



Flavour Profile of Ghee by Gas Chromatography Mass Spectrometry (Gc-Ms): A Mini Review

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RRS, RNB, TB and KJT conceptualized and drafted the manuscript. Author MR provided the necessary guidelines and contributed critically to revise the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Ghee, a clarified milk fat, is known for its rich and complex flavour, which arises from a diverse array of volatile compounds formed during heat clarification. This article explores the flavour profile of ghee using Gas Chromatography-Mass Spectrometry (GC-MS) and identifies key volatile components including carbonyls, lactones, free fatty acids (FFAs), esters, and miscellaneous compounds. The formation of these volatiles is influenced by factors such as the method of preparation, temperature and duration of clarification, acidity of raw materials, and microbial activity during fermentation. Traditional ghee, especially that prepared from ripened cream or butter, shows a higher concentration and diversity of flavour compounds compared to industrial counterparts. Compounds such as methyl ketones, δ -lactones, and specific FFAs are found to be primary contributors to the characteristic aroma and taste of ghee. The study also examines how feed, season, and enrichment with aromatic herbs affect ghee's volatile profile. These findings underscore the intricate biochemical transformations responsible for ghee flavour and highlight the potential of GC-MS as a powerful tool for its characterization.

Keywords: *Desi ghee; starter culture; bael fruit; GC-MS.*

1. INTRODUCTION

"Milk fat is the source of most of the flavour compounds in dairy products including ghee. Milk proteins and lactones also play an important role in producing flavour compounds during the heat desiccation of *makkhan*, butter or cream. The flavour of ghee is known to be affected by various other technical parameters like the method of preparation, the temperature of clarification, holding time, and storage period" (Bhide, 2014). "Some of the commercial products like anhydrous milk fat, butter ghee, and butter oil fit to the definition of ghee based solely on their physical characteristics. Whilst these are similar in some of their characteristics, these products do not have the same flavour profile to that of traditional indigenous ghee. Good ghee has been defined as having a pleasant, nutty, lightly cooked, or caramelized flavour. The flavour of ghee cannot be attributed to a single compound, but rather to a large number of compounds, alcohols, aldehydes, carboxylic acids, fatty acids, ketones and lactones" (Su et al., 2024). "Among these groups of compounds, carbonyls and lactones play a major role in imparting typical ghee flavour" (Yadav & Srinivasan, 1987). Deosarkar et al. (2016) reported that "the important flavour components of ghee were free fatty acids (FFAs) (6–12mg/g), methyl ketones (6–12ppm), and a complex mixture of 44 lactones, both 5-lactones and γ -lactones (29–43ppm), aldehydes, ketones, and alcohols. About 34 monocarbonyls were found in the flavour volatiles of ghee".

"These flavour compounds are generated during the heating process due to reactions between protein and lactose, protein and lactose degradation products, lipid oxidation, and degradation of FFAs" (Newton et al., 2012). "Ghee prepared at 120°C or above has an intense flavour which is usually referred to as cooked or burnt flavour. In contrast, ghee prepared at around 110°C has a somewhat mild flavour, often referred to as curdy. The presence of maltol and furans has been linked to significant differences in flavours. These products, along with other pyrans, various ketones, and aldehydes attribute to Maillard reaction in products" (Newton et al., 2012). "The acidity of the raw materials like cream or butter also affects the flavour of ghee. Sweet cream-butter yields ghee with a flat flavour whereas, cream or butter having an acidity of 0.15 to 0.25% lactic acid as in the case of ripened cream/butter, produces ghee with a more acceptable flavour" (Achaya, 1997).

The flavour of ghee can be enhanced by using ripened raw materials (*dahi*/butter/cream) which might be ascribed to the metabolic activity of the background microbiota on the various constituents of the raw material like lactose, citrate, and glucose. The unique ghee flavour and texture are obtained from microbial fermentation (Feng et al., 2021) "In a study, it was observed that the ghee flavour score for ripened cream ghee with *Streptococcus lactis* DRC-I was the highest as compared to the ghee made from washed cells of the starter culture and control unripened ghee. The enhancement of flavour score was due to the metabolic

products formed as a result of the activity of starter culture and not due to the cell mass obtained by its growth which was confirmed by the incorporation of washed cells of the starter culture into the fresh cream. The flavour compounds produced during the ripening process by starter activity get incorporated into the final product during the clarification process. In addition, it is likely that the lower pH produced by the starter activity also helps in increasing the intensity of chemical reactions like caramelization, during heat clarification. These reactions are reported to contribute to the flavour components in ghee" (Yadav & Srinivasan, 1987). Moreover, it was found that volatile compounds like alcohols, esters, aldehydes, and ketones got accumulated throughout the process of fermentation and the time of fermentation influenced the nature of the ghee aroma, short fermentation time have more fruity aroma while long fermentation time processed more buttery and cheesy aroma (Liang *et al.*, 2025). These volatile flavour compounds can be identified using GC-MS, an analytical method which uses gas chromatograph and mass spectrometer that identify substances in a much finer degree. The compounds identified to be associated with the ghee flavour are discussed below.

Carbonyls:

Carbonyl flavour compounds like acetaldehyde, acetone, formaldehyde, and butanone are naturally found in milk (Subrahmanyam *et al.*, 1991). Various sources contribute to carbonyl compounds in ghee. It is known to be formed as a microbial metabolism of various milk constituents, heat decomposition of milk carbohydrates and fats, oxidation of milk lipids, and from chemical reactions. Both monocarbonyls (alkan-2-ones, alk-2-enals, alkanals, alk-2,4-dienals) and dicarbonyls (diacetyl, furfural, methyl glyoxal, α -ketoglutaric acid, hydroxy-methyl furfural) have been identified in ghee (Yadav & Srinivasan, 1992). These compounds are associated with the development of ghee flavour. Other carbonyls associated with it are methyl ketones (2-pentanone, 2-heptanone, 2-undecanone, 2-tridecanone, 2-nonanone, 2-pentadecanone) (Wadodakar *et al.*, 1996). Methyl ketones are formed by hydrolysis of beta-ketoglycerides to keto-acids followed by decarboxylation during heat process (Wadodakar *et al.*, 1996) or due to oxidation of unsaturated fatty acid to beta-ketoacids followed by decarboxylation (Yadav & Srinivasan, 1992). Aldehydes and other

monocarbonyls are formed by autoxidation of unsaturated fatty acids; and polar carbonyls, viz., beta-ketoacids and glyoxal formed due to metabolism of milk constituents, viz., lactose, amino acids and citrate or from browning reaction (Yadav & Srinivasan, 1992). Gaba and Jain (1974) reported that the total carbonyl content of cow ghee prepared from ripened butter was significantly higher, i.e., 9.66 $\mu\text{M/g}$ fat, than that prepared from fresh butter which was 7.26 $\mu\text{M/g}$ of fat. However, no significant differences were observed in the total carbonyl content of buffalo ghee prepared from fresh and ripened butter. The volatile carbonyl contents of fresh ghee (cow's and buffalo's) prepared from ripened butter (0.42 $\mu\text{M/g}$ fat and 0.28 $\mu\text{M/g}$ fat, respectively) was higher than that prepared from fresh butter (0.33 $\mu\text{M/g}$ fat and 0.26 $\mu\text{M/g}$ fat, respectively).

Lactones:

Lactones impart a coconut-like aroma to ghee. These are formed by hydrolysis of lactogenic glycerides to hydroxy acids during heat processing followed by ring closure or lipolysis of glycerides before ring closure (Yadav & Srinivasan, 1992). Delta lactones constitute the major compounds of ghee volatiles whereas; gamma lactones constitute only 5-10% of the total lactone content. Moreover, saturated lactones constitute 65% while the remaining 35% are mainly unsaturated lactones (Yadav & Srinivasan, 1992). δ -lactones, δ -octalactones (C_8), δ -decalactones (C_{10}) and delta dodecalactones (C_{12}) are the most important compounds influencing the flavour of ghee (Urbach & Gordon, 1994). The level of lactones increases with the increase in temperature of clarification of ghee without any qualitative difference (Wadhwa & Jain, 1984). The lactone potential of milk fat is also affected by feed and season, Griffith and Hammond (1989).

Free Fatty Acids:

FFAs are formed by hydrolysis of fat during heat processing (Wadodakar *et al.*, 1996) or by lipolysis of fat by naturally occurring lipases (Yadav & Srinivasan, 1992). Various FFAs are known to occur in ghee, viz., butyric acid, caproic acid, capric acid, caprylic acid, lauric acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid and linoleic acid (FSSAI, 2021). FFA contributes significantly to ghee flavour. However, some of the FFA like the short-chain homologues is undesirable in milk fat

as they are responsible for the rancid flavour of ghee and also found to catalyze fat oxidation (Munro *et al.*, 1992). "From nutritional point of view, a lower proportion of saturated FAs (SFA) and a higher proportion of unsaturated FAs (UFA), especially polyunsaturated FAs (PUFA) n-3, is desirable; and from a unsability point of view, higher proportions of UFA are preferred (i.e., easier spreadability of butter is desirable for consumers). However, there are also problems associated with having high UFA content in milk fat, including its lower stability and the accompanying phenomena such as oxidation and possible sensory changes making desirable changes to the FA profile" (Hanus *et al.*, 2018).

"Some factors affecting the FA profile of milk are altitude, breed, lactation order (parity), lactation stage, and diet. A high proportion of green pasture or preserved forage as compared to grain concentrates and increasing the proportion of oilseeds in feed concentrates as compared to non-oleaginous seeds in dairy cow feeding rations improves the milk FA profile by increasing UFA and rumenic acid, viz., conjugated linoleic acid (CLA) in milk fat" (Hanus *et al.*, 2018). "Incorporation of dried plants into the liquid ghee also showed some significant differences in CLA. The percentage of CLA was significantly higher in ghee samples macerated with rosemary (1.50 ± 0.07) or with clove (1.24 ± 0.06) added at a rate of 6% (w/w) over that of the control ghee, while the other fatty acids were remained same" (Maiza *et al.*, 2020). "Palmitic and oleic acids were the dominant fatty acids in ghee, with 37.14 to 41.58% concentration followed by oleic acid i.e., 19.49 and 25.12%" (Dorni *et al.*, 2018). "The variation in FA composition might be related to differences in feeding practices, seasonal and climatic conditions, and other factors" (Najafi *et al.*, 2015). "FAs, viz., hexanal, heptanal, 2-nonenal, 2,4-decadienal, 2,6-nonadienal, and 1-octen-3-ol which gives a metallic, tallowy, and buttery taste are the products of auto-oxidation of USFAs and on prolonged storage of butter in cold storages" (Erfani *et al.*, 2020). "In camel milk ghee, a total of 35 different fatty acids are found. Important ones being α -linolenic, caproic, capric, linoleic, myristic, palmitic, pentadecylic, petroselinic, oleic and stearic acids. Type of FAs in ghee depends on the breed of camel from which milk was procured" (Deshwal *et al.*, 2022).

Esters:

"Esters may originate from the esterification of short-chain alcohols and FFA by the action of

bacterial esterases present in LAB" (Hosona *et al.*, 1974).

Miscellaneous Substances:

"Decomposition of the milk constituents like fat, protein, lactose, and glucose during the clarification process result information of dimethyl sulphide, alcohols, acrolein, diols, and denatured protein compounds. Indole and skatole present in butter may pass over into ghee and influence its flavour" (Sarkar *et al.*, 1993). "Alcohols are formed by reducing aldehydes and diols are formed as a result of lipid oxidation" (Wadodakar *et al.*, 1996).

"The aromatic compounds of ghee can vary due to differences in the feed, season of the year, production method, raw materials (milk, cream, butter, and yoghurt), butter clarification temperature, environmental temperature and storage conditions" (Azzara *et al.*, 1992). In a GC-MS study, Erfani *et al.* (2020) found compounds such as butyric acid, dodecane, acetone, hexanoic acid, 2-heptanone, 2-pentanone, and 2-undecanone, which accumulated as a result of oxidative, hydrolytic, or microbial activities, and contributed to the overall flavour of ghee. Specific volatile compounds produced by microbial enzymes are reported as hexanoic acid, octanoic acid, decanoic acid, heptanoic acid, butyric acid, 2-pentatone, tetradeconic acid aldehyde, 2-nonanone, 2-heptatone, 2-docecanone, 2-undecanone, octanoic acid and ethyl esters which impart different odour quality like buttery, sweaty, cheesy, milky, fruity, nutty, soapy, etc.

"The high concentrations of short-chain fatty acids like butyric acid can be associated with the activities of LAB as well as lipase" (Yadav & Srinivasan, 1992). "The variations in the short-chain fatty acids in ghee samples might be due to high temperatures used in the clarification process for butter, which caused the formation of short-chain fatty acids" (Wadodakar *et al.*, 1996).

"The ghee prepared from naturally fermented sour cream butter was rich in volatile compounds, particularly short-chain fatty acids. GC-olfactometric study of the ghee volatile compounds that contribute to its typical flavour was mainly composed of SFA approximately 34.77%, methyl ketones 16.11%, aldehydes 9.13%, and alcohols 5.4%" (Edris, 2014).

“Gas Chromatography-Mass Spectrometry (GC-MS) study of *desi* ghee prepared from fermented cream by heat clarification method revealed more volatile compounds (36 numbers) than the industrial ghee samples which had only 22-29 numbers. Of the identified compounds, maltol, 1,3-butanediol, 1-octanol, 5-hydroxymethyl furfuraldehyde, and dihydrodihydroxypyranone were documented to be present only in *desi* ghee. The concentration of acetic acid was found to be remarkably higher in *desi* ghee volatiles than in industrial ghee. In addition to these, the levels of identified fatty acids, methyl ketones, lactones, aldehydes, and alcohols were found to be higher in *desi* ghee volatiles compared with industrial ghee. In total, 62 compounds were

detected, which included 6 aldehydes, 12 ketones, 8 FAs, 4 each of esters, lactones and alcohols, hydrocarbons or other compounds, and 12 unidentified compounds” (Wadodkar *et al.* 2002).

Bhide (2014), however, documented that “ghee made from cow's milk-fed on a regular diet and prepared by the direct cream method has some of the volatile flavour compounds such as methyl mercaptan, furan, acetol, 2-methylfuran, formic acid, methyl pentanoate, furfuryl alcohol, cyclopent-2-en-1,4-dione, 2-acetyl furan, 5 methyl furfural, gamma-butyrolactone, and 2(5H)-furanone. These compounds are the derivatives of components of solids-not-fat, the

Table 1. Volatile compounds in ghee identified by GC-MS*

Compound Formation	Volatile Compounds	Odour Quality
Naturally occurring in milk	Dodecane Tetradecane Octadecane Hexadecane	Sweet Peppery, pungency Sweet Sweet
FFA Microbial, enzymatic reactions	Acetic acid Butyric acid Hexanoic acid Heptanoic acid Octanoic acid Decanoic acid Tetradecanoic acid	Sour, pungent, vinegar-like, grassy Buttery, sweaty Pungent, musty Cheesy, rancid Cheesy, rancid, musty Cheesy, rancid Cheesy
Aldehydes Lipid oxidation of unsaturated fatty acids during storage of butter or preparation of ghee	2-Methyl pentanal acetaldehydehexanal 2-heptenal 2-decenal 5-hydroxynethyl furfuraldehyde	Rancid
Ketones Breakdown of β -ketoacids through thermal processes or enzymatic activities	Acetone 2-Pentanone 2-Heptanone 8-Nonen-2-One 2-Nonanone 2-Undecanone 4-Hepten-3-one diethyl 2-Dodecanone	Sweet Creamy, green, milky, soapy Blue cheese, fruity, dairy-like Indolic, floral, honey, skatole Cooked Green, nutty Oxidized Green, nutty
Lactones Hydrolysis of lactogenic glycerides into hydroxy acids through a thermal process	Delta nonalactone Delta decalactone Delta dodecalactone Gamma-lactone	Coconut- and peach- like aroma Coconut-like, peach, flower-like Peach -
Esters Esterification of fatty acids and short-chain alcohols by the esterase activity of LAB	Diethyl phthalate (environmental pollution) Octanoic acid Ethyl ester (ethyl octanoate)	Bitter, slightly fruity Cheesy, rancid Fruity

* Wadodkar *et al.* (2002); Erfani *et al.* (2020)

fraction which either gets discarded during churning or is digested during fermentation in traditional method of ghee production method. These derivatives are absent in ghee samples made from cultured cream. On the contrary, chloroform, 2-hexanone, and hexanal are found only in ghee prepared from cultured cream. Acetone, 2-butanone, 2-pentanone, 2-heptanone, 2-nonanone, and 2-undecanone are present in all three kinds of ghee; significantly higher levels are, however, reported in ghee made by traditional and cultured cream methods. Higher concentrations of these compounds indicate that their levels increased upon fermentation". Bhide (2014) found similar results for organic grass-fed cow milk ghee. Duhan *et al.* (2019) found volatile flavour compounds of δ -dodecalctone (C12), δ -tetradecalctone (C14), 3-ethyl-3-methyl heptanes in cow milk ghee.

Enrichment of ghee with dried aromatic plants of rosemary and clove by hot maceration can have a marked effect on its volatile profile and therefore on its flavour. Maiza *et al.* (2020) found 47 volatiles compounds in aromatic plant macerated ghee, among which were eugenol and α -humulene in clove macerated ghee and 1,8-cineole, camphor and α -pinene in rosemary macerated ghee. The components found in the ghee, were originally present in the respective plants that were added to the ghee.

2. CONCLUSION

The flavour of ghee is a result of complex biochemical reactions occurring during its production, notably influenced by the heating process, microbial fermentation, and the nature of the raw materials used. GC-MS analysis reveals that carbonyls, lactones, FFAs, esters, and miscellaneous volatile compounds are key contributors to its characteristic aroma. Traditional methods, particularly those involving fermentation with lactic acid bacteria, yield a richer and more desirable flavour profile than modern industrial methods. Factors such as feed, lactation stage, and seasonal variations further influence the fatty acid composition and resultant flavour volatiles. Additionally, fortification with aromatic herbs like rosemary and clove can significantly enhance the sensory appeal of ghee. These insights not only support the importance of traditional ghee preparation methods but also offer avenues for developing premium ghee products with improved flavour and potential health benefits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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