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A review on understanding the subterranean insights in nature of South Indian ghee with its biological and physiochemical properties

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Abstract
Ghee is recognized as an important food product in Indian diet due its high nutritive values, pleasant aroma, taste and unique texture. India produces 900,000 tonnes of marketed ghee with a value of 85, 000 million. In tropical country like India spoilage of ghee is mainly due to oxidative rancidity. Ghee is manufactured by different method namely, desi methods, direct creamery method, creamery butter method, pre-stratification method and continuous method. Each method certainly pertains to quality and flavor imparting strategy in ghee. Several innovations are endeavored to increase fat concentration and reduction of fat loss during manufacturing process and high temperature heat treatment of ghee has significantly improved the overall quality of ghee. In this review, we have discussed the physiochemical properties, and effect of natural antioxidant and free fatty acid on thermal oxidation and storage of ghee. In addition, we have discussed the biological importance ghee.

Keywords: Ghee, physicochemical property, rancidity, antioxidant, biological importance, physic-chemical nature, desi method, natural antioxidant, flavor

1. Introduction

Ghee is one of the major dairy products that has place an important role in the diet of the people of the Indian sub-continent due to its good flavor, and its aroma. The Ghee is evolved from Sanskrit word ghruta. It is known by its various names in various languages however its common Indian name is represented as clarified butter fat. Ghee is a one of the good sources of energy, lipid nutrients including fat soluble vitamins and essential fatty acid and contains butyric acid, conjugated linoleic acid and phospholipids etc., hence it has been recognized to possess several therapeutic properties. It increases the digestive fire and improves absorption and assimilation, develops memory and strengthens the brain and nervous system. It lubricates the connective tissues and notably utilized in Ayurveda for numerous medical applications including the treatment of allergy, skin and respiratory diseases. Many Ayurvedic medicines are prepared by utilizing considerable amount of ghee because it acts as drug carrier molecules for transporting herbal medicines to the deeper tissues layer of the body (Lad 1998). Food Safety and Standards Authority of India (FSSAI) (2011) defined ghee is a purified form of fat derived solely from milk or curd and chemically defined as complex lipids of triacylglycerol consists of small number of sterols, hydrocarbons, carbonyl compounds, fat soluble vitamins (A, D, E, and K), carotenoids pigments, moisture and trace elements like copper and iron (Kapadiya and Aparnathi2017) [19].

Ghee forms the largest segment of the milk products in India where about 28% of the milk is utilized for ghee production in India. In 2001 it estimated that the annual growth rate of 5% ghee production is raised to 1.3 million tons valued at Rs.130 billion. The presence of good flavor, color and texture of ghee vary from region to regions in India (Table.1). The major component of ghee is glycerides which constitute 98% of total composite materials and remaining 2% consist of sterols most commonly cholesterol (Kumar *et al.,* 2016) [3, 20]. The quality of ghee is depending on the quality of milk, cream, curd, butter and the methods of preparation of above-mentioned, temperature, conditions of storage and the type of animal feed, animal breed influence the quality of ghee. Basic parameters such as peroxide value, free fatty acid value, flavor and acidity determine the quality of ghee. Temperature is one of the important parameters, it highly influences the quality of ghee as it controls the intensity of flavor ghee (Kumar *et al.,* 2016) [3, 20]. The conversion of milk into curd is the important stage in ghee production. Globular proteins casein is the major protein of milk. Due to the chemical reaction between the lactic acid's bacteria and casein, milk converted into curd. During fermentation process, lactic acid bacteria utilize enzymes to produce ATP molecules from lactose and lactic acid is produced as byproduct of ATP production. Then the lactic acid acts on the globular protein casein and leads denaturation process subsequently destroys the tertiary and quaternary structures of proteins which converted casein into fibrous proteins thus giving a thick texture to the milk due to coagulation of the proteins. Then coagulated milk subjected the next process Churning, where the curd is agitated and eventually butter granules formed in the end of the process there are two phases are formed such as semisolid mass of butter and liquid butter milk. Then butter is melted under continuous heat to separate ghee and its residues. Below mentioned figure 1 shows that, schematic representation of various stages of reactant and product that derives from milk to ghee that provides various products and from each stage.

2. Chemical nature of ghee

The chemical composition of ghee varies based on the preparation method. The chemical composition of cow and buffalo ghee varies slightly and represented in Table 2.

2.1 Lipids

Lipids are considered as the total lower chain fatty acids C4 to C12 (5.3%) with total saturated fatty acids 10.1% and more of unsaturated fatty acids (66.8%) in comparison to those of ghee. Basically, fatty acids are made up of phospholipids, shows that it has no fatty acids lower than 12 carbon atoms. The higher number of phospholipids present in ghee decreases the heating period required to the transfer of phospholipids from ghee-residues to ghee. While heating cream/butter, only a small fraction of the phospholipids gets transferred to ghee, most of the phospholipids remain with the residue because of their polar character. The differences observed in the physio-chemical constant, fatty acids and PUFA contents between lipids of ghee-residue and ghee are due to the high phospholipid content of ghee-residue.

2.2 Sensory aspects

In India ghee is greatly assed by its characteristic flavor. The flavor of the ghee is highly dependent on the method of preparation. The high score of flavors of ghee prepared from camel, cow, and buffalo milk was 33.73, 44.78 and 41.97 (out of 50) respectively compared to this goat milk does not have flavor hence it is not suitable for manufacture of ghee (Parmar2013). However, the pleasant flavor only presents in cow and buffalo milk, it shows unpleasant flavor and salty in camel milk (Parmer, 2013; Mal and Pathak 2010; Hamid 1993, Park and Haenlein, 2006). Next to flavor, color plays a key role in quality of ghee. The color of cow ghee varies from deep yellow to straw tallow while that of buffalo (Bharwade *et al.*, 2017). The physical nature of camel ghee consists of a mixture of higher softening point fats in crystalline from dispersed in the liquid lower softening points fats and this gives the ghee a somewhat granular appearance. The texture or granularity of the ghee is also quality parameters which are identified by the presence of uniform size grains with very little liquid fat is desirable characteristic of food quality.

2.3 Fat oxidation in ghee

The chemical composition of ghee revealed that lipid is the major constituents and this oxidation process play a key role in quality of ghee. The oxidation of fat differs from that of bulk lipids because it highly interacts between ingredients and the partitioning of ingredients between the oil, aqueous and interfacial region due to the presence of hydrophobic and hydrophilic groups. During this process, free radicals are produced which are unstable and readily react with oxygen, moisture or fat during processing or storage. Fat oxidation mainly depends on the several steps such as selection, storage, refining and manufacturing. Fatty acids a basically long aliphatic chain consist of carbon and hydrogen. In food products, fatty acids are found in lipid complexes such as triglycerides. Among them some fatty acids are saturated, while others have different types of unsaturated fatty acids. Polyunsaturated fatty acids mainly involved in oxidation due to the presence of two or more number of double bonds, risk of oxidation increases with the number of double bonds present in the fatty acids.

3. Main imperfections in ghee due to oxidation

Rancidity is the most serious defects of ghee. There are two types of rancidity namely hydrolytic and oxidative rancidity. Basically, it develops in ghee during storage and due to overheating in the freshly prepared ghee. Mainly it caused by the formulation of volatile compounds which exhibit unpleasant odors and adversely affect the nutritive value of ghee. Hydrolytic rancidity is mostly occurring in butter oil. Fat splitting enzyme, lipoprotein lipase is responsible for hydrolysis of milk fat and responsible to produce lower molecular weight fatty acids such as butyric, caproic and caprylic acids responsible for unpleasant odor in ghee. However hydrolytic rancidity is not of much problem in ghee because during manufacturing of ghee it is subjected to high heat treatment which inactivates the lipase enzyme. (Deeth and Fitz-Gerald 1983).

4. Preventive Methods

Autoxidation is one of the most common defects may produce adverse effect in serum lipid profile and toxic biochemical reaction at subcellular and vascular endothelial levels. Hence there is need an alternative strategy to reduce the toxic effects of thermally oxidized dietary lipids (Alam Zeb and Islam Uddin 2017). Several workers have studied to improve the stability of ghee against autoxidation through feeding specific feed to various milch animals (Tandon1997) or altering the processing parameters (Singh *et al.,* 1979), and using proper packaging materials and storage conditions (Amr1990). Addition of synthetic antioxidants also significantly reduces the oxidation in ghee by incorporating natural antioxidants from edible plant materials, species and condiments, aromatic herbs. In India BHA is legally approved as an antioxidant to improve the quality of ghee. Natural antioxidants have significant effect to prevent rancidity in ghee, but no commercial trial has been tired till date to evaluate the natural antioxidants in ghee. Ahmad *et al.,* (1960) studied the antioxidant properties of Amla juice (*Emblica officinalis*), it retards the onset of rancidity to the same extent as did 0.1 % propyl gallate and 0.01 % citric acid and it was attributed to its high ascorbic acid and gallates content. Nilakkanth (2012) reported that the addition of ethanolic extract of Shatavari (0.5%) in ghee significantly reduce the formation of peroxides, free fatty acids, conjugated dines and thiobarbituric acid value as compare to control sample of ghee during accelerated storage of 80±1°C. The study concludes that the main antioxidative compounds gave the maximum stabilizing effect to ghee against oxidative deterioration (Dinesh *et al.*, 2000). The active component of turmeric volatile and curcumin showed moderate pro-oxidant activity and exhibited slight pro-oxygenic activity. Himesh *et al.*, (2011) supported that the above study, the findings reported that the addition of curcumin powder at 0.4% gave ghee higher flavor value and lower peroxide value as compare to control sample during accelerated storage and reported that addition of curcumin had not create any color defect in ghee. Other herbs such as onion Arjuna bark extract (Parmar *et al.,*2013) Coriander extract (Patel *et al.,*2013), Gehan *et al*. (2014) evaluated that the different extracts from peanut skins (PS), pomegranate peel (PP) and olive pomance (OP) as source of natural antioxidant which improve the quality ghee. The results revealed that that the ethanolic extract could effectively use to retard fat auto-oxidation in ghee.

5. Physicochemical parameters

Flavor, color and texture are main characteristics which a mostly consumer judge the quality and accepts. Several physical methods can be used to check the purity of ghee on basis of various physical properties such as melting point, solidification point, BR reading, refractive index (Kumar *et al.,*2016) [3, 20]. FSSAI and AGMARK have been used some important analytical constants or standards for providing the genuine quality of ghee to the consumer are Butyro-refractometer reading (BR) at 40°C, Reichert Meissl (RM) value, Polenske value, Baudouin test and free fatty acid (FFA). The saponification value and Butyro-refractometer reading give an overall average nature of the constituent fatty acids present (Rangappa and Achaya 1974) [37].

5.1 Reichert-Meissl (Rm) Value

RM values are one of the most widely used analytical parameters to identify the quality of ghee. RM values is the number of 0.1 N alkali solution required to neutralize the steam volatile, water soluble fatty acids distilled from 5 g of fat under specified condition significantly measure the butyric (C4:0) and caproic (C6:0) acid. While measuring RM value of ghee primarily, it is subjected to saponification and heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulfuric acid. The volatile acids which present in ghee are measured by immediately steam distilled and the soluble volatile acids in the distillate are then filtered out and estimated by titration with standard sodium hydroxide solution. Ghee mainly contains butyric & caproic acid glycerides. RM value of milk fat ranges from 17 to 35, which is well above the value (generally 1) for all other fats and oils except coconut oil and palm kernel oil for which the value ranges between 4 to 8. Palm olein and sheep body fat added individually could be detected only at 15 per cent levels in pooled cow and buffalo ghee samples based on RM value determination. Mixture of palm olein and sheep body fat was detectable at 6+14 (20) and 9+21 (30) per cent levels Recently Amit Kumar *et al.*, (2016) [3, 20] studied the average value of three ghee samples among them sample 1 and 2 were found 28 and 29, which meet the required standard value. Whereas, RM value of sample 3 (40.2) was found out of the standard range.

5.2 Polenske Value

Polenske value also denotes the number of milliliters of 0.1 N alkali solutions required to neutralize the steam volatile and water insoluble fatty acids distilled from 5 g of fat under specified conditions. However, this value substantially a measure of caprylic (C8:0) and capric (C10:0) acid. The Polenske value of milk fat ranges from 1.2 to 2.4. This value for other oils and fats is also less except the coconut oil (15-20) and palm kernel oil (6-12).

5.3 Iodine Value

Iodine value measure the extent of unsaturation in milk fat with minimum range from 27-35. Iodine value represents the number of grams of iodine absorbed by 100g of fat under specified condition. Compared to vegetable fat animal body fat shows slightly higher iodine value. Lea (1939) [22] reported a decrease in iodine absorption in oxidized fats. Stored fat has showed considerable amount of iodine values, the liquid fraction obtained because of ghee storage at 30 to 35º C for one month showed higher iodine value than solid fraction.

5.4 Saponification Value (Sv)

Saponification value which denotes the number of milligrams of KOH required saponifying one gram of fat and it indirectly measure the average molecular weight of fatty acid present. For milk fat, animal body fat, vegetable oils and hydrogenated fats, the value ranges from 210 to 233, 192 to 203, 170 to 197 and 197 to 199 respectively. Feeding of cottonseeds to milch animals lowers this value by 7 units (Rangappa and Achaya, 1974) [37]. Autooxidation may cause partial hydrolysis of fats into fatty acids and glycerol's. Glyceride under oxidation gives low molecular weight fatty acids and oxidation of unsaturated fatty acids to hydroxy acids and peroxides, which lead to increase in potash absorption of fats (Lea 1939) [22].

5.5 Iodine value (IV)

The extent of unsaturation in milk fat can be detects by Iodine value measurement. It ranges from 27– 35 with an average value of 30. 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. The iodine value of cow (31.1) and buffalo (32.2) ghee were reported and the same study was carried out by Singh and Guptha (1982) and reported the iodine values of 34.05, 0.46 for cow and buffalo ghee respectively.

5.6 Butyro-refractometer (BR) reading

BR Reading is the index of purity of ghee. An increase value of BR reading indicates the adulteration of ghee with other fat like vegetable oil and animal fat. 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. Singh and Singh (1960) reported that the BR values of cow ghee in the range of 40.35-44.8, while that of buffalo ghee in the range of 40.6 – 44.3. BR reading of ghee shows variation and therefore the specification by PFA for this characteristic varies from state to state (PFA 2005).

5.7 Melting Point

Melting point is also known as melting interval and solidification point commonly used for the determined characteristics of milk fat. The temperature at which the solidified fat transforms completely into liquid state represents melting point. If melting occurs over a temperature range, it is called the melting interval. The study carried out by Godbole and Sadgopal (1939) [13] reported 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 appear to have a melting point of about 36.5°C. (Bhalerao *et al.*, 1947) [6]. Another study, Changade *et al.*, (2006) [10] reported the highest average melting point 33.19 C of ghee which prepared by desi method. There was no much difference in melting points of ghee prepared by direct boiling of cream or by creamery butter method. Rajorhia *et al.,* (1993) [36] reported average analytical constants of Ghee which is given in the Table 3.

5.8 Peroxide value and conjugated Dienes
Hydroperoxides is one the primary byproduct of oxidation processes. The unsaturated fatty acids of fat undergo oxidation which produces hydroperoxides as the primary oxidation products. Though the peroxides as such are not responsible for the development of off-flavors, their measurement gives a good indication to assess the degree of autoxidation. The rate of autoxidation may one of the good indicators for the keeping quality of ghee. Naturally after peroxide formation the non-conjugated double bonds are present in natural unsaturated lipids are converted to conjugated double bonds. These conjugated bonds significantly strongly absorb UV at 233 nm. Later stages it undergoes break down into secondary products which are not absorbing UV light strongly which leads to a decrease in absorbance. BHA (0.02%) delayed the development of conjugated dienes in comparison with the control ghee sample which showed progressive development of conjugated dienes throughout 21 days of storage at 80º C (Hirabhai 2006) [18].

6. Preparation of ghee
India is the largest milk producing country in the world. 155.49 million tons have been reached in total milk production during 2015-16 (National Dairy Development Board). Among the indigenous milk products ghee production forms, the largest segment. Its economic significance to the dairy industry can be realized from the fact that more than 30% of milk produced in India is converted to ghee. India produces 900,000 tons of marketed ghee, valued at Rs. 85,000 million. The market penetration of ghee is about 37% in urban areas and about 21% in rural areas (Tanmay Hazra and Pankaj Parmar 2014) [16]. Basically, two methods namely traditional and industrial methods are used for the production ghee. The industrial method includes creamery-butter method, direct cream method, pre-stratification method, and continuous method (Rajorhia 1993) [36]. In India ghee production is highly depends on the indigenous milk butter method, however the creamery butter methods one of the often used most efficient method and most dairies today using this method. In this method, butter is subjected to melting processes at 60°C which eventually give steam pressure and then rise to 90°C and keep constant if moisture is being released. The desi method accounts for more than 97% of ghee manufactured. The creamery butter and direct cream methods are more suitable for commercial operations because the loss of fat is less (Figure 2a). Direct cream method is reportedly most economical for preparing ghee and product obtained has better keeping quality (Guriye *et al.,* 1949) [14]. However, with industrial interest, the creamery butter and direct cream methods are increasing (Figure 2b). For large scale production of ghee, cow milk collected from the dairy institute and then separated into cream (40 % fat) and stored at 10°C for 12 hours 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 followed to settle and filtered through a double folded muslin cloth. The samples were filled in clean and dry glass stopper bottles, cooled to room temperature and thereafter stored in refrigerator. However, each method has its own merits and demerits. Table 4. Shows relative merits of different method discussed by Aneja *et al.,* (2002) [5].

7. Methods based on physical properties
Several methods have been used to detect the physical Properties of ghee. Oils and fats are important parameters which judging the quality of ghee and used for determining their authenticity. The following methods are widely used to detect the authenticity of ghee based on physical properties.

7.1 Opacity Test
Opacity test was developed by Singhal (1980) to detect the adulteration of ghee with animal or vegetative oils. He studied adulteration of different breed ghee such as buffalo, goat and sheep body fats at 5 percent level and above could be safely detected by opacity test. However, the limitations of this test are that the detection of pig body fat up to 10 percent level is difficult and ghee from cotton tract area also cannot be distinguished. This test also failed to detect the body fat in ghee in the presence of vegetable oils (Singhal1987) [41]. Almost similar results were reported by Sharma and Singhal (1996) [38] at 25°C for pure fats (ghee, body fats and Vanaspati) and adulterated ghee samples at 5, 10 and 20% level.

7.2 Critical Temperature of Dissolution (CTD)
CTD is a temperature at which turbidity appears on gradual cooling of the fat dissolved in a warm solvent or solvent mixture and one of the characteristics of a fat which depends the nature of the solvent, nature and amount of most insoluble glycerides present in a fat the mutual solubilizing power exerted on these glycerides by the soluble glycerides (Boghra *et al.*, 1982) [9]. Bhide and Kane (1952) [8] observed the CTD values for ghee and Vanaspati in the range of 39 to 45°C and 62 to 72°C, respectively, another study Kumar (2013) [3, 20] and Upadhyay (2014) [44] reported that CTD value about 51.6 to 54.6 with average range of 54.3 for pure cow ghee whereas 52.4 to 56.2 with average value of 54.3 for buffalo ghee by using CTD approach.

7.3 Fractionation of milk fat
In Fractionation test milk fat is subjected to controlled temperature at specific time to allow a portion of milk fat to crystallize. Then the crystals are separated by physical methods such as vacuum filtration, pressure filtration, centrifugation etc. Panda and Bindal (1997) [28] studied the crystallization behavior at 17°C of fat dissolved in a solvent mixture of acetone and benzene in the proportion of 3.5:1 and reported that ghee, 10% ghee adulterated with body fats and 10% ghee adulterated with vegetable oils and fats took 19 min, 3 to 15 min and 22 to 23 min to crystallize. The result of their study concluded that the low-level adulteration of animal body fats and vegetable oils and fats could be detected in ghee.

8. Biological importance of Ghee

In India traditional system of medicine evolved over age and has been recognized as Indian medicine for media extraction, absorption and assimilation of any medicine. Many cases it acts as good media for absorption of lipid soluble vitamins and other active principles. It has good membrane permeability effect hence it used as carrier molecules to facilitate the transport of active principles across the cell membrane, which is only permeable to lipid molecules, e.g., in blood-barrier, where transport of "medhya" which promote intellect and memory and are processed in lipid media (Prasanna2007) [35]. Ghee is good for growth of all the seven dhatus in the body (body fluids, blood, flesh, fat, bones, marrow and semen). Another important component of ghee Conjugated linoleic acid (CLA) is a mixture of both positional and geometric isomers of linoleic acid with conjugated unsaturation. Due this unique nature it may acts as anticarcinogenic, antiatherogenic, anti-obesity and antidiabetic agents (Menard *et al.*, 2010).

9. Conclusion

Ghee is a most widely consumed edible fat in India has a plethora of beneficial effects. Its ability to contact within the body tissues make an ideal base to prepare Ayurvedic formulations targeting many diseases including the treatment of allergy, skin and respiratory diseases and known to retard the undesirable effects of drugs besides cancelling the effect of toxins in the body. Adulteration is the major social issue that consequently diminishes the quality of ghee which may affect the health of consumers. Therefore, to understand the basic physicochemical properties of ghee has been deliberately associated with improved consumer knowledge towards the adverse effects of adulteration and developing good sense of choosing the preferable product. Several methods based on the physicochemical characteristics of oils and fats have been developed to detect the various types of adulterants in fats and need to be further concentrated towards improving the value of product. In this study we have discussed the physicochemical nature of ghee and their detecting methods and the biological importance of ghee. Complementing the usage of ghee among the Indian local markets and consumers has seen an authentic and antique pathway but the altering the methods and channelizing a streamlined process in manufacturing, transporting, distributing should be emphasized indebting the large population consuming ghee. Ghee has been a remarkable identity in Indian cuisine denoting ethnic, authentic and holding several therapeutic values.

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