EVALUATION OF QUALITY OF MARKET GHEE

THESIS SUBMITTED TO THE
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IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR
THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE
IN
DAIRYING
(DAIRY CHEMISTRY)

BY
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B.Tech. (Dairy Technology)

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

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CERTIFICATE

This is to certify that the thesis entitled, **'EVALUATION OF QUALITY OF MARKET GHEE'** submitted by **Mr. Parmar Satishkumar** towards the partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE IN DAIRYING (DAIRY CHEMISTRY)** of the **NATIONAL DAIRY RESEARCH INSTITUTE (Deemed University),** Karnal (Haryana), INDIA, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

(Dr. B. Surendra Nath)
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(Parmar Satishkumar)

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ABSTRACT

Ghee is a popular dairy product due to its characteristic flavour and texture. The major analytical characteristics like Reichert-Meissl (RM) value, Polenske value (PV), iodine value (IV), saponification value (SV) and butyrorefractometer (BR) reading are quite distinctive for ghee as it has a unique fatty acid composition. However, the natural variations in the fatty acid composition and conditions of manufacture can considerably alter the sensory and physico - chemical quality of market ghee. Ghee samples from both the organized (n, 17) and unorganized (n, 7) sectors from the Bangalore market were collected and evaluated for their quality. The price of ghee from the organized sector ranged from Rs.78 to 99 for 500 ml while that of the ghee from the unorganized sector ranged from Rs.35 to 90 for the same quantity. While wide variations were observed in the sensory quality of all the samples, the ones from the organized sector scored better for the sensory properties. Physico-chemical characteristics such as RM value, PV, IV, SV and BR reading of the samples from the organized sector were within the natural range. All the samples met the PFA standards for moisture, BR reading and free fatty acids. Though only 5 samples were graded as AG mark special and one sample as AG mark general, all the samples fulfilled the requirements of AG Mark special grade standards with respect to moisture, BR reading and free fatty acids. However, one sample failed to meet the RM value standard of PFA and AG mark. Three out of the seven samples of ghee from the unorganized sector had shown very low RM value and PV and marginally high BR reading indicating gross adulteration of these samples. All the samples from this sector had moisture and free fatty acids within the PFA and AG mark standards.

In addition to the physico-chemical characteristics, the samples were also subjected to specific tests for detection of adulteration. All the samples were negative to Baudouin test. Two samples each from the organized and unorganized sectors were found to contain added colours. One sample from unorganized sector was found to be added with mineral oil. Peroxide values were slightly higher in the samples from the unorganized sector. Though 4 out of 7 samples from the unorganized sector had the physico-chemical characteristics like RM, PV, SV, and BR within the normal range, crystallization and fractionation tests showed that they were highly adulterated with foreign fats. Since 90 % of the ghee production is from this sector, regular monitoring of the product for quality evaluation and corrective actions are essential to safeguard the interest of the public.

सारांश

घी अपने गुण, स्वाद एवं रचना के कारण एक प्रचलित एवं प्रमुख देशी उत्पाद है । प्रमुख विश्लेषित गुणों जैसे - रिचर्ट मीसल (RM) मूल्य, पोलेन्सके मूल्य (PV) आयडीन मूल्य (IV) सेपिनिफिकेशन मूल्य (SV) एवं बुटिरोरिफ्रेक्टोमीटर (BR) गणना घी के लिए विशेष है क्योंकि इसमें खास वसा अम्ल संगठित होता है । परन्तु, वसा अम्ल संगठन की प्राकृतिक भिन्ता एवं निर्माण की अवस्था बाजारी घी के सांवेदिक एवं भौतिक-रसायनिक गुण को विशेष रुप से बदल सकता है । बेंगलूर के बाजार से संगठित एवं असंगठित दोनों सेक्टरों से घी के नमूने संग्रहित किए गए एवं उनके गुण का मूल्यांकन किया गया । संगठित सेक्टरों के घी के दामों की रेंज 500 मि.लि. के लिए रु.78 से 99 थी, जबकि असंगठित सेक्टरों के घी के दामों की रेंज उतनी ही मात्रा के लिए रु 35 से 90 थी । सभी नमूनों के सांवेदिक गुण में विस्तृत भिन्नता को देखा गया, संगठित सेक्टरों के नमूनों में सांवेदिक लक्षण अच्छे पाए गए । संगठित सेक्टरों के नमूनों की भौतिक-रसायनिक गुण जैसे आर एम मूल्य, पी वी, आइ वी एवं बी आर गणना सहज रेंज में थी । सभी नमूनों में आर्द्रता, बी आर गणना एवं मुक्त वसा अम्ल पी एफ ए मानक से मिले जबकि केवल 5 नमूने विशेष ए जी मार्क और एक नमूने को सामान्य ए जी मार्क वर्गीकृत किया गया । आर्द्रता, बी आर गणना एवं मुक्त वसा अम्ल के संदर्भ में सभी नमूनों ने विशेष ए जी मार्क वर्ग मानक की आवश्यकता पूर्ति की । परन्तु एक नमूने पी एफ ए के आर मूल्य मानक एवं ए जी मार्क को पाने में असफल हुआ । असंगठित सेक्टरों के सात में से तीन नमूनों ने निम्न आर एम मूल्य एवं पी वी एवं थोड़े से उच्च बी आर गणना को दिखाते हुए इन नमूनों के ठोस मिश्रण की ओर इंगित किया । इस सेक्टर के सभी नमूनों में आर्द्रता और मुक्त वसा अम्ल पी एफ ए एवं ए जी मार्क मानक के अधीन थे ।

भौतिक -रसायनिक गुणों के साथ, इन नमूनों में मिलावट का पता लगाने के लिए विशेष परीक्षण भी एक शर्त थी । सभी नमूने बाउदोइन (Baudouin) परीक्षण के लिए अस्वीकार्य थे । दोनों संगठित एवं असंगठित सेक्टरों के दो नमूनों में रंग की मिलावट पाई गई । असंगठित सेक्टरों एक नमूनों में खनिज तेल का मिश्रण पाया गया । असंगठित सेक्टर के नमूनों में पिरोक्साइड़ मूल्य थोड़ उच्च था । असंगाठित सेक्टर के 7 नमूनों में से 4 में भौतिक रसायनिक गुण जैसे आर एम, पी वी, एस वी, और बी आर रेंज में थे एवं मानक, दानेदार गुण एवं पृथकीकरण परीक्षणों ने दिखाया कि असंगठित सेक्टरों के अधिकतर नमूनों में अत्याधिक विदेशी वसा से मिश्रित थे । चुंकि 90% घी उत्पाद इन सेक्टरों से होता है, गुण मुल्यांकन के लिए उत्पाद का नियमित अनुवीक्षण और आम लोगो के समर्थन की रक्षा के लिए सही कार्रवाही अनिवार्य है ।

1.0 INTRODUCTION

Ghee, a major dairy product, is the usual Indian name for clarified butter fat made from cream, desi butter or creamery butter. It has an important place in the diet of the people of the Indian sub-continent due to its good flavour, pleasant aroma and semi-solid (granular) texture. The rich pleasing flavour of ghee cannot be duplicated by other fats. This unique sensory property of ghee makes it a supreme cooking and frying medium. Besides being an energy dense food, ghee is a source of lipid nutrients such as fat soluble vitamins and essential fatty acids. Ghee is also a good source of butyric acid, conjugated linoleic acid and phospholipids etc, which have been recognized to possess therapeutic properties. The ancient ayurvedic literature and the recent research findings have recognized the positive role that ghee plays in our nutrition and health.

Ghee forms the largest segment of the milk products in India. Nearly 28 % of milk produced in our country is utilized for the production of ghee. Rising at an annual growth rate of 5%, ghee production has been estimated in 2001 to exceed 1.3 million tonnes, valued at Rs.130 billion. About 10% of this production comes from the organized sector, half of which is contributed by the co-operative sector.

The preferences for flavour, colour and texture of ghee vary from region to region in our country. The desired properties are usually achieved by way of selection of materials and manipulating the conditions of manufacture.

The physico-chemical quality of ghee is usually assessed by analysing certain characteristics such as Reichert-Meissl value, Polenske value, Butyro-refractometer reading, iodine value and saponification value. These analytical characteristics are mostly the reflections of the fatty acid composition of the product. Since ghee is unique among natural fats having a wide range of fatty acids, its characteristics are quite distinctive. However, the fatty acid composition is subjected to wide variation due to many factors. As a consequence, physico-chemical characteristics of ghee also cover a wide range permitting fairly high degree of adulteration while still keeping them within their natural limits.

The variation in the analytical characteristics coupled with expensive nature of ghee makes it susceptible to be adulterated with cheaper fats, mineral oils, colours, artificial flavours etc. by the unethical traders. The so-called specific tests are not sensitive or are difficult to perform as they involve usage of costly equipment and laborious procedures.

Further, large scale production by unorganized sector and lack of regular monitoring of quality may lead to the presence of sub-standard ghee in the market affecting the nutrition and health of public. There are very few published reports on the quality of ghee available in Bangalore market. Therefore, the present study was undertaken with the following objective:

- Assessment of the quality of ghee samples of organized and unorganized sectors, from the Bangalore market.

2.0 REVIEW OF LITERATURE

Traditional criteria of quality in ghee centre around flavour, texture and colour. These attributes of ghee have been serving to characterise and identify ghee. Many factors influence the sensory quality of ghee. Fats and oils exhibit certain physical and chemical characteristics, which have been employed for their characterization and differentiation. The determined analytical characteristics are reflections of the nature of the constituents, which make up the fat. Fats are almost entirely triglycerides of fatty acids. Since glycerol only constitutes about 10 per cent, it is the nature of fatty acids and the manner of distribution of these acids as glycerol triesters, which determine the various characteristics exhibited by the whole fat. Ghee is unique among natural fats in that it contains a large proportion of fatty acids of chain length lower than 12 carbon atoms and in consequence many of its characteristics are quite distinctive. Therefore, analysis of ghee for these characteristics provides useful information on the purity of ghee.

Prevention of Food Adulteration Act and AG Mark have also specified some of these characteristics as standards in order to check the quality of ghee mainly with reference to purity. A review of the quality of the ghee with reference to the sensory quality, physico-chemical characteristics and detection of adulteration is presented here.

2.1. SENSORY PROPERTIES

The consumer judges the quality and accepts it on the basis of three main attributes: flavour, granularity and colour. However, the preference for these characteristics of ghee varies considerably from region to region.

2.1.1. Flavour

Ghee flavour is often described as pleasant, nutty, lightly cooked or caramalized aroma. It is best described as lack of oiliness or of blandness, sweetly rather sharply acid. Local preferences exist in parts of India both for a raw, buttery, undercooked flavour and for a distinctly overcooked, slightly burnt one (Rangappa and Achaya, 1974). The flavour of ghee is influenced by many factors such as the quality of raw materials, method of preparation, ripening of cream, temperature of clarification.

In keeping with the size of the dairy industry and in view of the importance of flavour in maintaining the value of the product, a vast amount of work has been done by dairy chemists to delineate the chemistry of ghee flavour. Davies (1940) suggested that it was the charring of the casein in the boiling process, which was responsible for the ghee flavour. According to Ramaswamy and Banerjee (1948), the flavour in ghee was developed during the heating of butter through possible interaction among proteins (probably casein degradation products), reducing sugars (probably lactose) and minerals. Based on the work carried out in the ghee flavour, Wadhwa and Jain (1990) opined that ghee flavour is not due to a single constituent, or a class of constituents. An optimum blend of various compounds is responsible for the typical ghee flavour. Natural compounds present in milk, cream or butter and compounds produced during fermentation and clarification are partly transferred to ghee phase to make it flavoured. The important constituents of ghee flavour were identified to be fatty acids, carbonyls, lactones, browning compounds etc.

Flavour characteristics of ghee develop at almost all the stages of processing concerned with ghee making. However, fermentation of milk (as followed in the indigenous process) or cream and heat clarification of cream/butter influences, to the highest degree, the development of flavour in ghee.

2.1.1.1. Free fatty acids

Ghee owes its pleasing flavour to several free fatty acids (FFA). These compounds are produced from fatty acid glycerides by the mechanism involving lipolysis (through lactic streptococci)/hydrolysis during the fermentation of milk or cream and/or processing treatments while preparing ghee (Law, 1981; Wadhwa and Jain, 1989). The FFA make up of ghee has revealed the presence of 16 fatty acids C_4 to $_{C18:2}$ (Singhal and Jain 1973; Sharma and Bindal, 1987). The FFA level of fresh ghee was reported to be 6-12 mg/g. The lower fatty acids up to C_{12} though present in low concentration (0.4-1mg/g) accounting 5-10% of total FFA, contribute significantly to the ghee flavour. The FFA level of cow ghee (8.53 mg/g) was higher than that of buffalo ghee (6.89 mg/g). Desi ghee contained the highest level of FFA (6.62 mg/g), followed by creamery butter ghee (4.41 mg/g) and direct creamery ghee (4.38 mg/g) (Gaba and Jain, 1973).

2.1.1.2. Carbonyls

Carbonyls play an important role in contributing to the flavour of ghee. This class of compounds includes, broadly, monocarbonyls and dicarbonyls. Monocarbonyls further constitute alkan-2-ones (90%), alkanals (6%), alk-2-enals (2%) and alk-2, 4-dienals (2%). Alkan-2-ones are produced by the hydrolysis of ketonogenic glycerides (β-keto glycerides) followed by decarboxylation of β- keto carboxylic acids during various processing treatments involved in the preparation of ghee (Wadhwa and Jain, 1989). Also, lypolysis of triglycerides through penicillium moulds during fermentation of milk or cream may be the source of alkan-2-ones. Penicillium lipases liberate free saturated fatty acids and further oxidize them at β-position to form β-keto acids, which on decarboxylation yield methyl ketones (Law, 1981). Aldehydes, namely n-alkanals, alk-2-enals and alka-2, 4-dienals are formed by the autoxidation of unsaturated fatty acids of milk fat (Wadhwa and Jain, 1989).

Gaba and Jain (1975) reported that the total carbonyl content of cow and buffalo ghee made from fresh butter was 7.20 and 8.64 μ moles/g respectively. Gaba and Jain (1974, 1975) have identified various monocarbonyls responsible for ghee flavour. Rao and Rama Murthy (1984) quantified and identified polar carbonyls in ghee. Ghee prepared at clarification temperature of 100° and 120°C for 10 min contained about 1.9 mg and 31.5 mg/100 g of polar carbonyls respectively in direct cream ghee prepared from fresh cream. The content of polar carbonyls was found to increase in ghee made from ripened cream, the levels being 6.1 mg and 75.8 mg/100g of ghee clarified at 100° and 120°C respectively. The levels were lower (1.4 and 3.2 mg/100 g) in ghee from sweet butter.

Thus a definite blend of carbonyls in quantitative and qualitative terms appears to be critical as far as the characteristic ghee flavour is concerned.

2.1.1.3. Lactones

Lactones were reported to be responsible for the pleasing flavour of ghee. These compounds are produced by the hydrolysis of lactonegenic glycerides followed by dehydration (lactonization) of hydroxy acids (Wadhwa and Jain, 1989). Wadhwa *et al.* (1979, 1980 a, 1980 b) and Wadhwa and Jain (1984 a) isolated lactones from ghee using different methods. Later, Wadhwa and Jain (1984 b) quantified the individual lactones in ghee by GLC. The levels of lactones and factors influencing the lactone profile of ghee were also studied (Wadhwa and Jain, 1984c, 1985, 1986). Based on the results of these studies, Wadhwa and Jain (1990) reported that lactones were important flavour constituents, delta lactones being the dominant ones. The changes in the flavour quality of ghee due to temperature of clarification, method of preparation and storage were closely related with the corresponding changes in the lactone level of ghee.

2.1.2. Texture (Granularity)

Along with natural, sweet and pleasant aroma and an agreeable taste, presence of uniform size grains with very little liquid fat is a desirable characteristic of good quality ghee. Granularity in ghee is considered by Indian buyer to be an important criterion of quality and even purity. Ghee, which is hard, greasy, or having waxy texture is not liked by the consumers. Paul and Suri (1949) reported that greatest number of large grains in ghee was obtained when the ghee was melted at 60°-100°C and maintained at 29°C for cow ghee or 31°C for buffalo ghee. The maximum size of grains in case of buffalo and cow ghee were found to be 0.54 mm and 0.34 mm respectively.

Arumughan and Narayanan (1979) studied the granulation of buffalo and cow ghee at 29°C. Grains of buffalo ghee were irregular clusters whereas those of cow ghee were smaller and comprised spheroids of fine divergent mono crystals. Joshi and Vyas (1976 b) and Nagesh (1981) studied various conditions affecting the granulation of ghee. Singhal *et al.* (1973) studied that layer formation in ghee could be prevented by storing it at 20°C or below immediately after preparation. Ghee thus solidified could subsequently be stored at higher temperatures without formation of layers.

Grain formation is one of the critical attributes affecting consumer acceptability of ghee. The fatty acid profile of milk fat, temperature of clarification, followed by the rate at which it is cooled, seeded and held under quiescent storage before packing, and temperature of ghee storage in the marketing network are various factors that contribute towards graininess in ghee. In general, heat clarification at 110°C for 5 minutes, slow cooling to 29°C, seeding at the rate of 2%, followed by quiescent storage for 72 hours at 25°C is optimum for proper grain formation (Aneja *et al.*, 2002).

Though flavour and texture are the important attributes of ghee, preferences of varying degree exist in the different areas and regions. Aneja *et al.* (2002) summarized the regional preferences for flavour and texture in ghee and the information is given in the Table A.

Table: A. Regional preferences for ghee flavour and texture (Aneja, 2002)

Region	**Flavour, Texture Preferences**
Northern	Flavour: Slight acidic, mildly curdy Texture: Fine to medium size grains (1/2 –3/4 solid portion)
Western	Flavour: Mildly curdy (Strongly curdy in Saurashtra) Texture: Coarse grains of 0.3 to 0.6 mm size
Southern	Flavour: Mildly to highly cooked, aromatic, higher level of FFA (preferences for special herb flavours in TN and Karnataka). Texture: Medium size (TN), Coarse grains (AP & Karnataka)
Eastern	Flavour: Slightly to definitely cooked flavour Texture: Medium grains (1/4 liquid, 3/4 solid)

2.1.3. Colour

A bright yellow colour caused by the presence of carotenoid pigments is associated with cow ghee. Buffalo ghee lacks carotene, and is whitish with slight greenish tinge or pale-cream in colour. The colour of fats always appears deeper in eyes when melted than when in solid form.

The natural colour in milk fat is due to the presence of carotenoids and similar compounds. Nearly 85-95% of total fat-soluble pigment is β-carotene (Frankel *et al.*, 1958).

The intensity of cow ghee varies considerably as the carotene level is affected by many factors such as breed, season, stage of lactation, feed (Narayanan *et al.*, 1956; Kehar *et al.*, 1956; Narayanan and Anantakrishnan, 1959,1960).

The colour of ghee varies with the heat treatment. Overcooking imparts a brown 'burnt' cast, particularly noticeable in buffalo ghee which is nearly colorless. The browning may, in moderate heating, be a casein-lactose reaction giving rise to complex coloured products, or in the extreme to a scorching in which carbon is released (Richards, 1965). Colour intensification does not occur if the serum from melted butter is discarded before final boiling down (Fahmi and Fahmi, 1955).

The colour of ghee was also seen to be influenced by the souring process. Srinivasan and Banerjee (1946) noticed that the ghee made from milk soured for 6 h was lighter in colour than that derived after longer souring periods. Lalitha and Dastur (1956) demonstrated the presence of greenish yellow pigment in buffalo butterfat isolated from sour milk. Chandravadana and Dastur (1976) have tentatively identified the blue-green pigment as biliverdin and attributed the occurrence of a greenish yellow pigment in buffalo ghee (buffer fat) to the conversion of biliverdin to bilirubin during storage and souring of milk. Later, Kumar *et al.* (1985) identified and confirmed the greenish yellow pigment as bilirubin.

2.2. CHEMICAL CONSTITUENTS OF GHEE

Chemically ghee is nothing but ~ 99.5% milk fat. It is a complex lipid of glycerides, free fatty acids, phospholipids, sterols, sterol esters, fat soluble vitamins, tocopherol, carbonyls, hydrocarbons, carotenoids, small amounts of charred casein, traces of minerals like calcium, phosphorous, iron, copper etc. Moisture is always present in ghee, since it cannot be removed merely by boiling.

Apart from the above constituents, moisture; free acids like formic, acetic, propionic and lactic; fat breakdown products like fat hydroperoxides, free aldehydes and ketones, lactones etc.; bound aldehydes; minerals like calcium, magnesium, copper, iron etc were also reported to be present in ghee (Sharma, 1981).

The chemical composition of ghee as reviewed by Sharma (1981) is given in the Table B.

Table: B. The major and minor constituents of buffalo and cow ghee

Constituents	Buffalo Ghee	Cow Ghee
Triglycerides		
Short chain (%)	45.3	37.6
Long chain (%)	54.7	62.4
Trisaturated (%)	40.7	39.0
High melting (%)	8.7	4.9
Partial glycerides		
Diglycerides (%)	4.5	4.3
Monoglycerids (%)	0.6	0.7
Phospholipids (mg, %)	42.5	38.0
Total cholesterol (mg, %)	275.0	330.0
Lanosterol (mg %)	8.27	9.32
Lutein (µg / g)	3.1	4.2
Squalene (µg / g)	62.4	59.2
Carotene (µg / g)	0.0	7.2
Vitamin A (µg / g)	9.5	9.2
Vitamin E (µg / g)	26.4	30.5
Ubiquinone (µg / g)	6.5	5.0

2.2.1. Fatty acid composition

Among all natural fats, milk fat is the most unique with regard to its chemical characteristics. Milk fats derived from ruminant animals, contain an exceptional number and variety of fatty acids from $C_{4:0}$ to $C_{26:0}$ and from $C_{10:1}$ to $C_{22:6}$ combined as triglycerides. Jensen and Clark (1988) detected over 400 distinct fatty acids in bovine milk fat. Other land animal fats like tallow and lard contain mostly palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids, while vegetable oils like ground nut, sesame or cottonseed consist mainly of palmitic, stearic, oleic and linoleic ($C_{18:2}$) acids. Coconut oil is the best known exception, containing lauric ($C_{12:0}$) and myristic ($C_{14:0}$) acids in very large amounts (Rangappa and Achaya, 1974).

Some Indian workers (Rama Murthy and Narayanan, 1971; Arumughan and Narayanan, 1982; Lakshminarayana and Rama Murthy, 1985) reported the fatty acid composition of milk fats of cow and buffalo. The work reported by them definitely established that the fatty acid composition of buffalo milk fat distinctly differed from that of cow milk fat. It was observed that buffalo milk fat was richer in butyric ($C_{4:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids as compared to cow milk fat. The levels of the short chain fatty acids, caproic ($C_{6:0}$) to myristic ($C_{14:0}$), were significantly higher in cow ghee than buffalo ghee. The triglyceride structure of buffalo milk fat was also shown to be different from that of cow milk fat (Rama Murthy and Narayanan, 1975). The typical fatty acid composition of cow and buffalo milk fats as reported by Rama Murthy and Narayanan (1971) is shown in Table C.

Table: C. The fatty acid composition of cow and buffalo milk fat

Fatty acids	Buffalo milk fat	Cow milk fat
	(Wt%)	
C4:0 butyric	4.36	3.20
C6:0 caproic	1.51	2.11
C8:0 caprylic	0.78	1.16
C10:0 capric	1.28	2.57
C10:1 decinoic	trace	0.31
C12:0 lauric	1.78	2.78
C14:0 myristic	10.81	11.93
C14: 1myristoleic	1.27	2.12
C15:0	1.29	1.23
C16:0 Br.	0.18	0.30
C16:0 palmitic	33.08	29.95
C16:1 palmitoleic	1.99	2.16
C17:0	0.58	0.34
C18:0 Br.	0.24	0.35
C18:0 Stearic	11.97	10.07
C18:1 oleic	27.15	27.42
C18:2 linoleic	1.51	1.49
C18:3 linolenic	0.47	0.59

2.2.2. Variations in fatty acid composition

Fatty acid composition of milk fat is subjected to wide variations. Factors such as breed, feed, season and stage of lactation have a great influence on fatty acid composition. The effect of dietary roughage on fatty acid composition was assessed by several workers (Batch *et al.*, 1952 and Brown *et al.*, 1962) who found that there was an increase in the concentration of short chain fatty acids of milk fat with feeding of higher amount of roughage supplements to the animals. Addition of high amounts of fat to the diet decreased the levels of short chain fatty acids of milk fat (Parry *et al.*, 1964).

The fatty acid composition of milk fat as influenced by season was investigated by many workers (Patton *et al.*, 1960a; Jenson *et al.*, 1962; Rama Murthy and Narayanan, 1971; Joshi and Vyas, 1976 a). In general, it was found that during summer months, the milk fat contained higher amounts of $C_{18:0}$ and $C_{18:1}$ acids and lower amounts of short chain fatty acids than during winter months.

2.2.3. Polyunsaturated fatty acids

The composition of polyunsaturated fatty acids of bovine milk fat was determined by many workers (Smith and Jack, 1954; Smith, 1961; Boatman *et al.*, 1965) by using alkali isomerization method. Rama Murthy and Narayanan (1972) made a comparative study of the contents of the PUFA of cow and buffalo milk fats and the data are shown in Table D.

Table: D. Polyunsaturated fatty acid contents of cow and buffalo milk fat

PUFA		Cow milk fat	Buffalo milk fat
		(Wt%)	
Dienoic	Conj.	0.821	0.725
	Non-conj.	1.100	0.942
	Total.	1.921	1.667
Trienoic	Conj.	0.027	0.025
	Non-conj.	0.538	0.519
	Total	0.565	0.544
Tetraenoic	Conj.	0.003	0.006
	Non-Conj.	0.131	0.196
	Total.	0.134	0.202
Pentaenoic	Conj.	0.000	0.002
	Non-Conj.	0.062	0.105
	Total	0.062	0.107

Among the diene conjugated fatty acids, the major constituent is conjugated linoleic acid (CLA). CLA is a mixture of isomers of linoleic acid, the main component being Cis-9, trans –11-octadeca-dienoic acid. The presence of CLA in milk fat was first established by Booth *et al.* (1935). Aneja and Murthy (1990) reported that they were also formed during fermentation of milk and heating of milk fat in presence of milk proteins. Several workers have shown anticarcinogenic, antiatherogenic and immunomodulating activities of CLA (Parodi, 1994; Vissonneau *et al.*, 1996). Pariza (1991) described CLA as a new cancer inhibitor in dairy products. Of all the natural fats, milk fat is a rich source of CLA giving it a nutritional supremacy (Neelam and Tilak, 2001).

2.2.4. Moisture

It is almost impossible in practice, to remove all traces of moisture from ghee. If high moisture is present in ghee as a result of improper clarification, the keeping quality of ghee would be adversely affected. The presence of moisture accelerated the hydrolysis of fats, and thereby released free fatty acids, which were prone to quicker autoxidation than intact glycerides (Lalitha and Dastur, 1953).

2.2.5. Cholesterol

Cholesterol is the major constituent of the unsaponifiable matter of milk fat. It exists both in the esterified and in free forms. Bindal *et al.* (1972) determined the proportions of free and esterified cholesterol in *desi* ghee by TLC method. Later, Bindal *et al.* (1973) reported that cholesterol content was higher in cow ghee (330 mg/100g) than in buffalo ghee (275 mg/100g). The above authors also observed that the total cholesterol content was significantly influenced by season. Antila and Antila (1976) reported that cholesterol content of milk fat ranged from 241.6 to 386.5 mg/100 g, with a mean value of 302.2 mg/100g.

2.2.6. β-Carotene and Vitamin A

Average values of β-carotene and vitamin A for cow and buffalo ghee respectively were: carotene 4.8 and nil μg/g, and vitamin A 25.9 and 25.9 IU/g (Narayanan *et al.*, 1956). Kehar *et al.* (1956) reported that carotene content of milk fat of Indian breeds of cattle ranged from 2.56 to 4.03 μg/g. The vitamin A content ranged from 20.1 to 38.9 IU per gram of Murrah buffalo ghee. The β-carotene content of Sahiwal ghee ranged from 5.98 to 7.01 μg/g while vitamin A ranged from 26.8 to 56.20 IU/g (Becon and Bector, 1998).

In 58 samples of market ghee, Kehar *et al.* (1956), found no carotene at all and vitamin A content of only 4.0 – 4.8 IU/g.

2.3. PHYSICO-CHEMICAL CHARACTERISTICS

Fats and oils exhibit certain physical and chemical characteristics that have been employed for their characterization and differentiation. Six analytical characteristics may be considered of basic importance. Of these, three determinations, the Reichert value, the Polenske value and the iodine value, measure certain specific constituents of milk fats. The other characteristics, the saponfication value, the butyrorefractometer reading and the melting range, give an indication of the overall average nature of the constituent fatty acids present (Rangappa and Achaya, 1974). However, these characteristics show variations due to factors such as species, breed, feed, stage of lactation as the fatty acid composition is affected by these factors.

2.3.1. Reichert – Meissl (RM) value

RM value is substantially a measure of the short chain fatty acids of milk fat, viz., butyric, caproic and caprylic acids. The extent of these acids contributing to RM value of butterfat was found to be 65.3%, 30.1% and 4.6% respectively (Sengupta *et al.*, 1958). A considerable work on the RM value of ghee of different breeds of cow and buffalo was carried out (Doctor *et al.*, 1940; Singh *et al.*, 1946; Singh and Singh, 1960; Basu *et al.* 1948; Javeed *et al.*, 1986).

While extreme values of 14 to 44 were reported in the literature for genuine cow and buffalo ghee samples, the average value for ghee (milk fat) samples reported was 22.6 – 34.5 (Arumughan and Narayanan, 1982; Laksminarayana and Rama Murthy, 1985).

Kehar *et al*. (1956) reported that the average RM value of cow and buffalo ghee collected from 12 different parts of India was 24.29 and 29.44 respectively. Singh and Gupta (1982) reported RM value of 28.52 ± 0.35 and 30.84 ± 0.68 for cow ghee and buffalo ghee respectively. RM value of 24.6 and 30.9 for cow and buffalo ghee was reported by Lakshminarayana and Rama Murthy (1985). Sampath and Anantakrishnan (1956) studied the seasonal variation in the RM value of cow, buffalo and mixed ghee. Joshi and Vyas (1976 a) also studied the seasonal variation in RM value of buffalo ghee.

RM value is one of the specifications of PFA and AG Mark imposed for ghee. Since RM value is subjected to variation by feed and breed, the PFA specification for this characteristic varies from state to state.

2.3.2. Polenske value (PV)

Polenske value is a measure of medium chain fatty acids of milk fat. Sengupta *et al*. (1958) reported that caproic, caprylic, capric and lauric acids contributed to 0.6, 22.1 and 76.7 and 0.6% respectively to Polenske value of butterfat. Various workers have studied the Polenske value of ghee of different breeds of cows and buffaloes (Doctor *et al.*, 1940; Singh *et al.*, 1946; Singh and Singh, 1960; Basu *et al.*, 1948; Javeed *et al.*, 1986). The PV of cow and buffalo ghee collected from 12 different parts in India respectively was 1.77 and 1.78 as reported by Kehar *et al.* (1956). Average Polenske values of 1.2 and 1.5 were reported for buffalo and cow ghee respectively by Lakshminarayana and Rama Murthy (1985). Joshi and Vyas (1976 a) studied the seasonal variation in PV of buffalo ghee. While extreme values of 0.35 to 5.3 were reported for ghee samples, average PV of many works was reported to be between 0.7 and 2.0 (Katrak *et al.,* 1946 and Singh and Singh, 1960; Arumughan and Narayanan, 1982 and Laksminarayana and Rama Murthy, 1985).

Since the presence of lower fatty acids is peculiar to ruminant milk fats, RM and PV are important characteristics of ghee. Of the common vegetable oils, only coconut and palm oil contain steam volatile acids and both exhibit an RM of 7 and PV of 13 (Rangappa and Achaya, 1974).

2.3.3. Saponification value (SV)

Saponification value is an indirect measure of average molecular weight of component fatty acids. Earlier, Doctor *et al.* (1940) and Basu *et al.* (1948) studied the saponification value of ghee of different breeds of cattle and Murrah buffaloes. SV for cow and buffalo ghee as determined by Singh and Gupta (1982) were 234 .12 ± 3.52 and 236.60 ± 2.45. Joshi and Vyas (1976 a) studied the seasonal variation in saponification value of buffalo ghee. Since the SV is inversely proportional to the molecular weight, a higher SV for ghee will result from either an increase in the proportion of lower fatty acids, or a decrease in that of higher acids like palmitic, stearic and oleic, either individually or collectively (Rangappa and Achaya, 1974).

2.3.4. Iodine value (IV)

Iodine value is a measure of the extent of unsaturation in milk fat. It ranges from 27– 35 with an average value of 30. Doctor *et al.* (1940) and Singh *et al.* (1946) reported iodine value ranging from 30.7 to 39.1 for ghee of Indian breeds of cows and 29.5 to 37.4 for ghee of Murrah buffaloes. Lakshminarayana and Rama Murthy (1985) reported the average iodine values of 31.1 and 32.2 for buffalo and cow ghee respectively. Singh and Gupta (1982) also reported the iodine values of 34.05 ± 0.31 and 32.01 ± 0.46 for cow and buffalo ghee respectively. Sampath and Anantakrishnan (1956) and Joshi and Vyas (1976 a) studied the seasonal variation in the iodine value of cow and buffalo ghee.

2.3.5. Butyro-refractometer (BR) reading

The BR reading of ghee of Indian breeds of cows and Murrah buffaloes was in the range of 43 to 43.7 and 42.5 (Doctor *et al.*, 1940). Singh *et al.* (1946) reported BR reading of 44.1 to 44.5 for ghee of Indian breeds of cows and 43.6 for ghee of Murrah buffaloes. Singh and Singh (1960) found BR of cow ghee in the range of 40.35 – 44.8 while that of buffalo ghee in the range of 40.6 – 44.3. Lakshminarayana and Rama Murthy (1985) observed a Refractive Index of 1.4533 for buffalo ghee and 1.4543 for cow ghee. Like the RM value, BR reading of ghee shows variation and therefore the specifications by PFA for this characteristic varies from state to state (PFA, 2005). Most oils and fats have a BR value of about 75, i.e. a refractive index of 1.4575. An increase of BR in ghee will be caused by a decrease in the content of lower fatty acids, or by an increase either in higher saturated or unsaturated fatty acids, particularly the latter (Rangappa and Achaya, 1974).

The major physico- chemical characteristics are interrelated with each other. Indications of some degree of correlation between the various analytical characteristics of ghee are often apparent on examining the results of individual analysis. A high RM value is frequently found associated with a high PV and SV, and a low IV and BR. Katrak *et al*. (1946) found all the characteristics to be distinctly correlated. Mitra (1964) stated that minimum SV values for RM values of 28, 26, 24 and 21 were 222, 220, 219 and 213 respectively.

2.3.6. Melting Point

Melting point, melting interval and solidification point are the commonly determined characteristics of milk fat. The temperature at which previously solidified fat transforms completely into liquid state represents melting point. If melting occurs over a particular temperature range, it is called the melting interval.

Data from the studies of Godbole and Sadgopal (1939) showed that the melting point for cow ghee varies from 28.5° to 42.0°C and for buffalo ghee it ranged from 32.0° to 43.5°C. Both cow and buffalo ghee appears to have a melting point of about 36.5°C (Bhalerao *et al*., 1947).

Since the melting point of various oils and fats vary over a wide range, this property has been employed for checking the adulteration of milk fat. Body fats (36°-51.3^{O} C) and vanaspati (37.8-38^{0}C) have slightly higher melting point (Winton and Winton, 1999) while vegetable oils have lower melting point than milk fat (28-41^{O}C) (Kumar Arun, 2002). Sharma and Singhal (1995) reported that adulteration of ghee with body fats (buffalo, goat, pig and sheep) up to 20% level does not make significant change in the melting point of the sample; therefore, the method was not found to be useful for the detection of adulteration.

2.4. KEEPING QUALITY

Unlike all other indigenous milk products, ghee has a remarkably long keeping quality. The keeping quality of ghee is adversely affected by increase in acidity and development of oxidised off-flavours. Autoxidation is considered to be one of the main causes for flavour defects in dairy products. Therefore, the flavour stability of ghee is usually assessed by estimating free fatty acids and peroxide value.

2.4.1. Free fatty acids

Small proportion of fatty acids is always present in fresh milk fat; higher levels are found in the milk fat isolated from milk or cream that has been subjected to bacterial action or in fat that has been stored for some time. The presence of free acids in unlipolyzed fat may be the result of an incomplete esterification of glycerides in mammary gland (Kurtz, 1980). Small amount of free fatty acids in ghee (~0.15% oleic acid) did not affect its keeping quality. In high acid ghee samples, there is tendency for rapid development of acidity. Paul and Anatakrishnan (1949) showed that longer storage of curd led to greater development of acidity in ghee.

2.4.2. Peroxide value

The unsaturated fatty acids of fat undergo oxidation to produce hydroperoxides as the primary oxidation products. Though the peroxides as such are not responsible for the development of off-flavours, their measurement gives a good indication to assess the degree of autoxidation. The rate of autoxidation was reported to be affected by several factors which influence the keeping quality of ghee.

Ghee made by desi method had a tendency to develop peroxides more rapidly than creamery ghee (Lalitha and Dastur, 1953). Ghee clarified at higher temperature (120°C) developed peroxide at a significantly slower rate as compared to those clarified at lower temperature (Narayanan *et al*., 1966).

Temperature of storage, antioxidants, metal contamination, dissolved oxygen, exposure to light are some other factors which influence the keeping quality of ghee (Ray, 1957; Mukherjee, 1950; Vachha *et al.,* 1958).

2.5. DETECTION OF ADULTERATION

2.5.1. Detection of vegetable oils/ animal body fat

Detection of adulteration of milk fat with animal body fat/vegetable oil has been of concern to chemists for more than a century. Several methods are available for the detection of adulteration of milk fat by foreign fat(s). These tests are mainly based on the differences in the nature and contents of major/minor components of milk fat and adulterant fats/oils. But there is no single general method available for detecting milk fat adulteration, which is applicable to all possible adulteration situations; rather a number of methods have to be applied to detect the presence of adulterants in ghee.

2.5.1.1. Physico-chemical characteristics

Five analytical characteristics may be considered of basic importance. Of these, three determinations, the RM value, the Polenske value and the iodine value measure certain specific constituents of milk fats. Two other characteristics, the saponification value and the BR reading or RI give an indication of the overall average nature of the constituent acids present. Milk fat is unique in that it contains a large proportion of fatty acids of chain length lower than 12 carbon atoms, and in consequence many of its characteristics are quite distinctive. The determination of these analytical characteristics, therefore, is a useful tool and commonly practiced to detect adulteration of ghee. However, these characteristics of ghee cover a very wide range, permitting fairly high degree of adulteration while still keeping within the natural limits. The feeding of oil cakes can also alter the individual analytical characteristics of ghee so as to bring perfectly genuine specimens under suspicion of adulteration (Rangappa and Achaya, 1974).

2.5.1.2. Crystallization test

Panda and Bindal (1998) developed a test known as crystallization test to detect the adulteration of ghee with animal body fats and vegetable oils separately or in combination. This test measures the time taken for the crystallization of melted, filtered fat (0.8 ml), which is treated with 2.5 ml of solvent mixture (acetone: benzene

3.5:1) at 17 ^{O}C. The crystallization time for normal ghee was 19 min., while adulterated samples showed lower or higher values. Ghee adulterated with body fats took 4-12 min while that with vegetable oils took 23-27 min for crystallization. However, ghee from cotton tract area was being suspected for adulteration with animal body fat when the test results were applied. Accordingly they have modified the test to remove this ambiguity. They concluded that even a low level of adulteration of ghee with animal body fats (5%) and vegetable oils/fats including coconut oil (10%) could be detected using this method.

2.5.1.3. Opacity test

Singhal (1973) developed an opacity test to detect the adulteration of ghee with animal body fats based on the solidification time of the melted fat samples at 23°C at 570 nm in a colorimeter (>0.5 OD). Normal ghee took more than 35 min, where as animal body fats (buffalo, goat and sheep) took only 10-20 s to become opaque. By this test, the adulteration of ghee with buffalo, goat and sheep body fats at 5% level and above could be safely detected (Sharma and Singhal, 1996). But it failed to detect pig body fat up to 10% level of adulteration as well as an admixture of body fat and vegetable oil (Singhal, 1987). Panda and Bindal (1998 a) modified the procedure and found that the modified test could detect the vegetable oils including coconut oil even at 5 % level of adulteration.

2.5.1.4. Fractionation of ghee

Shipe (1955) suggested a method for the identification of vegetable and animal body fats in butter fat by urea fractionation. It was an empirical method and deviation from the outlined procedure may alter the nature of the fractionation. Moreover, it would be difficult to detect by this method coconut oil or a synthetic fat mixture having the similar fatty acid composition as butter fat. Bhalerao and Kummerow (1954, 1956 a, b) separated fat into solid (30 %) and liquid (70 %) fractions after dissolving it in hot absolute alcohol and maintaining the same at 20^{O} C for 2 h. The solid fraction was further fractionated using acetone at 0^{O} C and subsequently subjected to refractive index measurement. Adulteration of vegetable oils in butterfat up to 10 % could be detected. Selective solidification was used by several workers

(Krienke, 1953) for the detection of butter fat adulteration. Farag *et al.* (1983) carried out the fractional crystallization of fat (pure and adulterated samples) dissolved in silver nitrate-saturated methanol/acetone (70:30) in a ratio of 1:10 at 22, 7 and -8^{O} C and the three fractions obtained were subjected to GLC for their fatty acid profile. They reported that for detecting adulteration, 18:0 and 18:1 fatty acids were of great importance in first fraction, whereas 22:0 is important in second and third fraction. Fractionation approach for enriching the solid fraction with body fats and hydrogenated fats, and liquid fraction with vegetable oils is expected to hold a good potential to be exploited for solving the problem of adulteration (Kumar *et al.*, 2002).

2.5.1.5. Methods based on thin layer chromatography (TLC)

Mc Gugan (1959) could detect the adulteration of ghee with vegetable oils or body fats (10% level) over silica gel G layer using 5 % ethyl acetate in n-hexane. But coconut oil examined could not be detected even at 20 % level. The method reported by Roos (1966) to detect vegetable oils in milk fat was based on the identification of phytosteryl acetate band at low Rf value compared to that of cholesterol over silica gel G thin layer. Roose (1963) also 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 using reverse phase system consisting of undecane/acetic acid acetonitrile saturated with undecane.

Rama Murthy *et al.* (1967) described a TLC method using glass slides coated with a slurry of calcium carbonate and soluble starch, impregnated with liquid paraffin and a solvent system consisting of methanol: acetic acid: water (20:5:1,v/v) as a developer. Coconut oil at 25% in ghee could be detected by this method. Chakrabarty *et al.* (1968,1969) employed TLC technique for the detection of adulteration of milk fat with hydrogenated groundnut oil, tallow and mohua oil. Adulterated butter fat had a higher number of components than pure butter fat, the number depending on the type of adulterant. The lower limit of detection by this method was 5 %. Maglitto *et al*. (1970) recognized butter adulteration with synthetic esterified fat at 10 % level using unsaponifiable matter over silica gel plates.

Parodi (1972) distinguished synthetic milk fat (a mixture of beef tallow, coconut oil and tributyrin with subsequent inter-esterification) from butterfat over TLC using silica gel G layers developed in solvent mixture of hexane: di iso propyl ether 80:20 (v/v), and 93:7 (v/v) respectively for examining free and esterified sterols. Carisano and Riva (1976) described a method for detecting adulteration of butter using silica gel TLC. The procedure is based on TLC separation of a carbon tetrachloride solution of butterfat on silica gel into long and short-chain triglycerides, selective lipolysis of each fraction with pancreatic lipase and determination of the C_{18}:C_{16} ratio. They detected as low as 5% beef tallow in butter fat. They failed to detect 20% adulteration of a mixture of hydrogenated coconut (7%) palm (63%) and groundnut oil (30%). Mathew and Kamath (1978) developed a TLC method for the detection of vegetable oils/vanaspati in ghee. The method was sensitive enough to enable the detection of adulteration down to 2% level.

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) mixture as the solvent system and reported that ghee samples adulterated with 10% body fats resulted in the appearance of an extra spot due to dihydro cholesterol present in body fats. He could also detect 10% coconut oil by this technique.

2.5.2. Detection of mineral oil

Mineral oil or solid paraffin is considerably cheaper than ghee and encountered by city analysts. However, the information regarding the adulteration of ghee with mineral oil is not available in literature. Unlike oils and fats, mineral oils are not saponifiable by alkali. On this has been based a test for the presence of adulterant mineral oil in ghee.

The test sample (1 ml) was saponified with 5 ml of 1.4 N alcoholic potash; to 1 ml of the solution in a clean test tube 10 ml of 50 % alcohol was added followed by thorough shaking. Appearance of turbidity indicates the presence of mineral oil. The test is sensitive to the presence of even 1 per cent of mineral oil in ghee (Venkatachalam and Sundaram, 1957).

2.5.3. Detection of added colours

Despite the ban, yellow colours such as annatto, turmeric and coal tar dyes are sometimes added to buffalo ghee so as to pass it off as cow ghee, which fetches a higher price. Various methods have been suggested for examining such colours.

The ghee (5 g) was added with 10 ml of a solvent mixture of carbon disulphide and alcohol (2:15) in a separating funnel and the contents were shaken. The partition of colour to the upper alcohol layer indicated the presence of added colours such as annatto, turmeric, ceres yellow 39, ceres orange and sudan 1. Carotene and browning pigments remain with the fat (Roy and Sen, 1961).

2.6. QUALITY OF MARKET GHEE

Ghee is an expensive product, costing three times as much as edible vegetable oils. Thus its adulteration is a very profitable proposition and one widely practiced. Indeed adulteration is the lesser evil; even complete substitution of ghee with other fats is not unknown. Information regarding the quality of market ghee is scanty. A few surveys have been carried out all over India to check the quality of ghee sold in market. Subrahmanyam *et al.* (1952) reported that of a large number of samples drawn from the Bangalore and Mysore markets, one –third contained no ghee at all and one-half were grossly adulterated.

The work carried out on the quality evaluation of ghee from Bombay city revealed that of the samples collected, 19.0 % failed with respect to moisture, 18% failed with respect to RM and PV values when compared with PFA and AGMark standards. Baudouin test was found positive in 20 % of the samples (Sharma and Zariwala, 1978).

Ghatak and Bandyopadhyay (1989) assessed quality of ghee marketed by organised and unorganized dairies in Calcutta and Kalyani (West Bengal). Flavour, colour and texture of ghee from organised dairies were superior to that from unorganized dairies. Levels of moisture, free fatty acids and peroxide value in ghee from unorganized dairies were much higher than the ghee from organised dairies. Some samples of ghee from unorganised dairies did not confirm to PFA standards with respect to moisture and RM value. None of the ghee samples was found to be adulterated with animal body fat. On the other hand, vanaspati was detected in 11 ghee samples from unorganized dairies (Ghatak and Bandyopadhyay, 1989).

Rao *et al.* (2004) analysed the ghee samples from organised and unorganised market of Hyderabad and found that many ghee samples from unorganized market did not confirm the PFA and AG Mark standards with respect to moisture, free fatty acids and RM value. Thirty five per cent of the samples from local market were tested positive for Baudouin test. However, samples from the organised dairies (private + co-operative) were found to meet the PFA standards.

3.0 MATERIALS AND METHODS

3.1. COLLECTION OF MARKET GHEE SAMPLES

Market samples of ghee from organised and unorganized sectors were collected from the various parts of the Bangalore city. The samples of ghee were repacked in glass bottles and stored in refrigerator.

3.2. PREPARATION OF GHEE

Cow milk collected from the Experimental Dairy of the Institute was separated into cream (40% fat). Cream was aged at 10° C for 12 h and was churned into butter using a domestic mixer grinder (National). Butter so obtained was washed gently with cold water and then heated in a stainless steel vessel to remove moisture. Heating was continued till the curd become golden brown and the final temperature was not allowed to exceed 120°C. The prepared ghee was allowed to settle and filtered through a double folded muslin cloth. The samples were filled in clean and dry glass stoppered bottles, cooled to room temperature and thereafter stored in refrigerator.

3.3. SENSORY EVALUATION

All the market samples of ghee were evaluated for their sensory characteristics on a 9 point hedonic scale by a panel of judges. The samples were evaluated for their appearance and colour, flavour, body and texture and overall acceptance. The 9 point hedonic scale score card for sensory analysis is given in the Appendix.

3.4. SOLVENTS

AR grade reagents were used. The unspecified grade solvents were purified as per prescribed procedures.

3.5. DETERMINATION OF PHYSICO-CHEMICAL CHARACTERISTICS

3.5.1. Reichert Meissl value and Polenske value

Reichert-Meissl and Polenske values were determined by the method specified in BIS: 3508. Ghee (5 g) was saponified using glycerol and 50 % NaOH, diluted with water and acidified, and thereafter steam- distilled in a glass apparatus at a controlled rate. The condensed and cooled distillate is filtered and the water-soluble acids that pass through were estimated by titration with 0.1N NaOH to give the Reichert Meissl value. While the water-insoluble acids collected on the filter paper were dissolved out in alcohol and titrated to give the Polenske value. The blank tests were conducted in the same way without using ghee sample.

Reichert- Meissl value = 1.10 (T1-T2)

Polenske value = (T3-T4)

Where T1= volume in ml of 0.1N NaOH used for sample for titration (water soluble)

T2= volume in ml of 0.1N NaOH used for blank (water soluble)

T3= volume in ml of 0.1N NaOH used for sample (water insoluble)

T4= volume in ml of 0.1N NaOH used for blank (water insoluble)

3.5.2. Saponification value

Saponification value was determined by the method specified in BIS: 3508. Ghee sample (2 g) was saponified with and excess of alkali (0.5N KOH) dissolved in alcohol. Some of the alkali was used up in saponifying the fat to soap of its fatty acids and glycerol; the remainder was estimated by titrating against standard acid (0.5N

HCl) using 1% phenolphthalein as indicator. A blank determination was made upon the same quantity of the KOH solution at the same time under the same conditions. Saponification value was determined by the following formula

$$\text{Saponification value} = \frac{28.05\,(T2 - T1)}{W}$$

Where T2= volume in ml of 0.5 N acid required for the blank

T1=volume in ml of 0.5 N acid required for the sample, and

W= weight (g) of the sample taken

3.5.3. Iodine value

Iodine value was determined by the method specified in BIS: 3508 (Wij's method). Clear ghee (0.40-0.45 g) taken in a dried conical flask was added with 15 ml of carbon tetrachloride and 25 ml of Wij's reagent. The contents in the flask were kept in dark for 1 h. Potassium iodide solution (20 ml) and distilled water (150 ml) were added and mixed. The contents were titrated against 0.1 N sodium thiosulphate solution using 2 ml of 1 % starch solution as indicator. A blank test was carried out using the same quantities of the reagents. Iodine value was determined by the following formula:

$$\text{Iodine value} = \frac{12.69\,(B-S)\,N}{W}$$

Where B= volume in ml of standard sodium thiosulphate solution required for blank

S= volume in ml of standard sodium thiosulphate solution required for sample

N= normality of the standard sodium thiosulphate solution, and

W= weight in g of material taken for the test

3.5.4. Refractive Index

Refractive index of all the ghee samples was determined as per ISI procedure (1981) using Abbe refractometer (Carl-Zeiss).

3.5.5. Butyro-refractometer reading

The butyro refractometer reading was measured by using butyrorefractometer.

3.5.6. Melting point

Melting range of ghee samples was determined by slip point method of ISI (1981).

3.5.7. Moisture content

The moisture content was determined as per ISI procedure (1981).

3.5.8. Free fatty acids

The level of free fatty acids in ghee samples was determined as per ISI procedure (1981) and was expressed as percentage of oleic acid.

3.5.9. Peroxide value

The peroxide value was determined as per the ISI procedure (1981).

3.5.10. Total carbonyl value

Total carbonyl compounds in the ghee samples were determined by the modified method of Rama Murthy and Jain (1973).

3.5.10.1. Preparation of carbonyl free hexane

Contaminant carbonyl compounds in hexane were removed using the method of Schwartz and Parks (1961). One gram of 2,4-dinitrophenyl hydrazine (DNPH) was dissolved in 12 ml of 85% o-phosphoric acid by grinding in a 6 ” mortar. About 8 ml of water was added to the clear yellow solution and the precipitate formed was dissolved by further grinding. Then 20 g of celite-545 was added and ground until a homogenous damp preparation was obtained. The bright yellow 2,4-DNPH impregnated celite was poured into a chromatographic column (20x25 cm) containing a plug of glass wool at the constricted portion of the tube and filled with hexane. Five milliliters of hexane was allowed to pass through the column by opening the stopcock. The slurry was packed by gentle pressure using a flattened glass rod. The hexane in the column was drained and 100 ml of benzene was flushed through the column material using gentle pressure in order to remove the colour impurities, which behaved like aliphatic monocarbonyls DNP hydrazones. Hexane was stirred with excess DNPH reagent for 15 min and an aliquot was removed to serve as blank. The treated hexane was passed through the column at flow rate of 4 ml per min. the first 50 ml of the effluent was discarded and the remaining portion of the effluent was collected and distilled to get carbonyl free hexane.

3.5.10.2. Preparation of 2,4-DNPH-celite powder

A column of celite impregnated with 2,4-DNPH, phosphoric acid and water was prepared as described above. The column was flushed with 50 ml of benzene followed by carbonyl free hexane until a colourless effluent was obtained. The celite-DNPH powder present in the column was flushed out of the column, using pressure, into a glass container. The material was dispersed into a free flowing powder.

3.5.10.3. Spectrophotometric measurement

An aliquot (0.1 g) of the milk fat sample was weighed in glass stoppered conical flask. It was dissolved in 0.5 ml of carbonyl free hexane followed by addition of 0.5 g of DNPH-celite powder. The flask was stoppered, shaken well and was allowed to stand overnight at room temperature. Then the volume was made up to 10 ml using carbonyl free hexane. The optical density of the solution was read at 345 nm against the control prepared in a similar manner but without fat. The total carbonyl value was calculated using the formula:

$$\text{Total carbonyl value, } \mu\text{m/g fat} = \frac{\text{Optical density x total volume (ml)}}{\text{22.5 x weight (g) of sample taken}}$$

3.6. SPECIFIC TESTS TO DETECT ADULTERATION

3.6.1. Detection of presence of added colours

Ghee (5 g) was added with 10 ml of a solvent mixture of carbon disulphide and alcohol (2:15) in a separating funnel and the contents were shaken. The partition of colour to the upper alcohol layer indicated the presence of added colours such as annatto, turmeric, ceres yellow 39, ceres orange and sudan 1. Carotene and browning pigments remain with the fat (Roy and Sen, 1961).

3.6.2. Detection of presence of mineral oil in ghee

The test sample (1 ml) was saponified with 5 ml of 1.4 N alcoholic potash; to 1 ml of the solution in a clean test tube 10 ml of 50 % alcohol was added followed by thorough shaking. Appearance of turbidity indicated the presence of mineral oil. The test is sensitive to the presence of even 1 per cent of mineral oil in ghee (Venkatachalam and Sundaram, 1957).

3.6.3. Detection of presence of vanaspati (Baudouin test)

Baudouin test was carried out by the procedure given by BIS: 3508. Melted ghee (5 ml) was taken in a glass stoppered test tube and 5 ml of hydrochloric acid and 0.4 ml of furfural solution were added to it. A glass stopper was inserted to the tube and the contents were shaken vigorously for two minutes. The layers of the mixture were allowed to separate. The development of a pink or red colour in the acid layer indicated the presence of vanaspati.

3.6.4. Detection of dissolved soaps

The presence of dissolved soaps was determined as per ISI procedure (1981).

3.6.5. Crystallization pattern of ghee samples

Crystallization pattern of ghee was studied employing the method of Panda and Bindal (1998).

Clear melted fat (0.8 ml) was transferred with the help of 1 ml pipette to the glass tube ($L=10 \pm 0.1$ cm; $OD=1.2 \pm 0.02$ cm; $ID=1.0 \pm 0.02$ cm). To this, 2.5 ml of solvent mixture (acetone: benzene; 3.5:1) was added. The contents in the glass tube were mixed thoroughly and placed in a water bath for 3 min, maintained at 20°C to equilibrate the contents. Thereafter, the tube was placed in a water bath maintained at 17±0.2°C. Then the time required for crystallization to occur in pure ghee and adulterated samples was noted.

The results were expressed as below:

a) Negative crystallization	-
b) Slight crystallization	+
c) Fair crystallization	++
d) Excellent crystallization	+++

3.6.6. Fractionation of ghee

Melted ghee samples were taken in centrifuge tubes and incubated at 25°C for 12 h in a BOD incubator. Incubated ghee samples were centrifuged in a centrifuge (Remi) at 2000 rpm for 20 min at 25°C. The liquid and solid fractions were separated and their yield was measured.

4.0 RESULTS AND DISCUSSION

In the present study, ghee samples (24) from the Bangalore market were collected and evaluated for quality as per the objective outlined in Chapter 1. The results of the study along with their interpretation are presented in this chapter.

4.1. INFORMATION ABOUT MARKET GHEE SAMPLES

The samples of ghee for the study were procured from the Bangalore market. Bangalore, being one of the fastest growing cities of India, is a good source of many brands of ghee samples made within as well as outside the city. However, the availability of unbranded samples is limited probably due to the market being urban in nature. Seventeen samples from the organised sector and seven samples from the unorganised sector were collected and analysed.

It may be seen from the Table 1 that of the samples collected from the organised sector, two samples belonged to two different milk cooperatives and the rest were manufactured by private agencies. Two samples were packed in bottles and the rest were packed in flexible pouches. The price of ghee ranged from Rs.78 to 99 for a quantity of 500 ml. As per the label, five brands were of AG Mark special grade, one brand belonged to Agmark general grade and the rest of 11 samples did not declare the grade.

Based on the label information, the keeping quality of most of the samples was 6 months from the date of manufacture. Manufacturers of sample No. 2 and 4 declared a shelf life of 5 and 9 months respectively, while those of sample No. 13 declared shelf life of 12 months. The samples from the unorganized sector were sold loosely and were priced from Rs. 35 to 90 for a quantity of 500 ml. The variation in the price of ghee from unorganized sector was very high (Table 2).

Table: 1. Information about ghee samples from the organized sector

Sample Code	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Quantity (ml)	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
Packaging Material	FP	FP	FP	FP	FP	FP	FP	FP	FP	FP	Glass bottle	FP	FP	FP	FP	Plastic bottle	FP
Grade	AG (S)	AG (S)	-	AG (S)	AG (S)	-	-	-	-	AG (S)	-	-	AG (G)	-	-	-	-
Price Max. Rs.	78	90	91	79	99	82	82	90	87	83	95	90	90	87	86	78	78
Shelf life (Months)	6	5	6	9	6	6	6	6	6	6	6	6	12	6	6	6	6
Manufacturer	Co-op.	Pvt	Pvt	Co-op.	Pvt	Pvt	Pvt	Pvt	Pvt	Co-op	Pvt	Pvt	Pvt	Pvt	Pvt	Pvt	Pvt

FP=Flexible pouch; AG (S)=AG Mark Special; AG (G)=AG Mark General; Co-op.=Cooperative Dairy; Pvt= Private Dairy

Table: 2. Information about ghee samples from the unorganized sector

Sample Code	01	02	03	04	05	06	07
Quantity (ml)	500	500	500	500	500	500	500
Packaging Material	-	-	-	-	-	-	-
Grade	-	-	-	-	-	-	-
Price Max. Rs.	90	73	55	87	35	70	83
Shelf life (Months)	-	-	-	-	-	-	-
Manufacturer	-	-	-	-	-	-	-

4.2. SENSORY EVALUATION OF GHEE SAMPLES

The ghee samples were subjected to sensory evaluation on a nine-point hedonic scale for appearance and colour, flavour, body and texture and overall acceptance by a panel of judges. The data on the mean scores of sensory evaluation for the samples from the organised and unorganized sector are presented in Table 3 and 4 respectively. The samples from the organised sector were having light to deep yellow colour. Some samples were having brownish colour, which may be due to high clarification temperature. Three samples were found having ghee residue. As may be seen from the Table 3 that the scores for the colour were in the range of 6 to 8. The scores for ghee samples from the unorganized sector were in the range of 4 to 7 (Table 4). One sample (No. 3) out of 7 samples was having greenish yellow colour, which may be due to possible adulteration with added colour.

It may be seen from Table 3 that ghee samples from the organised sector have scored for flavour in the range of 4 to 8 with majority of the samples being liked by the judges. Some samples of ghee scored low (4 - 6) for flavour due to oxidized off-flavours. Ghee samples from the unorganized sector scored for the flavour in the range of 3 to 7 (Table 4). Out of the 7 samples from unorganized sector only one sample was disliked for flavour, whereas, two samples (No. 4 and 6) were found having oxidised flavour.

An observation of the findings revealed that the flavour scores for the organised ghee samples were higher than those for the samples from the unorganized sector. Ghatak and Bandyopadhyay (1989) also made a similar observation in their study on the quality evaluation of ghee samples from the Calcutta market.

Most of the ghee samples from the organised sector exhibited granular texture with fine to coarse grains. The scores for body and texture of ghee samples from the organised sector were in the range of 6 to 8 (Table 3). The scores for body and texture of the samples from the unorganized sector were in the range of 4 to 7. Two samples (Nos. 2 and 4) were found to have waxy texture (Table 4). In general, it was observed that the ghee samples from the organised sector scored better in sensory evaluation, when compared to the samples form the unorganized sector.

Table: 3. Sensory scores* of ghee samples from the organized sector

Sample Code	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
Appearance and colour	8	8	8	6	8	7	8	8	6	6	7	7	8	6	7	8	8
Flavour	8	7	7	6	8	7	8	7	4	7	6	8	6	5	6	5	7
Body and texture	7	8	8	7	8	6	8	7	6	7	8	7	8	7	7	7	8
Overall acceptance	8	7	7	6	8	6	8	7	4	7	7	8	8	6	7	6	7

Table: 4. Sensory scores* of ghee samples from the unorganized sector

Sample Code	01	02	03	04	05	06	07
Appearance and colour	7	7	5	7	6	7	4
Flavour	7	7	6	6	4	3	5
Body and texture	7	6	5	6	6	7	4
Overall acceptance	7	7	5	6	5	6	5

*** Scores are averages of the values given by a panel of judges on a 9 point hedonic scale**

4.3. GRANULATION BEHAVIOUR OF GHEE SAMPLES

As may be seen from the data in the Table 3 and 4, the texture of market ghee samples varied widely. It was reported earlier by Paul and Suri (1949), Arumughan and Narayanan (1979) and Rama Murthy (1980) that temperature of incubation was an important factor in inducing grain formation in ghee. The history of storage temperature of the samples collected in the present study, however, is not known.

Therefore, identical incubation conditions were created to test the granulation behaviour of the ghee samples.

Ghee samples were melted at 60°C and 50 g each of the samples taken in glass bottles was stored at 28°C for 12 h. The samples were examined for the grainy texture and the data are presented in Table 5 and 6. It was observed that the samples exhibited varying grainy texture with respect to size and quantity. Depending on the quantity and nature of the grains, the samples were arbitrarily classified to be having 3/4 solid and 1/4 liquid, 1/4 solid and 3/4 liquid, all solid, all liquid and waxy texture. From Table 5, it may be seen that out of seventeen ghee samples from the organized sector, eight were having good granulation with approximately 3/4 solid and 1/4 liquid part. Seven samples attained granulation with approximately 3/4 liquid and 1/4 solid part. Two samples got granulation without liquid portion. None of the samples was found with waxy texture. It may also be seen from Table 6, that out of seven samples from the unorganised sector, four samples showed granulation without any liquid part. One sample did not attain any granulation, whereas two samples were found with waxy texture.

From the above observation, it is evident that the ghee samples from the organized sector, in general, were better in grainy texture compared to ghee samples from the unorganized sector when identical condition were provided.

The grainy texture of ghee is reported to be influenced by the fatty acid composition (Joshi and Vyas, 1976 b), and the fatty acid composition is influenced by seasonal factors (Singh and Singh, 1960; Rama Murthy and Narayanan, 1971; Joshi and Vyas, 1976a). The varying granular texture of market ghee samples observed in the present study could be due to variation in the fatty acid composition. The variation observed in the physico-chemical characteristics of these samples (Tables 7 and 8) indicates a probable variation in the fatty acid composition of the market ghee samples.

Table: 5. Granulation behaviour of ghee samples from the organized sector incubated at 28°C for 12 h

Granulation (3/4solid-1/4liquid)	Granulation (1/4solid-3/4liquid)	Granulation (All solid)	No Granulation	Waxy texture
Sample code				
01,07,08,09,10,11, 12,14	02,03,05,06,15,16,17	04,13	-	-

Table: 6. Granulation behaviour of ghee samples from the unorganized sector incubated at 28°C for 12 h

Granulation (3/4solid -1/4liquid)	Granulation (1/4solid -3/4liquid)	Granulation (All solid)	No Granulation	Waxy texture
Sample code				
-	-	02,03,05,07	06	01,04

4.4. PHYSICO-CHEMICAL PROPERTIES OF GHEE SAMPLES

Fats and oils exhibit certain physical and chemical characteristics, which have long been employed for their characterization and differentiation. For experimental determination of these analytical characteristics many statutory bodies have laid exact procedures over the world. The Bureau of Indian Standards published the best known methods for the determination of these characteristics, which are accepted as standard procedures in India (ISI, 1981).

The determined analytical characteristics are of course reflections of the nature of the constituents, which make up the fat. Fats are almost entirely triglycerides of fatty acids. Since glycerol only constitutes about 10 %, it is the nature of the fatty acids and the manner of distribution of these acids as glycerol triesters, which determine the various characteristics exhibited by the whole fat.

Six analytical characteristics may be considered of basic importance. Of these, three determinations, the Riechert-Meissl value (RM), the Polenske value (PV) and the iodine value (IV) measure certain specific constituents of milk fats. Three other characteristics, the saponification value (SV), the butyrorefractometer reading (BR) or refractive index (RI) and the melting point (MP) give an indication of the overall average nature of the constituent fatty acids present. Milk fat is unique in that it contains a large proportion of fatty acids of chain length lower than 12 carbon atoms, and in consequence many of its characteristics are quite distinctive. Therefore, some of its characteristics like RM value and BR reading find place in the PFA standards. In addition to these two characteristics, AG Mark has specifications for PV in its standards for ghee. The market ghee samples collected from the organised and unorganized sectors were analysed for their physico-chemical characteristics and the results are presented in Tables 7 and 8 respectively.

4.4.1. Reichert-Meissl value

Since the presence of lower fatty acids is peculiar to ruminant milk fats, the RM value is considered to be an important characteristic to detect adulteration of ghee with foreign oils/fats.

It may be seen from the Table 7 that RM values of the market samples from the organised sector ranged from 23.54 to 33.99. Of the 17 ghee samples analysed, only one sample (No. 2) missed marginally the PFA standards of Karnataka i.e. a minimum of 24. Though sample No. 2 was labeled as AG Mark (Special), it is not meeting the standards with respect to RM value. However, considering the wide variation observed in RM values and many factors that affect this characteristic, the sample need not necessarily be suspected for adulteration based on this characteristic alone. While extreme values of 14 to 44 were reported in the literature for genuine cow and buffalo ghee samples, the average value for ghee (milk fat) samples reported was 22.6 – 34.5 (Arumughan and Narayanan, 1982 and Laksminarayana and Rama Murthy, 1985).

Of the ghee samples analysed from the unorganised sector, the RM value ranged from 0.22 to 30.58 showing wide variation (Table 8). The three samples (No.

3, 5 and 7) that failed to meet the standards showed extremely low RM values of 0.22, 1.1 and 13.09. This clearly indicates the gross adulteration these samples were subjected to. A similar trend was reported in the published information on the quality evaluation of market ghee. Sharma and Zariwala (1978) reported that 18 per cent of ghee samples failed with respect to RM value when compared with the PFA and AG Mark standards. They reported RM value for market ghee samples in the range of 1.0 to 34.9. Ghatak and Bandyopadhyay (1989) reported the RM value in the range of 28.3 to 31.6 (average 29.4) and 23.5 to 39.1 (average 27.8) for market ghee samples from organised and unorganized dairies respectively. Recently, Rao *et al.* (2004) analyzed market ghee samples from different sources viz., cooperative dairies, private dairies, sweetmeat shops and local market of Hyderabad city. They observed low RM values than prescribed by the PFA for the ghee samples from the local market.

4.4.2. Polenske value

Polenske value is one of the specifications of AG Mark. The data presented in Table 7 show that PV of ghee samples from the organised sector ranged from 1.2 to 2.0. Thus all the samples pass the test for AG Mark standards (1 to 2). While extreme values of 0.35 to 5.3 were reported for ghee samples, average PV of many works was reported to be between 0.4 and 2.0 (Katrak *et al.*, 1946; Singh and Singh, 1960; Arumughan and Narayanan, 1982 and Laksminarayana and Rama Murthy, 1985).

Polenske value of the samples from the unorganised sector ranged from 0.4 to 1.8 (Table 8). Of the 7 samples analysed, three samples (No. 3, 5 and 7) showed values of 0.4, 0.6 and 0.7. The low RM and Polenske values clearly indicate that the sample Nos. 3, 5 and 7 were grossly adulterated. Sharma and Zariwala (1978) reported the Polenske value of market ghee samples in the range of 0.2 to 1.76. Ghatak and Bandyopadhyay (1989) also reported Polenske value in the range of 1.1 to 1.5 (average 1.3) for ghee samples from organised dairies and 0.6 to 3.01 (average 1.8) for ghee samples from unorganized dairies.

4.4.3. Saponification value

In the present study, the saponification values of the market ghee samples from the organised sector were in the range of 220.0 to 242.61 (Table 7). The saponification values of the market ghee samples from the unorganized sector were in the range of 201.34 to 237.02 (Table 8). Here again the sample Nos. 3, 5 and 7 exhibited low SVs of 207.57, 201.34 and 203.74 which confirm that the samples were adulterated. The average saponification values as reported in the literature ranged from 219 to 232 (Arumughan and Narayanan, 1982 and Laksminarayana and Rama Murthy, 1985). Since ghee contains a high proportion of fatty acids of low molecular weight, its SV is exceptionally high. Most other fats, which contain palmitic, stearic, oleic and linoleic acids, have SVs of about 190; coconut oil is an exception having SV of 255, due to its high content of lauric and myristic acids.

Ghatak and Bandyopadhyay (1989) analysed the ghee samples from Calcutta and Kalyani market and reported that the samples from the organised dairies had higher SVs than the ghee samples from the unorganized dairies. They reported SV in the range of 213.4 to 227.6 (average 219.1) and 174.7 to 217.30 (average 194.7) for market ghee samples from the organised and unorganized sectors respectively.

4.4.4. Iodine value

The iodine value for ghee samples from the organised sector ranged from 25.68 to 35.28 (Table 7). IV, a measure of the extent of unsaturation of milk fat, was reported to be between 26 and 41 for ghee (Laksminarayana and Rama Murthy, 1985). Iodine value of ghee samples from the unorganised sector as may be seen from the Table 8, were in the range of 28.03 to 82.19. Three samples (Nos. 3, 5 and 7) were found to have iodine values of 82.19, 56.50 and 53.85. This showed that these samples were possibly adulterated with some vegetable oils.

4.4.5. Butyro-refractometer reading at 40°C/Refractive Index

BR reading of the ghee samples from the organised sector ranged from 41.0 to 43.0 while the refractive index ranged from 1.4531 to 1.4546 (Table 7). These values were within the average values reported for ghee by earlier workers (Ghatak and Bandyopadhyay, 1989; Rao *et al.,* 2004). The samples from the unorganised sector showed BR ranging from 42.0 to 46.0 and the RI of 1.4538 to 1.4565 (Table 8). Three samples (Nos. 3, 5 and 7) exhibited higher BR/RI of 45.5/1.4563, 46.0/1.4565 and 45.5/1.4564. These samples exhibited a very low RM, Polenske and saponification values and a high iodine values. However, the BR/RI did not show a corresponding increase for these samples.

4.4.6. Melting range

In the present study the melting range of different ghee samples from the organized sector was varying from 23°-39°C (Table 7). For ghee samples from the unorganized sector, the range was found to be 22° - 38°C (Table 8). Three samples (Nos. 3, 5 and 7) in general showed a lower melting range.

Table: 7. Physico-chemical characteristics of ghee samples from the organised sector

Sample code	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
Reichert-Meissl value	28.38	23.54	28.93	32.56	33.44	33.99	27.5	32.67	24.97	28.05	32.67	28.27	30.53	25.96	28.71	28.49	29.81
Polenske value	1.9	1.7	1.5	1.2	2.0	1.9	1.7	1.4	1.9	1.6	1.9	1.9	1.7	1.8	1.8	1.5	1.7
Saponifi-cation value	242.61	233.28	233.06	231.59	230.42	229.71	229.11	227.89	226.09	228.49	223.18	226.93	228.78	229.12	220.0	232.37	230.50
Iodine value	32.93	35.28	34.04	29.35	31.05	30.52	32.68	31.76	32.00	29.50	29.30	29.99	29.40	25.68	30.69	29.99	32.32
RI/(BR) at 40° C	1.4545 (43.0)	1.4545 (43.0)	1.4544 (42.5)	1.4545 (43.0)	1.4546 (43.0)	1.4531 (41.0)	1.4541 (42.5)	1.4537 (42.0)	1.4534 (41.5)	1.4545 (43.0)	1.4538 (42.0)	1.4531 (41.0)	1.4545 (43.0)	1.4541 (42.5)	1.4534 (41.5)	1.4545 (43.0)	1.4538 (42.0)
Melting range(°C)	25-38	25-37	24-38	24-36	23-37	23-38	25-37	25-38	25-38	24-37	24-38	26-37	25-38	24-36	25-39	25-36	24-37
Moisture (%)	0.11	0.10	0.10	0.05	0.15	0.06	0.12	0.13	0.09	0.05	0.21	0.05	0.16	0.20	0.18	0.07	0.09

Table: 8. Physico-chemical characteristics of ghee samples from the unorganized sectors

Sample Code	01	02	03	04	05	06	07
RM value	27.2	28.2	1.1	29.7	0.22	30.58	13.09
Polenske value	1.5	1.8	0.4	1.7	0.6	1.7	0.7
Saponification value	235.62	225.76	207.57	237.02	201.34	234.21	203.74
Iodine value	29.89	30.10	82.19	29.61	56.50	28.03	53.85
RI/(BR) at 40° C	1.4545 (43.0)	1.4538 (42.0)	1.4563 (45.5)	1.4541 (42.5)	1.4565 (46.0)	1.4545 (43.0)	1.4564 (45.5)
Melting range (°C)	24-38	25-37	26-38	26-37	22-28	24-37	22-29
Moisture (%)	0.15	0.18	0.05	0.20	0.06	0.20	0.11

4.4.7. Moisture content

The moisture content of the ghee samples from the organized sector was in the range of 0.05 to 0.21 % (Table 7). A similar trend was exhibited by the samples from the unorganised sector (Table 8). Thus all the samples met the standards for moisture by PFA (max. 0.5 %) as well as AG Mark (Max. 0.3%). Earlier, Sharma and Zariwala (1978) reported moisture content of 0.02 to 0.7 % for market ghee samples. An average moisture content of 0.22 and 0.29 % was reported for ghee samples of the organised and unorganised sector reported by Ghatak and Bandyopadhyay (1989). Rao *et al.* (2004) reported the average moisture content of 0.22, 0.23, 0.27 and 0.28 for the ghee samples from the cooperative, private dairies, sweetmeat shops and local market respectively.

4.4.8. Free fatty acids

In the present study, the ghee samples collected from the organized sector were found having the free fatty acids (FFA) in the range of 0.16 to 1.22 % in terms of oleic acid (Table 9). The ghee samples from the unorganized sector were found having the FFA in the range of 0.23 to 1.46 % as oleic acid (Table 10). The above results showed that the FFA contents of the samples from both the organsied and unorganized sectors were within the limit prescribed by the PFA and the AG Mark standards. These results are in the agreement with the findings of other workers (Ghatak and Bandyopadhyay, 1989; Rao *et al.,* 2004).

Small proportion of fatty acids are always present in fresh milk fat; larger percentages are found in the milk fat isolated from milk, cream or butter that has been subjected to bacterial action or in fat that has been stored for sometime. Small amounts of free fatty acids in ghee (~ 0.15 % oleic) did not affect its keeping quality, but high acid ghee deteriorated faster (Lalitha and Dastur, 1953).

4.4.9. Peroxide value

The peroxide values in the market ghee samples from the organized and unorganized sectors were found to be in the range of 0.09 to 2.75 mM of O_2/kg and 0.42 to 4.76 mM of O_2/kg respectively (Table 9 and 10). It was observed that the ghee samples from the unorganized sector were having higher peroxide values than ghee samples from the organized sector. Ghatak and Bandyopadhyay (1989) reported the peroxide value of 0.2 to 0.7 and 0.6 to 3.10 (mM of O_2/kg) for ghee samples from organized and unorganized sectors respectively. It may be seen from the data that all the samples had shown the development of peroxides. The age of the samples at the time of the analysis was in the range of 1 to 4 months. The samples that showed oxidised off-flavour on sensory evaluation (Table 3 and 4) were found to contain higher peroxide values (>1.0).

4.4.10. Total carbonyl value

Carbonyls present in ghee are significantly affected by manufacturing conditions such as temperature of clarification, method of preparation and initial quality of raw material. Carbonyl compounds play a significant role in the flavour of dairy products. Several carbonyl compounds have been shown to be natural (~ 4 μM/g fat) constituents of milk fat. Further increase in the level and type of these compounds would cause the development of oxidative rancidity.

The total carbonyl value in the ghee samples from the organized sector was found to be in the range of 4.84 to 11.26 μM/g fat (Table 9), whereas those for samples from unorganized sector were in the range of 5.58 to 11.66 μM/gm fat (Table 10). Gaba and Jain (1975) reported that the total carbonyl content of cow and buffalo ghee made from fresh butter was 7.20 and 8.64 μM/g respectively. Bhat *et al.* (1980) reported a total carbonyl value of 4.1 μM/g in cow ghee and 7.0 μm/g in buffalo ghee. Rama Murthy and Jain (1973) observed that the total carbonyls in cow and buffalo ghee ranged from 4.2 to 4.5 μM/g and 6.3 to 6.8 μM/g fat respectively.

Table: 9. Free fatty acids, peroxide value and total carbonyl value of ghee samples from the organized sector

Sample Code	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
FFA (% oleic acid)	0.61	0.40	0.63	0.16	0.32	0.26	1.09	0.39	0.44	0.33	1.16	0.47	1.22	0.51	0.68	0.63	0.89
Peroxide value (mM of O_2 /kg fat)	0.09	0.34	0.29	0.53	0.49	0.43	0.39	0.54	1.12	0.82	1.27	0.63	1.47	0.92	1.17	2.75	1.42
Carbonyl value (μ M /g fat)	6.62	7.34	5.82	7.70	7.18	5.90	4.84	7.24	9.40	7.76	11.26	7.56	6.82	7.04	6.04	7.14	9.34

Table: 10. Free fatty acids, peroxide value and total carbonyl value of ghee samples from unorganized sector

Sample Code	01	02	03	04	05	06	07
FFA (% oleic acid)	1.46	1.36	0.24	1.08	0.97	1.21	0.23
Peroxide value (mM of O_2 /kg fat)	0.89	0.56	1.22	0.42	4.76	0.49	1.72
Carbonyl value (µM /g fat)	8.74	9.60	5.58	11.66	6.30	9.82	9.34

4.5. QUALITY OF GHEE WITH REGARD TO PFA AND AG Mark STANDARDS

The data on quality of ghee samples from the organised sector with regard to PFA and AG Mark specifications are given in Table 11. It may be seen from the Table that all the samples met the specifications with regard to moisture, FFA, PV, Baudouin test and BR reading. Only one sample (6 %) did not meet the specification with regard to RM value. However, this sample fell short of the specifications marginally and all four physico-chemical characteristics were within the range. Therefore, the low RM value of this sample may not be due to adulteration. Similar data for the samples from unorganised sector are presented in Table 12. It may be seen that while all the samples met the PFA specification for moisture, FFA and Baudouin test forty three per cent of the samples failed to meet the specifications for RM value and BR reading. Though the samples were not graded with AG Mark label, all the samples met the standards for moisture and Baudouin test. However, as may be seen from the table, considerable proportion of the samples from this sector did not meet the specifications of AG Mark for RM value, PV and BR reading. This indicated that these samples were adulterated.

Table: 11. Quality of ghee samples from the organized sector with regards to PFA and AG Mark Standards

Parameter	Range	PFA Standard	AG Mark Standard	% Samples meeting PFA standards	% Samples meeting AG Mark (Special) Standards
Moisture (%)	0.05 - 0.21	0.5 (Max.)	0.3 (Max.)	100	100
RM value	23.54 - 33.99	24 (Min.)	28 (Min.)	94	76
Polenske value	1.2 - 2.0	-	1.0 –2.0	-	100
BR reading	41.0 - 43.0	40.0 – 43.0	40.0 - 43.0	100	100
FFA (% oleic acid)	0.16 - 1.22	3.0 (Max.)	1.4 (Max.)	100	100
Baudouin test	Negative	Negative	Negative	100	100

Table: 12. Quality of ghee samples from the unorganized sector with regards to PFA and AG Mark Standards

Parameter	Range	PFA Standard	AG Mark Standard	% Samples meeting PFA standards	% Samples meeting AG Mark (Special) Standards
Moisture (%)	0.05 - 0.20	0.5 (Max.)	0.3 (Max.)	100	100
RM value	0.22 - 30.58	24 (Min.)	28 (Min.)	57	76
Polenske value	0.4 -1.8	-	1.0 –2.0	-	57
BR reading	42.0 - 46.0	40.0 – 43.0	40.0 - 43.0	57	57
FFA (% oleic acid)	0.23 - 1.46	3.0 (Max.)	1.4 (Max.)	100	86
Baudouin test	Negative	Negative	Negative	100	100

4.6. DETECTION OF ADULTERATION IN GHEE SAMPLES

Ghee is an expensive product, costing three times as much as edible vegetable oils. Thus its adulteration is a very profitable proposition and one widely practiced. Once ghee has been isolated, a wide variety of materials are employed for adulteration. Various vegetable oils viz. coconut oil, palm oil, etc. are in common use. Tallow or other animal body fats are to be had from slaughterhouses, and being hard and firm cannot be detected visually in ghee even when present in considerable proportion. In fact blends of tallow and vegetable oil can be prepared which look remarkably like ghee. Mineral oils or solid paraffin are considerably cheaper than ghee and can be used as adulterants. The hydrogenated fats are also used to adulterate ghee. Despite the ban, yellow colours are sometimes added to buffalo ghee so as to pass it of as cow ghee, which fetches a higher price. Where cow ghee preferred over buffalo ghee, colours like annatto, turmeric and coal-tar colours such as ceres yellow 39, orange may be added to ghee. Sometimes the dissolved soaps could also be found in ghee. Keeping in mind the possibility for the presence of the above adulterants, all the ghee samples were subjected to tests to detect vanaspati (Baudouin test), added colours, mineral oils and dissolved soap.

The ghee samples were also subjected to crystallization and fractionation to detect the presence of foreign fats.

4.6.1. Detection of added colours

According to the PFA (2004) Rules, the ghee should be free from added colours. When the ghee samples from the market were tested to detect the presence of added colours, two samples, No.5 and 7 (Table 13), from the organized and two samples, No. 3 and 5 (Table 14), from the unorganised sector were found to have added colours.

As per the literature information, earlier works on the quality evaluation did not perform this test to detect added colours.

4.6.2. Detection of mineral oil

Unlike oils and fats, mineral oils are not saponifiable by alkali. On this basis there was a test to detect the presence of mineral oil in ghee (Venkatachalam and Sundaram, 1957).

None of the ghee samples from the organized sector (Table 13) tested positive with respect to mineral oil, whereas sample No.3 (Table 14) from the unorganized sector was tested positive.

4.6.3. Detection of vanaspati (Baudouin test)

In the present study, none of the samples from the organized and unorganized sector was found positive to Baudouin test (Table 13 and 14). However, Sharma and Zariwala (1978) found 20 % of the ghee samples added with vanaspati. Of the samples analyzed (47) from unorganized sector, 11 were adulterated with vanaspati (Ghatak and Bandyopadhyay, 1989). Rao *et al*., (2004) found 35% of the ghee samples from local market were added with vanaspati.

4.6.4. Detection of dissolved soaps

From the results it is evident that none of the samples both from organized and unorganised sector was tested positive with respect to the presence of dissolved soap (Table 13 and 14).

Table: 13. Presence of added colours, mineral oil, vanaspati and dissolved soaps in ghee samples from the organized sector

Sample Code	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
Added colours	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Mineral oil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vanaspati	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dissolved soaps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: Present; -: Absent

Table: 14. Presence of added colours, mineral oil, vanaspati and dissolved soaps in ghee samples from the unorganized sector

Sample Code	01	02	03	04	05	06	07
Added colours	-	-	+	-	+	-	-
Mineral oil	-	-	+	-	-	-	-
Vanaspati	-	-	-	-	-	-	-
Dissolved soaps	-	-	-	-	-	-	-

+: Present; -: Absent

4.6.5. Crystallization test

Crystallization test was developed by Panda and Bindal (1998) for the detection of foreign fats in buffalo ghee. They observed that the crystallization time for pure buffalo ghee was 19-20 min. Adulteration of buffalo ghee with other vegetable oils enhanced the time for crystallization whereas the presence of animal body fats reduced the time for crystallization.

Based on above work, the ghee samples were subjected to crystallization test, with cow ghee as control ghee. Results of the present study revealed that cow ghee took ~ 16 to 20 min for crystallization which is almost similar to that of buffalo ghee. From the Table 15, it is seen that majority of the samples from organised sector crystallize out within the same range i.e. 16 to 20 min. On the contrary, 6 ghee samples out of 7 from the unorganised sector crystallized very fast taking only few seconds to 13 min (Table 16). One sample (No. 6) did not show any crystallization even after 30 min of incubation at 17°C. This indicated that the samples from the unorganised sector could be suspected for adulteration. Based on the physico-chemical characteristics, it was found that the samples Nos. 3, 5 and 7 had low RM, Polenske and saponification values and high iodine values (Table 8) indicating a possible adulteration with vegetable oils. The rest of the samples exhibited a normal range of these characteristics. However, the crystallization time of these samples was so less that the samples (Nos. 1, 2, 3, 4, 5 and 7) could be suspected for adulteration with animal body fats. Probably these samples were having blends of vegetable oils and animal body fats. This could be the reason for a semi-solid consistency exhibited by these samples. The reasonably good scores that the samples obtained for flavour on sensory evaluation (Table 4) could be due to the presence of some ghee or artificial ghee flavour.

Table: 15. Crystallization time (min) for samples from the organized sector

Degree of crystalli-zation	**Sample code**																
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Negative	15.5	-	9.5	7.5	15	18.5	15.5	16.5	9	15	7.5	9	15	13	9.5	10	9
Slight	16	18	10	8	16	19	16	17	10	16	8	10	16	14	10	12	11
Fair	17	-	13	9	17	-	17	18	17	18	9	11	18	16	16	15	13
Excellent	19	-	17	13	19	-	19	-	19	19	12	13	19	19	18	-	16

Table: 16. Crystallization time (min) for ghee samples from the unorganised sector

Sample code	**Degree of crystallization**			
	Negative	**Slight**	**Fair**	**Excellent**
	Time (min)			
01	0.5	1.0	2.0	3.0
02	8.0	9.0	11.0	13.0
03	3.5	4.0	5.0	6.0
04	4.5	5.0	6.0	7.0
05	Crystallization before keeping at 17°C temperature			
06	No crystallization even after 30 min.			
07	Crystallization before keeping at 17°C temperature			

4.6.6. Yield of solid and liquid fractions of ghee at 25°C

Fractionation of milk fat into fractions is of great analytical value in characterizing the nature of fat. Lakshminarayana and Rama Murthy (1985) studied the fractionation of buffalo milk fat and the physico-chemical properties of different fractions. Kumar *et al*. (2002) mentioned in his review article that fractionation approach (for enriching the solid fraction with body fats and hydrogenated fat, and liquid fraction with vegetable oils) is expected to hold a good potential to be exploited for solving the problem of adulteration.

In the present study, samples from market were subjected to fractionation at 25°C for 12 h and the yield of liquid and solid fractions were measured. The control ghee, after fractionation, found to have about 15 % liquid fraction and 85 % of solid fraction. Table 17 shows the fractionation behaviour of the ghee samples from organised sector. The yield of liquid and solid fractions of ghee samples from the organised sector showed a similar range; liquid fractions ranged from 9 – 18 % and solid fractions ranged from 82 – 91 %. This indicated that these ghee samples might not have been adulterated. On the other hand, six samples of the unorganised sector showed a very less liquid fraction and one sample (No.6) showed a very high proportion of liquid fraction (Table 18). These observations matched with those observed for crystallization behaviour of the same samples, confirming the possible adulteration.

Table: 17. Yield of fractions of ghee samples from the organized sector obtained at 25°C

Sample Code	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
% Solid Part	88	86	90	91	88	84	82	86	88	90	84	88	82	90	86	85	84
% Liquid Part	12	14	10	09	12	16	18	14	12	10	16	12	18	10	14	15	16

Table: 18. Yield of fractions of ghee samples from the unorganized sector obtained at 25°C

Sample Code	01	02	03	04	05	06	07
% Solid Part	100	90	100	100	98	10	96
% Liquid Part	00	10	00	00	02	90	04

5.0 SUMMARY AND CONCLUSION

Seventeen brands of ghee from the organized market and seven samples from the unorganized sector were collected. The price of the ghee from the organized sector ranged from Rs.78 to 99 for 500 ml while it ranged from Rs. 35 to 90 for the same quantity of ghee from the unorganized sector. Of the 17 ghee samples from the organized sector only 5 were of AG mark standard and one was of AG mark general grade. Fifteen out of the seventeen samples from this organized sector were packed in flexible pouches and the other two in bottles. The shelf life declared on the labels ranged from 4 to 12 months for these samples. The samples from the unorganized sector were sold loosely.

The market samples from both the sources were subjected to sensory evaluation on a 9 point hedonic scale. The ghee samples were further analysed for their physico-chemical characteristics and presence of adulterants.

In general, it was observed that the ghee samples from the organized sector scored better for sensory attributes, when compared to the samples from the unorganized sector. Some samples from both the sources showed oxidized off flavours.

When these ghee samples were subjected to identical conditions of incubation at 28°C for 12 h to see the granulation behaviour, samples from the organized sector were found to have better grainy texture than those of samples from the unorganized sector. The varying granular texture of market ghee samples observed in the present study could be due to variation in the fatty acid composition. Based on the results regarding physico-chemical characteristics of the ghee samples, it is evident that most of the samples from the organized sector were meeting the standards prescribed by PFA, while three out of seven samples, from the unorganized sector were found to be grossly adulterated with foreign fats. The RM value, Polenske value, saponification, iodine value, BR reading and melting range of the ghee samples from the organized sector were found to be in the range of 23.54 to 33.99, 1.2 to 2.0, 220.0 to 242.61 25.68 to 35.28, 41.0 to 43.0 and 23 – 39°C respectively. The samples from the unorganized sector showed the above values in the range of 0.22 to 30.58, 0.4 to 1.8, 201.34 to 237.02, 28.03 to 82.19, 42.0 to 46.0 and 22 – 38°C respectively.

The moisture content of the ghee samples from the organized and unorganized sector were found to be in the range of 0.05 to 0.21% and 0.05 to 0.21 % and 0.05 to 0.20 respectively. All the samples have passed the PFA requirement with respect to moisture content. The free fatty acids content of the ghee samples from the organized sector ranged from 0.16 to 1.22 % where as that of samples from unorganized sector ranged from 0.23 to 1.46 %, as oleic acid, meeting the standards prescribed by the PFA and AG Mark. The peroxide values of the ghee samples from the organized sector ranged from 0.09 to 2.75 mM of 0_2/Kg fat where as the sample from the unorganized sector had higher peroxide value ranging from 0.42 to 4.76 mM of 0_2/kg fat for the samples from unorganized sector. The carbonyl contents of the ghee samples from the unorganized sector were also higher than those from the organized sector.

The ghee samples were also subjected to different specific tests to detect the presence of added colours, mineral oil, vanaspati and dissolved soaps. The results revealed that two samples each from the organized sector and unorganized sector found to be adulterated with added colours. One sample from the unorganized sector was found to have mineral oil. None of the samples from both the sectors was tested positive for the presence of vanaspati and dissolved soaps.

The crystallization and fractionation behaviour of the ghee samples were also analysed to assess the degree of adulteration of ghee analysed. Results showed that the samples from the organized sector were not found adulterated with any foreign fats. However, the crystallization time of the samples from the unorganized sector was so less that six samples (No.1, 2,3,4,5 and 7) could be suspected for adulteration with animal body fats. However, results of the physico-chemical characteristics showed that only 3 samples had low RM and Polenske values and moderately high BR reading a probably these samples were having blends of vegetable oils and animal body fats.

The yield of solid and liquid fractions of genuine ghee were found to be 15 and 85 % respectively. The fractions of the samples from the organized sector also showed a similar trend. On the other hand, six samples from the unorganized sector showed a very less liquid fraction and one sample showed a very high proportion of liquid fraction. These observations matched with those observed for crystallization behaviour of the same samples, confirming the possible adulteration.

Overall findings of the study revealed that the ghee samples from the organized sector were superior in quality than those from the unorganized sector. Since 90 % of the ghee production is from this sector, regular monitoring of the product for quality evaluation and corrective action are essential to safeguard the interest of the public.

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Appendix

Sensory evaluation score card for ghee samples

Name of the judge: Date:
Time: Batch no:

Ghee sample(s) is/are served to you for organoleptic evaluation. Please judge the product on 9-point hedonic scale. Please write your valuable comments below in the remarks column.

	I	II	III	IV	V	VI
1. Appearance and colour						
2. Flavour						
3. Body and texture						
4. Overall acceptance						

Scale:

9. Liked extremely
8. Liked very much
7. Liked moderately
6. Liked slightly
5. Neither liked nor disliked
4. Disliked slightly
3. Disliked moderately
2. Disliked very much
1. Disliked extremely

Signature

Remarks: