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REVIEW ARTICLE

# Recent innovations in functionality and shelf life enhancement of *ghee*, clarified butter fat

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**Abstract** *Ghee* (clarified butter fat) is a well relished traditional fat rich dairy product. *Ghee* preparation involves concentration of milk fat using of different techniques, followed by heat treated at 110–120 °C for 10–20 min. During this process, moisture evaporates from the system with simultaneous changes in protein, lactose, fat and minerals. Interaction among these thermally altered species results into the development of characteristic '*ghee*' flavor. But, the presence of unsaturated free fatty acids makes it highly susceptible to oxidative spoilage. Efforts have been made to increase the shelf life and functionality of *ghee* by adding many functional ingredients and natural antioxidants from different sources. This review deals with the different process employed for *ghee* preparation and the attempts made in the past two decades years to increase the functionality and shelf life of *ghee*. Also, the changes taking place during *ghee* preparation and flavour generation has been discussed in this review.

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**Keywords** *Ghee* preparation · Flavor generation · Functionality · Shelf life enhancement

**Abbreviations**

| | |
|---|---|
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| ETP | Effluent treatment plant |
| FFA | Free fatty acids |
| PPM | Parts per million |
| PV | Peroxide value |
| SNF | Solids-not-fat |
| TBHQ | Tertiary butylhydroquinone |
| TBA | Thiobarbituric acid |
| TPC | Total phenolic content |

## Introduction

*Ghee* could be defined as a thermally processed purified fat-rich product derived solely by milk or its products into which no preservative or coloring agents has been added. It is consumed in different parts of the under different names, viz., *maslee* and *samna* in the middle-east; *roghan* in Iran; *meshho* in Aramea; *samin* in Sudan; *samuli* in Uganda. It is used for preparation of sweetmeats; cooking and frying of different products, topping for sauces and coffee, feeding children, etc. (Sserunjogi et al. 1998). India is one of the larger producer and exporter of *ghee*. Approximately 30–34% of milk produced in India is converted into *ghee* (Lodh et al. 2018). According to Poonia and Pandhi (2016), about 9 million tonnes of *ghee* is marketed annually in India. Chemically, *ghee* is a complex mixture of different triacylglycerols, free fatty acids, phospholipids, sterols, low quantities of burnt caseins and minerals. *Ghee* is different from other milk fat based products like anhydrous fat and butter oil in terms of method of preparation and its unique flavor profile. *Ghee* could be prepared using milk or milk based fat rich products, however white butter is the most commonly used raw material at the industrial scale for *ghee* preparation. One of the common step in all the *ghee* making methods is a thermal treatment of white butter at 110–120 °C for 10–20 min, during which the heat catalyzed reactions results into generation of distinctive volatile aromas that imparts the distinctive flavour to *ghee* (Aneja et al. 2002). Because of the unique rich flavor, *ghee* is consumed by large populace of all age categories and it is also used for frying and preparation of different sweetmeats. But, the presence of unsaturated free fatty acids makes it highly susceptible to oxidation (Kumar et al. 2010). The condition becomes more crucial as almost all of the *ghee* is packaged hygienically but not under modified atmospheric conditions. Presence of oxygen in the head space along with iron, which might have migrated into *ghee* during the boiling process induces oxidative changes and limits the shelf life of *ghee* (Choe and Min 2006; Mehta 2006; Kapadiya and Aparnathi 2018; Lodh et al. 2018). Oxidation not only decreases the shelf life of fat rich foods, but it is also associated with various neuro-degeneration, diabetes, cancer, cardiovascular and anti-inflammatory diseases (de Mello Andrade and Fasolo 2014).

Increasing health awareness among the common people has enhanced the demand for functional foods. Functional foods could be referred to as those foods which provide health benefits beyond providing the basic nutrition. A common strategy to induce functionality in a product is by adding a functional ingredient (mainly polyphenols) into it. The polyphenols possess multiple unsaturation sites and exert their antioxidant property by different mechanisms, viz., free radical scavenging activity, decreasing lipids peroxidation, affecting the enzyme activity, etc. (Sobhani et al. 2020; Meena et al. 2021). Looking at the wide preference of *ghee*, many researchers have used a variety of natural ingredients for increasing the functionality and shelf life of *ghee*, however a compiled information pertaining to these works in the present times is not available. Considering this, the present review includes the different methods for *ghee* preparation used at the commercial and domestic level, and the works carried out on functionality and shelf life enhancement of *ghee* in the last 20 years.

## Methods of *ghee* preparation

*Ghee* preparation involves concentration of milk fat and suitable heat treatment for imparting the characteristic rich flavour in it. There are four basic methods for *ghee* processing viz., indigenous (*desi*), direct cream, creamery butter and pre-stratification process (Aneja et al. 2002). Each of this method starts with a raw material containing different level of milk fat and involves a thermal treatment (*ghee* boiling) step during which moisture evaporates, protein and lactose degradation occurs which result into flavor generation. At the end, heat treated milk fat is clarified from the residual milk solids-not-fat (SNF) to obtain *ghee*. A brief about all these methods is provided in the following section.

### Indigenous (desi) method

This is the traditional method of *ghee* preparation and is used at the domestic and small scale dairies for *ghee* preparation. It involves collection of cream from the milk by regularly transferring the top layer of milk fat from undisturbed milk. The collected milk fat (cream) is then fermented using lactic acid bacteria. After fermentation, the cream is churned using wooden beater to obtain butter. The butter so obtained is heated on an open fire in metal container until almost all of the moisture evaporates and dark brown colored particles (ghee residue) appears due to Maillard's reaction and caramelization of milk SNF (Newton et al. 2012). Frequency of frothing is often used to judge when to finish the heating process (Fig. 1). Then the contents are left undisturbed for about an hour. The pure fat is carefully decanted as the curd particles settle at the bottom because of the density difference. The collected fat (*ghee*) is then transferred to other container for storage and use. Product obtained using this method has very rich flavor, but this method generates a significant amount of buttermilk of differing consistency and often contributes to low (75–80%) fat recovery (Aneja et al. 2002).

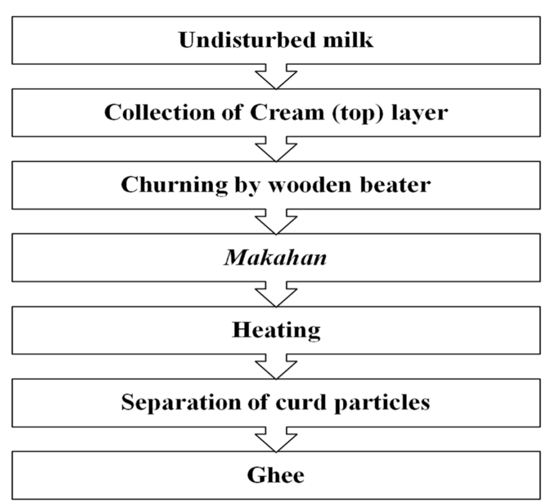


**Fig. 1** Indigenous (*desi*) method

### Direct cream method

In this method, cream is first separated from the milk using centrifugal separators and the cream is then directly used for *ghee* preparation. For *ghee* preparation, cream is transferred to a stainless-steel jacketed kettle (*ghee* boiler) which is equipped with an agitator, steam valve as well as pressure and temperature gauges. During the process, cream is heated and maintained at about 115 °C temperature and the heating is terminated as soon as the milk SNF color changes to light brown. This is followed by clarification of heat treated milk fat (*ghee*) from the remaining contents (Fig. 2). Drawbacks of this method includes long heating duration, as the entire moisture (about 40–50%) present in the cream is to be removed through open pan heating. In addition, high serum solid content imparts strong caramelized flavor in the product and results into large amount of *ghee* residue production. Also, in this method around 4–6% butterfat is lost along with the *ghee* residue. Finally, this method is less labor intensive than indigenous (*desi*) method but lower fat recovery and mildly greasy *ghee* texture are the demerits of this method (Deosarkar et al. 2016).

### Creamery butter method

White (unsalted) butter is used as the raw material in this method of *ghee* preparation. Cream obtained from centrifugal separation of milk is cooled to 6–8 °C and aged for 12–16 h. The aged cream is then churned in butter churn to obtain white butter. The white butter is then transferred to a butter melting vat and heated to 60 °C, followed by pumping the contents into *ghee* boiler. In the *ghee* boiler, contents are heat treated in a similar manner as that in the case of direct cream method. As the moisture evaporation takes place, temperature of the contents remain constant at about 105–110 °C, but as soon at the moisture is completely evaporated, a rapid increase in the temperature occurs. At this point, heating is carefully regulated by regulating the steam valve. The characteristic *ghee* flavor is generated at this point. The boiling process is terminated at the absence of effervescence, presence of finer air bubbles and browning of the curd (SNF) particles, usually this takes place at 115–120 °C. The *ghee* is then screened and upon cooling to 60 °C, filtration is done to remove *ghee* residue and obtain *ghee* (Fig. 3). Increasing the fat concentration by churning cream into white butter helps in reducing the thermal load on *ghee* boiler as less amount of moisture is to be evaporated. Also, less amount of SNF in white butter results into lesser amount of *ghee* residue generation and fat losses with it (Aneja et al. 2002; Ray 2019).

### Pre-stratification method

This approach makes use of gravity based separation of moisture and milk SNF from white butter, saves significant amount of thermal energy needed to remove the moisture. In this method, white butter is the starting material for *ghee* preparation. The white butter obtained after churning the cream is melted in a butter-melting vat by heating it to 80 °C. This is followed by pumping the melted butter into a vertical jacketed storage tank and allowed to stand undisturbed for about 30 min. When the melted butter is left undisturbed at 70–80 °C temperature, the contents get stratified into three distinct layers, viz., an upper layer of denatured curd flakes, a middle layer of fat, and a lower part of buttermilk. Buttermilk, present in the lower strata, comprises 60–70% of total milk SNF and > 80% of the moisture normally found in white butter. Without disturbing the top and middle layers, buttermilk is manually

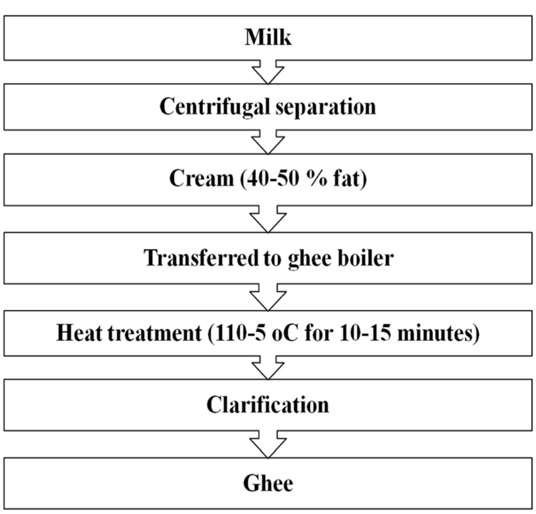


**Fig. 2** Direct cream method

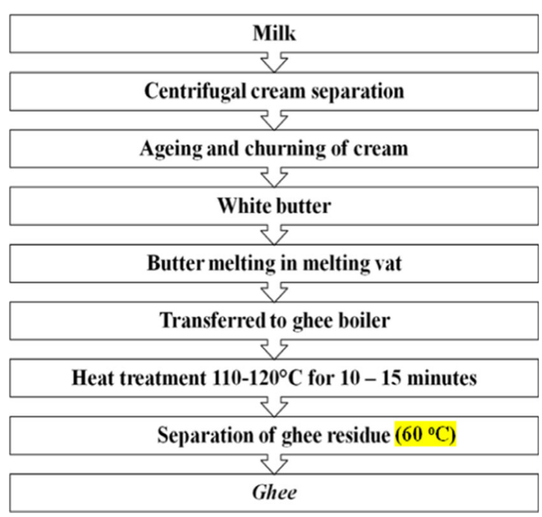


**Fig. 3** Creamery butter method

removed from the bottom. The remaining contents are then transferred to *ghee* boiler, where they are processed in a similar fashion as that in creamery butter method (Fig. 4). This technique provides the benefits of saving up to 35–50% of fuel usage, reducing up to 45% of time and effort, and processing *ghee* with reduced levels of free fatty acid (Aneja et al. 2002).

## Changes taking place during *ghee* preparation

From the previous section, it could be observed that *ghee* preparation involves two processes, viz., fat concentration and thermal treatment. Fat concentration is important because direct desiccation of milk in the *ghee* boiler would result into a semi-solid product (*khoa*) because of the fact that milk contains higher amount of SNF as compared to fat. On the other hand, exposure of a minimal heat treatment to evaporate only the residual moisture from an isolated fat source will result into a butteroil or anhydrous milk fat like product, both of which lacks the characteristic *ghee* flavour. The most economic way to prepare *ghee* is to concentrate milk fat to about 90% by employing the pre-stratification technique of melted white butter, followed by thermal treatment of the fat layers to evaporate the remaining moisture and develop the characteristic '*ghee*' flavour.

Fat concentration is achieved through physical means, viz., centrifugal force during cream separation from milk and mechanical action during the churning process of aged cream. Changes take place in the milk system during these physical processes has already been covered in detail by Halder et al. (2020). On the other hand, changes taking place during the heating (*ghee* boiling) step are unique to this product and involves a variety of chemical reactions along with moisture evaporation. When cream is used as the raw material for *ghee* preparation (direct cream method), no separate serum part is visible during the initial stages when the heating is started; however as the moisture evaporates and fat concentration increases, milk SNF destabilizes and separate out as visible particles as a result of the heat treatment. In addition to this, continued heating of curd (SNF) particles in milk fat results into generation of the characteristic '*ghee*' flavour.

### Flavour generation

Flavour is one of the major acceptability parameter of *ghee* and it is greatly affected by various factors, such as cream or butter fermentation and the extent of thermal conditions employed during the *ghee* boiling step. Yadav and Srinivasan (1992) and Sserunjogi et al. (1998) reported that *ghee* should have rich nutty, pleasant and slightly caramelized flavour. Heat treatment during the *ghee* boiling step results into thermal decomposition of lactose, fat, proteins and amino acids. This results into the generation of carbonyls, free fatty acids (FFAs) and lactones, which are responsible for the development of *ghee* flavor (Newton et al. 2012). Different pathways for flavor development in *ghee* are provided in Fig. 5. Maillard's browning and caramelization are reported to be the main reactions responsible for the generation of these flavours compounds

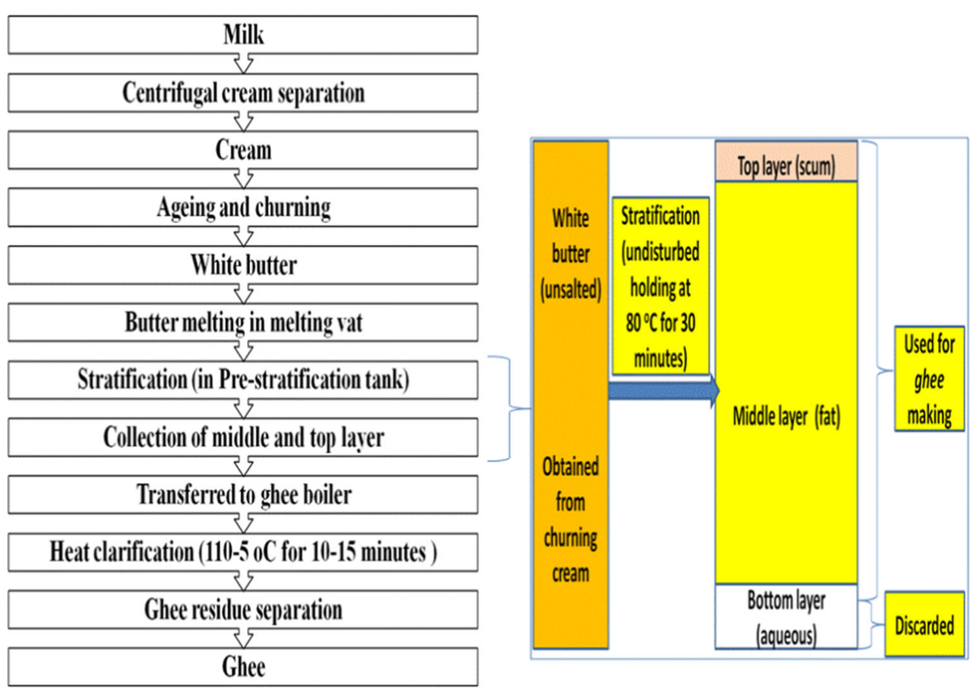


**Fig. 4** Pre-stratification method

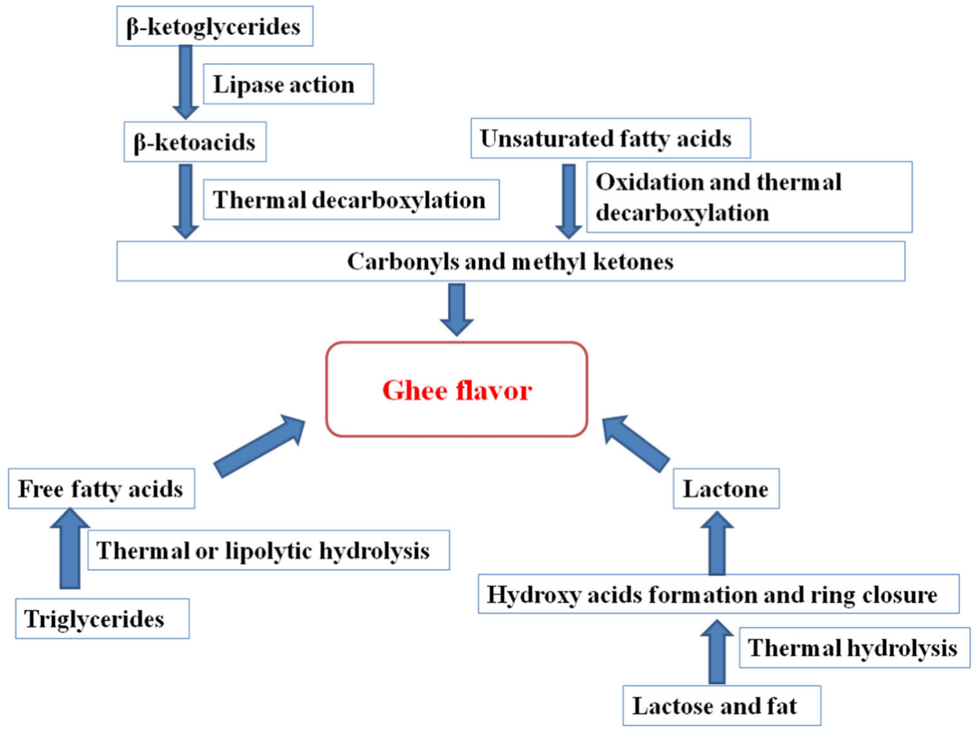


**Fig. 5** Pathways for flavour generation in *ghee*

(Duhan et al. 2020). Rate of these reactions increases with a decrease in moisture content as a result of continued *ghee* boiling for about 12–14 min, particularly when almost all of the moisture is evaporated and the contents are held at around 115 °C (Wadhwa and Jain, 1990; Wadodkar et al. 2002). Butyrolactone was found to decline with an increase in time as well as temperature of *ghee* boiling, while generation of Maillard's reaction derivatives (furfural, 2(5H)-furanone, maltol) were not much affected during the heat treatment at 120–125 °C but there was a distinct rise in their intensity as the temperature increased to 135 °C (Newton et al. 2012). Rao and Ramamurthy (1984) reported that the amount of polar carbonyls increased by ripening the cream and increasing the temperature of *ghee* clarification. Wadodakar et al. (2002) reported that maltol and furans are the products of lactose degradation, and their presence is directly linked with the *ghee* flavour. Edris (2014) reported that cream maturation through *Streptococcus lactis* and *Streptococcus diacetylactis* improved flavour of *ghee* but the cream fermented by *Streptococcus thermophillus* and *Lactobacillus bulgaricus* had no major influence on the flavour of *ghee*. In addition, the author reported that acetic acid contributes towards large proportion of *ghee* volatiles (about 30% of the total short chain fatty acid content). Ongol and Asano (2009) reported that higher proportion of acetic acid could be attributed to the incidence of growth of *Acetobacter* species. Carbonyls (aldehydes and ketones) as well as lactones are reported to play an important role in *ghee* flavour (Yadav and Srinivasan 1992). Assessment of volatile components by GC–MS of *ghee* revealed about the existence of 62 components, which included 8 fatty acids, 12 ketones, 6 aldehydes, 8 alcohols and 8 lactones, as well as 4 esters (Wadodkar et al. 2002). Duhan et al. (2020) reported 39 volatile compounds in *ghee* which included δ-lactones, tetradecanal, 2-nonadecanone, 2-pentadecanone, butyl isodecyl phthalate, glycerol 1-myristate, 2,3-dihydroxypropyl ester. Kwak et al. (2013) evaluated the fatty acid profile of white butter and *ghee* and reported that stearic, palmitic and myristic acids were the predominant saturated fatty acids present in higher amount *ghee* as compared to white butter. On the other hand, short chain fatty acids (such as butyric, capric, caprylic even caproic acid) were found in trace levels in *ghee* in comparison to white butter. Pathania et al. (2021) prepared cow and buffalo milk *ghee* by heating to the contents to different temperatures (110, 130 and 150 °C) during ghee boiling. No significant difference ($p > 0.05$) was obtained in the fatty acid profile of the samples heat treated to different temperatures. Lactose contributes to *ghee* flavor either by participating in the Maillard's reaction or by undergoing thermal degradation (caramelization). Heating above 100 °C results into isomerization of lactose into lactulose, which further degrades to galactose, formic acid and other 5–6 carbon compounds (Berg and van Boekel 1994). Yadav and Srinivasan (1992) reported about the contribution of aldehydes and ketones (carbonyl compounds) in *ghee* flavour. The main group of mono-carbonyls identified in *ghee* is alkan-2-ones which arise as a consequence of microbial metabolism of different components from milk, lipid oxidation, thermal decomposition of lactose and fat (Wadhwa and Jain 1984). Whitfield and Mottram (1992) reported that heating fat results into the production of volatile compounds such as methyl ketones, aldehydes and FFA, which reacts with Maillard reaction products or amino acids to produce flavoring compounds. Joshi and Thakar (1994) reported that when *ghee* is prepared from fermented cream, a variety of compounds are produced by lipid oxidation, namely keto-glycerides as well as carbonylic compounds. Lactones provide a coconut-type aroma synonymous with the distinctive flavor of *ghee* (Schlutt et al. 2007). Wadodkar et al. (2002) reported that δ- and γ-lactones are the predominant lactones present in *ghee*. Wadhwa and Jain (1985) reported that δ-lactones are the primary flavoring lactones in *ghee*, among which δ-decalactone, δ-dodecalctone and δ-tetradecalctone are important. Wadhwa and Jain (1984) reported that lactone concentration in *ghee* increased with an increase in the *ghee* boiling temperature. The total amount of lactone in *ghee* was about double to that of butter, which indicated that heat plays a major role in lactone production in *ghee*. Urbach and Gordon (1994) reported that the amount of saturated δ-lactones was higher in cow milk *ghee* than buffalo milk *ghee*. Rao and Ramamurthy (1984) reported that higher amount of lactone derivatives are formed via hydrolysis of lactogenic (hydroxyl) glycerides preceded through dehydration (lactonization) of hydroxy acids. It is also interesting to note that FFA, which are associated with rancidity and off-flavor in fat rich products, is also a contributing factor in *ghee* flavor (Yadav and Srinivasan 1992). Whitfield et al. (1992) reported about the interaction between FFA and Maillard's reaction products, which results into removal of rancid compounds from the product, primarily because of the interaction between the two. Sserunjogi et al. (1998) reported that *ghee* boiling not only results into development of characteristic *ghee* flavor, but also drives out the off-flavour which might have developed in the white butter during storage.

## Functionality and shelf life extension of *ghee*

*Ghee* is a microbiologically stable product due to its very low moisture content (< 0.5%, w/w), which is influenced by the time and duration of the heat treatment employed during its preparation, also the absence of milk SNF does not support bacterial growth in the *ghee*. Spoilage in *ghee* occurs primarily due to the oxidative degradation of fatty acids resulting into detrimental changes in the sensory attributes and nutritive value. Hydroperoxides are produced during the initial (primary) stages of lipid oxidation, which further undergoes degradation to produce secondary oxidation products like peroxides, oxidized sterols, malonaldehyde, and mono, di and poly cyclic aromatic compounds. Although the primary oxidation products are tasteless but presence of secondary oxidation products severely affects the quality attributes of *ghee* (Mehta et al. 2015). Oxidative changes are mainly due to auto-oxidation of lipids, because of the fact that once the oxidative deterioration is initiated, it continues on its own because of being a self-catalyzed reaction (Kapadiya and Aparnathi 2018). Changes taking place during lipid oxidation have been reported in detail by Choe and Min (2006). Different antioxidants of natural origin have been explored to increase the storage stability and functionality of *ghee*, a brief of which is presented in the following section.

Pawar et al. (2012) and Kapadiya and Aparnathi (2018) reported that antioxidants could delay the lipid oxidation by either restricting the free radicals formation and/or decreasing their propagation by mainly four mechanisms, viz., (a) reducing the free radical species that initiate the lipid oxidation; (b) decreasing the ability of metal ions to generate free radicals by forming complex with them; (c) binding and thus reducing the oxygen free radicals from generating peroxides and (d) disrupting the oxidative chain reaction. In the early ear of 2000s, research conducted on the health implications of synthetic antioxidants, viz., butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) revealed about their mutagenic and carcinogenic effects in experimental animals. Regular intake of synthetic antioxidants has been reported to cause teratogenic and carcinogenic effects in experimental animals, leading to initiation and propagation of cancer cells, chromosomal aberrations and tissue damage (Puravankara et al. 2000). This has led the researchers to look towards natural plant-based resources as a naturally occurring and safer source of polyphenols and antioxidants. Studies conducted in the last two decades on application of different plant as a source for increasing antioxidant activity in *ghee* is discussed in the following section and in Table 1.

### Herbs and spices

Bipinbhai and Aparnathi (2017) optimized the process for curry leaves (*Murraya koenigii*) added *ghee* preparation and reported that curry leaves addition at later stages of heat clarification (at 105 °C) was more effective for retaining the antioxidant potential as compared to addition at earlier stages. Surprisingly, the authors reported that *ghee* added with 0.3% curry leaves had higher resistance to oxidative changes as compared to 0.4% curry leaves added sample, which was attributed to pro-oxidant behavior of polyphenols at higher concentration because of their auto-

**Table 1** Studies on functionality and shelf life enhancement of *ghee*

| Source | Form* | Level of addition | Findings | References |
|---|---|---|---|---|
| Tulsi (*Ocimum sanctum Linn*) | Ethanolic extract of 'Krishna' and 'Shri' variety of *Tulsi* | 0.6% (w/v) level | Krishna *tulsi* extract had higher (154.13 mg/g) phenolic content than Shri *tusli* extract (107.53 mg/g)<br>Krishna tulsi extract addition increased oxidative stability similar to BHA (at 0.02%) in delaying the autoxidation to 192 h at 80 °C | Merai et al. (2003) |
| Ragi | Methanolic extract of husked ragi powder | 0.1, 0.25 and 0.5% | Increased phospholipids and water extractable phenolics content<br>0.5% extract added sample had higher anti-oxygenic indices than 200 ppm BHA added sample | Mehta (2006) |
| Shatavari (*Asparagus racemosus*) | Ethanolic and aqueous extract | Aqueous extract at 1% and ethanolic extract at 0.5% | Ethanolic extract of *shatavari* possessed superior antioxidant activity than aqueous extract<br>Order of DPPH free radical scavenging activity at the end of 21 days storage (at 80 °C): aqueous *shatavari* extract < ethanolic *shatavari* extract < BHA < green tea < TBHQ ~ rosemary extract | Pawar et al. (2012) |
| Coriander | Oleoresin and steam distilled extract | 0.5% | Addition of coriander extract significantly improved the oxidative stability of *ghee*<br>Steam distilled extract of coriander was more effective in delaying oxidation than its oleoresin extract | Patel et al. (2013) |
| *Arjuna* | Ethanolic extract of Arjuna bark | 7% | *Arjuna* bark extract addition increased the resistance towards generation of free fatty acids and reduced the DPPH free radicals<br>Extract added samples were more stable (10 days) than the control *ghee* (2 days) at 80 °C storage temperature | Parmar et al. (2013) |
| Peanut skins, pomegranate peels and olive pomace | Ethanolic, ethyl acetate and n-hexane extract | 200, 400 and 600 ppm | Antioxidant potential increased with increasing extract level in *ghee*<br>Ethanolic extract possessed higher amount of polyphenols and anti-oxidative capacity<br>Order of *ghee* stability added with ethanolic extract: peanut skins > pomegranate peels > olive pomace | El-Shourbagy and El-Zahar (2014) |
| Clove, Ashwagandha, Green tea, Shatavari, Coriander and Vidarikand | Extract | 0.5% | Herbal extract addition resulted into significant increase in the antioxidant activity in *ghee*, in the order: *Shatavari* (33.74%) < *Ashwagandha* (34.25%) < *Vidarikand* (38.72%) < Coriander (52.65%) < Clove (79.84%) < Green tea (80.39%), in terms of DPPH free radical scavenging activity<br>Sample added with clove and green tea extract was most stable after five times frying (each of 180 °C for 7 min) as compared to all other samples | Patel et al. (2014) |
| Clove | Clove extract (oleoresin and essential oil) | @ 0.5% | Order of antioxidant potential and oxidative stability: BHA < clove essential oil < clove oleoresin<br>Order of thermal stability: clove essential oil > BHA > clove oleoresin | Shende et al. (2014) |
| Shatavari (*Asparagus racemosus*), Vidarikand (*Pueraria tuberosa*), and Ashwagandha (*Withania somnifera*) | Aqueous and ethanolic extract | Aqueous extract at 1% and ethanolic extract at 0.5% | Ethanolic extract of the herb possessed higher antioxidant activity than their aqueous extract<br>Order of antioxidant activity: *vidarikhand* > *ashwagandha* > *shatavari*<br>Among all the studied extracts, ethanolic extract of *vidarikhand* resulted exhibited highest antioxidant activity | Pawar et al. (2014) |
| Tomato | Lycopene | 30, 60, 90, 120 and 150 ppm | Lycopene at 150 ppm had stability at par with 200 ppm BHA added *ghee* | Siwach et al. (2016) |
| Orange | Ethanolic extract of peel powder | 1.0% | Order of antioxidant potential: orange peel powder > BHA (200 ppm) > control *ghee*<br>Changes in *ghee* quality were more rapid at higher temperature | Asha et al. (2015) |

**Table 1** continued

| Source | Form* | Level of addition | Findings | References |
|---|---|---|---|---|
| Arjuna | Aqueous and ethanolic extract | 4, 5, 6 and 7% | Ethanolic extract had higher yield and sensory attributes, but lower phytosterol content<br>*Ghee* samples added with ethanolic extract at different level (i.e., 5, 6 and 7%) had similar sensory attributes, but the sample added with 7% ethanolic extract had highest phytosterol content (0.47 mg/g) | Parmar and Khamrui (2017) |
| Curry leaves | Dried powder | 0.1, 0.2, 0.3 and 0.4% | Curry leaves powder at 0.3% had highest resistance to oxidative changes<br>Curry leaves addition at later stages of heat clarification (at 105 °C) was more effective for retaining the antioxidant potential<br>*Ghee* added with 0.3% curry leaves had higher resistance to oxidative changes as compared to 0.4% curry leaves added sample | Bipinbhai and Aparnathi (2017) |
| Shatavari, lemon grass, drumstick leaves, jequirity, dill, tulsi leaves, curry leaves and betel leaves | Aqueous-methanolic (1:4, v/v) extract | 0.5% | *Shatavari*, lemon grass, drumstick leaves, jequirity, dill and *tulsi* leaves extract exhibited pro-oxidant activity<br>Curry leaves and betel leaves extract exhibited strong antioxidant activity and decreased the production of free fatty acids in *ghee* during storage at 80 °C for 12 days | Kapadiya and Aparnathi (2018) |
| Rosemary | Commercially available rosemary extract | 0.1% | Rosemary extract addition increased the antioxidant potential without affecting the physicochemical and sensory attributes of *ghee*<br>Rosemary extract addition increased the thermal stability of *ghee* by decreasing the production of cholesterol oxidation products during frying<br>Shelf life of rosemary extract added *ghee* was 210 and 36 days higher than 200 ppm BHA added *ghee* (90 and 12 days) at 37 and 60 °C temperature, respectively | Rahila et al. (2018) |
| Orange peel | Dried powder | 1, 1.5 and 2% | Increasing peel powder concentration decreased the oxidative changes during storage<br>Sample added with 2% orange peel powder had lowest peroxide value while the sample added with 200 ppm BHA had lowest TBA value | Manjunatha et al., (2019) |
| *Mentha spicata* L, *Salvia officinalis* L, *Mentha longifolia* L and *Cuminum cyminum* L | Essential oil | 20 ppm | Essential oil addition increased the antioxidant potential<br>Order of Antioxidant potential: *Salvia officinalis* L > *Mentha longifolia* L > *Mentha spicata* L > *Cuminum cyminum* L | Pajohi-Alamoti et al. (2019) |
| Ajwain, betel and curry | Dried powder form | 0.5% | Order of cholesterol reduction in *ghee*: betel leaves (10.53%) > *ajwain* (4.63%) > curry leaves (4.50%)<br>Herb addition at later stages of heat decreased more amount of cholesterol | Shingala et al. (2019) |
| Shatavari (*Asparagus racemosus*) and Ashwagandha (*Withania somnifera*) | Ethanolic extract | 0.5% (individually) and combination (1:1 ratio) | Herb addition decreased oxidative changes in *ghee* during storage at 37 °C for 4 months<br>Higher antioxidant activity was observed in *ghee* sample added with combination of herbal extracts<br>Herbal extract addition increased the antioxidant activity in *ghee*, but it was lower than BHA (at 200 ppm) | Deshmukh et al. (2019) |
| Piper betel leaves | Fresh leaves | 1.0, 1.5 and 2.0% | Antioxygenic indices in *ghee* increased with an increase in the piper betel leaves concentration<br>*Ghee* prepared from 1.5% piper betel leaves was most preferred by the sensory panelists | Baburao et al., (2020) |
| Rosemary and clove | Dried powder form | 6% | Rosemary and clove added *ghee* samples had carnosic, rutin and gallic acid as the major polyphenolic compounds<br>Rosemary added sample had higher resistance against oxidative changes<br>Rosemary and clove added *ghee* samples had higher retinol concentration (1.3–3.05 mg/kg) and possessed superior wound healing ability | Maiza et al. (2020) |

**Table 1** continued

| Source | Form* | Level of addition | Findings | References |
|---|---|---|---|---|
| *Harde*, Tamarind, *Catechu, Nagkesar, Jamun, Chicory, Pomegranate*, Areca nut, Mango, Hibiscus, *Goloy*, spiked ginger lily, Banyan tree, *Jivanti*, *Brahmi* | Dried powder form | 0.5% | Some plants increased while others decreased the susceptibility of oxidation in *ghee*<br>Order of oxidative changes in *ghee*: *harde* ∼ *tamarind seeds* ∼ *catechu* ∼ *nagkesar* < *jamun seeds* < *chicory* < control sample < pomegranate peel < areca nut ∼ mango seeds ∼ hibiscus < *goloy* ∼ spiked ginger lily < banyan tree root ∼ *jivanti* < *brahmi*<br>*Ghee* added with tamarind seed, *nagkesar, harde* and *catechu* were sensorially acceptable | Patel and Balakrishnan (2021) |

*Form: powder/extract/isolated compound

oxidation and production of reactive species. In addition, flavor scores of the curry leaves added samples was lower than control samples, primarily due to higher levels of cadinol, pinene, caryophyllene, sabinene and cadinene which altered the natural flavor profile of *ghee*. Similarly, Baburao et al. (2020) prepared herbal *ghee* by adding *Piper betel* leaves into white butter and heating the contents to 115 °C. The authors observed that anti-oxygenic indices in *ghee* increased with an increase in the *Piper betel* leaves concentration but the *ghee* prepared using 1.5% *Piper betel* leaves was most preferred by the sensory panelists due to masking of natural *ghee* flavor in the samples added with 2.0% betel leaves. Shingala et al. (2019) used different herbs for decreasing cholesterol content in *ghee* by mixing aj*wain* (*Trachyspermum ammi*), betel *(Piper betle)* and curry leaves (*Murraya koenigii*) at 0.5% into *ghee* at 80 °C and held for 30 min. The authors reported that cholesterol reduction in the samples were in the order of betel leaves (10.53%) > ajwain (4.63%) > curry leaves (4.50%). Also, it was observed that herb addition at later stages of heat clarification (at 90 °C, after boiling at 105 °C) was found to decrease more amount of cholesterol as compared to herb addition at earlier stages of *ghee* preparation. Maiza et al. (2020) prepared herbal *ghee* by macerating rosemary (*Salvia rosmarinus*) and clove (*Syzygium aromaticum*) in *ghee* at 60 °C for 30 min. It was observed that herbal *ghee* samples had higher retinol concentration (1.30–3.05 mg/kg) and possessed superior wound healing ability by increasing the migration of fibroblast to the affected site. Ambhore et al. (2020) studied the effect of turmeric (*Curcuma longa* L.) powder incorporation on the sensory quality of *ghee* prepared using fermented traditional (*desi*) method. The authors added turmeric powder at three different levels (0.5, 1.0 and 1.5. %, weight basis) during three stages of *ghee* preparation i.e., directly to milk, curd and butter. It was observed that turmeric addition resulted into bitterness perception and increased yellowness in the samples, and the *ghee* prepared from the milk into which turmeric powder was added was scored highest by the panelists, but it was still lower than the control *ghee* scores. Patel and Balakrishnan (2021) used 14 nonconventional plants as a source of natural antioxidants for increasing the antioxidant potential in *ghee* and reported that some plants increased while others decreased the susceptibility of oxidation in *ghee*. The order of oxidative changes in *ghee* during storage were in the order of harde ∼ tamarind seeds ∼ catechu ∼ nagkesar < jamun seeds < chicory < control sample < pomegranate peel < areca nut ∼ mango seeds ∼ hibiscus < goloy ∼ spiked ginger lily < banyan tree root ∼ jivanti < brahmi. Besides this, *ghee* samples added with tamarind seed, nagkesar, harde and catechu were sensorially acceptable to the panelists.

From the above studies, it appears that addition of antioxidants rich agricultural ingredients results into an increase in the antioxidant potential in *ghee*. But this is also accompanied with significant changes in sensory attributes, viz., color and flavor of the product. The change in sensory attributes could be in part due to the polyphenols and also because of other bio-polymers (viz., proteins, minerals and carbohydrates) that would have migrated into *ghee* along with the polyphenols. In addition, the amount of fat adhered to these materials (in addition to *ghee* residue) results into increased losses during clarification. Based upon these facts, researchers have focused upon application of plant extracts, instead of the plant material itself, to minimize these changes. It is also important to mention here that the intense heating (110–115 °C) affects the antioxidant activities of the polyphenols. Studies are available which indicates about decreased activities of polyphenols upon exposure to higher temperatures during *ghee* preparation (Lodh et al. 2018). Shingala et al. (2019) reported that herb addition at later stages of heat clarification (at 90 °C, after boiling at 105 °C) decreased more amount of cholesterol as compared to herb addition at earlier stages of *ghee* preparation.

## Application of extracts

This section deals with functionality and shelf life extension of *ghee* using plant extracts. Different solvents have been used to extract polyphenols, viz., methanol, propanol, hexane, water, etc. Further, physiologically active compounds like curcumin, lycopene, eugenol, etc. isolated from the source plant material have also been explored for functionality and shelf life extension of *ghee*.

Selection of solvent primarily depends upon the polyphenols extraction efficacy of solvents and ease of solvent removal from the polyphenol—solvent mixture. Sandhya et al. (2018) reported that ethanolic extract of pomegranate peel contained higher amount of polyphenols as compared to aqueous extract. Ahmed et al. (2018) studied the extraction of essential oil from *Coriandrum sativum* using different solvents and reported that highest yield was obtained from petroleum-ether (8.82%), followed by chloroform (7.07%) and methanol (6.72%). For the removal of solvent from polyphenol—solvent mixture, the contents are heat treated to temperatures above the boiling point of the solvent. Solvents with higher boiling point need to be heated to higher temperature, which requires higher amount of energy. This emphasizes on the need for solvents with lower boiling point to conserve energy. Although, almost all of the solvent is evaporated during the heating of polyphenol—solvent mixture, but the traces of solvent that are still present in the final product is always a source of concern for the food industries. Food Safety and Standards Authority of India (FSSAI), an autonomous body under the Government of India which deals with the safety and quality of food products, has allowed a maximum of 5 mg/kg of hexane as residual solvent in the oils extracted using solvent extraction with proper labeling on the package as 'solvent extracted oil'. Sicaire et al. (2015) reported that hexane is registered as category 2 reprotoxic under the European regulation 'Registration, Evaluation, Authorization and Restriction of Chemicals' (REACH) and also classified as category 2 aquatic chronic toxic. Crinnion (2010) reported that short term exposure of these solvents through inhalation or absorption through skin might lead to irritation in eye and skin, nausea and dizziness; while long term exposure may lead to malfunctioning and disorders in the central nervous system, kidney and liver.

This has led to increased preference for environment friendly and lesser toxic solvents for extraction of polyphenols. These solvents are derived from bio-degradable feedstock which will not remain for long in the environment and formation of hazardous products during their preparation or processing is negligible. Because of the fact that they are prepared using chemical that are rapidly degraded in the environment, they are eco-friendly and do not adversely affect the human health. Examples of such solvents include ethanol, di-methyl carbonate, methyl ether, ethyl acetate, etc. (Anastas and Kirchhoff, 2002).

### *Herbs and spices*

Tulsi (*Ocimum tenuiflorum*) is a widely used herb in the south Asian region because of its functional activities. Merai et al. (2003) isolated antioxygenic compounds from the ethanolic extract of two varieties of *tulsi* (*Shri* and *Krishna*) powder and reported that '*Krishna*' variety contained higher amount of phenolics (154.13 mg/g) as compared to '*Shri*' variety (107.53 mg/g). It was observed that addition of '*Krishna tulsi*' extract at 0.6% (w/v) level into creamery butter *ghee* had antioxygenic effect similar to that of BHA (at 0.02%) in delaying the auto-oxidation to 192 h at 80 °C. Kapadiya and Aparnathi (2018) compared the anti-oxidative activity of different herbs in delaying the oxidative changes during the shelf life of *ghee*. The authors mixed 0.5% (w/w) aqueous—ethanolic extract of each herb with *ghee* at 80 °C for 30 min, followed by filtration through six layered muslin cloth. It was observed that *shatavari* (*Asparagus racemosus*), lemon grass (*Cymbopogon* spp.), drumstick leaves (*Moringa oleifera*), jequirity (*Abrus precatorius*), dill (*Anethum graveolens*) and *tulsi* (*Ocimum tenuiflorum*) leaves exhibited pro-oxidant activity, while curry leaves (*Murraya koenigii*) and betel leaves (*Piper betle*) exhibited strong antioxidant activity and decreased the production of free fatty acids in *ghee* during storage at 80 °C for 12 days. Lodh et al. (2018) optimized the processed for curcumin fortified buffalo *ghee* preparation by varying curcumin levels, heating time and temperature. The authors reported that increasing curcumin level increased the antioxidant potential, while increasing heat treatment decreased the same. Also, increasing curcumin level and heating temperature increased the sensory attributes of *ghee*. Curcumin (300 ppm) and heat treatment of 115 °C for 17.89 min yielded most suitable *ghee*. Works related to functionality and shelf life extension of *ghee* using herbs and spices extract is provided in Table 1.

### *Fruits*

Fruits are known to possess high amount of polyphenols. This has been linked with their growth promoting and disease preventing activity. For the same reason, they have also been explored as source for natural antioxidants for increasing the functionality and shelf life of *ghee*. Siwach et al. (2016) studied the effect of lycopene addition at 30, 60, 90, 120 and 150 ppm on the storage stability of cow milk *ghee* at 30 °C for 12 months and reported that lycopene addition at 150 ppm level resulted into stability of *ghee* at par with the BHA (200 ppm) added sample. Similarly, Aditya and Divya (2018) used aqueous and alcoholic extract of guava (*Psidium guajava*) leaves for increasing the oxidative stability in *ghee*. The authors reported that addition of guava leaves extract increased the antioxidant potential in *ghee* and the optimized level of aqueous and alcoholic extract in *ghee* were 1.0 and 0.25%, respectively. It was found that the optimized extract added *ghee* samples were more stable (60 days) than the control *ghee* sample (40 days) at 60 °C.

### *Cereals*

Cereals are commonly used as an economic and rich source of energy (carbohydrates). In addition they also contain significant amount of protein and polyphenols, primarily in the husk and germ region. This contributes to their antioxidant and free radical scavenging activity. Kaur et al. (2001) reported that addition of sorghum (*Sorghum bicolor* Linn) grain powder at 1% level during *ghee* boiling resulted into increase in the phenolic content. Similar results were reported by Mehta (2006) for methanolic extract of husked *ragi* (*Eleusine coracana* L) powder.

### *Agricultural by-products*

By-products are obtained during the course of processing agricultural produce and occur in large quantities. They could arise from peels, seeds, etc. Compositional analysis indicates about the presence of abundant polyphenols in the agricultural by-products. Processing these by-products in an Effluent Treatment Plant (ETP) before disposal is essential to prevent environment pollution. Utilizing these by-products for food applications has been an emerging trend with a major objective to diversify the source of polyphenols and decrease the cost of processing in ETP. Orange peel (comprising of about 50% of the total fruit) is an important by-product obtained from the orange processing industries. Puravankara et al. (2000) utilized the mango (*Mangifera indica* Linn) seed kernels as an economic source of polyphenols and phospholipids for increasing the shelf life of *ghee*. The authors reported that higher shelf life was obtained in the samples containing both polyphenols and phospholipids, indicating about their synergistic activity in extending the shelf life of *ghee*. Higher shelf life was obtained at 5% level of mango seed kernel extract (polyphenols and phospholipids) as compared to 200 mg/Kg BHA. El-Shourbagy and El-Zahar (2014) used agricultural byproducts, viz., peanut (*Arachis hypogaea*) skins, pomegranate (*Punica granatum*) peels and olive (*Olea europaea*) pomace for increasing the antioxidant potential in *ghee*. It was found that ethanolic extract (80%) possessed higher amount of polyphenols and anti-oxidative capacity then the extract obtained using ethyl acetate and n-hexane. Also, ethanolic extract of peanut skins resulted into highest increase in the stability of *ghee*, followed by pomegranate peels extract and olive pomace. Asha et al. (2015) added ethanolic extract of orange (*Citrus sinensis*) peel powder at 1.0% (w/w) level in *ghee* and monitored the changes in oxidation and sensory attributes during storage at 6, 32 and 60 °C for 21 days. The authors reported that changes in *ghee* quality were more rapid at higher temperature and sample added with orange peel extract experienced least changes in oxidation status, followed by BHA (200 ppm) added sample and control *ghee*. Abdel-Ghany (2018) added ethanolic extract of taro (*Colocasia esculenta*) peels at 200, 400 and 600 ppm in *ghee* and reported that taro peel extract addition resulted into increase in the oxidative stability of *ghee* at 63°C for 21 days storage. Ali et al. (2019) reported that addition of rice bran methanolic extract resulted into an increase in the resistance against oxidative changes in *ghee* without causing any major changes in the fatty acid profile of *ghee*. Manjunatha et al. (2019) added orange peel powder at 1, 1.5 and 2% (w/w) into white butter and heated the contents at 115 °C for *ghee* preparation, and changes in peroxide value and thiobarbuturic acid content were monitored at 60 °C for 16 days. At the end of storage study, the authors reported that sample added with 2% orange peel powder had lowest peroxide value while the sample added with 200 ppm BHA had lowest thiobarbuturic acid content. Similarly, Srivastava and Singh (2019) reported that addition of pomegranate, apple, banana, orange peel ethanolic extract and *tulsi* leaves resulted into an increase in the oxidative stability of *ghee* at 63 °C during 6 months duration.

Although many studies have been reported on functionality and shelf life extension of *ghee* using plant extracts (Table 1); however, it is important to mention here that the constituents (minerals, proteins and carbohydrates) present in the plant materials synergistically increases the anti-oxidative activity of polyphenols and proposed that application of plant extract instead of plant material could not yield superior antioxidant activity in the product (Bipinbhai and Aparnathi, 2017).

## Conclusion

*Ghee* is prepared using four different methods. During the heat treatment, protein, fat and lactose degrades and their interaction results into the development of characteristic *ghee* flavor. Presence of unsaturated and free fatty acids makes it susceptible to oxidation. Agricultural commodities viz., herbs, spices, fruits, bark, peel, by-products, etc. contain high levels of antioxidants. Application of plant based antioxidants resulted into an increase in the storage stability and functionality of *ghee*, but the sensory attributes are also significantly altered. Also, higher level of herb addition tends to decrease the antioxidant potential in *ghee*. Use of extract, in place of plant material, tends to minimize the changes in sensory attributes without much affecting the anti-potential.

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