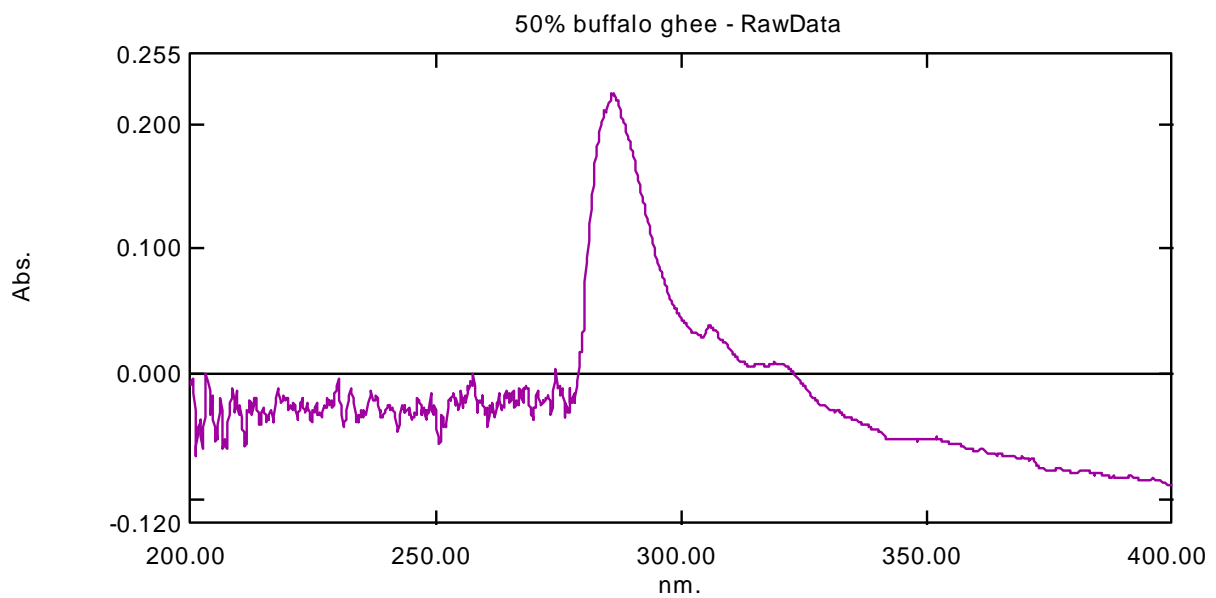
C- Buffalo tallow

Fig. 4.1 : Normal UV absorption spectra of pure buffalo ghee (A), pure cow ghee (B) and(C) Buffalo tallow.

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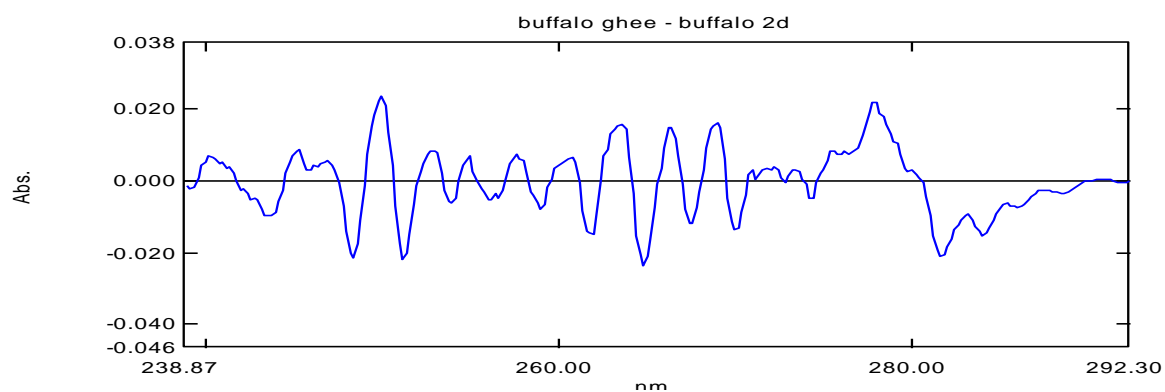


A- 50% adulteration of tallow in cow ghee

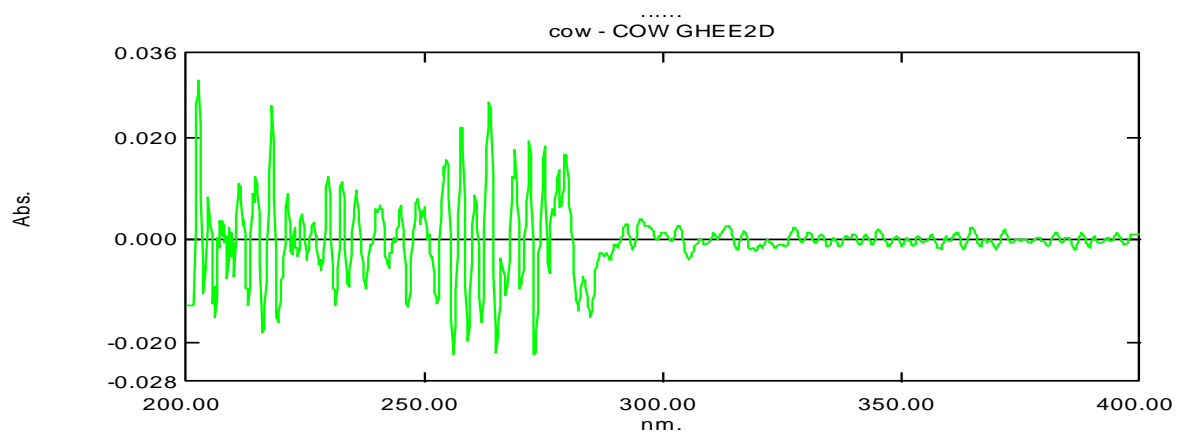


B- 50% adulteration of tallow in buffalo ghee

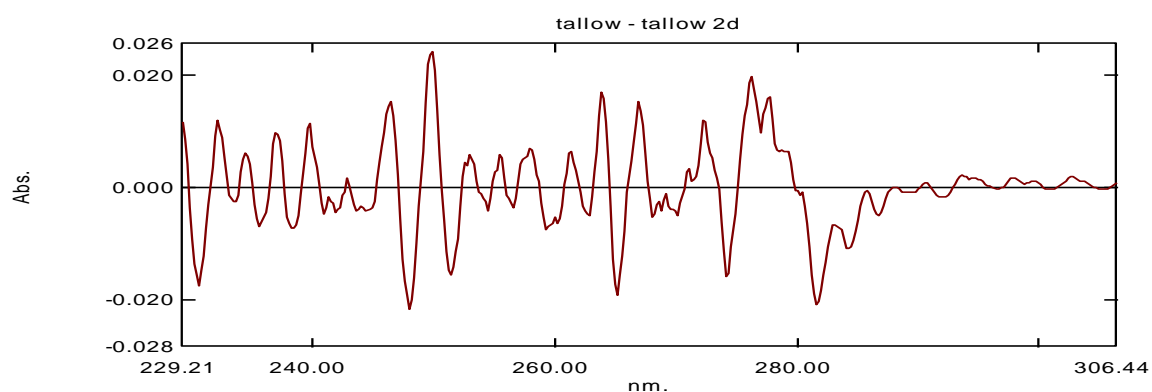
Fig. 4.2 : Normal UV absorption spectra of (A) 50% adulteration of tallow in cow ghee and (B) 50% adulteration of tallow in buffalo ghee



A- Pure buffalo ghee



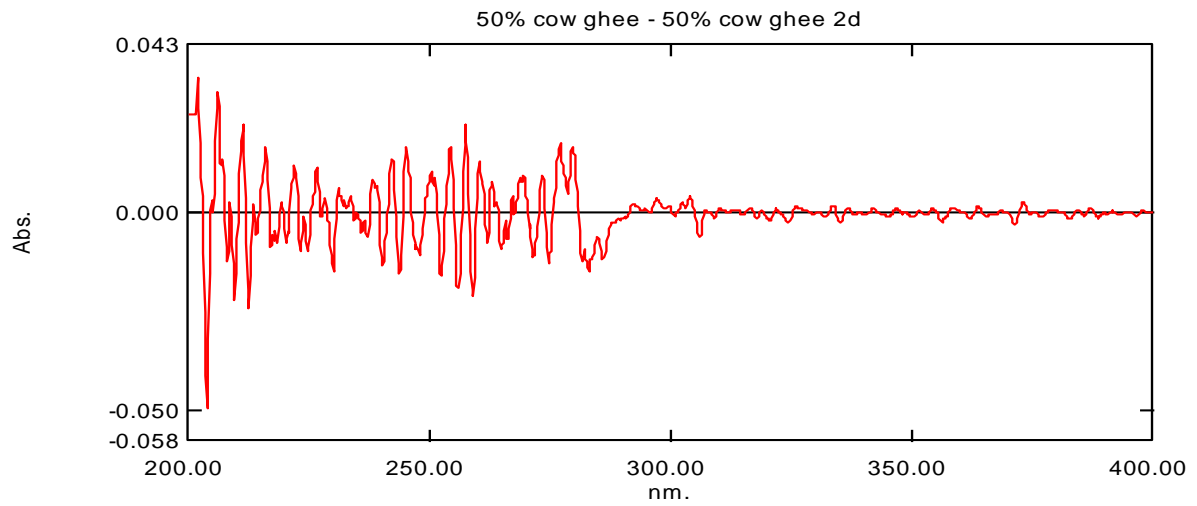
B- Pure cow ghee



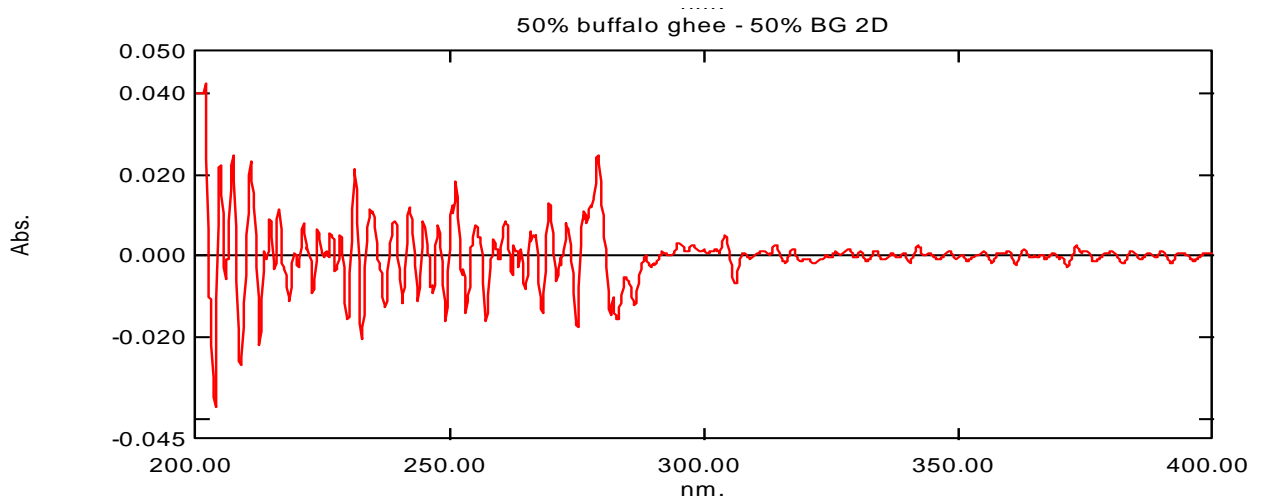
C- Buffalo tallow

Fig. 4.3 : Second order UV absorption spectra of pure buffalo ghee (A), pure cow ghee (B) and (C) buffalo tallow.

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A- 50% adulteration of tallow in cow ghee



B- 50% adulteration of tallow in buffalo ghee

Fig. 4.4 : Second order UV absorption spectra of 50% adulteration of tallow in cow ghee (A), 50% adulteration of tallow in buffalo ghee (B) .

4.2 To evaluate the quality of milk fat in market samples of butter, ghee and paneer

4.2.1 Analysis of milk fat samples adulterated individually with animal body fats and vegetable oil

In this section, samples of pure ghee (cow, buffalo) and ghee adulterated with animal body fats (buffalo, goat, pig) and vegetable oil at 5, 10 and 20% level, individually, were analyzed for physico-chemical constants. Similarly, buffalo paneer fat with vegetable oil and buffalo butter with margarine were adulterated at 5, 10 and 20% level, individually and analyzed for physico-chemical constants.

4.2.1.1 Physico-chemical constants of fats and oils

Physico-chemical constants such as Reichert- Meissl value, Polenske value, butyro-refractometer (BR) reading at 40°C and free fatty acid content of cow ghee, buffalo ghee and adulterant fats and oils were described in Table 4.3. For ghee adulterated with animal body fats and soybean oil and buffalo paneer fat adulterated with soybean oil and buffalo butter adulterated with margarine at 5, 10 and 20 percent levels, individually, the analysis of variance for these constants are presented in Tables 4.4 – 4.8.

4.2.1.1.1 Effect of milk fat adulteration with vegetable oil and body fats on RM-value

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. 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 is one of the quality parameters covered under FSSR Rules (2011) for ghee which varies from region to region including cotton tract area. The average RM value for cow ghee, buffalo ghee, buffalo butter and buffalo paneer fat were observed to be 28.66, 31.51, 31.12 and 29.13 respectively (Table 4.3). The average RM values for buffalo tallow, lard, goat tallow, margarine and soybean oil were observed to be 0.33, 0.44 and 0.45, 0.54 and 0.19, respectively (Table 4.3). RM value of buffalo

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paneer fat was found less as compared to buffalo ghee, the possible reason may be incomplete extraction of steam volatile water soluble short chain fatty acid ($C_{4:0}$ and $C_{6:0}$) in soxhlet extraction due to non-polar nature of chloroform and second reason may be the storage change in fatty acid profile of paneer as mentioned by Singh R. (2015).

Table 4.3 Physico-chemical constants of cow ghee, buffalo ghee, vegetable oil/fat and animal body fats

Control	RM value	Polenske value	BR at 40°C	FFA (% oleic acid)
Cow Ghee	28.66±0.12	1.46±0.03	42.4±0.05	0.25±0.017
Buffalo Ghee	31.51±0.19	1.32±0.06	41.23±0.08	0.27±0.03
Buffalo paneer	29.13±0.26	1.28±0.04	42.00±0.03	0.22±0.017
Buffalo butter	31.12±0.18	1.09±0.08	41.13±0.03	0.30±0.01
Soybean Oil	0.19±0.017	0.21±0.015	64.00±0.06	0.35±0.04
Margarine	0.54±0.046	0.27±0.03	48.1±0.05	0.32±0.02
Buffalo tallow	0.33±0.03	0.43±0.07	46.13±0.08	0.22±0.04
Lard	0.44±0.06	0.35±0.05	47.06±0.06	0.38±0.06
Goat tallow	0.45±0.04	0.38±0.03	43.00±0.03	0.19±0.01

Data are presented as Mean±SE (n=3)

Analysis of variance of data revealed that pure milk fat samples and adulterant fats and oil differ significantly ($p<0.05$) from each other with respect to RM value. Pure buffalo ghee showed significantly ($p<0.05$) higher RM value than pure cow ghee. Buffalo ghee has generally a slightly higher content of butyric acid and caproic acid (Ramamurthy and Narayanan, 1972; Bector and Narayanan, 1974a; Arumughan and Narayanan, 1979; Lal and Narayanan, 1984; Kumar, 2003) which could be responsible for its higher RM value than cow ghee.

The various body fats and vegetable oil/fat showed negligible RM values in comparison with ghee. From Table 4.4, it can be concluded that the RM value significantly decreased as level of adulteration increased. There was significant

difference in the RM values of adulterated samples. There was no significant difference in RM value of buffalo ghee as compared to buffalo butter but paneer fat isolated from paneer showed slightly lower RM-value. The possible reason may be incomplete extraction of steam volatile water soluble short chain fatty acid (C_{4:0} and C_{6:0}) in soxhlet extraction due to non-polar nature of chloroform and second reason may be the storage change in fatty acid profile of paneer as mentioned by Singh R. (2015). Therefore it is not preferable to use RM-value as a parameter to judge the quality of milk fat isolated by soxhlet method as it can give false positive results.

Addition of adulterant fats and oils such as body fats (buffalo, goat and pig) and vegetable oil individually at 5, 10, and 20 percent level decreased the RM values of cow and buffalo ghee, depending upon the level of adulteration. Higher the level of adulteration, greater was the decrease in the RM value of ghee samples.

Similarly, addition of soybean oil in buffalo paneer fat and addition of margarine in buffalo butter at 5, 10 and 20% revealed the decrease in RM value of both buffalo paneer fat and butter fat. Keeping in view the minimum standard value of 28 under FSSR (2011) for Haryana, it can be concluded from the Table 4.4 that adulteration of pure cow ghee with all animal body fats and vegetable oil at 10 percent and above this level of adulteration could be detected, whereas in case of pure buffalo ghee adulteration 20% percent and above could be detected, but in case of buffalo paneer fat adulterated with soybean oil and buffalo butter adulterated with margarine could be detected at 10% level. The results obtained in the present study on the RM value of various fats including milk fats are in general agreement with those reported by earlier workers (Singhal, 1973; Rangappa and Achaya, 1974; Sharma and Singhal, 1995). Animal body fats showed negligible RM values as against high RM values observed for cow and buffalo milk fats. Similar observations on negligible RM values have also been reported by earlier workers for body fats (Singhal, 1973; Sharma and Singhal, 1995). Animal body fats are not expected to have any RM value because this value is typical for milk fats only, and is represented by butyric (C_{4:0}) and caproic (C_{6:0}) acids which are synthesized in the cells of mammary glands of milch animals where *de novo* synthesis of fatty acids from C_{4:0} to C_{14:0} as well as part of C_{16:0} fatty acids takes place (Fox and McSweeney, 1998).

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Table 4.4 Effect of milk fat adulteration with vegetable oil and body fats on RM-value

Samples	Adulterants	RM-value			
		Level of adulteration (%)			
		0	5	10	20
Cow ghee +	Soybean	28.66±0.12 ^{aA}	27.73±0.10 ^{bB}	26.08±0.21 ^{cB}	23.76±0.20 ^{dA}
	Buffalo tallow	28.66±0.12 ^{aA}	28.11±0.12 ^{bA}	27.86±0.17 ^{cA}	23.7±0.23 ^{dA}
	Lard	28.66±0.12 ^{aA}	28.06±0.14 ^{bA}	25.76±0.20 ^{cB}	23.24±0.24 ^{dB}
	Goat tallow	28.66±0.12 ^{aA}	27.88±0.20 ^{bA}	26.00±0.23 ^{cB}	23.90±0.25 ^{dA}
CD value at 5%	0.355				
Buffalo ghee +	Soybean	31.51±0.19 ^{aA}	30.98±0.11 ^{bA}	28.24±0.20 ^{cD}	26.7±0.26 ^{dA}
	Buffalo tallow	31.51±0.19 ^{aA}	31.06±0.14 ^{bA}	30.96±0.12 ^{bA}	25.83±0.31 ^{cB}
	Lard	31.51±0.19 ^{aA}	30.94±0.08 ^{bA}	29.03±0.27 ^{cB}	25.8±0.23 ^{dB}
	Goat tallow	31.51±0.19 ^{aA}	30.74±0.12 ^{bA}	29.28±0.26 ^{cB}	25.81±0.27 ^{dB}
CD value at 5%	0.378				
Buffalo Paneer fat +	Soybean oil	29.13±0.26 ^a	28.66±0.11 ^b	26.88±0.22 ^c	24.92±0.27 ^d
CD value at 5%	0.449				
Buffalo Butter+	Margarine	31.12±0.18 ^a	30.47±0.18 ^b	27.94±0.26 ^c	24.48±0.17 ^d
CD value at 5%	0.470				

Data are presented as Mean±SE (n=3)

^{A-D} Mean within column with different upper case superscript are significantly different (p<0.05)

^{a-d} Mean within row with different lower case superscript are significantly different (p<0.05)

Some workers have reported that adulteration of ghee with body fats (Singhal, 1973 and 1980) at 20 percent level is not detectable, whereas the present investigation revealed that Reichert-Meissl value could detect animal body fat adulteration up to 10%, 20% and 20% level in cow ghee, buffalo ghee and fat extracted from paneer and butter, respectively. This may be possible because the RM values of ghee observed in the present study were close to the minimum value of RM as specified by FSSR (2011) standards for Haryana region. Kumar A. (2008) had reported that adulteration of pure cow ghee with all animal body fats and all vegetable oils 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. Patel (2011) reported that RM value could detect more than 5% adulteration in cow ghee while 25% in case of buffalo ghee depending on initial RM values of respective ghee samples. Upadhyay (2014) reported that groundnut oil when added singly in cow ghee could be detected as low as 10% level, however pooling of the respective data of cow and buffalo ghee revealed that detection of groundnut oil could be done at 15% and above level using R.M. value.

4.2.1.1.2 Effect of milk fat adulteration with vegetable oil and body fats on P-value

Polenske value is substantially a measure of steam volatile and water insoluble fatty acids such as caprylic (C_{8:0}) and capric (C_{10:0}) acids which contribute one fourth and three fourth, respectively, to this value. Again, like butyric acid and caproic acid, caprylic (C_{8:0}) and capric (C_{10:0}) acids are also mainly present in milk fat and they are not found in animal body fats and vegetable oils except palm kernel oil and coconut oil. These two exceptional oils are reported to show the average Polenske value of 6.12 and 15.20 (Winton and Winton, 1999), respectively, which are much higher than the average value of 1.7 and 2.0 reported for buffalo and cow milk fat (Rangappa and Achaya, 1974; Zachariah S., 2005).

It is evident from the Table 4.3 that the average Polenske values for pure cow ghee buffalo ghee, buffalo paneer fat and buffalo butter fat were observed to be 1.46, 1.32, 1.28 and 1.3, respectively. The average Polenske values for

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buffalo tallow, lard, goat tallow, margarine and soybean oil (Table 4.3) were observed to be 0.043, 0.35, 0.38, 0.27 and 0.21, respectively.

Analysis of data revealed that cow ghee, buffalo ghee, buffalo butter fat and buffalo paneer fat differed significantly ($p < 0.05$) from the adulterant fats and oil with respect to Polenske value. Cow ghee exhibited slightly higher Polenske value than the buffalo ghee. The various body fats showed much lower Polenske values in comparison with ghee. Table 4.5 revealed that as the level of adulteration increased Polenske value decreased. There was no significant difference in Polenske value of buffalo ghee as compared to buffalo butter fat and buffalo paneer fat. Addition of adulterant fats such as animal body fats (buffalo, pig and goat) individually at 5, 10, and 20 percent levels in cow and buffalo ghee showed a significant decrease ($p < 0.05$) in Polenske value at 10% level of adulteration or above, similar pattern was observed in case of vegetable oil and margarine adulteration in buffalo paneer fat and buffalo butter fat, respectively. Adulteration of milk fat samples with different adulterants, at all levels studied, caused a decrease in the Polenske value of adulterated milk fat samples but remained within the limits of prescribed range of Polenske value for milk fat and hence, it did not offer much help in detection of adulteration of milk fat.

The Polenske values of milk fat observed in the present study are in general agreement with the values reported in the literature (Singhal, 1980). Although there are no standards for Polenske value in the FSSR (2011), the values obtained in the present study were well within the range of 1.0 – 2.0 specified for ghee under AGMARK Standards (De, 2010) on all India basis. Earlier workers (Singhal, 1980; Zachariah, 2005) have also made similar observations that the Polenske value is not a good parameter to detect the adulteration in ghee. Kumar (2008) reported that on the basis of Polenske value, adulteration of cow, buffalo and mixed ghee with any of the adulterant animal body fats could not be detected at any of the levels studied. Patel (2011) reported that adulteration up to 5% in cow ghee with animal body fats and up to 20% in case of mixed ghee could not be possible by Polenske value.

Table 4.5 Effect of milk fat adulteration with vegetable oil and body fats on P-value

Samples	Adulterants	P-value			
		Level of adulteration (%)			
		0	5	10	20
Cow ghee +	Soybean	1.46±0.03 ^{aA}	1.41±0.017 ^{aA}	1.32±0.023 ^{bA}	1.22±0.02 ^{cA}
	Buffalo tallow	1.46±0.03 ^{aA}	1.43±0.015 ^{aA}	1.33±0.02 ^{bA}	1.16±0.046 ^{cA}
	Lard	1.46±0.03 ^{aA}	1.41±0.017 ^{aA}	1.29±0.02 ^{bA}	1.02±0.048 ^{cC}
	Goat tallow	1.46±0.03 ^{aA}	1.40±0.02 ^{aA}	1.3±0.04 ^{bA}	1.13±0.045 ^{cB}
CD value at 5%	0.0624				
Buffalo ghee +	Soybean	1.32±0.06 ^{aA}	1.30±0.008 ^{aA}	1.23±0.026 ^{aA}	1.09±0.05 ^{bA}
	Buffalo tallow	1.32±0.06 ^{aA}	1.30±0.012 ^{aA}	1.23±0.037 ^{aA}	0.95±0.092 ^{bB}
	Lard	1.32±0.06 ^{aA}	1.28±0.011 ^{aA}	1.11±0.026 ^{bC}	0.94±0.06 ^{cC}
	Goat tallow	1.32±0.06 ^{aA}	1.3±0.011 ^{aA}	1.2±0.02 ^{bB}	1.02±0.048 ^{cA}
CD value at 5%	0.0802				
Buffalo Paneer fat +	Soybean	1.28±0.04 ^a	1.22±0.011 ^a	1.18±0.011 ^b	1.02±0.05 ^c
CD value at 5%	0.080				
Buffalo Butter +	margarine	1.3±0.08 ^a	1.29±0.025 ^a	1.23±0.049 ^b	1.1±0.02 ^b
CD value at 5%	0.118				

Data are presented as Mean±SE (n=3)

^{A-D} Mean within column with different upper case superscript are significantly different (p<0.05)

^{a-d} Mean within row with different lower case superscript are significantly different (p<0.05)

4.2.1.1.3 Effect of milk fat adulteration with vegetable oil and body fats on BR-reading at 40°C

Butyro-refractometer (BR) reading or refractive index which measures the index of refraction between air and the liquid fat, and vary with the nature of the fat, is usually determined at 40°C. Refractive index is a characteristic for the particular liquid or solid, and is often considered as an important criterion for the evaluation of milk fat purity. BR reading is one of the quality parameters covered under FSSR (2011) for milk fat and it varies from region to region including cotton tract area. As per FSSR (2011), BR reading of pure milk fat 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.

The average BR reading for pure milk fat samples, animal body fats (buffalo, goat and pig) and vegetable oil/fat are shown in Table 4.3. The average BR reading for adulterated ghee samples at 5, 10, and 20 percent levels are presented in Table 4.6. The average BR reading for fresh pure cow ghee, buffalo ghee, buffalo butter and buffalo paneer fat were observed to be 42.4, 41.23, 41.13, and 42, respectively (Table 4.3). The mean values of BR reading for buffalo tallow, lard, goat tallow, soybean oil and margarine were observed to be 46.13, 47.06, 43.0, 64.0 and 48.1, respectively (Table 4.3). On comparing the data it was observed that milk fat samples and adulterant fats differed significantly from each other with respect of BR reading. It was also seen that pure milk fat samples did not show any significant difference from each other.

Among the animal body fats, lard showed the highest BR reading, whereas among the vegetable source, soybean oil showed the highest one. Pure cow and buffalo ghee adulterated with animal body fats (buffalo, goat and pig) and soybean oil, individually at 5, 10, and 20 percent levels, showed increased BR readings and the increase depended upon the amount of adulterants added to the ghee (Table 4.6). Similar pattern was observed in buffalo butter fat adulterated with margarine and buffalo paneer fat adulterated with soybean oil at 5, 10 and 20% level. Higher the level of adulterants added, greater was the increase in the BR reading of milk fat samples.

Table 4.6 Effect of milk fat adulteration with vegetable oil and body fats on BR-reading at 40°C

Samples	Adulterants	BR reading at 40°C			
		Level of adulteration (%)			
		0	5	10	20
Cow ghee+	Soybean	42.4±0.05 ^{aA}	42.55±0.06 ^{bB}	42.76±0.026 ^{cB}	43.4±0.09 ^{dC}
	Buffalo tallow	42.4±0.05 ^{aA}	42.46±0.011 ^{aA}	42.55±0.017 ^{bA}	42.9±0.023 ^{cA}
	Lard	42.4±0.05 ^{aA}	42.50±0.026 ^{aA}	42.67±0.052 ^{bB}	43.2±0.11 ^{cB}
	Goat tallow	42.4±0.05 ^{aA}	42.42±0.02 ^{aA}	42.5±0.024 ^{aA}	42.85±0.066 ^{bA}
CD value at 5%	0.101				
Buffalo ghee +	Soybean	41.2±0.08 ^{aA}	41.3±0.024 ^{aA}	41.64±0.052 ^{bC}	42.06±0.066 ^{cC}
	Buffalo tallow	41.2±0.08 ^{aA}	41.25±0.017 ^{aA}	41.36±0.021 ^{bA}	41.75±0.029 ^{cB}
	Lard	41.2±0.08 ^{aA}	41.32±0.024 ^{bA}	41.46±0.06 ^{cB}	42.16±0.057 ^{dC}
	Goat tallow	41.2±0.08 ^{aA}	41.24±0.04 ^{aA}	41.3±0.015 ^{bA}	41.60±0.046 ^{cA}
CD value at 5%	0.105				
Buffalo Paneer fat +	Soybean	42.0±0.03 ^a	42.11±0.016 ^a	42.43±0.033 ^b	42.78±0.075 ^c
CD value at 5%	0.103				
Buffalo Butter +	Margarine	41.1±0.03 ^a	41.17±0.014 ^a	41.37±0.032 ^b	42.0±0.05 ^c
CD value at 5%	0.0805				

Data are presented as Mean±SE (n=3)

^{A-D} Mean within column with different upper case superscript are significantly different (p<0.05)

^{a-d} Mean within row with different lower case superscript are significantly different (p<0.05)

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Using 40.0 to 43.0, range of BR readings of milk fat, as prescribed for Haryana under FSSR (2011), it was noticed that the adulteration of cow and buffalo ghee with animal body fats could not be detected at any of the levels studied. However, the adulteration of soybean oil in cow ghee could be detected at 20% level. While the adulteration of soybean oil in buffalo ghee and buffalo paneer fat could not be detectable even up to 20% level. Similarly, adulteration of buffalo butter with margarine could not be detectable up to 20%. The results obtained in the present study on the BR readings are in general agreement with those reported by earlier workers on ghee (Bector and Narayanan, 1974b; Lal and Narayanan, 1984, Kumar, 2003) and vegetable oils (Singhal, 1980; Rangappa and Achaya, 1974; Gunstone *et al.*, 1994;). However, in case of body fats, values of BR readings obtained in the present study were found to be slightly lower than that reported by Kumar (2003).

Sharma and Singhal (1995) reported that 10 percent levels of adulteration of body fats could be detected, whereas in the present study, butyro-refractometer (BR) reading could detect adulteration of cow ghee with lard at 20% level but in case of buffalo ghee values were within the range given by FSSR (2011). A similar observation as recorded in the present investigation was also made by Kumar (2003) and Pramod (2012) for animal body fats. Kumar (2008) reported that BR-reading could not detected adulteration of cow and buffalo pure ghee with animal body fats at any of the levels studied but it could detect the adulteration of soybean oil in cow ghee at 10 and 15% levels, while in buffalo ghee detection was possible at 15% level, whereas in the present study, BR-reading could detect the adulteration of soybean oil in cow ghee at 20% level but in case of buffalo ghee values were within the range. Patel (2011) concluded that BR-reading was not very useful in detection of adulterants. From the above result, it is clear that, body fats added to either cow or buffalo ghee could not be detected at any of the level except in case of pig body fat using BR reading as a base. Result of the present study also established that BR-reading is not affected by the fat isolation method i.e. soxhlet method unlike RM-value.

4.2.1.1.4 Effect of milk fat adulteration with vegetable oil and body fats on FFA value

Free fatty acid content is defined as percentage by weight oleic acid. FSSR (2011) has established a maximum limit of FFA, 3% as oleic acid in ghee. To ensure that all samples have been prepared by proper process, FFA content was measured. It is clearly evident from results that free fatty acid content within the prescribed limits.

FFA content of pure cow ghee, buffalo ghee, buffalo butter fat and buffalo paneer fat were 0.25%, 0.27%, 0.30% and 0.22 %, respectively. Buffalo tallow, lard, goat tallow, margarine and soybean oil had 0.22, 0.38, 0.19, 0.32 and 0.35 percent FFA content, respectively (Table 4.3). When adulterant fats and oil were added to pure milk fat samples, the FFA content had changed due to the initial FFA content of adulterant fats and oil. However, in any case FFA content did not change beyond the FSSR standards for ghee (Table 4.7). From the results discussed in section 4.2 and its sub-sections, it was established that fat isolated from soxhlet procedure could be used successively to paneer by judge the quality of milk fat by BR-reading. However, RM-value could give some false results.

4.2.2 Physico-chemical analysis of market samples of butter, ghee and paneer with special refrence to milk fat.

4.2.2.1 Physico-chemical constants of pure ghee and market samples of ghee from organized and unorganized sector of national capital region (NCR)

Milk fat is characterized by certain physico-chemical properties (RM-value, Polenske value, BR-reading, free fatty acid value, saponification value, iodine value) 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 type of component fatty acids present in fats. They also enable the detection of fat adulteration qualitatively and in some instances quantitatively.

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Table 4.7 Effect of milk fat adulteration with vegetable oil and body fats on FFA value

Samples	Adulterants	FFA(% Oleic acid)			
		Level of adulteration (%)			
		0	5	10	20
Cow ghee +	Soybean	0.25±0.017 ^{aA}	0.269±0.004 ^{bB}	0.285±0.004 ^{bB}	0.301±0.008 ^{cB}
	Buffalo tallow	0.25±0.017 ^{aA}	0.237±0.003 ^{aA}	0.231±0.001 ^{bA}	0.225±0.002 ^{cA}
	Lard	0.25±0.017 ^{aA}	0.261±0.002 ^{aB}	0.276±0.004 ^{bB}	0.296±0.002 ^{cB}
	Goat tallow	0.25±0.017 ^{aA}	0.237±0.006 ^{aA}	0.228±0.003 ^{bA}	0.214±0.001 ^{bA}
CD value at 5%	0.01774				
Buffalo ghee+	Soybean	0.27±0.03 ^{aA}	0.288±0.004 ^{aA}	0.292±0.0038 ^{aB}	0.31±0.005 ^{bB}
	Buffalo tallow	0.27±0.03 ^{aA}	0.256±0.004 ^{aA}	0.250±0.002 ^{aA}	0.245±0.003 ^{aA}
	Lard	0.27±0.03 ^{aA}	0.280±0.003 ^{aA}	0.292±0.002 ^{aB}	0.322±0.005 ^{bB}
	Goat tallow	0.27±0.03 ^{aA}	0.257±0.003 ^{aA}	0.248±0.004 ^{aA}	0.231±0.002 ^{bA}
CD value at 5%	0.03582				
Buffalo Paneer fat +	Soybean	0.22±0.017 ^a	0.236±0.002 ^a	0.259±0.002 ^b	0.282±0.004 ^c
CD value at 5%	0.0203				
Buffalo Butter+	Margarine	0.30±0.01 ^a	0.305±0.001 ^a	0.308±0.0008 ^a	0.31±0.001 ^a
CD value at 5%	0.0154				

Data are presented as Mean±SE (n=3)

^{A-D} Mean within column with different upper case superscript are significantly different (p<0.05)

^{a-d} Mean within row with different lower case superscript are significantly different (p<0.05)

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, season of the year, region of the country, feed of the animal etc. (Doctor *et al.*, 1940). FSSR (2011) have prescribed the requirements for two physico-chemical constants butyro-refractometer (BR) reading at 40°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) specified the physico-chemical constants i.e. BR reading, RM value and Polenske value, besides free fatty acid (% oleic acid) and moisture for the same purpose. The FSSR (2011) and AGMARK standards of ghee are presented in Appendix-I and II, respectively.

Table 4.8 Physico-chemical analysis of cow and buffalo pure ghee

Pure ghee	RM-value	Polenske value	BR at 40°C	FFA (% oleic acid)
Cow Ghee	28.66±0.12	1.46±0.03	42.4±0.05	0.25±0.017
Buffalo Ghee	31.51±0.19	1.32±0.06	41.23±0.08	0.27±0.03

Data is presented as Mean±S.E. (n=3)

Tables 4.8 to 4.10 show the results on physico-chemical constants such as Reichert-Meissl value, Polenske value, butyro-refractometer reading (at 40°C) and free fatty acid of pure lab made cow and buffalo ghee, and ghee samples collected from organized and unorganized dairies situated national capital region (NCR).

The ranges of RM-value, Polenske value, BR-reading at 40°C and free fatty acid for pure ghee of institute herd of cow ghee were observed 28.49 to 28.9, 1.4 to 1.5, 42.3 to 42.5 and 0.22 to 0.28, respectively. The corresponding values for pure ghee of institute herd of buffaloes were observed to be 31.35 to 31.72, 1.28 to 1.4, 41.1 to 41.3 and 0.22 to 0.34, respectively. Similar observations were reported by Kumar (2010)

Laboratory made pure ghee have indicated the average RM value was higher in buffalo ghee than in cow ghee. On the other hand, the average BR reading and Polenske value were higher in cow ghee as compared to buffalo ghee.

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Table 4.9 depicts the physico-chemical constants of market ghee samples from unorganized dairies of NCR in winter season. The ranges of RM value, Polenske value, BR reading at 40°C and free fatty acid for ghee samples of unorganized sector (UOS) were observed 0.27 to 30.33, 0.47 to 1.56, 42.1 to 55.2 and 0.14 to 1.75, respectively. Table 4.10 depicts the physico-chemical constants of market ghee samples from organized dairies of NCR in winter season. The ranges of RM value, Polenske value, BR reading at 40°C and free fatty acid for ghee samples of organized sector (OS) were observed 0.27 to 29.70, 0.51 to 1.67, 40.5 to 48.1 and 0.19 to 0.66, respectively. The analysis of market ghee samples of unorganized sector of NCR (Table 4.9) collected in winter season revealed that out of fifteen samples two samples (UOS-4, 11) showed very low RM and Polenske value and very high BR reading, while two samples (UOS-8, 13) showed slightly less RM and Polenske value and slightly high BR reading as compared to AGMARK standards. Two samples (UOS-4, 11) were found positive for Baudouin test (Plate 4.1). While rest of samples had physico-chemical constants within the range specified by FSSR (2011) and AGMARK (1981). Free fatty acid value for all the samples were in the range specified by FSSR (2011) and AGMARK (1981). The result of the study of market ghee samples from organized sector from NCR have been depicted in Table 4.10. It is evident from the observation that physico-chemical constants and free fatty acid value for all the samples except one sample studied were within the range prescribed by FSSR (2011) and AGMARK (1981). One sample (OS-11) from organized sector showed very low RM and Polenske value and high BR reading. Four out of eleven samples were found to be positive for Baudouin test (Plate 4.2).

4.2.2.2 Physico-chemical constants of pure butter and market samples of butter from organized and unorganized sector of NCR

As per FSSR (2011), butter means the fatty product derived exclusively from milk of cow and buffalo or its products principally in the form of water in oil type of emulsion. Butter is a raw material for ghee making. Pure and market samples of butter were analyzed for the same physico-chemical constants, which was prescribed by FSSR (2011) and AGMARK (1981) to evaluate the quality of butterfat.

Table 4.9 Physico-chemical analysis of market ghee samples from unorganized sector (UOS)

Physico-chemical constants					
Samples	RM-value	Polenske value	BR at 40°C	% FFA	Baudouin test
UOS-1	28.31± 0.1	1.2±0.04	42.4±0.08	0.33±0.03	Negative
UOS -2	28.52±0.03	1.27±0.06	42.6±0.05	1.75±0.04	Negative
UOS -3	29.57±0.26	1.53±0.04	44.2±0.11	0.48±0.03	Negative
UOS -4	0.27±0.03	0.57±0.04	55.2±0.08	0.16±0.03	Positive
UOS -5	29.03±0.28	1.23±0.06	43.1±0.05	0.62±0.02	Negative
UOS -6	29.51±0.19	1.26±0.05	42.5±0.05	0.46±0.04	Negative
UOS -7	28.20±0.10	1.2±0.02	42.5±0.03	0.39±0.034	Negative
UOS -8	25.63±0.27	0.63±0.04	45.23±0.14	0.28±0.02	Negative
UOS -9	30.11±0.14	1.55±0.05	42.5±0.11	0.37±0.02	Negative
UOS -10	28.71±0.16	1.21±0.04	43.1±0.05	0.31±0.02	Negative
UOS -11	0.49±0.03	0.47±0.03	54.4±0.08	0.14±0.01	Positive
UOS -12	30.33±0.13	1.56±0.05	42.2±0.1	0.33±0.02	Negative
UOS -13	26.97±0.14	0.9±0.03	43.1±0.05	1.22±0.02	Negative
UOS -14	28.45±0.15	1.2±0.02	43.3±0.11	0.27±0.01	Negative
UOS -15	28.35±0.12	1.03±0.08	42.1±0.08	0.45±0.01	Negative

Data are presented as Mean±S.E. (n=3)

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Physico-chemical constants					
Samples	RM-value	Polenske value	BR at 40°C	FFA%	Baudouin test
OS-1	28.28±0.06	1.34±0.03	42.5±0.07	0.56±0.03	Negative
OS -2	29.70±0.15	1.48±0.01	41.5±0.05	0.25±0.01	Negative
OS -3	28.56±0.03	1.53±0.05	42.4±0.05	0.22±0.02	Negative
OS -4	28.22±0.17	1.3±0.05	42.0±0.03	0.66±0.02	Negative
OS -5	29.49±0.15	1.67±0.01	41.4±0.05	0.31±0.03	Negative
OS -6	28.59±0.12	1.55±0.02	40.5±0.07	0.48±0.03	Negative
OS -7	28.77±0.17	1.54±0.04	41.2±0.08	0.33±0.02	Negative
OS -8	28.24±0.07	1.33±0.05	42.1±0.08	0.28±0.01	Positive
OS -9	28.32±0.14	1.27±0.01	42.2±0.08	0.45±0.03	Positive
OS -10	29.38±0.1	1.58±0.03	41.0±0.03	0.44±0.04	Positive
OS -11	0.27±0.03	0.51±0.02	48.1±0.03	0.19±0.01	Positive

Data are presented as Mean±S.E. (n=3)

Tables 4.11 to 4.13 show the results of physico-chemical constants such as Reichert-Meissl value, Polenske value, butyro-refractometer reading (at 40°C) and free fatty acid of pure buffalo butter and butter collected from organized and unorganized dairies of NCR.

Table 4.11 Physico-chemical analysis of pure buffalo butter fat

Pure ghee	RM value	Polenske value	BR at 40°C	FFA (% oleic acid)
Buffalo butter	31.12±0.18	1.3±0.08	41.13±0.03	0.30±0.01

Data are presented as Mean±S.E. (n=3)

The ranges of RM-value, Polenske value, BR-reading at 40°C and free fatty acid for pure ghee of institute herd of buffalo ghee were observed be 30 to 31.2, 1.28 to 1.4, 41.1 to 41.2 and 0.26 to 0.34, respectively. Physico-chemical analysis of buffalo butterfat indicated that the value of physico-chemical constants of buffalo butter was close to buffalo ghee.

Table 4.12 depicts the physico-chemical constants of market butter samples from unorganized dairies of NCR in winter season. The range of RM value, Polenske value, BR reading at 40°C and free fatty acid for butter samples of unorganized sector (UOS) were observed 1.77 to 30.41, 0.61 to 1.53, 40.1-47.6 and 0.25-1.33, respectively. Table 4.13 depicts the physico-chemical constants of market butter samples from organized dairies of NCR in winter season. The ranges of RM value, Polenske value, BR reading at 40°C and free fatty acid for butter samples of organized sector (OS) were observed 28.32 to 29, 1.32 to 1.61, 41.1-41.56 and 0.27-0.51, respectively. It was observed from the table 4.12 that the out of sixteen samples of butter from unorganized sector analyzed, the RM value of four samples (UOS-9, 10, 11, 14) showed the lower value as compared to standards prescribed by FSSR (2011) and AGMARK (1981). While data of BR reading revealed that the out of sixteen samples six samples (UOS-2, 9, 10,11, 12, 14) showed the higher BR reading as compared to standards specified by FSSR (2011) and AGMARK (1981). For Polenske value, one out of sixteen samples showed lower value, while rest of the samples were in general range of 1 to 2 (AGMARK, 1981).

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Table 4.12 Physico-chemical analysis of market butter samples from unorganized sector (UOS) of NCR.

Physico-chemical constants					
Samples	RM-value	Polenske value	BR at 40°C	FFA%	Baudouin
UOS-1	28.61±0.15	1.13±0.03	41.1±0.08	0.33±0.004	Negative
UOS -2	29.93±0.26	1.22±0.08	44.00±0.05	0.44±0.008	Negative
UOS -3	28.38±0.06	1.03±0.08	40.1±0.08	0.28±0.04	Negative
UOS -4	28.59±0.15	1.13±0.08	42.5±0.08	0.43±0.05	Negative
UOS -5	30.47±0.06	1.47±0.09	42.9±0.12	1.33±0.04	Negative
UOS -6	28.85±0.03	1.23±0.06	41.4±0.06	0.46±0.05	Negative
UOS -7	29.55±0.18	1.11±0.05	43.0±0.06	0.67±0.02	Negative
UOS -8	30.21±0.23	1.53±0.06	42.2±0.08	0.25±0.01	Negative
UOS -9	26.60±0.15	1.12±0.06	45.1±0.05	0.28±0.05	Negative
UOS -10	1.77±0.05	0.61±0.04	47.6±0.05	0.37±0.04	Negative
UOS -11	21.6±0.16	1.06±0.08	47.2±0.08	0.29±0.03	Negative
UOS -12	28.27±0.12	1.21±0.03	43.4±0.03	0.56±0.02	Negative
UOS -13	29.83±0.18	1.4±0.04	42.1±0.08	0.34±0.03	Negative
UOS -14	25.01±0.1	1.08±0.09	46.0±0.06	0.41±0.04	Negative
UOS -15	29.72±0.18	1.52±0.05	42.2±0.05	0.29±0.04	Negative
UOS-16	29.87±0.11	1.20±0.02	41.4±0.03	0.54±0.05	Negative

Data are presented as Mean±S.E. (n=3)

Samples of butter from unorganized sector were observed negative for Baudouin test (Plate 4.3). Free fatty acid values were in the range prescribed by both FSSR (2011) and AGMARK (1981). The study of market butter samples from organized sector revealed that all the physico-chemical constants and free fatty acid values studied were within the range specified by FSSR (2011) and AGMARK (1981). No samples found positive for Baudouin test (Plate 4.4).

Table 4.13 Physico-chemical analysis of market butter samples from organized sector (OS) of NCR.

Physico-chemical constants					
Samples	RM value	Polenske value	BR at 40°C	FFA%	Baudouin test
OS-1	29.0±0.14	1.45±0.05	41.5±0.05	0.38±0.03	Negative
OS -2	28.63±0.14	1.52±0.05	41.1±0.03	0.27±0.04	Negative
OS-3	28.32±0.11	1.32±0.06	41.4±0.06	0.57±0.02	Negative
OS-4	28.5±0.11	1.48±0.03	41.5±0.03	0.37±0.02	Negative
OS-5	28.82±0.15	1.61±0.04	41.56±0.08	0.46±0.05	Negative

Data are presented as Mean±S.E. (n=3)

4.2.2.3 Physico-chemical constants of pure and market samples of paneer from organized and unorganized sector of NCR.

Paneer is an important Indian traditional coagulated dairy product that provides sound nutrition, variety, safety, texture, portability and profitability to consumer. The demand of these products is increasing especially during festival season. To meet the increased demand, fraudulent traders take undue advantage of such a situation and admix cheaper fat in this novel food item, to meet the legal standards of fat for paneer as prescribed by FSSR (2011).

Pure and market samples of paneer with reference to fat were analyzed for the same physico-chemical constants, which was prescribed by FSSR (2011) and AGMARK (1981) to evaluate the quality of ghee.

In this study, a total of sixteen and seven market samples of paneer were collected from unorganized and organized sector of NCR, respectively. Fat was extracted from these samples with chloroform using soxhlet extraction method. The isolated fat after removing the solvent was examined for their physico-chemical parameters, such as RM value, Polenske value and BR reading and free fatty acid (% oleic acid).

Tables 4.14 to 4.16 depicts the results on physico-chemical constants such as Reichert-Meissl value, Polenske value, Butyro-refractometer reading (at

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40°C) and free fatty acid of pure buffalo paneer fat and paneer fat from organized and unorganized dairies NCR.

Table 4.14 Physico-chemical analysis of pure buffalo paneer fat

Pure ghee	RM value	Polenske value	BR at 40°C	FFA (% oleic acid)
Buffalo Paneer Fat	29.13±0.26	1.28±0.04	42.00±0.03	0.22±0.017

Data are presented as Mean±S.E. (n=3)

The ranges of RM value, Polenske value, BR reading at 40°C and free fatty acid for pure paneer fat were observed to be 28.93 to 29.44, 1.2 to 1.36, 42 to 42.1 and 0.19 to 0.25, respectively. Table 4.15 depicts the physico-chemical constants of fat isolated from market paneer samples from unorganized dairies of NCR. The ranges of RM value, Polenske value, BR reading at 40°C and free fatty acid for butter samples of unorganized sector (UOS) were observed to be 4.76 to 30.37, 0.59 to 1.64, 41.4 to 60.0 and 0.22 to 0.56, respectively.

It is evident from the data presented in table 4.15 that out of sixteen samples of paneer collected from unorganized sector, the RM value of three samples (UOS-4, 6, 15) found to be the lower for milk fat as compared to standards prescribed by FSSR (2011) and AGMARK (1981). BR reading data revealed that out of sixteen samples five samples (UOS-2, 4, 5, 8, 15) showed the higher BR reading as compared to standards specified by FSSR (2011) and AGMARK (1981). For Polenske value, one out of sixteen samples showed lower value, while rest of the samples were in general range of 1 to 2 (AGMARK, 1981). One sample (UOS-4) of paneer from unorganized sector was observed positive for Baudouin test (Plate 4.5). Free fatty acid values were in the range as prescribed by both FSSR (2011) and AGMARK (1981). Table 4.16 depicts the physico-chemical constants of market paneer samples from organized dairies of NCR in winter season. The ranges of RM value, Polenske value, BR reading at 40°C and free fatty acid for butter samples of organized sector (OS) were observed to be 29.21 to 31.3, 1.52 to 1.83, 41.1 to 42.2 and 0.22 to 0.58, respectively. No samples from organized sector were found positive for Baudouin test (Plate 4.6).

Table 4.15 Physico-chemical analysis of market paneer samples from unorganized sector (UOS) of NCR.

Physico-chemical constants				
Samples	RM value	Polenske value	BR at 40°C	FFA (% oleic acid)
UOS-1	30.37±0.07	1.4±0.05	42.2±0.08	0.27±0.04
UOS -2	28.93±0.18	1.26±0.03	44.36±0.07	0.34±0.06
UOS -3	28.56±0.14	1.15±0.09	43.00±0.03	0.38±0.07
UOS -4	4.76±0.26	0.59±0.02	60.0±0.06	0.47±0.04
UOS -5	28.54±0.12	1.17±0.07	43.5±0.05	0.31±0.01
UOS -6	26.8±0.2	1.43±0.06	43.0±0.03	0.40±0.02
UOS -7	28.13±0.17	1.03±0.06	42.6±0.06	0.47±0.03
UOS -8	29±0.23	1.53±0.06	43.26±0.06	0.28±0.01
UOS -9	28.8±0.20	1.64±0.06	41.4±0.03	0.23±0.02
UOS -10	28.6±0.1	1.16±0.08	42.43±0.08	0.56±0.01
UOS -11	29.23±0.20	1.34±0.05	42.4±0.05	0.22±0.01
UOS -12	29.56±0.20	1.3±0.05	42.48±0.04	0.34±0.04
UOS -13	29.13±0.14	1.43±0.07	41.6±0.04	0.48±0.07
UOS -14	29.1±0.17	1.58±0.07	42.23±0.08	0.34±0.05
UOS -15	27.30±0.25	1.53±0.04	44.23±0.08	0.50±0.05
UOS-16	28.55±0.21	1.13±0.03	42.5±0.05	0.48±0.07

Data are presented as Mean±S.E. (n=3)

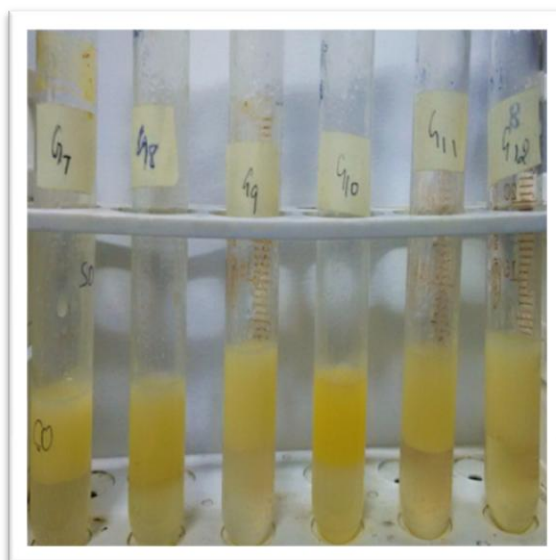
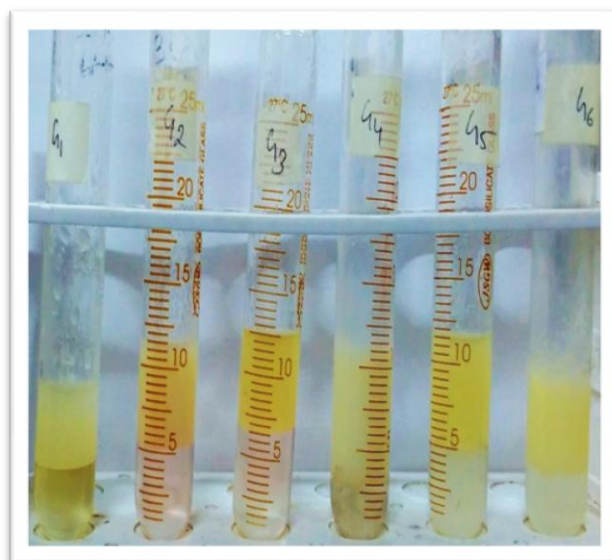
Table 4.16 Physico-chemical analysis of market paneer samples from organized sector (OS) of NCR.

Physico-chemical constants					
Samples	RM value	Polenske value	BR at 40°C	FFA%	Baudouin test
OS-1	29.74±0.16	1.52±0.04	42.2±0.05	0.30±0.03	Negative
OS-2	31.16±0.13	1.71±0.06	41.46±0.03	0.44±0.05	Negative
OS-3	31.3±0.24	1.54±0.08	41.1±0.08	0.30±0.01	Negative
OS-4	30.73±0.23	1.72±0.04	42.1±0.05	0.22±0.01	Negative
OS-5	29.66±0.24	1.63±0.03	41.1±0.05	0.38±0.08	Negative
OS-6	31.19±0.29	1.83±0.03	41.5±0.03	0.32±0.07	Negative
OS-7	29.21±0.20	1.67±0.01	42.1±0.08	0.58±0.09	Negative

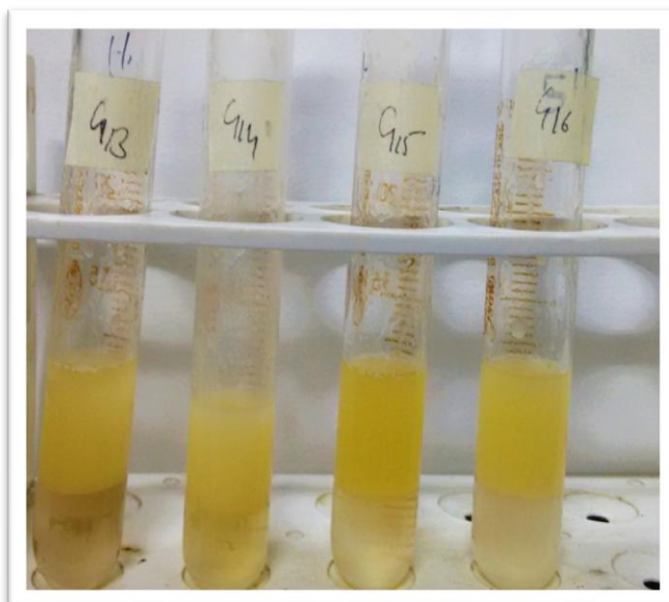
Data are presented as Mean±S.E. (n=3)

Data depicted in Tables 4.9 and 4.10 clearly revealed that out of eleven samples from organized sector, 9% samples failed to meet legal requirement of RM value, Polenske value and BR reading as specified in AGMARK (1981) and FSSR (2011), and 36.36% samples were found positive for Baudouin test. Similarly, that out of fifteen samples from unorganized sector, 26.6% samples failed to meet legal requirement of RM value, Polenske value and BR reading (Table 4.9) as specified in AGMARK (1981) and FSSR (2011), 13.3% samples were found positive for Baudouin test.

In case of butter samples (Table 4.13) all the samples from organized sector met the legal requirement as specified by AGMARK (1981) and FSSR (2011). However from unorganized sector 25 %, 6.25% and 37.5% samples could not meet specification of RM value, Polenske value and BR reading, respectively (Table 4.12). No samples found positive for Baudouin test.



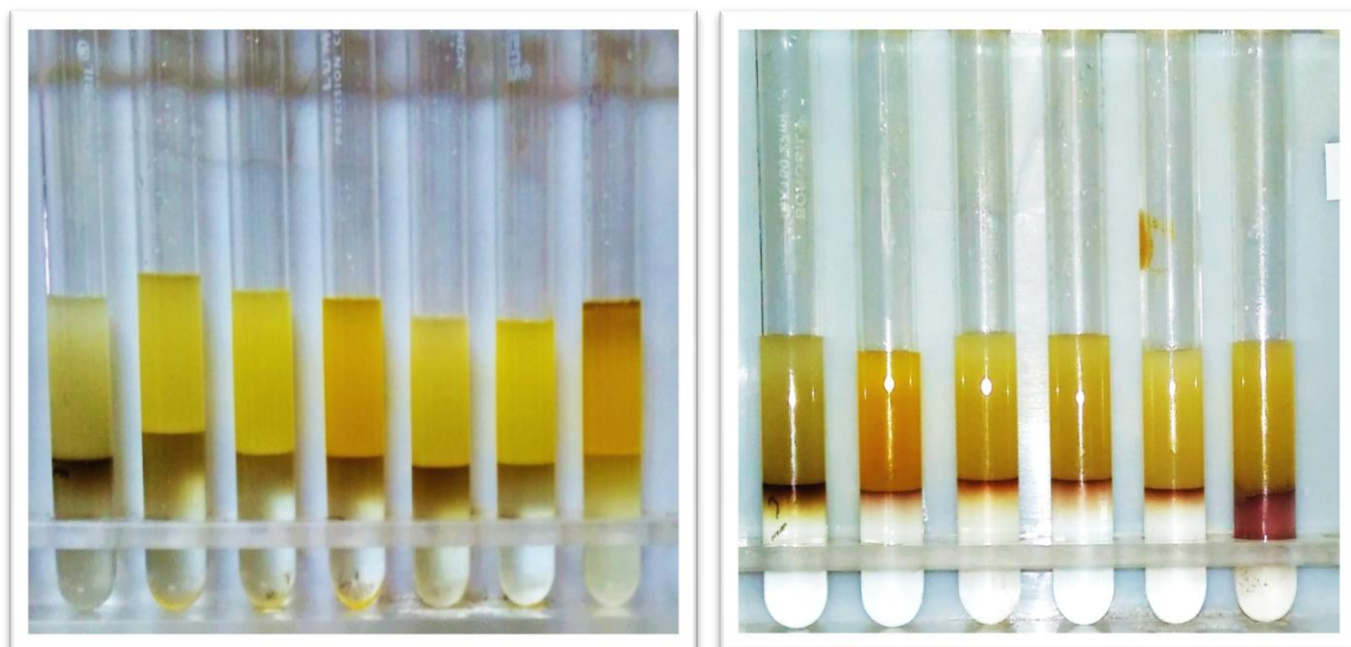
A B C D E F G H I J K L



M N O P

Plate 4.1 Baudouin test for detection of vanaspati in unorganized market ghee samples A: pure ghee; B: UOS-4, C:); D UOS-11: UOS-1; E: UOS-2; F: UOS-3; G: UOS-5; H: UOS-6; I: UOS-7; J: UOS-8; K: UOS-9; L: UOS-10; M: UOS-11; N: UOS-12; O: UOS-13; P: UOS-15

Results & Discussion



A B C D E F G

H I J K L M

Plate 4.2 Baudouin test for detection of vanaspati in organized market ghee samples: A: pure ghee; B: OS-1; C: OS-2; D: OS-3; E: OS-4; F: OS-5; G: OS-6; H: control; I: OS-7; J: OS-8; K: OS-9; L: OS-10; M: OS-11

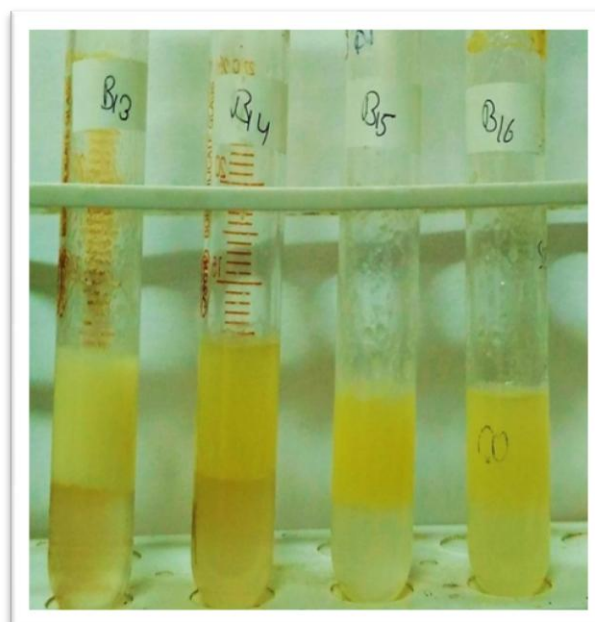
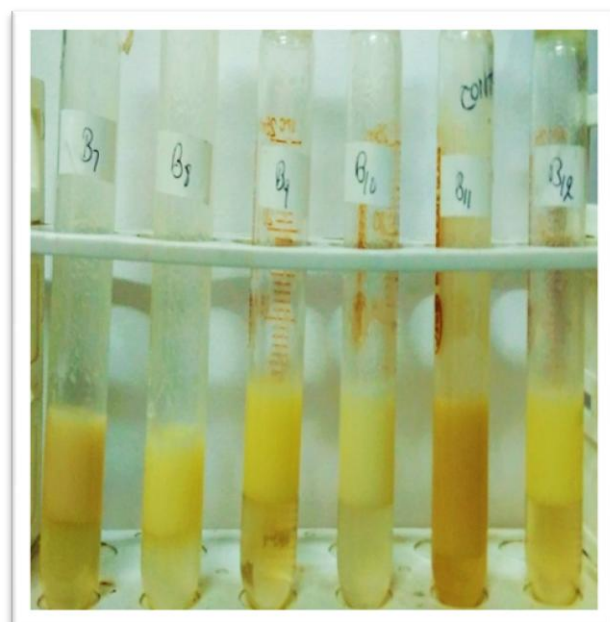
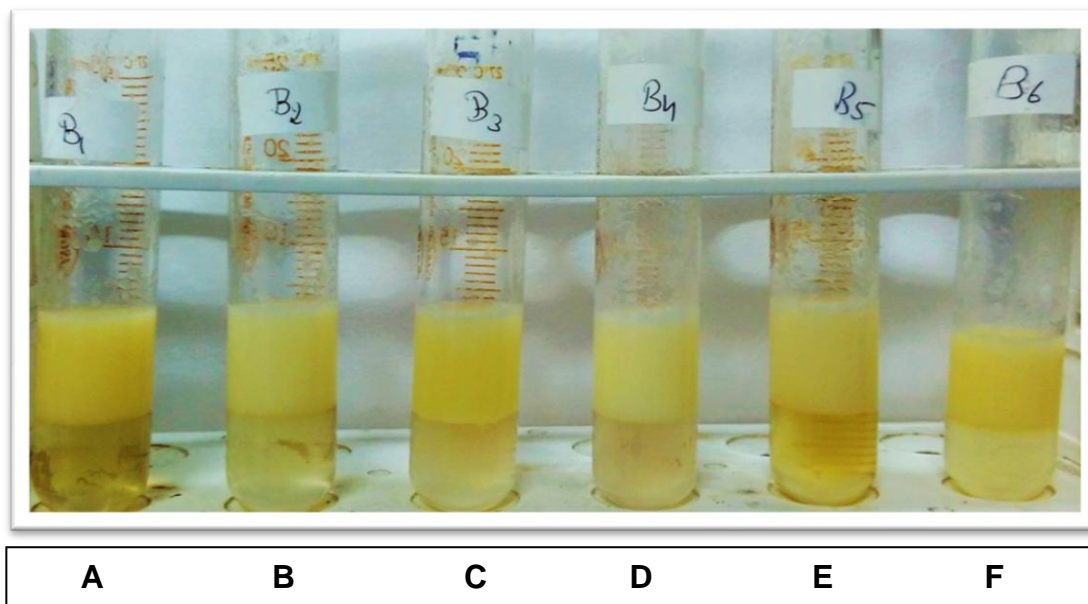
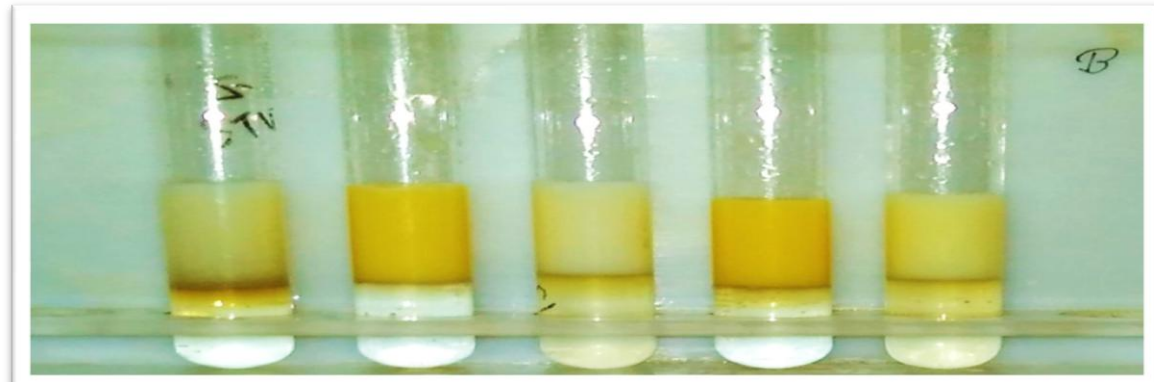


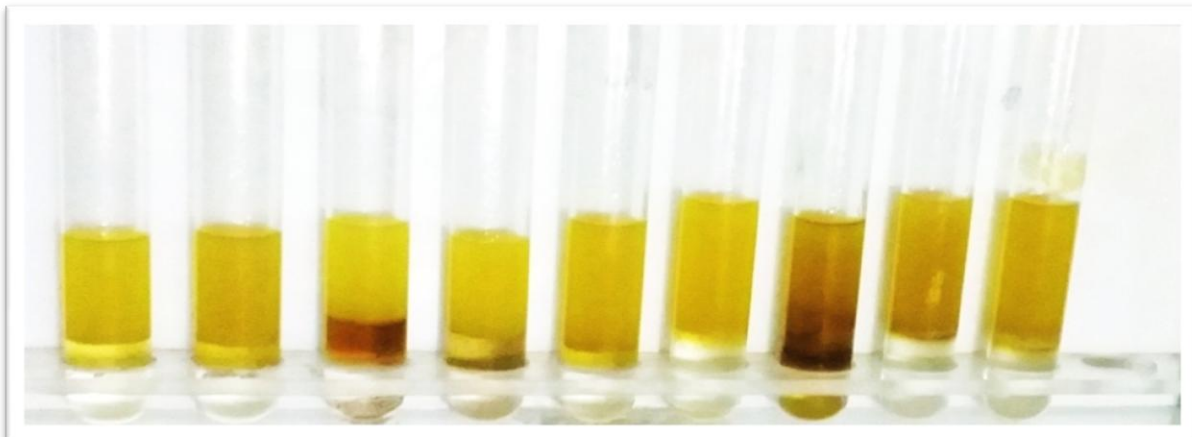
Plate 4.3 Baudouin test for detection of vanaspati in unorganized market butter samples: A: OS-1; B: OS-2; C: OS-3; D: OS-4; E: OS-5; F: OS-6 G: UOS-7; H: UOS-8; I: UOS-9; J: UOS-10; K: UOS-11; L: UOS-12; M: UOS-13; N: UOS-14; O: UOS-15; P: UOS-16

Results & Discussion

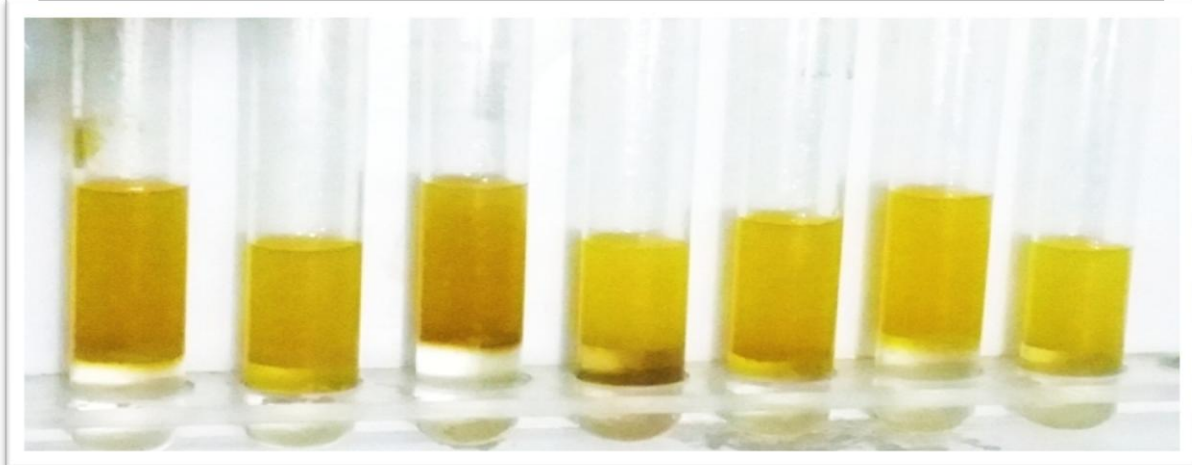


A B C D E

Plate 4.4 Baudouin test for detection of vanaspati in organized market butter samples: A: OS-1; B: OS-2; C: OS-3; D: OS-4; E: OS-5



A B C D E F G H I



J K L M N O P

Plate 4.5 Baudouin test for detection of vanaspati in unorganized market paneer samples: A: UOS-1; B: UOS-2; C: UOS-4; D: UOS-3; E: UOS-5; F: UOS-6; G: UOS-7; H: UOS-8; I: UOS-9 J: UOS-10; K: UOS-11; L: UOS-12; M: UOS-13; N: UOS-14; O: UOS-15; P: UOS-16

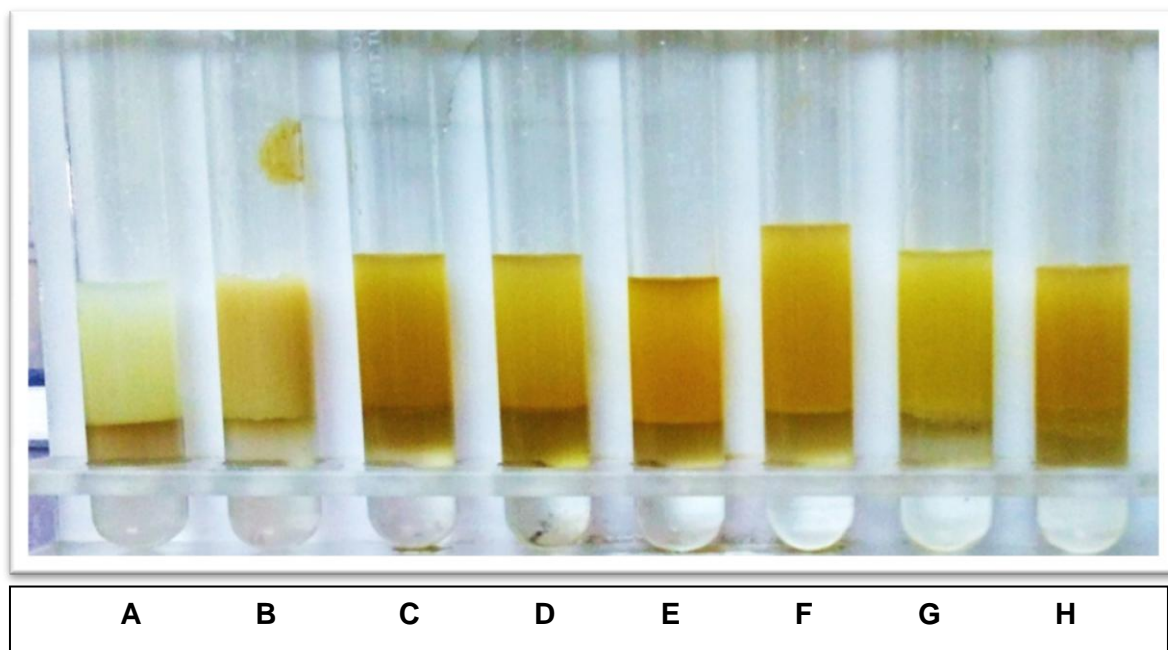


Plate 4.6 Baudouin test for detection of vanaspati in organized market paneer samples: A: control; B: OS-1; C: OS-2; D: OS-3; E: OS-4; F: OS-5; G: OS-6; H: OS-7

The analysis of market samples (Table 4.16) of paneer from organized sector revealed that all the samples from organized sector met the physico-chemical parameters of milk fat as specified by AGMARK (1981) and FSSR (2011). On the contrary, in case of unorganized sector samples 18.75 %, 6.25% and 31.25% samples failed on the basis of RM value, Polenske value and BR reading, respectively (Table 4.15). Baudouin test was found positive in milk fat of 6.25% of the total samples of paneer collected from unorganized sector.

It was also observed from the study of physico-chemical parameters that samples of particular unorganized dairy which were found adulterated for ghee, also found adulterated for butter and paneer. Similar pattern was also observed for organized dairies.

4.2.3 Reversed-phase thin layer chromatography of unsaponifiable matter of laboratory made samples of ghee, butter and paneer milk fat

In order to confirm the presence of vegetable oils in milk fat samples, RP-TLC of unsaponifiable matter was performed. Unsaponifiable matter from fat samples was extracted as per the method standardized by Sharma *et al.* (2009) and applied on RP-TLC plates as per the method described by Rani *et al.* (2015).

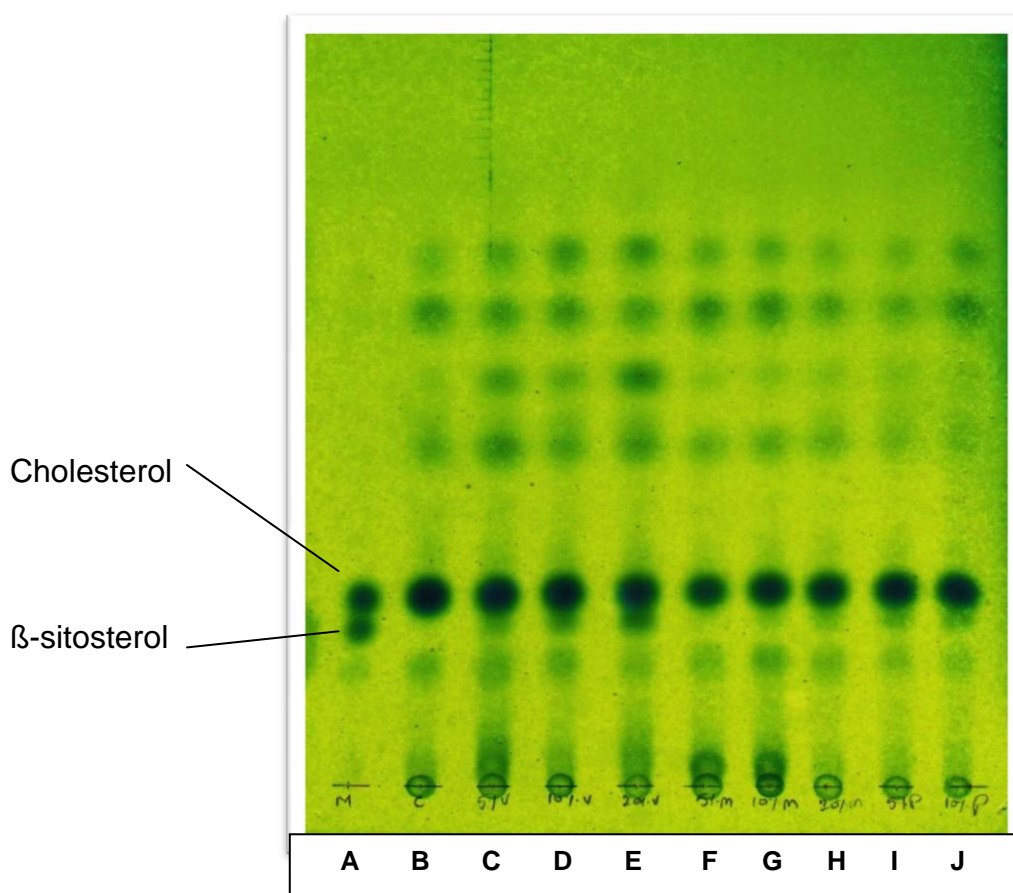


Plate 4.7 RP-TLC of USM ghee, butter and paneer sample adulterated with adulterant at different levels- A: mixture (cholesterol+ β -sitosterol); B: pure buffalo ghee; C: 5% soybean oil in buffalo ghee; D: 10% soybean oil in buffalo ghee; E: 20% soybean oil in buffalo ghee; F: 5% margarine in butter; G: 10% margarine in butter; H: 20% margarine in butter; I: 5% soybean oil in buffalo paneer fat; J: 10% soybean oil in buffalo paneer fat

The RP-TLC of USM extracted from control samples like 5, 10 and 20% level adulteration of soybean oil in buffalo ghee and paneer fat and 5, 10 and 20% level adulteration of margarine in butter were carried out. RP-TLC chromatogram (Plate 4.7), clearly showed that the adulteration of vegetable oil in both ghee and paneer fat could be detected at 5% level and adulteration of margarine in butter was easily detectable at 5% level. Results clearly demonstrated that fat extracted from paneer using soxhlet procedure can be used to confirm the presence of vegetable oil in milk fat using RP-TLC.

4.2.3.1 Reversed-phase thin layer chromatography of unsaponifiable matter of market samples of ghee from organized and unorganized sector of NCR

In this section the RP-TLC results of the organized (OS) and unorganized sector (UOS) ghee samples has been discussed.

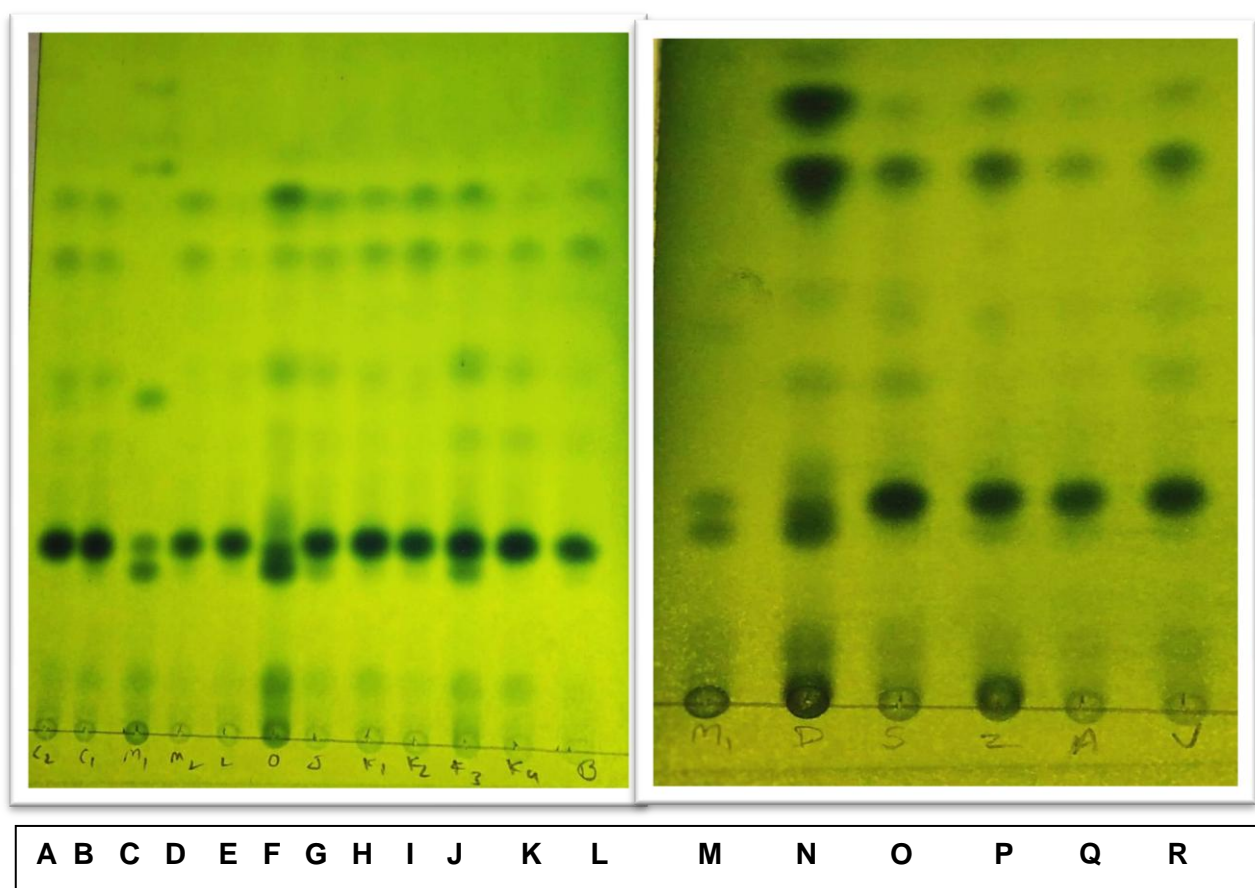


Plate 4.8 RP-TLC of unorganized market ghee samples: A: pure ghee; B: UOS-1, C: Mixture (β -sitosterol+cholesterol); D: UOS-2; E: UOS-3; F: UOS-4; G: UOS-5; H: UOS-6; I: UOS-7; J: UOS-8; K: UOS-9; L: UOS-10; M: Mixture (β -sitosterol+cholesterol); N: UOS-11; O: UOS-12; P: UOS-13; Q: UOS-14; R: UOS-15

Results & Discussion

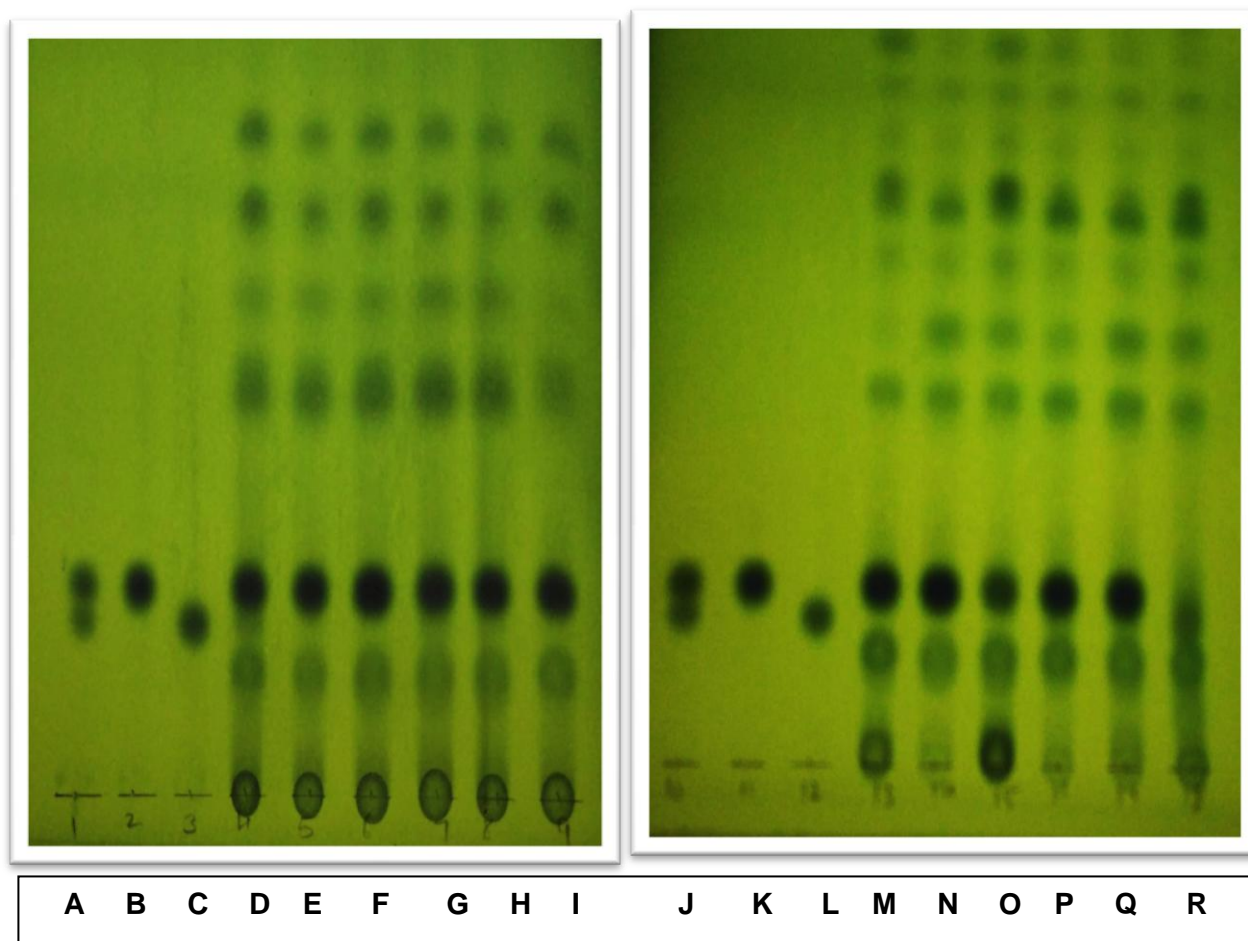


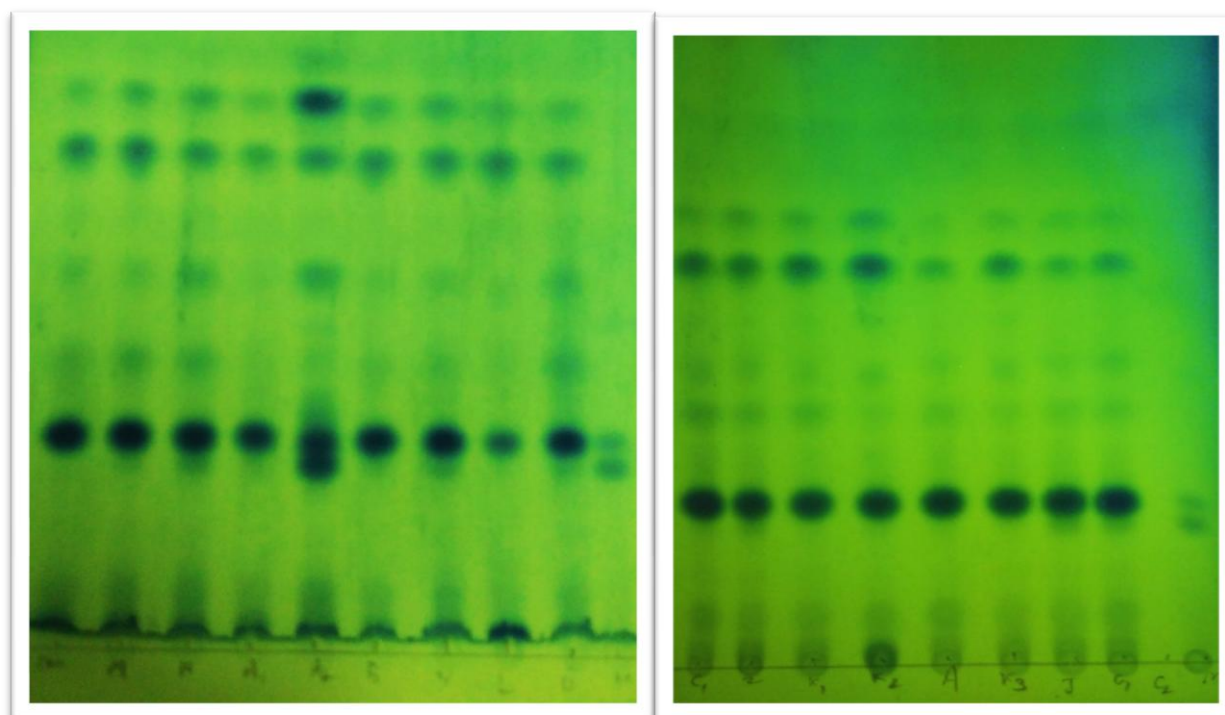
Plate 4.9 RP-TLC of organized market ghee samples: A: Mixture (β -sitosterol + cholesterol); B: cholesterol; C: β -sitosterol; D: OS-1; E: OS-2; F: OS-3; G: OS-4; H: OS-5; I: pure ghee; J: Mixture (β -sitosterol + cholesterol); K: cholesterol; L: β -sitosterol; M: OS-6; N: OS-7; O: OS-8; P: OS-9; Q: OS-10; R: OS-11

It was observed from the Plates 4.8 and 4.9 that the majority of ghee samples i.e. ~66.6% from unorganized sector showed a band corresponding to the band of β -sitosterol in their RP-TLC chromatograms. This clearly indicated that majority of samples from unorganized sector had some amount of added vegetable oils/fats. Similarly, six samples of organized ghee sector out of eleven samples i.e. 54.5% showed a band of corresponding to the band of β -sitosterol in their RP-TLC chromatograms. Similar finding had been reported by Rani (2013). She reported in her market survey that 80% samples of ghee from organized and 73% samples from unorganized sector were found adulterated with vegetable oils/fats through this technique. From the finding of the present investigation it was concluded that the quality of market ghee is a big issue and needs to be looked in to. It was also concluded that there is a need to include

test like RP-TLC of USM as one of the parameter to ensure the quality of ghee with respect to vegetable oil/fat/PHVO addition in ghee milk fat.

4.2.3.2 Reversed-phase thin layer chromatography of unsaponifiable matter of market samples of paneer fat from organized and unorganized sector of NCR

The unsaponifiable matter (USM) obtained from market paneer fat was used in the present investigation for carrying out RP-TLC analysis. The typical chromatogram depicting the separation of unsaponifiable constituents of pure buffalo paneer milk fat and USM of different market paneer samples along with standard compounds cholesterol, β -sitosterol are shown in Plate 4.10. The result showed that standard cholesterol and β -sitosterol showed two distinct bands, the former being slightly above the latter. Pure Buffalo paneer milk fat showed a prominent band with a similar distance moved from the spotting line as that of standard cholesterol. Buffalo paneer milk fat did not show any band of β -sitosterol.



A B C D E F G H I J K L M N O P Q R S

Plate 4.10 RP-TLC of unorganized market paneer samples: A: pure buffalo paneer fat; B: UOS-1; C: UOS-2; D: UOS-3; E: UOS-4; F: UOS-5; G: UOS-6; H: UOS-7; I: UOS-8; J: Mixture (β -sitosterol+cholesterol); K: UOS-9; L: UOS-10; M: UOS-11; N: UOS-12; O: UOS-13; P: UOS-14; Q: UOS-15; R: UOS-16; S: Mixture (β -sitosterol+cholesterol)

Results & Discussion

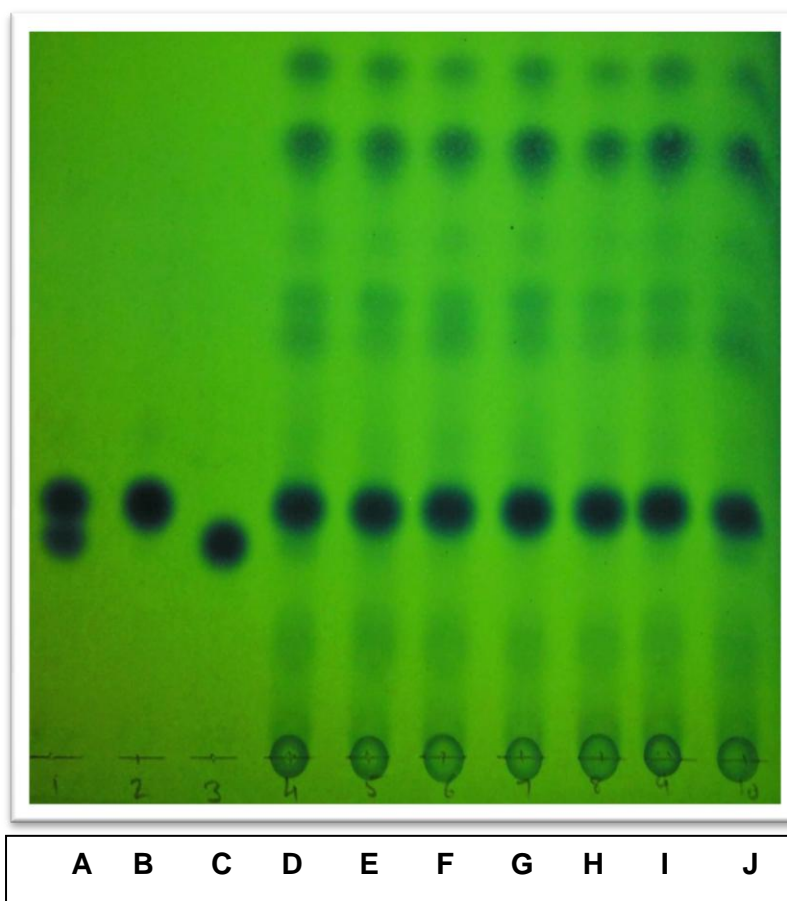


Plate 4.11 RP-TLC of organized market paneer samples: A: Mixture (β -sitosterol + cholesterol); B: cholesterol; C: β -sitosterol; D: OS-1; E: OS-2; F: OS-3; G: OS-4; H: OS-5; I: OS-6; J: OS-7;

It was observed from the plates 4.10 and 4.11 that the majority of paneer fat samples i.e. ~43.75% from unorganized sector showed a band corresponding to the band of β -sitosterol in their RP-TLC chromatograms. This clearly indicated that majority of samples from unorganized sector had some amount of added vegetable oils/fats. However, in case of samples of paneer collected from organized sector, two samples out of seven samples i.e. 28.57% showed a band of corresponding to the band of β -sitosterol in their RP-TLC chromatograms. Similar work had been reported by Aghav (2014). He reported that 53.3% samples of paneer were found adulterated with vegetable oils/fats through RP-TLC method.

4.2.3.3 Reversed-phase thin layer chromatography of unsaponifiable matter of market samples of butter from organized and unorganized sector of NCR

In this case RP-TLC analysis of fat isolated from butter samples of organized sector (OS) and unorganized sector (UOS) was carried out. The results have been depicted in Plates 4.12 and 4.13. It is evident from the RP-TLC analysis that the majority of butter samples i.e. ~43.75% from unorganized sector showed a band corresponding to the band of β -sitosterol in their RP-TLC chromatograms. This clearly indicated that majority of samples from unorganized sector had some amount of added vegetable oils/fats. Similarly, one sample out of five samples of butter collected from organized sector i.e. 20.0% showed a band of corresponding to the band of β -sitosterol in RP-TLC analysis.

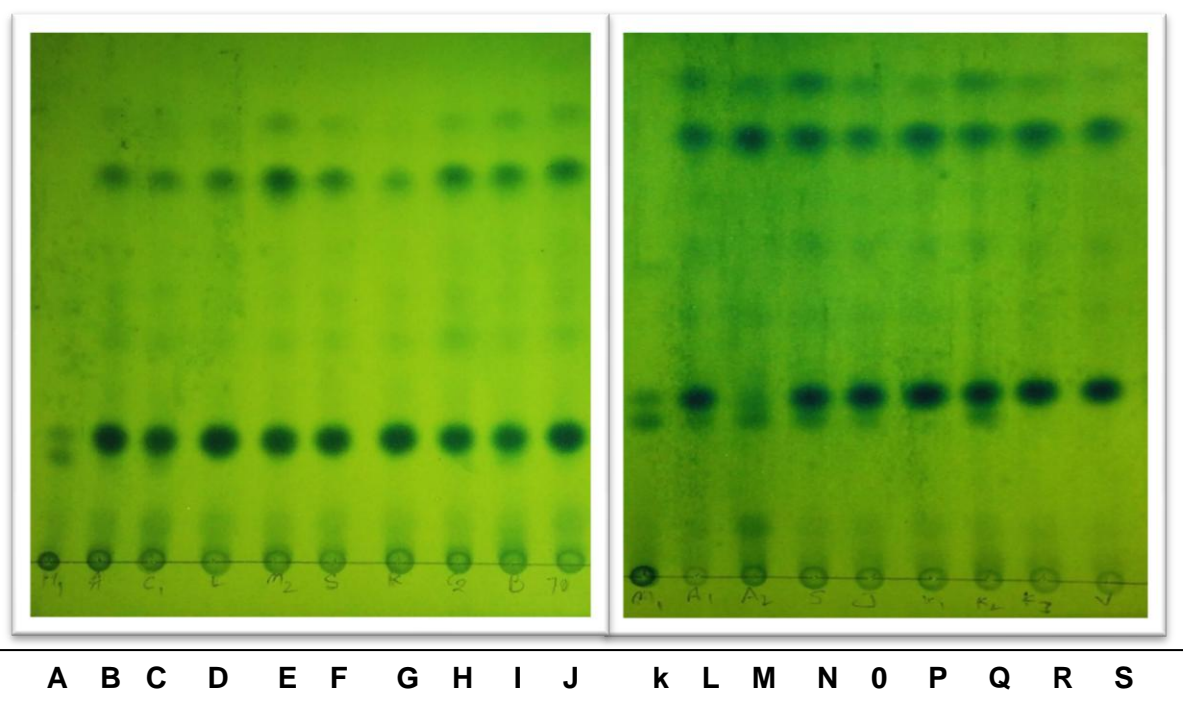


Plate 4.12 RP-TLC of unorganized market butter samples: A: Mixture (β -sitostero+ cholesterol); B: UOS-1; C: UOS-2; D: UOS-3; E: UOS-4; F: UOS-5; G: UOS-6; H: buffalo butter; I: UOS-7; J: UOS-8; K: Mixture(β -sitostero+ cholesterol); L: UOS-9; M: UOS-10; N: UOS-11; O: UOS-12; P: UOS-13; Q: UOS-14; R: UOS-15; S: UOS-16

Results & Discussion

It can be concluded from the above results that RP-TLC is a strong tool to detect adulteration of ghee and paneer fat with vegetable oil at 5% level. Similarly adulteration of butter with margarine was possible to detect at 5% level using RP- TLC. Results clearly demonstrated that fat extracted from paneer using soxhlet procedure can be used to confirm the presence of vegetable oil in milk fat using RP-TLC.

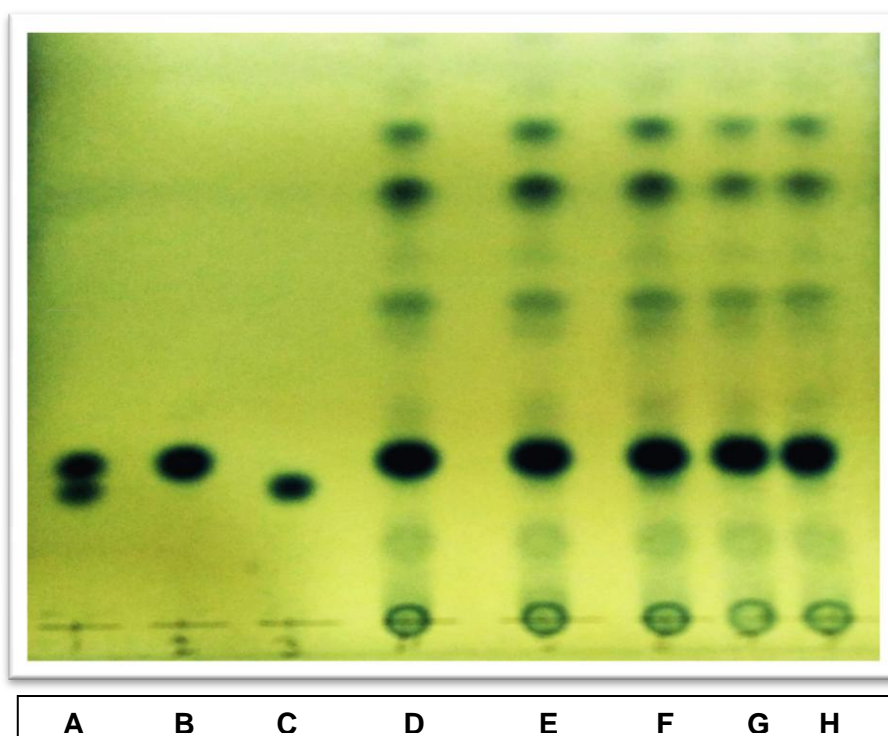


Plate 4.13 RP-TLC of organized market butter samples: A: Mixture (β -sitosterol + cholesterol); B: cholesterol; C: β -sitosterol; D: OS-1; E: OS-2; F: OS-3; G: OS-4; H: OS-5

RP-TLC profile of USM of ghee samples from organized sector revealed that 54.5% samples were found to contain vegetable oil/fat (Plate 4.9). RP-TLC profile of USM of ghee samples from unorganized sector revealed that 66.6% samples were found to contain vegetable oil/fat (Plate 4.8).

RP-TLC profile of USM of market samples of paneer fat from organized sector revealed that 28.5% samples were found to contain vegetable oil/fat (Plate 4.11). While RP-TLC profile of USM of samples of paneer fat from unorganized sector revealed that 43.75% were found to contain vegetable oil/fat (Plate 4.10).

RP-TLC profile of USM of market samples of butter from organized sector revealed that 40% samples were found to contain vegetable oil/fat (Plate 4.13). RP-TLC profile of USM of samples of butter fat from unorganized sector revealed that 43.75% were found to contain vegetable oil/fat (Plate 4.12).

It was also observed from RP-TLC profile of USM that samples of particular unorganized dairy which were found positive for ghee, also found positive for butter and paneer. Similar pattern was also observed for organized dairies.

4.2.4 Triacylglycerol (TAG) analysis of milk fat samples adulterated individually with animal body fats and vegetable oil using low-resolution GLC.

A new stage in the GLC of TAGs was represented by the introduction of capillary columns in the 1970s. Nearly all the commercially important fats and oils of animal and plant origin consist almost exclusively of the simple lipid class – triacylglycerol (TAG). This includes all the vegetable oils, such as those from corn (maize), olive, palm, and sunflower, and animal fats, such as tallow, lard and butter, as well as commercial products such as margarines. Very recently researchers reported that fatty acid analysis using GLC fail to detect foreign fat specially animal body fat in milk fat (Rebechi *et al.*, 2016).

The analysis of lipid compositions of milk fat is recommended by many researchers to detect foreign fat in milk fat (Alonso *et al.* 1997; Destailats *et al.*, 2006; Molquentin, 2007). TG determination has been used to detect foreign fats in milk fat (Precht, 1990; Contarini *et al.*, 1993 and Kala, 2013). Also profiling of triacylglycerol using GLC has been reported to be more effective than fatty acid analysis (ISO&IDF, 2010) but the method suggested by ISO & IDF (2010) has not been studied systematically in Indian conditions. TAG analysis was carried out on a GLC programme standardized in the lab by Hazra (2016), which was essentially based on the method of Kala (2013). Five reference standards (C24, C30, C36, C42, C48) of triacylglycerol (Figure 4.5) were used for identification of the particular TAG (Triacylglycerol) by comparing their retention time (Table 4.17) with that of obtained TAG (Triacylglycerol) profile of unknown sample.

Results & Discussion

4.2.4.1 TAGs profile of control ghee and fat extracted from pure buffalo paneer and butter

The result of TAG compositions determined by GC for control samples were as shown in Table 4.18. There were 16 major peaks (Figures 4.6 and 4.7) of even carbon number TAGs, corresponding to TG of 24 to 54 carbon number TAG, were identified in both cow or buffalo milk ghee. Similarly, 14 major peaks (Fig. 4.8 and 4.9) of even carbon number TAGs, corresponding to TAG of 28 to 54 carbon number TAG, were identified in both buffalo butter fat and paneer fat. However, some unidentified peaks were also observed, these peaks might be of odd carbon number TAG but Quantity of these TAGs were very low.

Table 4.17 Retention time of standard TAGs

TAGs	Retention time
C24	9.01±0.05
C30	14.34±0.04
C36	19.23±0.1
C42	23.42±0.04
C48	27.01±0.02

Data is presented as Mean±SD (n=5)

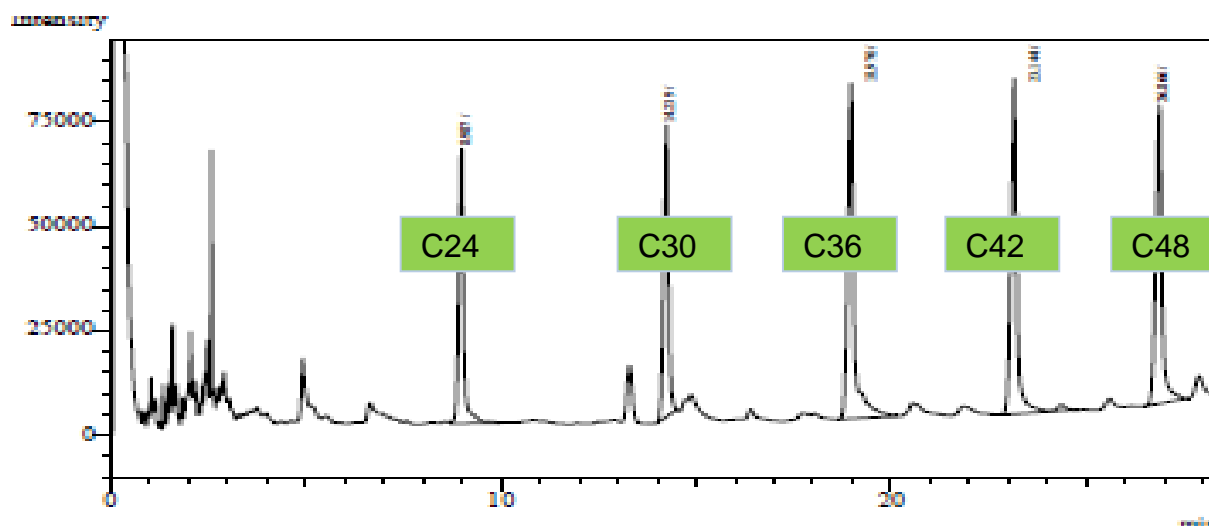


Fig. 4.5: Chromatogram of 5 TGs (C24, C30, C36, C42, C48) using standardized condition with split ratio (1:50)

In control samples of milk fat, there were six TAGs (Table 4.18) were predominant (C36, C38, C40, C48, C50 and C52), the quantity of these six TG were almost sixty percentage of total TG present in control samples. Presence of C24 carbon number TG was almost negligible (Table 4.18) in cow ghee and buffalo ghee. While C24 and C26 were absent in buffalo butter and paneer fat. Smaller carbon number TAGs like (C26, C28, C30, C32) found to be very nominal (Table 4.18), where as TAGs carbon number C34-C54 were the major TAGs found in control samples. It was also noted from Table (4.18) that summation of C50, C52 and C54 were higher in cow ghee as compared to buffalo ghee, buffalo butter and buffalo paneer fat. There was no significant difference in summation value (C50 + C52 + C54) of buffalo butter fat and fat isolated from paneer as compared to buffalo ghee. It was also observed from Table (4.18) that quantity of TAGs C52 and C54 were almost same in buffalo ghee, butter and paneer fat but slightly high in cow ghee as compared to buffalo ghee and its products. Hence results are in accordance with early observations of Kim *et al.* (2015) and Kala (2013) who reported that the major TAG of cow milk fat were C30 to C54. Destailats *et al.* (2006) who also identified 16 TG peaks (C24-C54) of pure cow milk fat on GLC using packed column. From fig. 4.10 it was clearly visible that small and medium carbon number TAGs (C26 to C36) were higher for buffalo ghee than cow ghee. It was also observed that small and medium carbon number TAGs (C26 to C36) were almost similar in buffalo ghee and buffalo based butter and paneer. Higher carbon number TAGs (C42 to C54) were higher in cow ghee than other control samples. Earlier Kala (2013) reported that higher carbon number TAGs were higher in cow ghee as compare to buffalo ghee. It was also observed from Figure (4.10) that higher carbon TAG content C50, C52 and C54 in buffalo ghee was significantly lower than cow ghee. The weight percentage and distribution of TAG in milk exhibited many structural similarities for different animal species (Christie, 1983). The range of summation of TAGs carbon number C50, C52 and C54 in control samples was found in between 26.31 to 30.1. Rabechi *et al.* (2016) reported that fatty acid analysis is not a good marker for detection of lard in milk fat, but TAGs analysis followed by summation of three TAGs (C50+C52+C54) could be efficiently use to detect lard in ghee to the tune of 5%.

Results & Discussion

4.2.4.2 TAGs profile of adulterants oils and fats using low-resolution GLC

Animal body fat (buffalo tallow, goat tallow and lard) and vegetable oils/fats (soybean oil, margarine, and coconut oil) were used as adulterants to spike the control samples.

Table 4.18 TAGs profile of control ghee and fat extracted from pure buffalo paneer and butter

TAGs	Cow ghee	Buffalo ghee	Buffalo butter fat	Buffalo paneer fat
C24	0.08±0.01	0.09±0.01		
C26	0.45±0.09	0.41±0.05		
C28	0.49±0.10	0.55±0.13	0.36±0.03	0.43±0.08
C30	0.79±0.23	0.59±0.07	0.53±0.04	0.69±0.15
C32	1.42±0.15	1.28±0.06	1.21±0.12	1.41±0.13
C34	3.68±0.59	4.06±0.20	4.10±0.36	4.19±0.45
C36	7.97±0.94	10.77±0.97	10.67±1.28	10.35±1.11
C38	11.56±1.02	12.83±0.62	14.84±1.40	14.31±1.62
C40	10.24±0.72	10.90±0.56	10.28±1.20	10.19±1.19
C42	5.89±0.53	3.96±0.02	4.61±0.57	5.12±0.81
C44	5.35±0.20	3.35±0.20	3.36±0.17	3.94±0.81
C46	6.29±0.24	4.25±0.33	4.55±0.59	4.93±0.39
C48	8.13±0.54	7.04±0.47	7.40±0.78	7.21±0.26
C50	11.31±1.13	10.99±0.58	11.34±0.87	10.43±0.81
C52	12.59±1.01	10.91±0.94	10.87±1.0	11.48±0.99
C54	6.20±0.88	4.87±0.36	4.10±0.88	4.65±0.46
C50+C52+C54	30.1	26.77	26.31	26.56

Data is presented as Mean±SD (n=3)

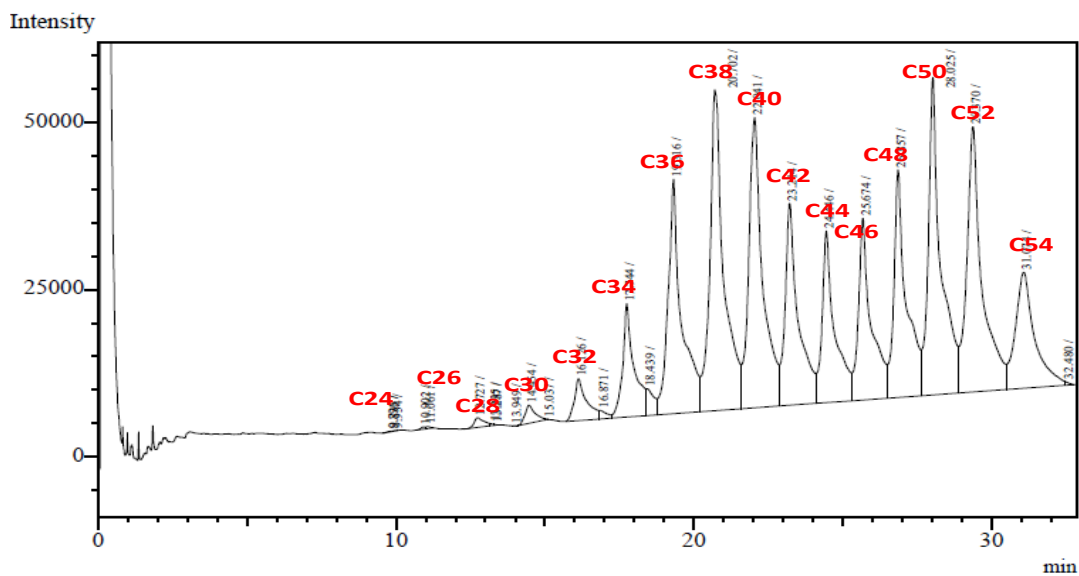


Fig. 4.6 TAGs chromatogram of cow ghee

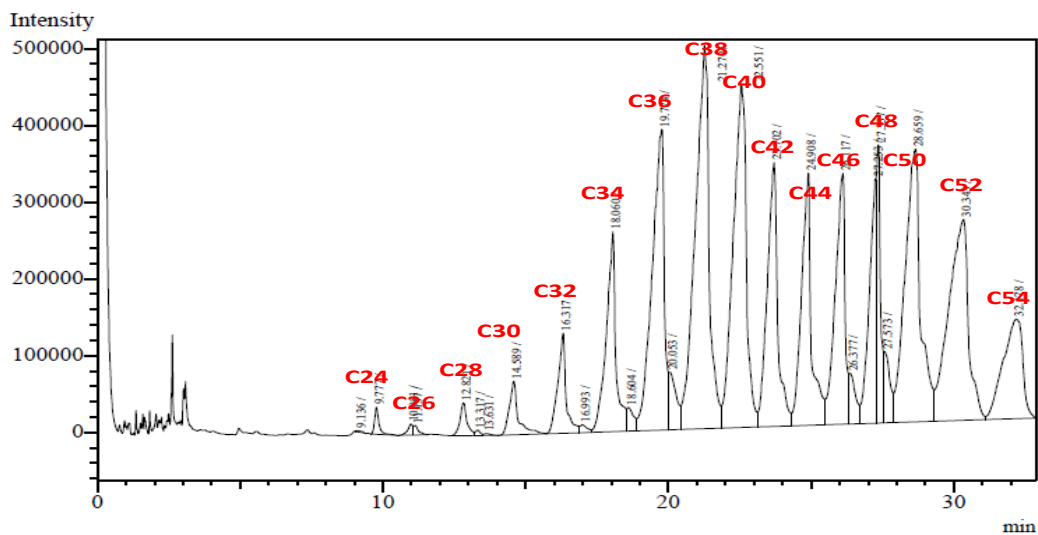


Fig. 4.7 TAGs chromatogram of buffalo ghee

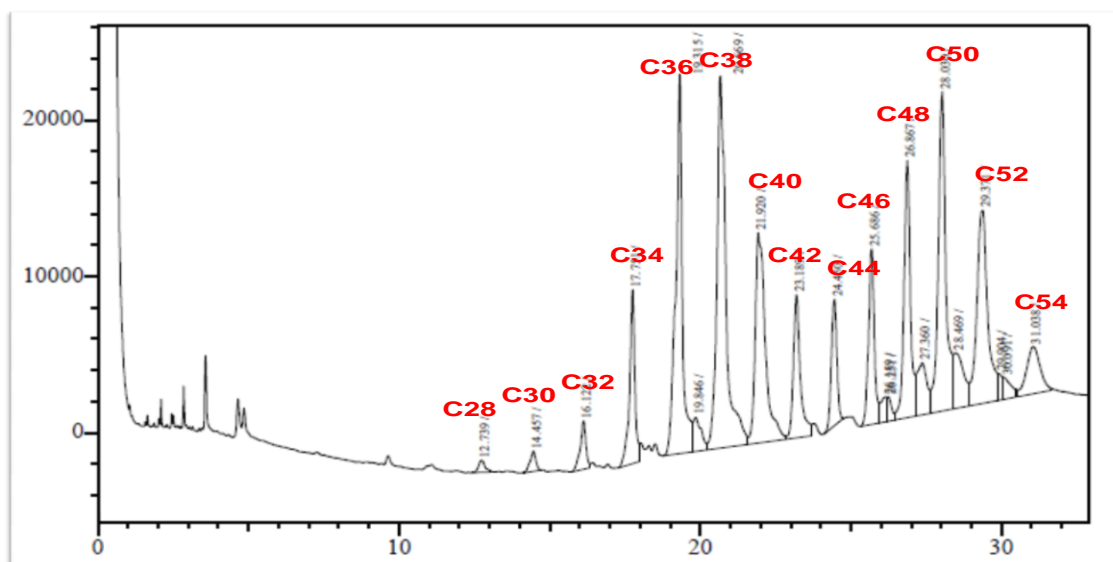


Fig. 4.8 TAGs chromatogram of buffalo butter

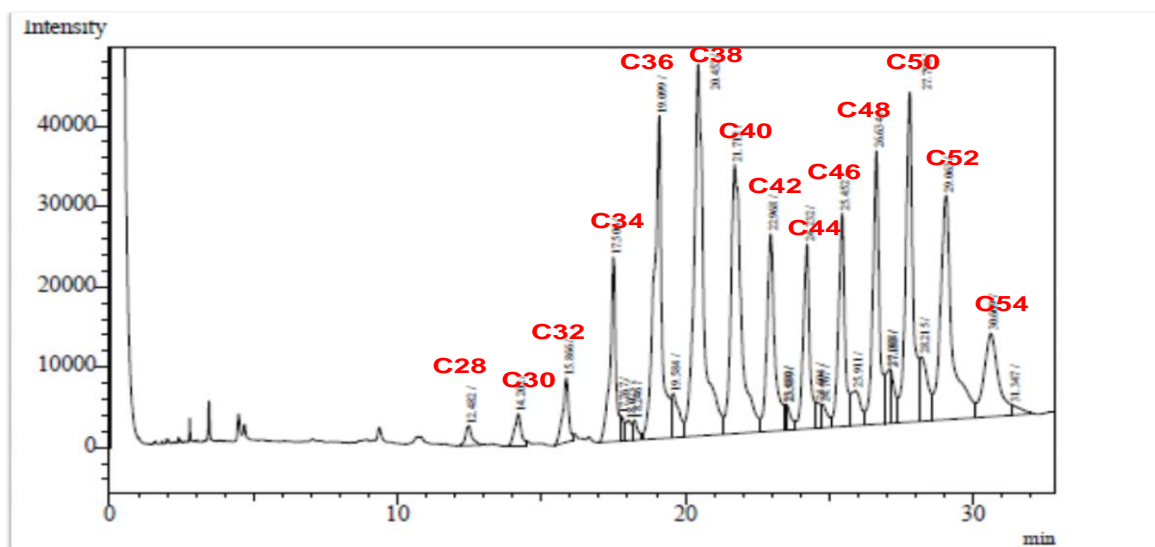


Fig. 4.9 TAGs chromatogram of fat isolated from buffalo paneer

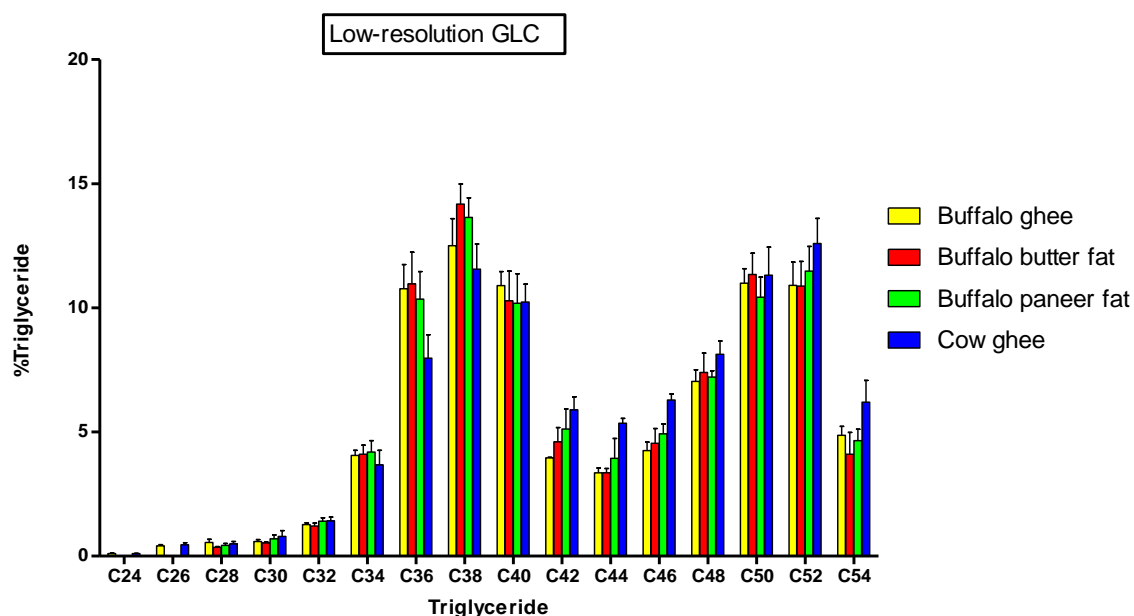


Fig. 4.10 Comparison of TAGs profile of control ghee and fat extracted from pure buffalo paneer and butter

From Table 4.19 it was observed that short and medium carbon number TAGs (C24 to C48) were absent in buffalo tallow, goat tallow, lard and soybean oil. TAGs profile of coconut oil revealed that coconut oil had C34, C36 and 38 TAGs content higher as compared to other fat TAGs. TAG profile of coconut oil indicated absence of C24, C26, C52 and C54. It was observed from TAG profile of margarine that TAGs from C24 to C30 were absent and concentration of C32 to C46 was less as compared to milk fat. The TAGs profile of buffalo tallow, goat tallow, lard and margarine had higher relative percentage of C50, C52, C54 TAGs, while soybean oil had C52 and C54 content higher as compared to other milk fat TAG. Similar type of observation were reported Kala (2013). Kim *et al.* (2015) reported that animal body fats contain higher amount of C50, C52 and C54 and these TAGs could be the potent biomarker to differentiate pure milk fat from the milk fat adulterated animal body fat. Kim *et al.* (2015) also reported that soyabean oil has no C24-C48 carbon number TAG and C54 was the main TAG identified in soya oil. It was concluded from table 4.18 that summation of TAGs C50, C52 and C54 was much higher in animal body fat, soybean oil and margarine except coconut oil than the range specified for control milk fat samples (26.31 to 30.1).

Results & Discussion

Table 4.19 TAGs profile of adulterants oils and fats using low-resolution GLC

TAG	Buffalo tallow	Lard	Goat tallow	Soybean	Coconut oil	Margarine
C24						
C26						
C28					0.40±0.08	
C30					2.86±0.31	
C32					12.88±0.06	0.64±0.09
C34					18.10±0.06	1.10±0.23
C36					20.00±0.23	2.35±0.40
C38					18.72±0.25	1.86±0.29
C40					11.20±0.18	0.91±0.02
C42					7.45±0.04	1.36±0.25
C44					5.14±1.10	1.15±0.20
C46					1.50±0.33	1.76±0.24
C48					0.97±0.39	8.97±0.48
C50	20.77±1.89	17.67±0.99	25.3±0.96	3.83±0.21	0.54±0.17	34.84±0.89
C52	37.61±1.09	57.1±1.89	37.13±1.56	30.95±0.59		35.25±0.56
C54	26.76±0.74	22.99±1.50	24.18±0.75	64.50±0.82		11.35±0.39
C50+C52+C54	85.14	97.76	86.61	99.28		81.44
C34+C36+C38	0	0	0	0	56.82	5.31

Data is presented as Mean±SD (n=3)

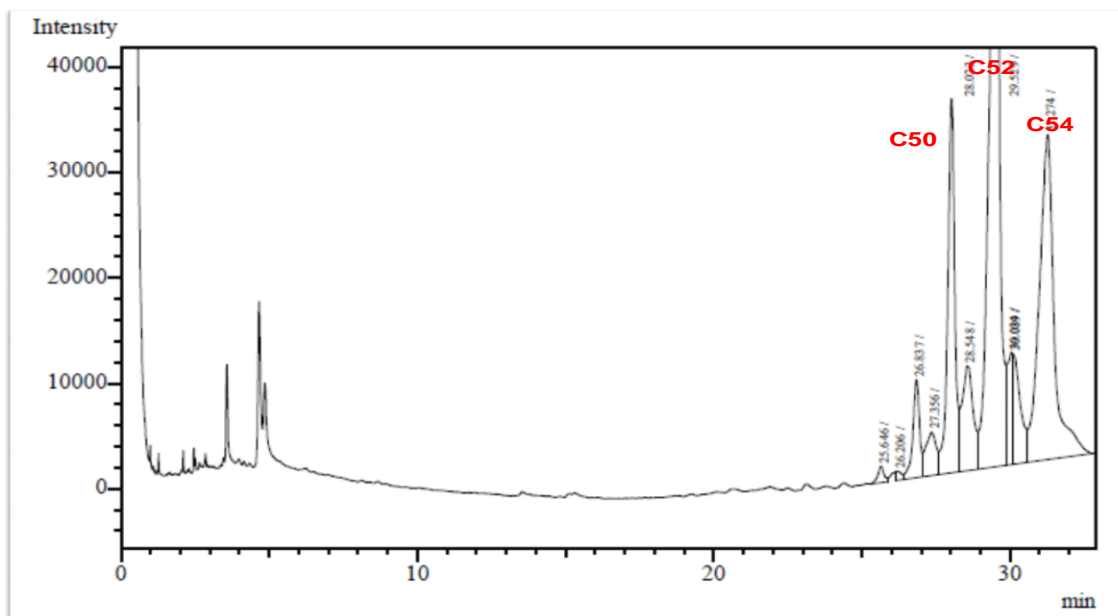


Fig. 4.11 TAGs chromatogram of buffalo tallow

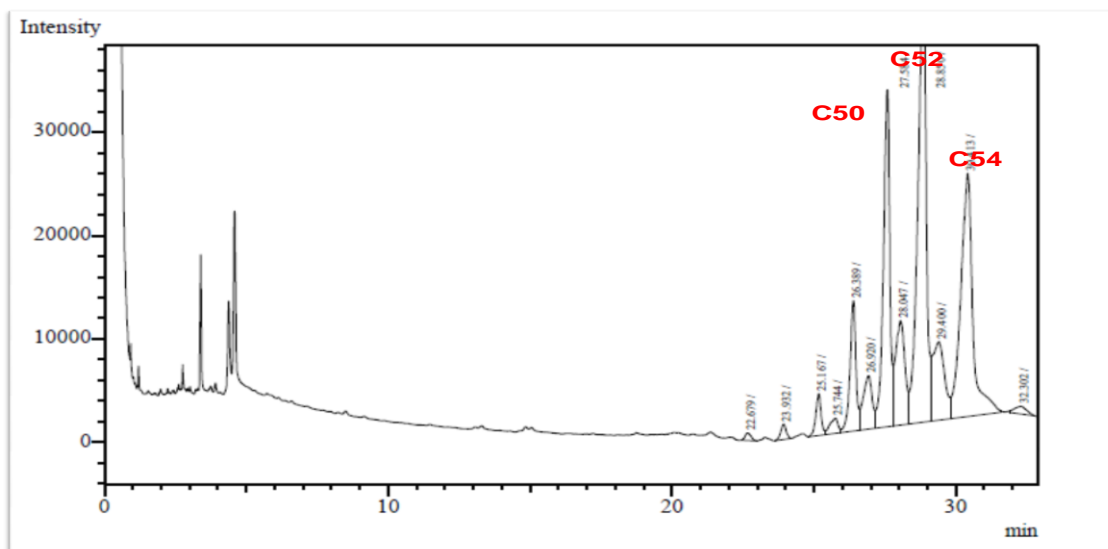


Fig. 4.12 TAGs chromatogram of goat tallow

Results & Discussion

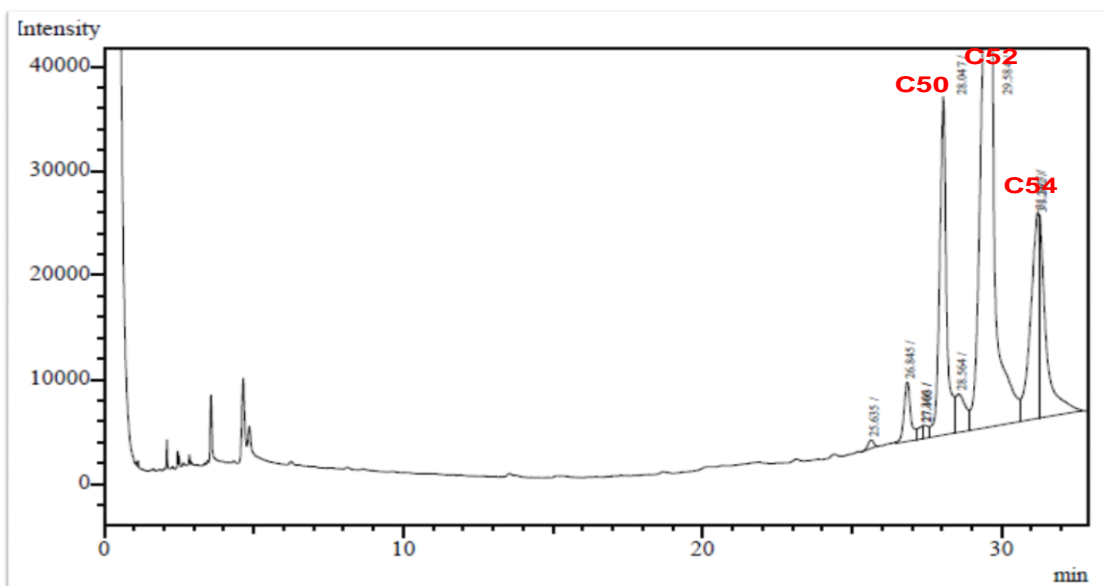


Fig. 4.13 TAGs chromatogram of lard

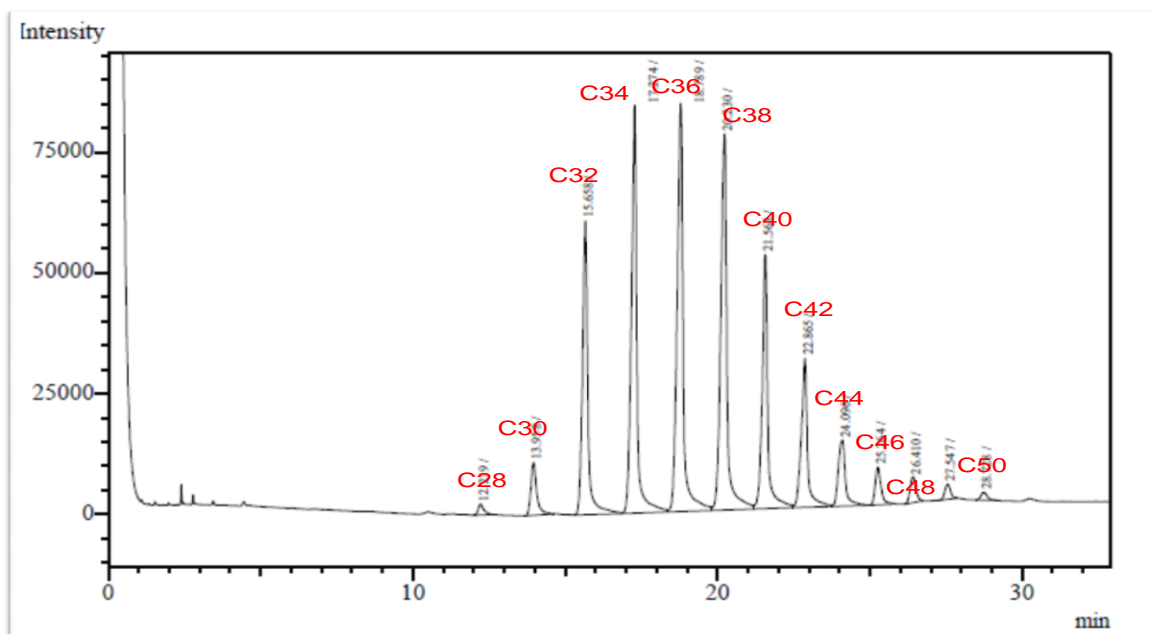


Fig. 4.14 TAGs chromatogram of coconut oil

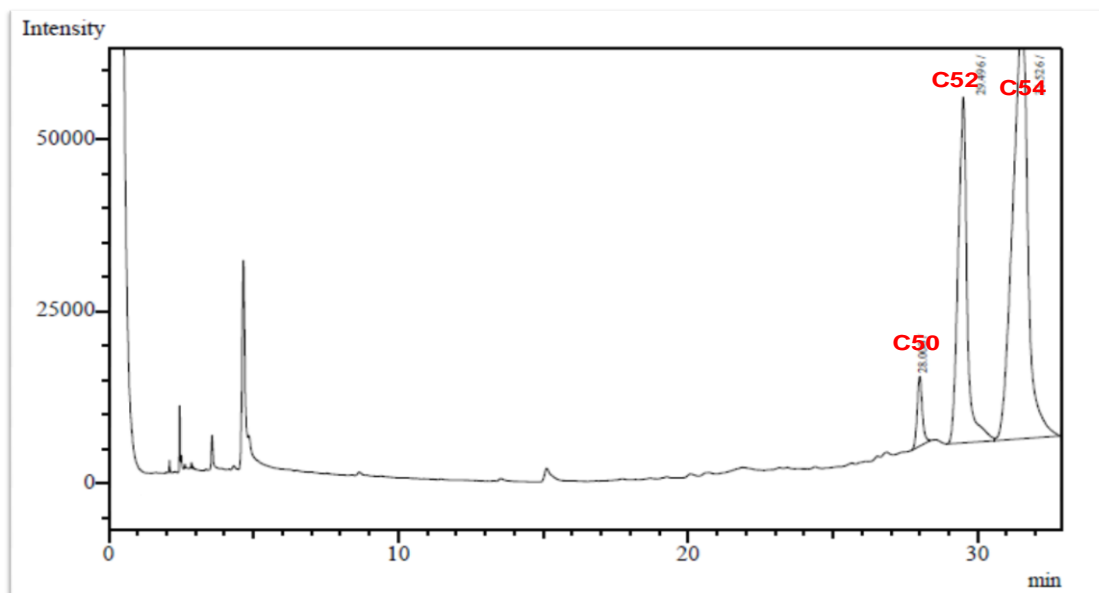


Fig. 4.15 TAGs chromatogram of soybean oil

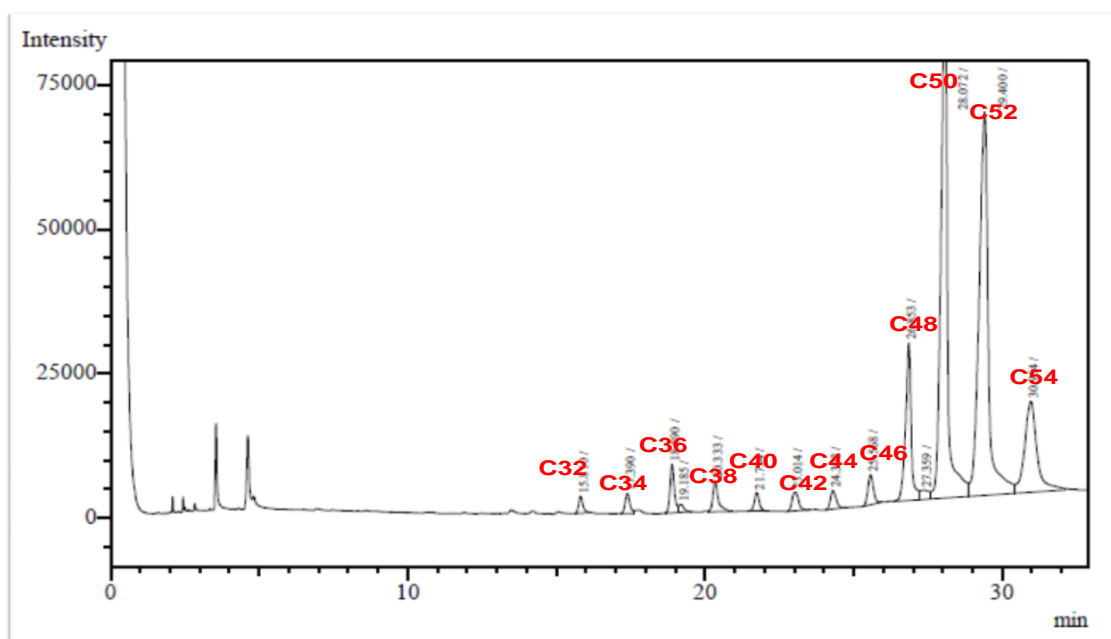


Fig. 4.16 TAGs chromatogram of margarine

4.2.4.3 TAGs profile of cow and buffalo ghee adulterated with lard

From Table 4.19 it was observed that TAG profile of lard had higher weight percentage of C50, C52 and C54 TAG, and TAGs from C24 to C48 were absent. It is clearly visible from the Table 4.20 and Fig. 4.17 and 4.18 that the TAG profile of cow and buffalo ghee adulterated with lard at 5, 10 and 20% level, individually, showed an increase in C50, C52 and C54 percentage. The TAGs profile of ghee spike with lard showed that with increase the level of adulteration, decrease in short and medium chain fatty acid. In lard C52 TAG was the main TAG being identified in present study. Kim *et al.* (2015) also reported that that C52 TAG was the major TAG found in lard. Kala (2013) also reported that addition of lard increases the C50, C52 and C54, weight percent. It was also revealed from Table 4.20 that summation of TAGs C50, C52 and C54 was increasing with increase in the level of adulteration of lard in cow and buffalo ghee. The adulteration of lard in both type of ghee (cow and buffalo) was easily detectable at 5% level. The range of summation value (C50+C52+C54) at 5% level of adulteration was higher than the value noted for control samples (26.31 to 30.1).

4.2.4.4 TAGs profile of cow and buffalo ghee adulterated with goat tallow

In case of goat tallow adulteration in cow and buffalo ghee a trend in TAGs change similar to the lard adulteration in cow and buffalo ghee was observed. Table 4.19 depicted that TAG profile of goat tallow had higher weight percentage of C50, C52 and C54, and TAGs from C24 to C48 were absent. It was clearly visible from the Table 4.21 and Fig. 4.19 and 4.20 that the TAG profile of cow and buffalo ghee adulterated with goat tallow at 5, 10 and 20% level, individually, showed an increase in C50, C52 and C54 weight percentage. Kala (2013) reported that addition of tallow increases the C50, C52 and C54, weight percent. It was also revealed from Table 4.21 that summation of TAGs C50, C52 and C54 was increasing with increase in the level of adulteration of goat tallow in cow and buffalo ghee. The adulteration of goat tallow in both type of ghee (cow and buffalo) was easily detectable at 5% level. The range of

summation value (C50+C52+C54) at 5% level of adulteration was higher than the value observed for control samples (26.31 to 30.1).

4.2.4.5 TAGs profile of cow and buffalo ghee adulterated with soybean oil

It can be seen from Table 4.19 that TAG profile of soybean oil had higher weight percentage of C52 and C54. TAGs having carbon number C24 to C48 were absent. It is evident From Table 4.22 and Fig. 4.21, 4.22 that the TAGs profile of cow and buffalo ghee adulterated with vegetable oil at 5, 10 and 20% level, individually, showed an increase in C52 and C54 weight percentage. The TAGs profile of ghee adulterated with soybean oil showed that with increase in the level of adulteration, a decrease in short and medium chain fatty acid was observed. It can be seen from Table 4.22 that summation of TAGs C50, C52 and C54 was increasing with increase in the level of adulteration in cow and buffalo ghee. The range of summation value (C50+C52+C54) at 5% level of adulteration was higher than the value observed for control samples (26.31 to 30.1). It was also depicted from Table 4.22 that increase of TAGs C52 and C54 were higher in cow ghee adulterated samples than buffalo ghee. Addition of soybean oil in cow and buffalo ghee showed the majority increase in C54. In soybean oil adulteration, the increase in C54 content could be considered as a better marker than the changes in any other TAG (Destailats *et al.*, 2006). Similar work for soybean oil was also reported by Kala (2013). Kuksis and McCarthy (1964) detected the presence of vegetable fat and lard in butterfat at 5 to 10% based on the increase of the TAGs, C52 and C54 content.

4.2.4.6 TAG profile of buffalo ghee adulterated with buffalo tallow

Table 4.19 depicted that TAGs profile of buffalo tallow had higher weight percentage of C50, C52 and C54 than buffalo ghee. TAGs from C24 to C48 were absent. It can be seen from the Table 4.23 and Fig. 4.23 that the TAGs profile of buffalo ghee adulterated with buffalo tallow at 5, 10 and 20% level, individually, showed an increase in C50, C52 and C54, weight percentage. The TAGs profile of ghee adulterated with buffalo tallow showed that with increase the level of adulteration, decrease in short and medium chain TAGs. Kala (2013) also reported that addition of beef tallow in ghee show an increase the C50, C52 and C54, weight percent.

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Table 4.20 TAGs profile of cow and buffalo ghee adulterated with lard

TAG	Buffalo ghee + lard				Cow ghee + lard			
	Buffalo ghee	5%LARD	10%LARD	20%LARD	Cow ghee	5%LARD	10%LARD	20%LARD
C24	0.09±0.01				0.08±0.01			
C26	0.41±0.05				0.45±0.09			
C28	0.55±0.13	0.45±0.04	0.49±0.07		0.49±0.10	0.44±0.14	0.38±0.22	0.59±0.04
C30	0.59±0.07	0.70±0.01	0.70±0.02	0.52±0.02	0.79±0.23	0.62±0.14	0.58±0.18	0.92±0.11
C32	1.28±0.06	1.47±0.06	1.35±0.02	1.11±0.02	1.42±0.15	1.16±0.28	1.08±0.05	1.93±0.45
C34	4.06±0.20	4.28±0.13	4.20±0.06	3.23±0.23	3.68±0.59	3.75±0.70	2.89±0.10	2.52±1.69
C36	10.77±0.97	10.51±0.25	10.39±0.16	8.65±0.71	7.97±0.94	10.04±0.66	6.24±0.03	5.84±1.68
C38	12.83±0.62	11.42±0.38	12.83±0.24	12.87±0.50	11.56±1.02	11.90±0.15	11.75±1.72	9.46±0.30
C40	10.90±0.56	10.40±0.81	9.64±0.03	8.19±0.67	10.24±0.72	10.12±0.63	8.49±1.88	8.85±0.17
C42	3.96±0.02	4.38±0.29	4.20±0.05	3.31±0.32	5.89±0.53	4.36±0.25	6.53±0.66	5.26±0.08
C44	3.35±0.20	2.96±0.20	2.98±0.02	2.29±0.12	5.35±0.20	3.24±0.37	5.91±0.10	3.59±0.21
C46	4.25±0.33	3.92±0.24	3.75±0.18	3.25±0.25	6.29±0.24	3.84±0.26	5.77±0.29	3.74±0.42
C48	7.04±0.47	6.50±0.51	6.21±0.36	6.00±0.31	8.13±0.54	6.37±0.50	7.21±0.87	5.83±0.02
C50	10.99±0.58	11.17±1.54	10.57±0.25	12.33±0.58	11.31±1.13	11.33±1.22	13.80±0.69	13.31±1.46
C52	10.91±0.94	14.75±0.33	15.28±0.38	20.76±1.42	12.59±1.01	15.39±0.40	18.00±0.84	26.08±2.64
C54	4.87±0.36	6.54±0.04	6.62±0.22	8.10±0.59	6.20±0.88	7.25±0.68	8.55±0.29	12.06±1.82
C50+C52+C54	26.77	32.46	32.47	41.19	30.1	33.97	40.35	51.45

Data is presented as Mean±SD (n=3)

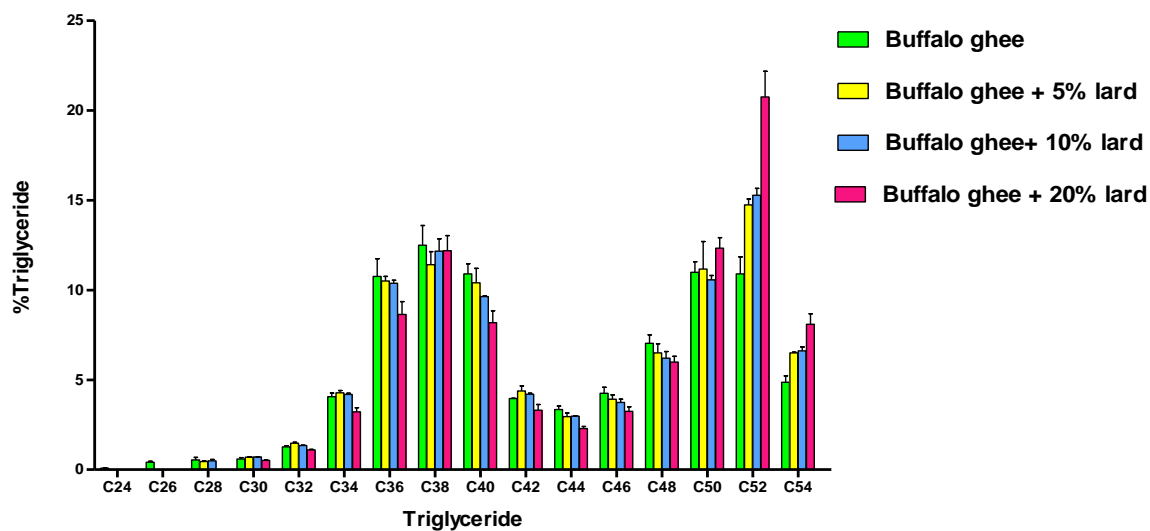


Fig. 4.17 TAGs profile of buffalo ghee adulterated with lard

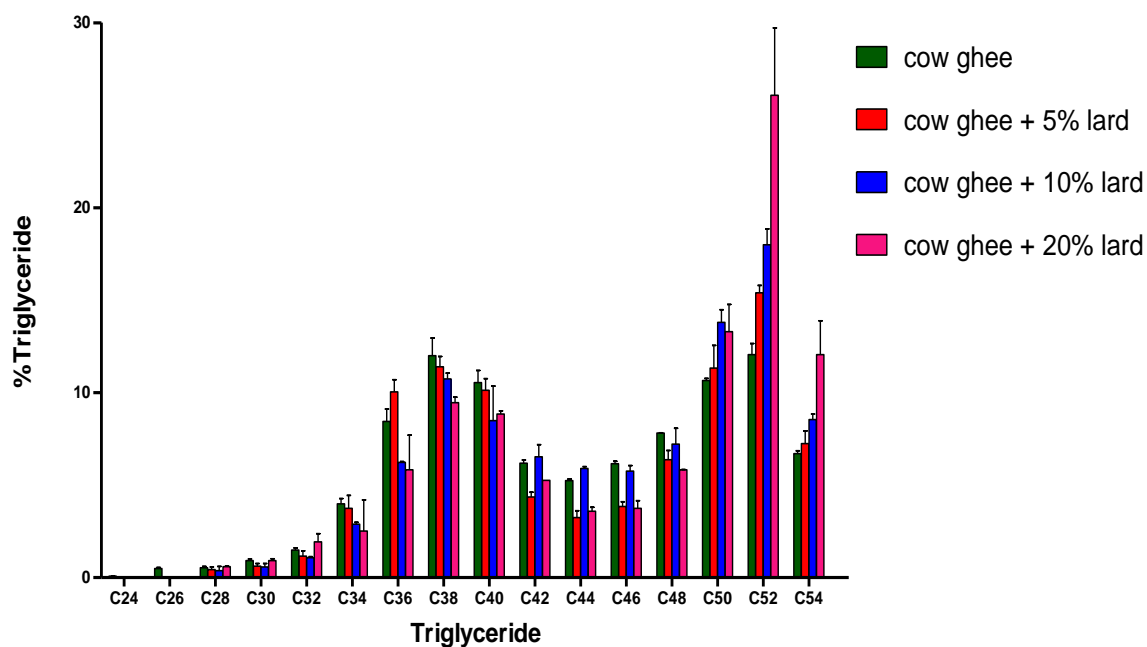


Fig. 4.18 TAGs profile of cow ghee adulterated with lard

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Table 4.21 TAGs profile of cow and buffalo ghee adulterated with goat tallow

TAG	Buffalo ghee + Goat tallow				Cow ghee + Goat tallow			
	Buffalo ghee	5% goat tallow	10% goat tallow	20% goat tallow	Cow ghee	5% goat tallow	10% goat tallow	20% goat tallow
C24	0.09±0.01				0.08±0.01			
C26	0.41±0.05				0.45±0.09			
C28	0.55±0.13	0.40±0.02	0.35±0.03	0.37±0.06	0.49±0.10	0.27±0.16	0.34±0.03	0.20±0.02
C30	0.59±0.07	0.58±0.01	0.53±0.01	0.63±0.16	0.79±0.23	0.44±0.21	0.64±0.17	0.48±0.07
C32	1.28±0.06	1.16±0.01	1.13±0.06	1.20±0.03	1.42±0.15	0.96±0.30	1.36±0.23	0.83±0.31
C34	4.06±0.20	3.82±0.05	3.61±0.18	3.77±0.31	3.68±0.59	3.29±0.82	3.13±0.97	1.75±0.60
C36	10.77±0.97	10.17±0.48	9.55±0.21	9.53±0.16	7.97±0.94	8.70±1.79	8.07±1.75	5.47±1.34
C38	12.83±0.62	12.05±1.56	11.28±0.63	10.52±1.15	11.56±1.02	11.74±0.73	10.05±0.84	9.62±0.52
C40	10.90±0.56	10.14±1.38	9.98±0.84	8.46±1.64	10.24±0.72	9.70±0.44	9.28±0.38	9.11±0.20
C42	3.96±0.02	3.90±0.78	4.26±0.20	3.77±0.13	5.89±0.53	4.11±0.90	4.33±0.42	5.52±0.37
C44	3.35±0.20	3.15±0.10	3.26±0.27	3.09±0.64	5.35±0.20	3.28±0.23	3.19±0.33	3.91±0.66
C46	4.25±0.33	3.93±0.41	3.87±0.12	3.62±0.25	6.29±0.24	3.93±0.38	4.23±0.32	4.13±0.97
C48	7.04±0.47	6.74±0.78	6.48±0.44	6.36±0.44	8.13±0.54	6.79±0.65	6.49±0.71	5.86±0.01
C50	10.99±0.58	12.14±2.97	10.93±0.35	15.20±0.72	11.31±1.13	12.80±0.90	11.90±0.81	14.03±0.45
C52	10.91±0.94	12.75±0.16	16.37±0.33	17.29±1.5	12.59±1.01	19.15±0.86	19.23±0.34	26.22±3.44
C54	4.87±0.36	6.70±0.49	7.80±0.50	7.89±0.19	6.20±0.88	10.48±0.64	11.47±0.28	12.10±1.76
C50+C52+C54	26.77	31.59	35.1	40.38	30.1	42.43	42.6	52.35

Data is presented as Mean±SD (n=3)

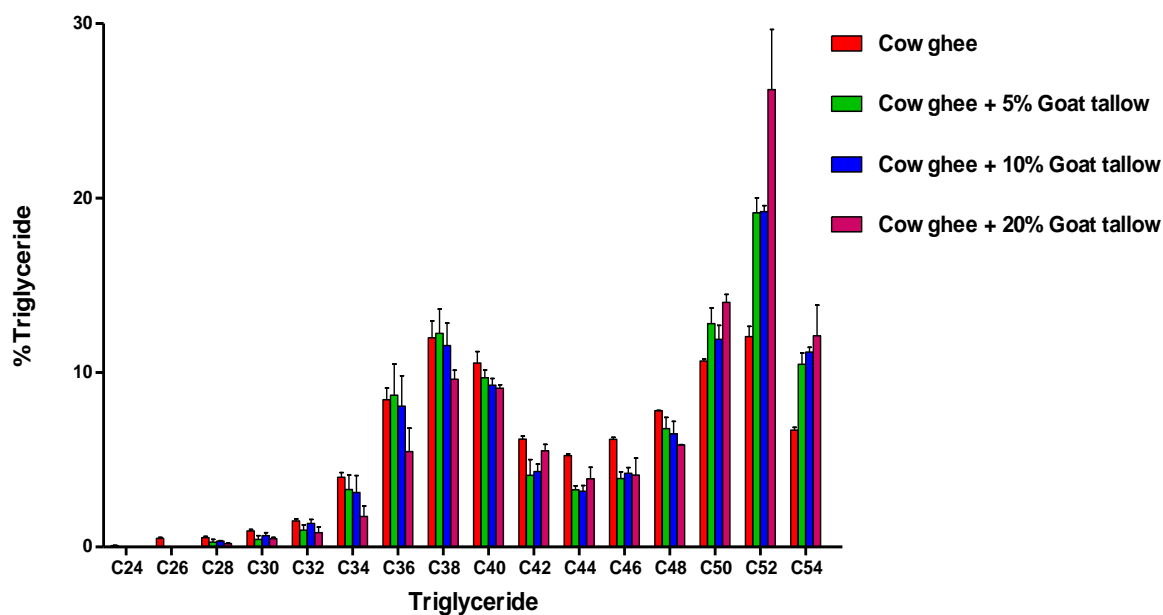


Fig. 4.19 TAGs profile of cow ghee adulterated with goat tallow

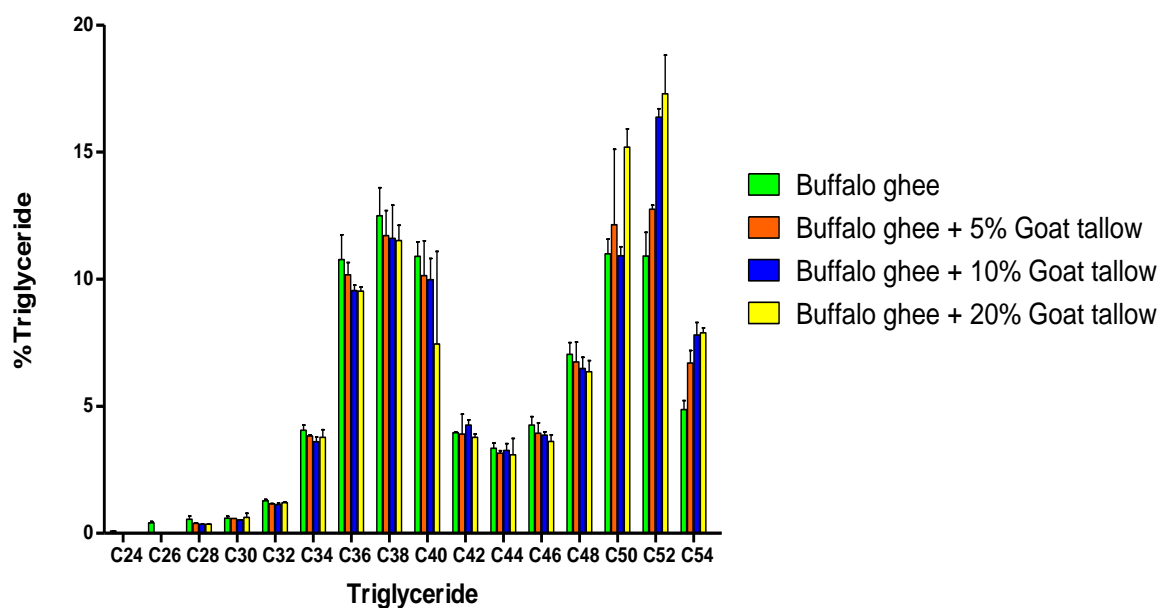


Fig. 4.20 TAGs profile of buffalo ghee adulterated with goat tallow

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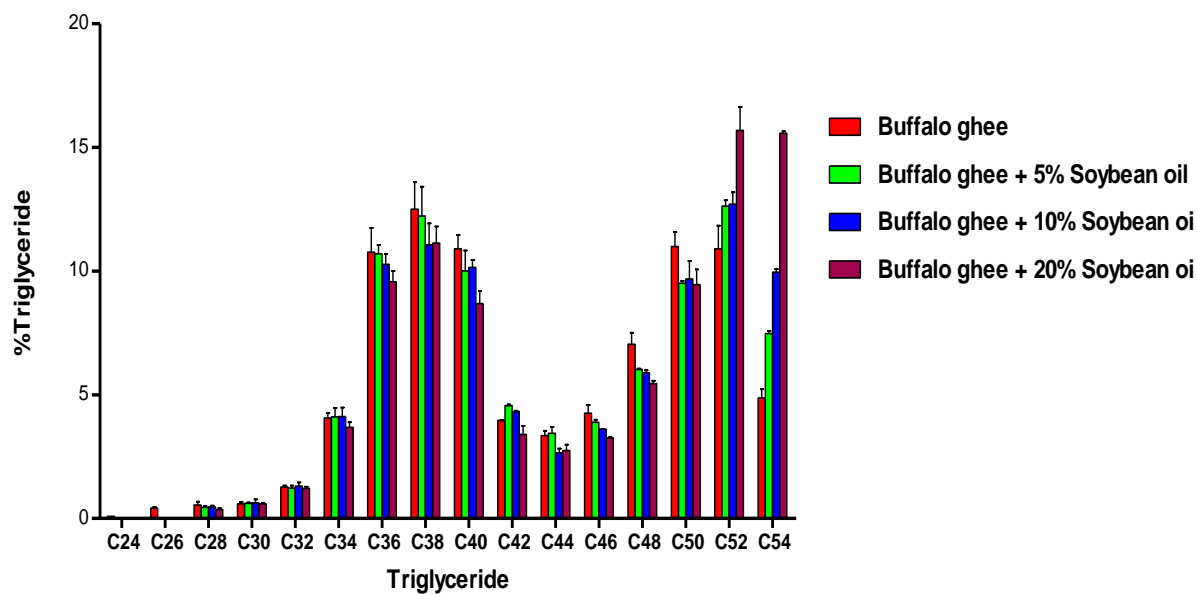


Fig. 4.21 TAGs profile of buffalo ghee adulterated with soybean oil

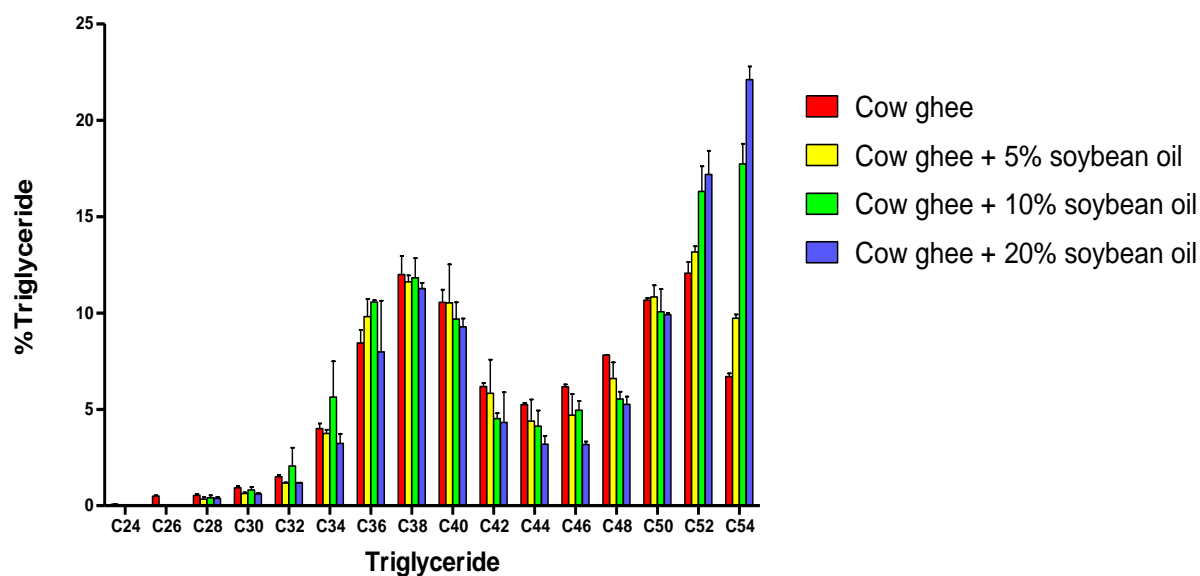


Fig. 4.22 TAGs profile of cow ghee adulterated with soybean oil

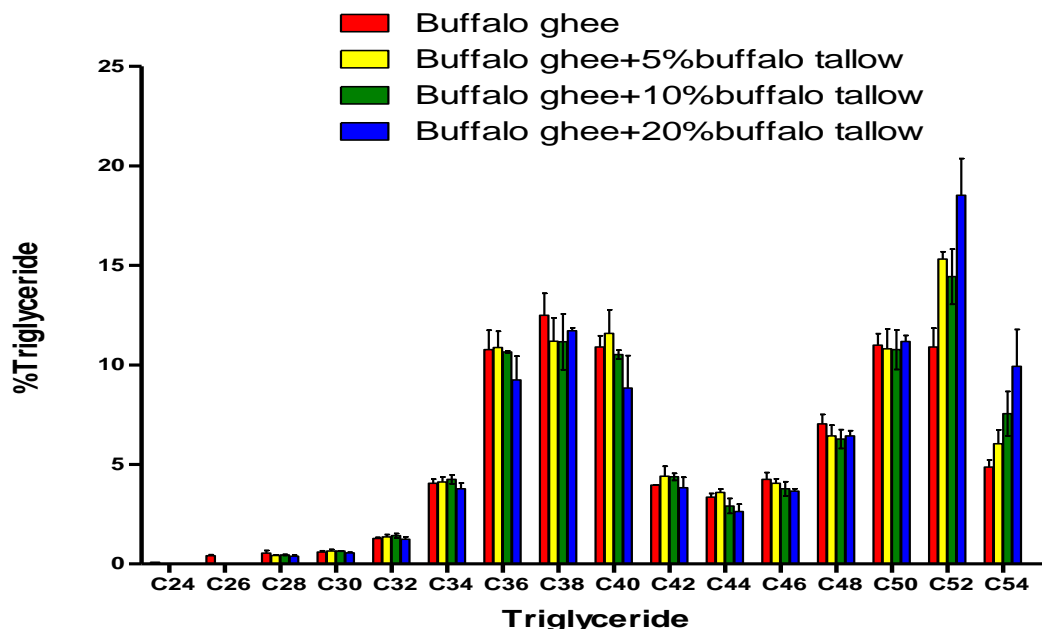


Fig. 4.23 TAGs profile of buffalo ghee adulterated with buffalo tallow

It was also revealed from Table 4.23 that summation of TAGs C50, C52 and C54 was increasing with increase the level of adulteration in buffalo ghee. The adulteration of buffalo tallow in buffalo ghee was easily detectable at 5% level. While Kala (2013) reported beef tallow at 6.56% level showed an increase in C50, C52 and C54, weight percentage. The range of summation value (C50+C52+C54) at 5% level of adulteration was higher than the value observed for control samples (26.31 to 30.1). Similarly, Guyot (1978) detected the adulteration of beef tallow and lard in butter on the basis of TAGs C50 and C52.

4.2.4.7 TAGs profile of buffalo ghee adulterated with coconut oil and mixture of coconut oil and lard.

From Table 4.19 it was observed that TAGs profile of coconut oil had C34, C36 and C38, weight percentage higher as compared to other milk fat TAGs. TAGs profile of coconut oil indicated absence of C24, C26, C52 and C54. It can be seen from the Table 4.24 and Fig. 4.24 that the TAGs profile of buffalo ghee adulterated with coconut oil at 5, 10 and 20% level, individually, showed an increase in C34, C36 and C38, weight percentage, While TAGs C50, C52 and C54 were found unaffected from coconut oil adulteration in ghee. It was also observed from Table 4.24 that when mixture of lard and coconut oil was used as adulterant in ghee, a slight increase was observed in C34, C36 and C38, while a significant increase was observed in C52 with increase in the level of adulteration. Hence, C34, C36 and C38 could be used as marker TAGs for detection of coconut oil in ghee.

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Table 4.22 TAGs profile of cow and buffalo ghee adulterated with soybean oil

TAG	Buffalo ghee + soybean oil				Cow ghee + soybean oil			
	Buffalo ghee	5% soybean oil	10% soybean oil	20% soybean oil	Cow ghee	5% soybean oil	10% soybean oil	20% soybean oil
C24	0.09±0.01				0.08±0.01			
C26	0.41±0.05				0.45±0.09			
C28	0.55±0.13	0.45±0.05	0.47±0.05	0.36±0.06	0.49±0.10	0.34±0.12	0.41±0.12	0.38±0.07
C30	0.59±0.07	0.61±0.03	0.64±0.13	0.59±0.05	0.79±0.23	0.63±0.06	0.82±0.15	0.60±0.06
C32	1.28±0.06	1.22±0.11	1.31±0.14	1.22±0.07	1.42±0.15	1.18±0.03	2.06±0.95	1.19±0.04
C34	4.06±0.20	4.11±0.36	4.12±0.36	3.68±0.21	3.68±0.59	3.76±0.19	5.63±1.86	3.24±0.48
C36	10.77±0.97	10.69±0.38	10.28±0.41	9.57±0.43	7.97±0.94	9.82±0.92	10.56±0.11	7.98±1.66
C38	12.83±0.62	11.89±0.04	11.73±0.71	10.46±0.10	11.56±1.02	11.62±1.74	11.82±1.81	10.27±1.71
C40	10.90±0.56	10.01±0.82	10.15±0.30	8.68±0.51	10.24±0.72	10.52±1.99	9.69±0.86	9.29±0.43
C42	3.96±0.02	4.57±0.04	4.32±0.04	3.40±0.34	5.89±0.53	5.83±1.76	4.52±0.27	4.32±1.56
C44	3.35±0.20	3.45±0.26	2.66±0.17	2.75±0.24	5.35±0.20	4.39±1.12	4.12±0.81	3.20±0.42
C46	4.25±0.33	3.89±0.10	3.61±0.01	3.26±0.02	6.29±0.24	4.70±1.08	4.95±0.48	3.18±0.16
C48	7.04±0.47	6.03±0.04	5.88±0.11	5.46±0.11	8.13±0.54	6.59±0.84	5.54±0.37	5.26±0.40
C50	10.99±0.58	9.51±0.09	9.68±0.73	9.45±0.62	11.31±1.13	10.83±0.60	10.06±1.17	9.91±0.09
C52	10.91±0.94	12.63±0.24	12.70±0.49	15.69±0.95	12.59±1.01	13.17±0.28	16.31±1.29	17.18±1.23
C54	4.87±0.36	7.47±0.10	9.95±0.13	15.57±0.08	6.20±0.88	9.73±0.19	17.74±1.05	22.11±0.68
C50+C52+C54	26.77	29.61	32.33	40.71	30.1	33.73	44.11	49.20

Data is presented as Mean±SD (n=3)

Summation of TAGs (C34+C36+C38) could be used as a marker to differentiate pure ghee from coconut oil adulterated ghee samples. It was observed from Table 4.24 that the summation value at 5% level of adulteration was significantly higher than control ghee sample. Kala (2013) also concluded that coconut oil had C34, C36 and C38, weight percentage higher as compared to other milk fat TAGs.

4.2.4.8 TAGs profile of fat extracted from buffalo paneer adulterated with soybean oil

It can be evident from Table 4.19 that TAGs profile of soybean oil had higher weight percentage of C52 and C54 carbon number, and containing TAGs from C24 to C48 were absent. From Table 4.25 and Fig. 4.26 it can be seen that the TAG profile of buffalo paneer fat adulterated with vegetable oil at 5, 10 and 20% level, individually, showed an increase in C52 and C54 weight percentage as was the case in buffalo ghee adulterated with soybean oil. It can be seen from Table 4.25 that summation of TAGs C50, C52 and C54 showed an increase with the increase in the level of adulteration in buffalo paneer fat. The range of summation value (C50+C52+C54) at 5% level of adulteration was higher than the value recorded for control samples (26.31 to 30.1). Addition of soybean oil in buffalo paneer fat showed the main increase in C54, like soybean oil adulteration in buffalo ghee (Table 4.22).

In soybean oil adulteration, the increase in C54 height and area could be considered as a better marker than the changes in any other TAG (Destailats *et al.*, 2006). Similar observations were reported for soybean oil adulteration in ghee by Kala (2013). Using GLC Kuksis and McCarthy (1964) detected the presence of vegetable fat and lard in butterfat at 5 to 10% based on the increase of the TAGs, C52 and C54 content. Hence, it was concluded from the results of present study that it was possible to detect adulteration of paneer fat using TAGs profiling approach.

4.2.4.9 TAG profile of buffalo butter adulterated with margarine

From table 4.19 it can be seen that TAGs from C24 to C30 were absent in margarine and concentration of C32 to C46 was less as compared to milk fat. It was also depicted that TAGs profile of margarine had higher relative weight percentage of C50, C52 and C54 TAGs. TAGs profile of buffalo butter adulterated with vegetable oil at 5, 10 and 20% level, individually, indicated an increase in C50 and C52 weight percent

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TAG	Buffalo ghee + buffalo tallow			
	Buffalo ghee	5% buffalo tallow	10% buffalo tallow	20% buffalo tallow
C24	0.09±0.01			
C26	0.41±0.05			
C28	0.55±0.13	0.43±0.03	0.45±0.03	0.38±0.06
C30	0.59±0.07	0.65±0.09	0.65±0.07	0.56±0.02
C32	1.28±0.06	1.36±0.12	1.42±0.11	1.24±0.12
C34	4.06±0.20	4.12±0.26	4.25±0.22	3.78±0.28
C36	10.77±0.97	10.87±0.82	10.66±0.04	9.25±1.19
C38	12.83±0.62	14.53±0.76	15.83±0.21	13.05±1.70
C40	10.90±0.56	11.59±1.18	10.52±0.22	8.84±1.63
C42	3.96±0.02	4.41±0.50	4.38±0.18	3.83±0.52
C44	3.35±0.20	3.59±0.17	2.92±0.37	2.63±0.37
C46	4.25±0.33	4.05±0.22	3.78±0.36	3.65±0.12
C48	7.04±0.47	6.43±0.55	6.27±0.47	6.43±0.26
C50	10.99±0.58	10.81±1.00	10.77±0.99	11.18±0.30
C52	10.91±0.94	15.32±0.36	14.43±1.38	18.53±1.84
C54	4.87±0.36	6.04±0.69	7.56±1.12	9.93±1.84
C50+C52+C54	26.77	32.17	32.76	39.64

Data is presented as Mean±SD (n=3)

Table 4.24 TAGs profile of buffalo ghee adulterated with coconut oil and mixture of coconut oil and lard.

TAG	Buffalo ghee + coconut oil				Buffalo ghee + coconut oil + lard		
	Buffalo ghee	5% coconut oil	10% coconut oil	20% coconut oil	5% Coconut oil + Lard	10% Coconut oil + Lard	20% Coconut oil + Lard
C24	0.09±0.01						
C26	0.41±0.05						
C28	0.55±0.13	0.43±0.05	0.42±0.05	0.59±0.15	0.29±0.06	0.37±0.06	0.33±0.07
C30	0.59±0.07	0.77±0.07	0.55±0.01	0.61±0.18	0.56±0.04	0.65±0.20	0.56±0.03
C32	1.28±0.06	1.19±0.08	1.19±0.08	1.37±0.28	1.06±0.26	1.11±0.06	1.75±0.71
C34	4.06±0.20	4.51±0.67	3.68±0.01	4.15±0.86	3.45±0.22	3.25±0.31	2.99±0.32
C36	10.77±0.97	11.37±0.38	12.34±0.07	12.21±1.50	9.64±0.67	10.11±0.68	10.41±0.60
C38	12.83±0.62	17.25±0.43	18.13±0.57	19.34±0.75	15.43±0.87	15.06±0.29	15.86±1.08
C40	10.90±0.56	10.92±0.68	10.77±1.42	11.41±0.07	10.25±0.84	10.06±0.87	9.49±0.64
C42	3.96±0.02	4.88±0.30	4.97±0.24	5.03±0.30	4.61±1.28	4.82±0.78	4.51±0.12
C44	3.35±0.20	3.39±0.42	3.28±0.12	3.59±0.06	3.44±1.21	3.52±0.68	3.49±0.46
C46	4.25±0.33	4.01±0.26	3.95±0.18	4.01±0.05	4.22±0.66	4.06±0.63	3.76±0.57
C48	7.04±0.47	6.33±0.77	6.15±0.38	6.10±0.12	6.67±0.11	6.18±0.29	5.68±0.47
C50	10.99±0.58	9.39±0.71	9.49±0.11	9.29±0.69	10.74±0.76	10.09±0.65	9.68±0.33
C52	10.91±0.94	10.12±0.67	10.42±0.41	9.49±0.57	14.30±1.89	13.49±2.23	15.08±1.72
C54	4.87±0.36	4.82±0.07	4.57±0.44	4.68±0.80	6.60±0.51	6.53±0.85	6.57±0.57
C34+C36+C38	27.66	33.13	34.15	35.7	28.52	28.42	29.27
C50+C52+C54	26.77	24.33	24.48	23.46	31.64	30.11	31.33

Data is presented as Mean±SD (n=3)

Results & Discussion

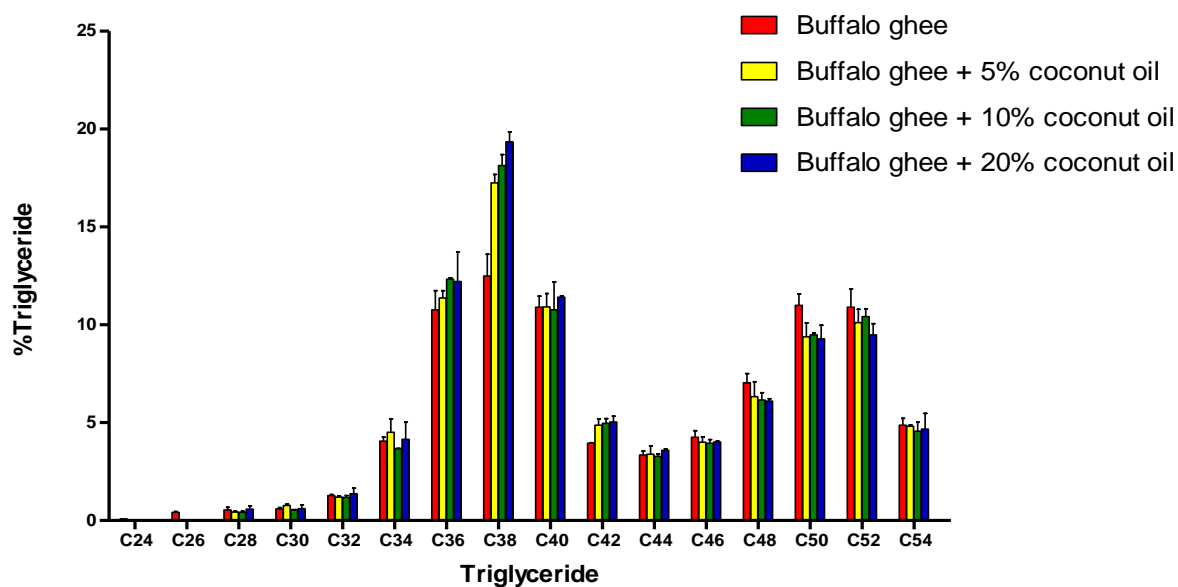


Fig. 4.24 TAGs profile of buffalo ghee adulterated with coconut oil

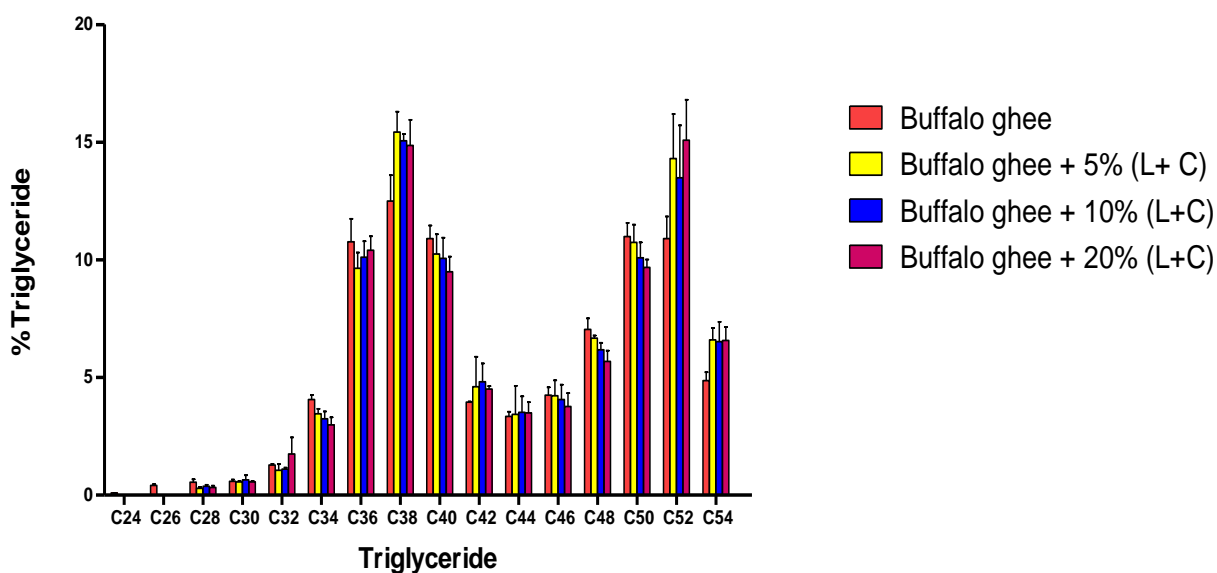


Fig. 4.25 TAGs profile of buffalo ghee adulterated with mixture of coconut oil and lard

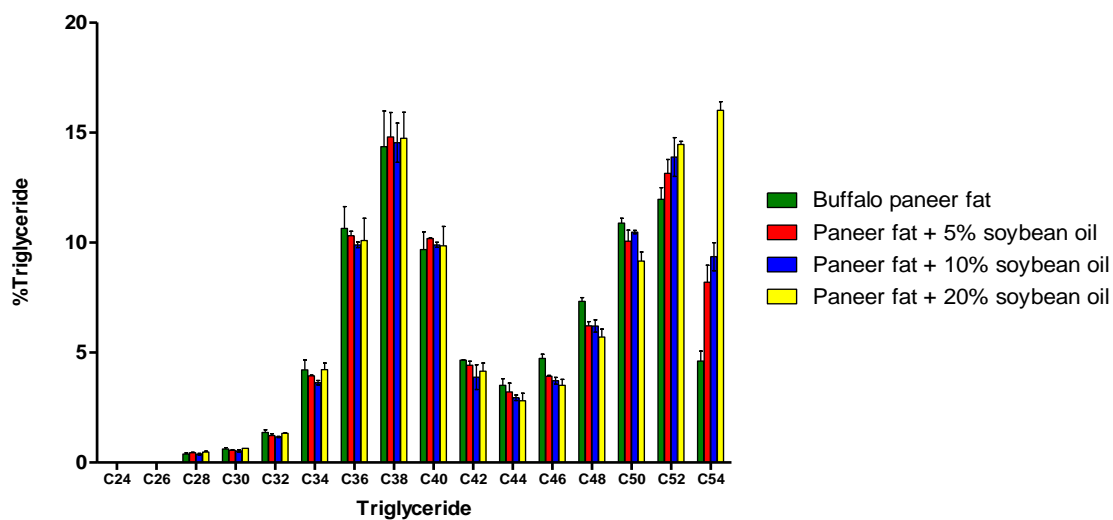


Fig. 4.26 TAGs profile of buffalo paneer fat adulterated with soybean oil

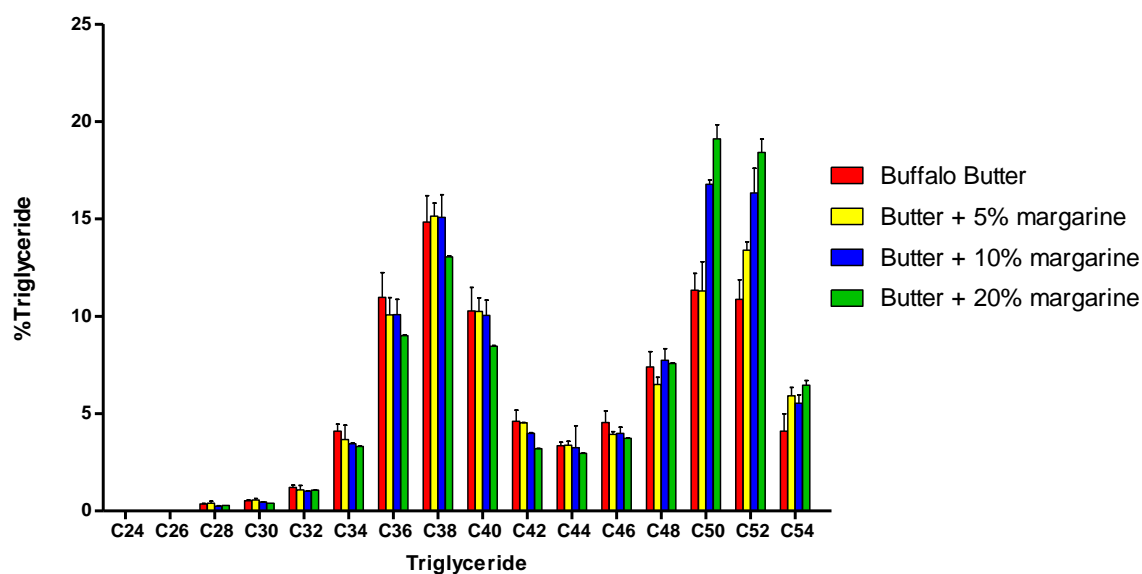


Fig. 4.27 TAGs profile of buffalo butter adulterated with margarine

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(Table 4.26 and Fig. 4.27). It can be seen from Table 4.26 that summation of TAGs C50, C52 and C54 showed an increase in the level of adulteration in buffalo butter. The range of summation value (C50+C52+C54) at 5% level of adulteration was higher than the value observed for control samples (26.31 to 30.1). Marjanovic *et al.* (1984) reported that adulteration of milk fat with margarine at 5 to 10% level could be detected on the basis that margarine had more triacylglycerol content from C48 to C54 than milk fat. Similarly, Luf *et al.* (1987) also concluded that it was possible to detect the adulteration of vegetable fat in butter based on C50, C52 and C54 TAGs.

4.2.4.10 TAGs profile of market samples of ghee from organized sector (OS) and unorganized sector (UOS).

The possibility of use of TAGs profiling in fat isolated from soxhlet method in paneer and by heat clarification in ghee and butter to detect the added foreign fats was established in previous section. The method was applied to check the quality of milk fat in market samples of ghee, paneer and butter. The results have been depicted in the section. From Tables 4.27 and 4.28, it can be seen that majority of ghee samples i.e. ~54.5%, from organized sector showed increase in C50, C52 and C54 weight percent. Summation value of TAGs (C50, C52 and C54) in six samples was found to contain higher value than the range observed for control milk fat (26.31 to 30.1). The relative TAGs weight percent of C50, C52, and C54 of market samples OS-1, OS-4, OS-8, OS-9, OS-10 and OS-11 were given in the Tables 4.27 and 4.28. The presence of bovine tallow and lard increased the relative TAG content from C50 to C54 and decreased the TAG content from C32 to C40 (Gutierrez *et al.*, 2009). Market sample OS-11 showed the absence of TAG from C24 to C46 and higher increase in TAG composition of C50 and C52. It was observed from Table 4.19 that among all the adulterant only lard showed the high TAG content of C52 (57.1%) and other adulterants showed the weight percent of C52 less than 38% in pure form. TAG weight percent of C52 in market sample OS-11 was found closer to lard. So, from above study it was concluded that Market sample OS-11 could be adulterated with concoction of vegetable fat and lard. The analysis of market samples of ghee from unorganized sector (UOS) are depicted in Tables 4.29 and 4.30.

Table 4.25 TAGs profile of fat extracted from buffalo paneer adulterated with soybean oil

TAG	Buffalo paneer + soybean oil			
	Paneer	5% soybean oil	10% soybean oil	20% soybean oil
C24				
C26				
C28	0.43±0.08	0.45±0.03	0.38±0.04	0.47±0.06
C30	0.69±0.15	0.55±0.05	0.52±0.05	0.64±0.02
C32	1.41±0.13	1.23±0.10	1.16±0.02	1.32±0.04
C34	4.19±0.45	3.94±0.05	3.63±0.10	4.22±0.43
C36	10.35±1.11	10.31±0.28	9.89±0.12	10.09±1.43
C38	14.31±1.62	14.79±1.58	14.54±0.89	14.73±1.67
C40	10.19±1.19	10.18±0.02	9.89±0.12	9.85±1.24
C42	5.12±0.81	4.42±0.25	3.88±0.56	4.15±0.52
C44	3.94±0.81	3.21±0.56	2.95±0.13	2.81±0.48
C46	4.93±0.39	3.92±0.05	3.72±0.16	3.51±0.38
C48	7.21±0.26	6.21±0.27	6.20±0.28	5.70±0.52
C50	10.43±0.81	10.06±0.72	10.48±0.07	9.15±0.59
C52	11.48±0.99	13.15±0.89	13.89±0.88	14.45±0.21
C54	4.65±0.46	8.20±1.10	9.36±0.64	16.01±0.55
C50+C52+C54	26.56	31.41	33.73	39.61

Data is presented as Mean±SD (n=3)

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TAG	Butter + margarine			
	Butter	5% margarine	10% margarine	20% margarine
C24				
C26				
C28	0.36±0.03	0.40±0.1	0.25±0.02	0.29±0.03
C30	0.53±0.04	0.56±0.07	0.46±0.01	0.40±0.01
C32	1.21±0.12	1.09±0.23	1.03±0.01	1.07±0.01
C34	4.10±0.36	3.67±0.74	3.46±0.03	3.32±0.02
C36	10.97±1.28	10.07±0.90	10.08±0.78	9.02±0.05
C38	14.18±1.40	15.14±0.67	15.08±1.17	13.06±0.07
C40	10.28±1.20	10.25±0.69	10.05±0.78	8.47±0.05
C42	4.61±0.57	4.53±0.01	3.98±0.04	3.21±0.02
C44	3.36±0.17	3.38±0.20	3.25±1.14	2.96±0.02
C46	4.55±0.59	3.94±0.14	3.99±0.31	3.74±0.02
C48	7.40±0.78	6.50±0.37	7.73±0.60	7.59±0.04
C50	11.34±0.87	11.30±1.50	16.78±0.23	18.91±0.88
C52	10.87±1.0	13.40±±0.43	16.34±1.27	18.21±0.85
C54	4.10±0.88	5.92±0.42	5.53±0.43	6.39±0.30
C50+C52+C54	26.31	30.62	38.65	43.51

Data is presented as Mean±SD (n=3)

Data clearly showed that majority of ghee samples i.e. ~66.6%, from unorganized sector showed increase in C50, C52 and C54 weight percentage. Summation value of TAGs (C50, C52 and C54) in eight samples was found higher than the range recorded for control milk fat (26.31 to 30.1) samples. Market samples (UOS-4, 11) from unorganized sector showed higher summation value of TAGs (C50, C52 and C54) than control milk fat. The relative TAGs weight percentage of C50, C52 and C54 of sample number 4 from UOS was 24.45%, 39.44%, and 31.89%, whereas that of sample number 11 from UOS was 26%, 36.92% and 29.23%, respectively. It was concluded from the study of control samples and several reports of researchers that sample number 4 and 11 from unorganized sector might be adulterated with mixture of body fat vegetable oils/fats. Kala (2013) reported the market survey of ghee based on triacylglycerol profile and standardised (S) values calculation revealed up to 43% branded ghee samples and 87.5% samples of unbranded ghee did not conform to triacylglycerol profiles of control ghee. Kala (2012) reported that among the khoa samples analysed, 57% showed fatty acid composition characteristic of milk fat. The GC profiles of 43% khoa samples showed the composition of fatty acids not conforming to milk fats. The khoa samples adulterated with non milk fats were further confirmed by cholesterol and triacylglycerol estimation. Kala *et al.* (2016) studied that the fat content of ghee based sweets accounts to nearly 50% of the edible portion through TAGs profiling using GC.

4.2.4.11 TAGs profile of market samples of butter from organized sector (OS) and unorganized sector (UOS).

It can be seen from Table 4.31 that three samples (60%) out of five samples from organized sector showed increase in C50, C52 and C54, weight percentage. The summation value of C50, C52 and C54 was also fit well in adulterated butter sample. Kala (2013) reported that the TAGs profile of beef tallow, lard and vegetable fat had higher relative weight percentage of C48, C50, C52 and C54 TAGs. Sample number 2 and 3 of butter from organized sector showed increase in C54, it might be possible to adulterate with vegetable oil. From Tables 4.32 and 4.33, data clearly showed that majority of butter samples i.e. ~43.75%, from unorganized sector showed increase in C50, C52 and C54. Summation value of TAGs (C50, C52 and C54) in seven samples was found to contain higher value than the range observed for control milk fat (26.31 to 30.1).

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Samples number 2 and 10 of butter from unorganized sector showed more increase in C50 and C52 and little increase in C54, from this it was concluded that these two samples were adulterated with concoction of vegetable oil/fat and lard.

While remaining five adulterated butter samples (7, 9, 11, 12 and 14) showed higher percentage of C54 TAG, from this it was clear that these samples were adulterated with vegetable oil. Luf *et al.* (1987) concluded that it was possible to detect the adulteration of vegetable fat in butter based on C50, C52 and C54. Using GLC Kuksis and McCarthy (1964) detected the presence of vegetable fat and lard in butterfat at 5 to 10% based on the increase of the TAGs, C52 and C54 content. The summation value of C50, C52 and C54 was also fit well in adulterated butter samples from unorganized sector. Summation value could be used as a potent marker to detect the authenticity of milk fat samples.

4.2.4.12 TAGs profile of market samples of paneer from organized sector (OS) and unorganized sector (UOS).

From Table 4.34, it can be observed that two samples (28.5%) out of seven samples of paneer from organized sector showed increase in C52 and C54. Both the adulterated samples indicated higher increase in C54, hence paneer samples number 1 and 7 were found adulterated with vegetable oil. Summation value of TAGs (C50, C52 and C54) was also fit well in case of adulterated paneer fat sample. Similarly TAG profile of seven samples (43.75%) out of sixteen samples of paneer indicated higher percentage of C52 and C54. It was concluded from Table 4.35 and 4.36 that TAGs profile of all the adulterated samples showed higher weight percentage of C54. In vegetable oil adulteration, the increase in C54 height and area could be considered as a better marker than the changes in any other TAG (Destailats *et al.*, 2006). Kala (2013) reported that addition of beef tallow and lard increases the C50, C52 and C54, weight percent; the vegetable oil addition also increases the same TG compositions, with a majority rise in C54. TAG profile of market sample number 4 from unorganized sector showed high level of adulteration of vegetable oil in paneer fat. Hence, it was concluded from the above research that majority of paneer samples were found adulterated with vegetable oil/fat.

Table 4.27 TAGs profile of market samples of ghee from organized sector (OS)

Triacylglycerols (TAGs)	Samples					
	OS-1	OS-2	OS-3	OS-4	OS-5	OS-6
C24			0.08±0.01			
C26			0.5±0.05			
C28	0.34±0.02	0.37±0.02	0.54±0.08	0.27±0.02	0.31±0.01	0.27±0.01
C30	0.42±0.04	0.54±0.02	0.92±0.09	0.38±0.01	0.54±0.02	0.52±0.03
C32	1.06±0.04	1.31±0.15	1.50±0.11	1.13±0.23	1.15±0.10	1.22±0.15
C34	3.28±0.13	3.93±0.23	4.00±0.27	3.10±0.14	4.02±0.22	3.62±0.19
C36	8.54±0.35	10.70±0.64	8.44±0.67	9.17±0.42	10.54±0.58	9.59±0.49
C38	10.74±0.44	15.73±0.94	11.99±0.97	14.49±0.66	13.22±0.73	12.46±0.64
C40	8.39±0.34	10.69±0.64	10.55±0.66	9.97±0.46	10.40±0.57	10.19±0.52
C42	4.14±0.17	4.90±0.29	6.19±0.17	4.12±0.19	5.32±0.29	5.67±0.29
C44	3.25±0.13	3.76±0.22	5.24±0.09	3.06±0.14	4.29±0.24	4.87±0.25
C46	4.30±0.18	4.85±0.29	6.16±0.13	4.42±0.20	5.29±0.29	5.68±0.29
C48	7.81±0.32	7.57±0.45	7.82±0.01	7.57±0.35	7.66±0.42	7.82±0.40
C50	14.40±0.59	11.56±0.69	10.66±0.11	13.42±0.61	10.81±0.60	11.57±0.59
C52	16.65±0.68	10.95±0.77	11.56±1.30	15.71±0.72	10.29±0.57	12.51±0.64
C54	8.06±0.33	4.88±0.29	4.70±1.26	6.85±0.31	5.08±0.28	4.61±0.24
C50+C52+C54	39.11	27.39	26.92	35.98	26.18	28.69

Data is presented as Mean±SD (n=3)

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Table 4.28 TAG profiles of market samples of ghee from organized sector (OS)

Triacylglycerols (TAGs)	Samples				
	OS-7	OS-8	OS-9	OS-10	OS-11
C24					
C26					
C28	0.24±0.03	0.27±0.02	0.35±0.01	0.22±0.02	
C30	0.44±0.02	0.37±0.03	0.47±0.01	0.31±0.03	
C32	1.36±0.89	0.93±0.15	1.19±0.13	0.77±0.04	
C34	2.22±0.08	2.99±0.12	3.78±0.22	2.47±0.14	
C36	6.67±1.10	9.08±0.35	9.73±0.55	6.98±0.40	
C38	16.09±1.07	15.72±0.61	11.82±0.67	11.45±0.65	
C40	10.34±0.39	10.63±0.41	8.16±0.47	8.13±0.46	
C42	3.52±0.13	3.16±0.12	3.78±0.22	4.38±0.25	
C44	5.32±0.20	2.42±0.09	3.34±0.19	3.68±0.21	
C46	6.11±0.23	3.68±0.14	4.69±0.27	4.94±0.28	
C48	8.09±0.31	6.77±0.26	8.18±0.47	9.38±0.54	7.75±0.09
C50	11.16±0.42	12.63±0.49	14.82±0.84	16.39±0.94	40.19±0.44
C52	10.75±0.41	16.97±0.66	16.10±0.92	17.06±0.97	40.62±0.45
C54	3.56±0.14	8.47±0.33	6.28±0.36	6.23±0.36	10.67±0.12
C50+C52+C54	25.47	38.07	37.20	39.68	91.48

Data is presented as Mean±SD (n=3)

Table 4.29 TAG profiles of market samples of ghee from unorganized sector (UOS)

Triacylglycerols (TAGs)	Samples							
	UOS-1	UOS-2	UOS-3	UOS-4	UOS-5	UOS-6	UOS-7	UOS-8
C24								
C26								
C28	0.26±0.03	0.38±0.02	0.44±0.03	0.27±0.01	0.37±0.02	0.47±0.02	0.32±0.03	0.25±0.03
C30	0.40±0.03	0.55±0.02	0.71±0.03	0.50±0.02	0.57±0.01	0.59±0.02	0.76±0.04	0.51±0.02
C32	1.21±0.24	1.20±0.05	1.47±0.12		1.31±0.04	1.40±0.22	2.59±0.52	1.287±0.15
C34	3.73±0.32	3.77±0.16	5.15±0.55		4.42±0.37	5.25±0.46	4±0.61	4.42±0.47
C36	10.12±0.86	10.29±0.44	12.76±1.36		10.46±0.87	12.69±1.12	8.77±0.85	12.04±1.29
C38	12.58±1.07	16.28±0.69	15.57±1.66		12.38±1.04	14.20±1.25	14.24±1.19	14.64±1.57
C40	9.33±0.79	11.59±0.49	10.52±1.12		7.96±0.66	9.09±0.80	9.26±0.76	9.13±0.98
C42	4.45±0.38	4.63±0.20	5.23±0.56		4.13±0.34	4.86±0.43	4.47±0.90	4.47±0.48
C44	3.58±0.30	3.29±0.14	3.49±0.37	0.25±0.01	3.29±0.27	4.08±0.36	3.16±0.68	3.06±0.33
C46	5.12±0.44	4.23±0.18	5.06±0.54		4.47±0.37	5.44±0.48	4.27±0.57	4.03±0.43
C48	8.22±0.70	6.83±0.29	8.15±0.87	3.61±0.17	7.65±0.64	8.15±0.72	8.18±0.70	6.24±0.67
C50	12.30±1.05	10.98±0.47	11.91±1.27	24.45±0.18	13.33±1.11	11.20±0.99	13.82±1.20	7.7±0.83
C52	11.95±1.02	13.56±0.58	10.73±1.14	39.44±0.29	13.67±1.14	9.67±0.85	15.08±1.37	13.57±1.46
C54	6.52±0.55	6.63±0.28	4.41±0.47	31.89±0.23	7.54±0.63	4.01±0.35	7.16±0.95	13.34±1.04
C50+C52+C54	30.77	31.17	27.05	95.78	34.54	24.88	36.06	34.61

Data is presented as Mean±SD (n=3)

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Table 4.30 TAGs profile of market samples of ghee from unorganized sector (UOS)

Triacylglycerols (TAGs)	Samples						
	UOS-9	UOS-10	UOS-11	UOS-12	UOS-13	UOS-14	UOS-15
C24							
C26							
C28	0.33±0.03	0.28±0.04	0.28±0.04	0.35±0.04	0.44±0.04	0.27±0.03	0.32±0.03
C30	0.54±0.05	0.48±0.02	0.54±0.05	0.49±0.10	0.52±0.04	0.41±0.02	0.53±0.04
C32	1.25±0.13	1.17±0.11		1.25±0.08	1.36±0.14	1.04±0.16	1.26±0.14
C34	4.51±0.44	3.88±0.37	0.24±0.02	4.54±0.39	4.91±0.57	3.37±0.27	3.91±0.32
C36	12.08±1.17	10.57±1	0.40±0.01	11.3±0.97	11.78±1.38	9.29±0.74	10.62±0.86
C38	16.10±1.08	15.44±0.80	0.52±0.09	12.60±1.08	13.88±1.62	10.38±0.83	13.81±1.11
C40	10.20±0.33	9.36±0.223	0.19±0.02	7.72±0.66	8.76±0.63	6.09±0.48	10.60±0.86
C42	4.63±0.45	4.41±0.42	0.55±0.04	4.26±0.37	4.73±0.55	3.27±0.40	4.89±0.39
C44	4.33±0.42	3.10±0.29		3.50±0.30	3.67±0.43	2.79±0.78	3.75±0.30
C46	5.47±0.53	4.85±0.45		4.91±0.42	4.75±0.56	5.20±0.42	4.61±0.37
C48	8.26±0.80	8.16±0.77	6.55±0.12	8.10±0.70	7.38±0.86	10.18±0.82	7.37±0.59
C50	12.12±1.17	12.70±1.20	26.00±0.48	12.90±1.11	11.47±1.34	16.36±1.31	11.23±0.91
C52	11.52±0.92	15.05±1.42	36.92±0.69	11.22±0.96	11.46±1.34	15.13±1.21	12.23±0.99
C54	3.88±0.37	6.19±0.58	29.23±0.54	4.16±0.30	7.57±0.77	6.82±0.55	6.74±0.54
C50+C52+C54	27.52	33.94	92.15	28.28	30.51	38.31	30.2

Data is presented as Mean±SD (n=3)

4.2.4.13 Summary of quality of milk fat in market samples of butter, ghee and paneer in totality.

- In the end, it may be concluded from the Table 4.37 that market ghee samples eleven from organized and fifteen from unorganized sector were first analysed for their physico-chemical constants such as RM-value, P-value, BR-reading to check the authenticity of milk fat. Out of eleven samples from organized sector, 9% samples failed to meet legal requirement through RM value, Polenske value and BR reading as specified in AGMARK (1981) and FSSR (2011), and 36.36% samples were found positive for Baudouin test. RP-TLC and TAGs profile using GLC of ghee samples from organized sector revealed that 54.5% samples were found to contain vegetable oil/fat and may be concoction of vegetable oils and animal body fats.
- Similarly, from Table 4.38, it can be seen that out of fifteen samples from unorganized sector, 26.6% samples failed to meet legal requirement through RM value, Polenske value and BR reading as specified in AGMARK (1981) and FSSR (2011), 13.3% samples were found positive for Baudouin test. RP-TLC and TAGs profile of ghee samples from unorganized sector revealed that 66.6% samples were found to be adulterated with vegetable oils/fats and may be concoction of vegetable oils/fats and animal body fats.
- From Table 4.39, it can be observed that all the samples of butter from organized sector met the legal requirement as prescribed by AGMARK (1981) and FSSR (2011), however RP-TLC and TAGs profile of market samples of butter from organized sector revealed that 40% samples were found to be adulterated.
- From Table 4.40, data clearly showed that 25 % samples through RM value, 6.25% through Polenske value and 37.5% through BR reading analysis failed to meet legal requirements. None of the samples were found positive for Baudouin test. While RP-TLC and TAGs profile revealed that 43.75% samples of butter from unorganized sector were adulterated with foreign fats i.e. Which may be vegetable oil/fat, body fat or their admixture.

Results & Discussion

- The analysis of market samples of paneer from organized sector are depicted in Table 4.41. It can be observed that all the samples of paneer from organized sector met the legal requirement as prescribed by AGMARK (1981) and FSSR (2011). However, RP-TLC and TAGs profile of market samples of paneer from organized sector revealed that 28.5% samples were adulterated either with vegetable oils/fats and body fats or their concoction.
- From Table 4.42, it can be seen that 18.75 % samples through RM value, 6.25% through Polenske value and 31.25% through BR reading analysis were found failed to meet legal requirement. 6.25% samples found positive for Baudouin test. While RP-TLC and TAGs profile revealed that 43.75% samples of paneer from unorganized sector were adulterated. It was also observed from GLC analysis of TAGs that samples of particular unorganized dairy which were found positive for ghee, also found positive for butter and paneer. Similar pattern was also observed for organized dairies.
- Above study clearly indicated that tests like RP-TLC and TAGs profiling using GLC have high sensitivity and selectivity to check the authenticity of milk fat than the physico-chemical constants. So, they can be recommended to legal authorities like FSSR and BIS for its inclusion in test methods. However, to ensure the foolproof application of TAGs of milk fat by GLC, a detailed mapping of TAGs in milk fat of Indian origin is very much required. This will enrich the data base to enable us to validate or develop regression equation like ISO/IDF methods.

Table 4.31 TAGs profile of market samples of butter from organized sector (OS)

Triacylglycerols (TAGs)	Samples				
	OS-1	OS-2	OS-3	OS-4	OS-5
C24					
C26					
C28	0.38±0.01	0.33±0.08	0.27±0.02	0.30±0.02	0.27±0.03
C30	0.60±0.07	0.52±0.18	0.45±0.12	0.44±0.06	0.41±0.02
C32	1.37±0.06	1.15±0.37	1.18±0.16	1.08±0.02	1.10±0.18
C34	4.72±0.38	3.63±0.51	3.89±1.09	4.06±0.46	3.49±0.18
C36	11.29±0.82	10.63±0.11	8.74±0.58	10.34±1.18	9.88±0.50
C38	15.14±1.57	14.67±0.91	14.89±0.57	13.87±1.59	14.94±0.76
C40	10.54±0.47	10.62±0.36	9.79±0.82	9.92±1.14	10.37±0.53
C42	4.57±0.33	4.20±0.20	4.50±0.17	5.49±0.63	4.51±0.23
C44	3.62±0.39	3.45±0.64	2.96±0.11	3.85±0.44	3.53±0.18
C46	5.30±0.66	4.47±0.42	4.31±0.16	5.38±0.62	4.73±0.24
C48	8.28±0.63	8.03±0.98	7.46±0.28	8.57±0.98	7.81±0.40
C50	11.11±0.82	11.23±0.99	12.20±0.47	13.03±1.49	11.98±0.61
C52	11.00±1.32	13.80±1.23	15.59±0.60	13.04±1.49	11.44±0.74
C54	3.22±0.18	5.85±0.71	7.66±0.29	4.96±0.57	6.34±0.32
C50+C52+C54	25.33	30.88	35.45	31.03	29.76

Data is presented as Mean±SD (n=3)

Results & Discussion

Table 4.32 TAGs profile of market samples of butter from unorganized sector (UOS)

Triacylglycerols (TAGs)	Samples							
	UOS-1	UOS-2	UOS-3	UOS-4	UOS-5	UOS-6	UOS-7	UOS-8
C24								
C26								
C28	0.31±0.07	0.24±0.04	0.31±0.02	0.32±0.02	0.41±0.01	0.42±0.01	0.28±0.01	0.39±0.08
C30	0.50±0.05	0.41±0.11	0.49±0.11	0.53±0.02	0.66±0.07	0.61±0.03	0.46±0.04	0.59±0.10
C32	1.29±0.01	0.98±0.16	1.32±0.15	1.21±0.10	1.40±0.17	1.51±0.04	1.45±0.70	1.37±0.33
C34	4.73±0.39	3.62±0.27	4.89±0.38	3.57±0.19	5.79±0.58	6.10±0.69	3.46±0.49	5.57±0.62
C36	11.64±0.96	8.84±0.66	11.22±0.87	9.88±0.54	15.09±1.5	14.68±1	9.60±0.68	12.89±1.43
C38	13.23±1.09	9.87±0.73	13.58±1.06	11.40±0.78	16.25±1.62	16±0.50	15.77±0.66	13.91±1.54
C40	8.47±0.70	5.78±0.43	9.13±0.71	11.11±0.61	9.56±0.95	4.90±0.55	10±1.08	8.12±0.90
C42	4.64±0.38	2.90±0.22	5.49±0.43	5.11±0.63	4.91±0.49	3.84±0.43	4.72±0.69	6.12±0.68
C44	3.87±0.32	2.39±0.18	4.74±0.37	3.92±0.57	4.03±0.40	5.14±0.58	3.17±0.42	5.22±0.58
C46	5.34±0.44	3.82±0.28	6.07±0.47	4.49±0.24	5.53±0.55	4.48±1.86	4.21±0.55	6.56±0.73
C48	8.33±0.69	8.75±0.65	8.97±0.70	7.26±0.40	8.44±0.84	8.48±0.95	7.14±0.69	9.34±1.03
C50	11.45±0.95	19.84±1.48	11.95±0.93	11.81±0.64	11.13±1.1	11.71±1.32	12.16±0.46	12.01±1.33
C52	10.49±0.87	18.13±1.35	10.02±0.78	14.35±0.78	8.11±0.26	9.41±0.91	13.79±1.01	9.77±0.83
C54	5.07±0.42	6.65±0.63	3.38±0.26	3.46±0.19	2.64±0.26	3.33±0.38	8.38±0.94	3.42±0.38
C50+C52+C54	27.01	44.62	25.35	29.62	21.88	24.45	34.33	25.2

Data is presented as Mean±SD (n=3)

Table 4.33 TAG profiles of market samples of butter from unorganized sector (UOS)

Triacylglycerols (TAGs)	Samples							
	UOS-9	UOS-10	UOS-11	UOS-12	UOS-13	UOS-14	UOS-15	UOS-16
C24								
C26								
C28	0.29±0.03		0.29±0.01	0.35±0.01	0.32±0.01	0.27±0.01	0.28±0.01	0.26±0.02
C30	0.46±0.03	0.22±0.01	0.40±0.03	0.47±0.01	0.48±0.01	0.37±0.02	0.44±0.03	0.46±0.03
C32	1.15±0.08	0.26±0.03	1.18±0.91	1.22±0.12	1.20±0.06	1.34±0.42	1.25±0.21	1.41±0.28
C34	4.51±0.41	0.56±0.15	3.76±0.31	4.22±0.38	4.29±0.33	3.94±0.34	4.14±0.37	4.33±0.49
C36	11.01±1.01	0.71±0.40	9.51±0.77	12.19±1.09	10.83±0.83	10.64±0.93	10.96±0.97	11.57±1.31
C38	12.47±1.15	0.79±0.01	11.52±0.94	15.09±1.39	13.47±1.03	11.77±1.03	13.71±1.21	14.21±1.61
C40	7.61±0.70	0.63±0.22	7.30±0.59	9.45±0.83	8.94±0.68	7.17±0.57	4.59±0.41	4.86±0.55
C42	3.80±0.35	0.78±0.28	3.38±0.28	3.75±0.33	4.98±0.38	3.75±0.33	4.85±0.87	4.87±0.55
C44	3.04±0.28	0.66±0.14	2.41±0.20	3.63±0.32	3.98±0.30	2.92±0.25	4.09±1	4.96±0.56
C46	4.38±0.40	1.37±0.13	3.84±0.31	4.90±0.44	5.19±0.40	4.07±0.35	5.06±0.45	4.59±1.40
C48	7.02±0.65	11.10±0.63	6.61±0.54	7.74±0.69	8.10±0.62	6.62±0.58	8.08±0.71	5.36±0.85
C50	10.10±0.93	36.30±0.25	10.79±0.88	9.55±0.85	11.74±0.89	9.99±0.87	11.51±1.02	11.92±1.35
C52	13.88±0.76	35.34±0.24	16.04±1.31	13.94±1.24	11.66±0.9	13.47±1.17	10.69±0.94	11.08±1.26
C54	11.75±1.08	10.54±0.07	17.00±1.38	9.61±0.86	5.18±0.39	13.87±1.21	5.00±0.44	4.43±0.50
C50+C52+C54	35.73	82.18	43.83	33.1	28.58	37.33	27.20	27.43

Data is presented as Mean±SD (n=3)

Results & Discussion

Table 4.34 TAGs profile of market samples of paneer from organized sector (OS)

Triacylglycerols (TAGs)	Samples						
	OS-1	OS-2	OS-3	OS-4	OS-5	OS-6	OS-7
C24							
C26							
C28	0.36±0.03	0.33±0.02	0.48±0.01	0.52±0.02	0.30±0.01	0.43±0.02	0.34±0.02
C30	0.57±0.02	0.54±0.02	0.55±0.02	0.84±0.02	0.51±0.02	0.70±0.03	0.57±0.02
C32	1.23±0.04	1.29±0.05	1.49±0.07	1.54±0.09	1.12±0.18	1.53±0.06	1.10±0.04
C34	3.85±0.12	3.86±0.16	4.75±0.24	4.35±0.25	3.35±0.18	4.85±0.26	3.91±0.15
C36	10.19±0.32	9.95±0.41	11.92±0.60	10.18±0.58	9.53±0.51	12.09±0.66	10.54±0.39
C38	14.85±0.46	13.12±0.53	14.09±0.70	14.81±0.84	14.82±0.80	16.62±0.90	15.19±0.57
C40	10.06±0.31	9.15±0.37	9.42±0.47	11.67±0.66	10.83±0.58	10.90±0.59	10.04±0.37
C42	4.41±0.14	4.81±0.20	4.66±0.23	6.31±0.36	5.65±0.30	4.81±0.26	4.43±0.17
C44	3.43±0.11	3.93±0.16	3.85±0.19	5±0.28	4.38±0.24	3.33±0.18	3.45±0.13
C46	4.45±0.14	5.07±0.21	5.15±0.26	5.55±0.32	5.09±0.27	4.72±0.26	4.52±0.17
C48	7.09±0.22	7.72±0.31	7.80±0.39	7.29±0.42	7.45±0.40	7.43±0.40	7.20±0.27
C50	10.83±0.34	11.43±0.47	10.61±0.53	9.93±0.57	10.73±0.58	11.08±0.60	11.23±0.42
C52	13.13±0.41	12.87±0.52	9.69±0.48	10.96±0.62	12.39±0.67	11.88±0.64	13.35±0.50
C54	6.60±0.20	5.21±0.21	4.68±0.23	4.94±0.28	5.15±0.28	4.32±0.23	7.34±0.27
C50+C52+C54	30.56	29.51	24.98	25.83	28.27	27.28	31.92

Data is presented as Mean±SD (n=3)

Table 4.35 TAGs profile of market samples of paneer from unorganized sector (UOS)

Triacylglycerols (TAGs)	Samples							
	UOS-1	UOS-2	UOS-3	UOS- 4	UOS-5	UOS-6	UOS -7	UOS -8
C24								
C26								
C28	0.33±0.02	0.42±0.01	0.41±0.02		0.32±0.02	0.26±0.02	0.43±0.03	0.25±0.02
C30	0.51±0.02	0.52±0.02	0.59±0.01		0.53±0.04	0.38±0.01	0.60±0.02	0.35±0.02
C32	1.24±0.15	1.34±0.13	1.35±0.11	0.40±0.04	1.19±0.07	1.14±0.18	1.36±0.17	1.19±0.22
C34	3.62±0.28	5.25±0.43	5.29±0.52	0.44±0.02	3.66±0.18	3.37±0.12	5.06±0.48	4.06±0.34
C36	9.78±0.76	13.13±1.07	13.31±1.30	0.83±0.54	10.25±0.51	9.38±0.34	11.38±1.09	10.56±0.87
C38	13.91±1.08	13.99±1.14	16.36±1.59	1.36±0.22	15.75±0.79	13.90±0.51	14.40±1.38	13.12±1.08
C40	10.76±0.83	7.38±0.60	10.25±1	1.35±0.21	10.81±0.54	9.75±0.36	10.10±0.97	8.46±0.70
C42	4.46±0.35	4.50±0.37	5.04±0.49	0.86±0.47	4.39±0.22	4.81±0.18	5.56±0.53	4.03±0.33
C44	3.41±0.26	3.95±0.32	4.02±0.39	0.54±0.17	3.18±0.16	3.89±0.14	4.10±0.39	3.08±0.25
C46	4.55±0.35	5.26±0.43	5.22±0.51	0.72±0.18	4.20±0.21	4.82±0.18	5.52±0.53	4.41±0.36
C48	7.44±0.58	7.89±0.64	7.91±0.77	1.29±0.28	6.86±0.34	7.26±0.27	8.38±0.80	7.29±0.60
C50	11.94±0.93	11.01±0.90	9.68±0.94	4.43±0.03	11.28±0.56	10.67±0.39	11.48±1.1	11.09±0.91
C52	15.23±1.18	11.10±0.59	11.37±1.11	29.09±0.17	14.15±0.71	13.48±0.49	10.42±1	12.72±0.97
C54	7.44±0.58	9.78±0.80	5.11±0.50	58.42±0.35	7.36±0.37	8.58±0.31	4.52±0.43	7.78±0.64
C50+C52+C54	34.61	31.89	26.16	91.94	32.79	32.73	26.42	31.59

Data is presented as Mean±SD (n=3)

Results & Discussion

Table 4.36 TAGs profile of market samples of paneer from unorganized sector (UOS)

Triacylglycerols (TAGs)	Samples							
	UOS-9	UOS-10	UOS-11	UOS-12	UOS-13	UOS-14	UOS-15	UOS-16
C24								
C26								
C28	0.26±0.02	0.41±0.01	0.30±0.01	0.52±0.02	0.35±0.03	0.33±0.03	0.34±0.02	0.35±0.02
C30	0.43±0.02	0.57±0.04	0.41±0.02	0.62±0.02	0.62±0.02	0.51±0.02	0.47±0.02	0.56±0.02
C32	1.21±0.03	1.28±0.16	1.15±0.09	1.14±0.12	1.36±0.10	1.31±0.05	1.16±0.11	1.44±0.03
C34	4.53±0.41	5.03±0.51	4.18±0.4	5.14±0.41	5.05±0.57	4.93±0.47	4.46±0.52	5.34±0.36
C36	12.83±1.16	12.90±1.31	11.18±1.17	12.70±1.02	12.32±1.38	13.36±1.26	11.86±1.37	13.68±0.93
C38	14.41±1.30	14.41±1.46	14.26±1.1	14.45±1.16	14.41±1.61	14.74±1.39	13.69±1.58	14.05±0.95
C40	8.61±0.78	8.79±0.89	9.49±0.99	9.29±0.74	7.84±0.88	8.78±0.83	8.75±1.01	8.37±0.57
C42	4.43±0.40	4.55±0.46	4.44±0.46	4.84±0.39	4.77±0.53	4.39±0.41	4.28±0.49	4.51±0.31
C44	3.57±0.32	3.99±0.41	3.46±0.36	3.87±0.31	4.16±0.47	3.52±0.33	3.28±0.38	3.71±0.25
C46	5.08±0.46	5.56±0.57	4.92±0.51	5.29±0.42	5.58±0.62	4.98±0.47	4.67±0.54	5.09±0.35
C48	8.11±0.74	8.54±0.87	8.12±0.86	7.82±0.63	8.65±0.97	8.03±0.76	7.46±0.86	7.73±0.52
C50	11.36±1.03	11.57±1.18	12.60±1.32	11.04±0.88	12.26±1.11	11.71±1.1	10.89±1.26	10.36±0.70
C52	10.07±0.91	9.82±1.00	11.44±1.20	9.18±0.73	10.58±1.18	10.19±0.96	11.86±1.37	9.03±0.61
C54	3.74±0.34	3.21±0.25	3.82±0.36	3.77±0.30	3.53±0.33	3.94±0.37	8.48±0.98	4.05±0.27
C50+C52+C54	25.17	24.60	27.86	23.99	26.37	25.84	31.23	23.44

Data is presented as Mean±SD (n=3)

Table 4.37 Summary of quality of milk fat in market samples of ghee from organized sector (OS)

Samples	RM value	Polenske value	BR reading	Baudouin test	RP-TLC	GLC
OS-1	Negative	Negative	Negative	Negative	Positive	Positive
OS-4	Negative	Negative	Negative	Negative	Positive	Positive
OS-8	Negative	Negative	Negative	Positive	Positive	Positive
OS-9	Negative	Negative	Negative	Positive	Positive	Positive
OS-10	Negative	Negative	Negative	Positive	Positive	Positive
OS-11	Positive	Positive	Positive	Positive	Positive	Positive

Table 4.38 Summary of quality of milk fat in market samples of ghee from unorganized sector (UOS)

Samples	RM value	Polenske value	BR reading	Baudouin test	RP-TLC	GLC
UOS-1	Negative	Negative	Negative	Negative	Positive	Positive
UOS-2	Negative	Negative	Negative	Negative	Positive	Positive
UOS-4	Positive	Positive	Positive	Positive	Positive	Positive
UOS-5	Negative	Negative	Negative	Negative	Positive	Positive
UOS-7	Negative	Negative	Negative	Negative	Positive	Positive
UOS-8	Positive	Positive	Positive	Negative	Positive	Positive
UOS-10	Negative	Negative	Negative	Negative	Positive	Positive
UOS-11	Positive	Positive	Positive	Positive	Positive	Positive
UOS-13	Positive	Positive	Positive	Negative	Positive	Positive
UOS-14	Negative	Negative	Negative	Negative	Positive	Positive

Table 4.39 Summary of quality of milk fat in market samples of butter from organized sector (OS)

Samples	RM value	Polenske value	BR reading	Baudouin test	RP-TLC	GLC
OS-3	Negative	Negative	Negative	Negative	Positive	Positive
OS-4	Negative	Negative	Negative	Negative	Positive	Positive

Results & Discussion**Table 4.40 Summary of quality of milk fat in market samples of butter from unorganized sector (UOS)**

Samples	RM value	Polenske value	BR reading	Baudouin test	RP-TLC	GLC
UOS-2	Negative	Negative	Positive	Negative	Positive	Positive
UOS-7	Negative	Negative	Negative	Negative	Positive	Positive
UOS-9	Positive	Negative	Positive	Negative	Positive	Positive
UOS-10	Positive	Positive	Positive	Negative	Positive	Positive
UOS-11	Positive	Negative	Positive	Negative	Positive	Positive
UOS-12	Negative	Negative	Positive	Negative	Positive	Positive
UOS-14	Positive	Negative	Positive	Negative	Positive	Positive

Table 4.41 Summary of quality of milk fat in market samples of paneer from organized sector (OS)

Samples	RM value	Polenske value	BR reading	Baudouin test	RP-TLC	GLC
OS-1	Negative	Negative	Negative	Negative	Positive	Positive
OS-2	Negative	Negative	Negative	Negative	Positive	Positive

Table 4.42 Summary of quality of milk fat in market samples of paneer from unorganized sector (UOS)

Samples	RM value	Polenske value	BR reading	Baudouin test	RP-TLC	GLC
UOS-1	Negative	Negative	Negative	Negative	Positive	Positive
UOS-2	Negative	Negative	Positive	Negative	Positive	Positive
UOS-4	Positive	Positive	Positive	Positive	Positive	Positive
UOS-5	Negative	Negative	Positive	Negative	Positive	Positive
UOS-6	Positive	Negative	Negative	Negative	Positive	Positive
UOS-8	Negative	Negative	Positive	Negative	Positive	Positive
UOS-15	Positive	Negative	Positive	Negative	Positive	Positive

CHAPTER –5

Summary and Conclusions

SUMMARY AND CONCLUSION

In the present investigation, quality of milk fat in market samples of butter, ghee and paneer was evaluated. In order to meet the objective of the study, control samples of the three products were prepared in the laboratory. To study the quality of milk fat in market samples of paneer from both organized and unorganized sector, milk fat was extracted with the help of chloroform using soxhlet extraction method. Similarly, for analysis of quality of milk fat in market samples of butter, milk fat was extracted using the heat clarification method.

For market survey, 10 branded ghee samples, 7 branded paneer samples and 5 branded butter samples from organized dairies were collected from NCR. Beside this, 16 samples of each butter, ghee and paneer from unorganized dairies and road side dhabas of NCR were also collected. Therefore, a total of 70 samples of three products were analyzed in the present study.

Milk fat extracted from market samples of the said product collected from organized as well as unorganized sector along with control samples were analyzed for, such as:

- I. Reichert-Meissl value (RM-value)
 - II. Polenske value (P-value)
 - III. Butyro-refractometer reading at 40 (B.R.-reading)
 - IV. Free fatty acid value (FFA %)
 - V. Spectral analysis- i.e. Derivative spectroscopy to detect tallow in ghee
 - VI. Baudouin test for detection of vanaspati in ghee samples
 - VII. Reversed-phase thin layer chromatography of unsaponifiable matter of fat extracted from market samples to ascertain the presence of vegetable oil/fat in ghee.
 - VIII. Low-resolution gas liquid chromatography for triacylglycerol analysis
- 5.1** The study revealed that the 2nd order derivative UV spectroscopy did not offer any help in detecting the adulteration in ghee because no characteristic differences were observed in the absorption behavior of different oils and fats including milk fat studied in the UV (200-400 nm).

Range of absorption maxima in normal and 2nd order UV spectra was found 280 to 290 nm and 240 to 270 nm, respectively.

- 5.2** The study depicted that average RM value for pure cow ghee was 28.66 ± 0.12 , whereas that for pure buffalo ghee, buffalo butter fat and fat isolated from paneer were 31.51 ± 0.19 , 31.12 ± 0.18 and 29.13 ± 0.26 , respectively. There was no significant difference in RM value of buffalo ghee as compared to buffalo butter but paneer fat isolated from paneer showed slightly lower RM-value. The possible reason may be incomplete extraction of steam volatile water soluble short chain fatty acid ($C_{4:0}$ and $C_{6:0}$) in soxhlet extraction due to non-polar nature of chloroform. Therefore it is not preferable to use RM-value as a parameter to judge the quality of milk fat isolated by soxhlet method as it can give false positive results. The various animal body fats and soybean oil showed negligible RM values in comparison with ghee and fat isolated from butterfat and buffalo paneer. The results showed that adulteration of pure cow ghee with soybean oil at 5% level or above, whereas all animal body fats individually at 10% or above could be detected by using RM value. The adulteration of buffalo ghee with all the adulterants individually at 20% level or above could be detected by using RM value. The adulteration of buffalo paneer with soybean oil and buffalo butter with margarine could be detected at 10% level or above by RM value analysis. However, it is not preferable to use RM value as a parameter to judge the quality of milk fat in the soxhlet extracted fat
- 5.3** The average Polenske value for pure cow ghee and buffalo ghee were 1.46 ± 0.03 and 1.32 ± 0.06 , respectively. Whereas average Polenske value for buffalo butter fat and fat isolated from paneer were 1.09 ± 0.08 and 1.28 ± 0.04 , respectively. The animal body fats and soybean showed much lower polenske value in comparison with ghee and fat extracted from butter and paneer. It was observed that adulteration of cow ghee, buffalo ghee, butter and paneer with any adulterant fats and oil could not be detected at any of the studied levels by using Polenske value.
- 5.4** The study depicted that average BR reading at 40°C for pure cow ghee was 42.4 ± 0.05 , whereas that for pure buffalo ghee, buffalo butter fat and

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fat isolated from paneer were 41.23 ± 0.08 , 41.13 ± 0.03 and 42.00 ± 0.03 , respectively. The various animal body fats and soybean oil showed higher BR reading at 40°C in comparison with ghee and fat isolated from butterfat and buffalo paneer. It was revealed from the BR reading analysis of adulterated samples that cow ghee adulteration with lard and soybean oil below 20% level could not be detected. However, adulteration in case of buffalo ghee, paneer and butter with any adulterant fats and oil even at 20% level could not be detected at any of the studied level by using BR reading at 40°C .

- 5.5** FFA contents of adulterated samples were found within the limit prescribed by AGMARK (1981) and FSSR (2011).
- 5.6** Fifteen market ghee samples from unorganized and eleven from organized sector were analysed for their physico-chemical constants such as RM-value, Polenske value, BR-reading 40°C and Baudouin test to check the authenticity of milk fat. Out of eleven samples from organized sector, 9% samples failed to meet legal requirement on the basis of RM value, Polenske value and BR reading as specified in AGMARK (1981) and FSSR (2011), and 36.36% samples were found positive for Baudouin test. Similarly, it was observed that out of fifteen samples from unorganized sector, 26.6% samples failed to meet legal requirement of RM value, Polenske value and BR reading as specified in AGMARK (1981) and FSSR (2011), 13.3% samples were found positive for Baudouin test.
- 5.7** From the study of butter samples, it was observed that all the samples of butter from organized sector met the legal requirement as prescribed by AGMARK (1981) and FSSR (2011). From unorganized sector, it was depicted that 25 % samples could not meet specified RM value, 6.25% Polenske value and 37.5% BR reading. No samples found positive for Baudouin test.
- 5.8** Analysis of market samples of paneer from organized sector revealed that all the samples met the legal requirements as specified by AGMARK (1981) and FSSR (2011). From unorganized sector, it was depicted that

18.75 % samples could not meet specified RM value, 6.25% Polenske value and 31.25% BR reading. 6.25% samples found positive for Baudouin test. It was also observed from physico-chemical study that samples of particular unorganized dairy which were found positive for ghee, also found positive for butter and paneer. Similar pattern was also observed for organized dairies.

- 5.9** It was concluded from the study that RP-TLC protocol could detect the adulteration of ghee, paneer and butter with vegetable oil and margarine, respectively at 5% level of adulteration. Results clearly demonstrated that fat extracted from paneer using soxhlet procedure can be used to confirm the presence or absence of vegetable oil in milk fat using RP-TLC.
- 5.10** RP-TLC profile of USM of ghee samples from organized and unorganized sector revealed that 54.5% and 66.6% samples, respectively, were found to contain vegetable oil/fat. Similarly, RP-TLC profile of USM of market samples of paneer from organized sector and unorganized sector showed that 28.5% and 43.75% samples were adulterated with vegetable oil/fat. RP-TLC profile of USM of market samples of butter from organized sector and unorganized sector showed that 40% and 43.75% samples were found to contain vegetable oil/fat. It was also observed from RP-TLC profile of USM that samples of particular unorganized dairy which were found positive for ghee, also found positive for butter and paneer. Similar pattern was also observed for organized dairies.
- 5.11** Sixteen major peaks of even carbon number TAGs, corresponding to TAG of C24 to 54 carbon number TAG, were identified in both cow or buffalo ghee. Similarly, 14 major peaks of even carbon number TAGs, corresponding to TAG of C28 to 54 carbon number TAG, were identified in fat isolated from buffalo butter and paneer. It was also noted that summation of C50, C52 and C54 TAGs were higher in cow ghee as compared to buffalo ghee and fat isolated from buffalo butter and buffalo paneer. There was no significant difference in summation value (C50 + C52 + C54) of buffalo butter fat and fat isolated from buffalo paneer as compared to buffalo ghee. It was observed that small and medium carbon number TAGs (C26 to C36) were higher in buffalo ghee than cow ghee.

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The range of summation of TAGs carbon number C50, C52 and C54 in control samples (ghee, butter and paneer) was found in between 26.31 to 30.1.

- 5.12** It was observed that short and medium carbon number TAGs (C24 to C48) were absent in buffalo tallow, goat tallow, lard and soybean oil. TAGs profile of coconut oil revealed that it had C34, C36 and 38 TAGs content higher as compared to other fats TAGs. TAG profile of coconut oil indicated absence of C24, C26, C52 and C54. It was observed from TAG profile of margarine that TAGs from C24 to C30 were absent and concentration of C32 to C46 was less as compared to milk fat. The TAGs profile of buffalo tallow, goat tallow, lard and margarine had higher relative percentage of C50, C52, C54 TAGs, while soybean oil had C52 and C54 content higher as compared to other milk fat TAG. In soybean oil C54 and in lard C52 were found better marker to check the authenticity of milk fat. It was concluded from present study that summation of TAGs C50, C52 and C54 was much higher in animal body fat, soybean oil and margarine except in coconut oil than the range observed for control milk fat samples (26.31 to 30.1).
- 5.13** Adulteration of coconut oil alone and in conjunction with lard in buffalo ghee could be detected at 5% level by observing the increase in sum of TAGs (C34+C36+C38). While, in case of all the adulterants except coconut oil sum of TAGs (C50+C52+C54) increased as the level of adulteration increased in control samples. Adulteration of buffalo tallow, goat tallow and lard in both type of ghee (cow and buffalo) could be detected at 5% level by observing the increase in sum of TAGs (C50+C52+C54). Adulteration of soybean oil in buffalo paneer and margarine in butter also could be detected at 5% level by observing the increase in sum of TAGs (C50+C52+C54).
- 5.14** It was observed from the study that TAGs profile using GLC of ghee samples from organized sector and unorganized sector revealed that 54.5% and 66.6% samples, respectively, were found to contain vegetable oil/fat or their concoction with animal body fats. Similarly, TAGs profile using GLC of butter samples from organized sector and unorganized

sector revealed that 40% and 43.75% samples, respectively, were found to be adulterated with vegetable oil/fat or concoction of vegetable oils/fats and animal body fats. TAGs profile using GLC of paneer samples from organized sector and unorganized sector revealed that 28.5% and 43.75% samples, respectively, were found to be adulterated with the above said adulterants. It was also observed from GLC analysis of TAGs that samples of particular unorganized dairy which were found positive for ghee, also found positive for butter and paneer. Similar pattern was also observed for organized dairies.

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2nd order derivative UV spectroscopy did not offer any help in detecting the adulteration in ghee with tallow. Polenske value did not help in detection of soybean oil and animal body fats added to cow ghee and buffalo ghee at any of the levels studied (5, 10 and 20%). Adulteration of soybean oil and animal body fats added singly could not be detected at the lowest level of 5% used in the present study using analysis of physico-chemical constants. A slight decrease was observed in RM value of extracted milk fat of paneer, the reason could be improper extraction of water soluble fatty acid (C₄ and C₆) in soxhlet extraction method due to the non-polar nature of chloroform. So it is not preferable to consider RM value as an indicator to check the quality of milk fat extracted using soxhlet method, it may lead to false positive results. Results clearly demonstrated that fat extracted from paneer using soxhlet procedure can be used to confirm the presence or absence of vegetable oil in paneer and other milk based products using RP-TLC. Gas liquid chromatography and Reversed-phase thin layer chromatography can be considered as the more appropriate techniques of detection of adulteration as it was concluded from the present investigation that Gas liquid chromatography could detect the adulteration of soybean oil and animal body fats in milk fat at a level of 5% using summation of C₅₀, C₅₂ and C₅₄ as marker, while adulteration of coconut oil alone and in conjunction with lard in buffalo ghee could be detected at 5% level by observing the increase in sum of TAGs (C₃₄+C₃₆+C₃₈). Reversed-phase thin layer

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chromatography could detect adulteration of paneer and ghee with soybean oil, while butter with margarine at a level of 5% using β -sitosterol as marker. Above study clearly indicated that tests like RP-TLC and TAGs profiling using GLC have high sensitivity and selectivity to check the authenticity of milk fat than the physico-chemical constants. RP-TLC protocol used in the present study to detect adulteration of paneer and butter with vegetable oils/ fats is rapid and easy to perform in any laboratory assessing the quality of milk fat and does not require sophisticated equipment, so it can be recommended to the testing laboratories of law enforcing agencies as well as of dairy/food testing laboratories. Similarly, TAGs analysis by GLC can also be used in the testing laboratories to ensure the purity of milk fat isolated from paneer and butter as well as ghee. Using these techniques, it was found that 54.5%, 40% and 28.5% organized ghee, butter and paneer samples, respectively did not conform to RP-TLC chromatogram and triacylglycerol profile of control milk fat. Similarly, 66.6%, 43.75% and 43.75% market samples of ghee, butter and paneer, respectively from unorganized sector were found to contain vegetable oil/fat or their concoction with animal body fats.

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Appendices

Appendix I

Standards of Ghee under FSSR (2011)

Sr.No.	Name of the state & U.T.	BR at 40 °C	RM value (mini.)	FFA (% oleic acid, max)	Moisture (% max)
1	Bihar, Chandigarh, Delhi, Punjab, Haryana (areas other than cotton tract), West Bengal (areas other than Bishnupur Subdivision), Sikkim, Jharkhand.	40-43	28	3.0	0.5
2	Manipur, Meghalaya, Mizoram, Arunachal Pradesh, Orissa, Uttaranchal, Nagaland, Goa, Kerala, Himachal Pradesh, U.P., J&K, Rajasthan, (areas other than Jodhpur division), Haryana (cotton tract areas), Lakshadweep, Maharashtra (areas other than cotton tract areas).	40-43	26	3.0	0.5
3	Karnataka (Belgaum district), M.P. (areas other than cotton tract areas), Pondicherry, Chhattisgarh.	40-44	26	3.0	0.5
4	A.P., Daman & Diu, Dadar & Nagar Haveli, Karnataka (areas other than Belgaum Dist.)	40-43	24	3.0	0.5
5	Gujarat (areas other than cotton tract)	40-43.5	24	3.0	0.5
6	Gujarat (cotton tract areas), M.P. (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 area 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 (1981)

S.No	Specifications	All India	Regional and Seasonal*	
			Winter (Sept-Feb)	Summer (Mar-Aug)
1	Baudouin test	Negative		
2	Butyro refractometer reading at 40°C	40.0 – 43.0	41.5 – 44.0	42.5 – 45.0
3	Reichert – Meissl value	Not less than 28.0	Not less than 23.0	Not less than 21.0**
4	Polenske value	1.0 – 2.0	0.5 - 1.2	0.5 – 1.0
5	Moisture (%)	Not more than 0.3		
6	Percentage of free fatty acids (oleic)			
a	Special Grade	Not more than 1.4		
b	General Grade	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.