Indian J Dairy Sci 79(1): 18-24
https://doi.org/10.33785/IJDS.2026.v79i01.004

RESEARCH ARTICLE

Flavour profile of *desi* cow's milk ghee prepared with *bael* pulp extract

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Received: 11 August 2025 / Accepted: 13 October 2025 / Published online: 23 February 2026

Abstract: The study investigated the flavour enhancement and oxidative quality stabilization properties of selected starter cultures and pulp extract of *Aeglemarmelos* (bael) in*desi* cow ghee. Four treatment groups (T_1 - T_4) were designed using two culture types: a yoghurt culture containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (T_1 and T_2), and *Lactococcuslactis* ssp. *lactis*biovar*diacetylactis* (T_3 and T_4). Cultures were added at 3% (v/v), with or without 1%(v/ v) bael pulp extract. 1% (v/v) bael pulp extract. Ghee was prepared traditionally from *dahi* (curd) and stored for 6 months at 21–30°C for periodic analysis. Volatile flavour compounds were characterized using Gas Chromatography–Mass Spectrometry (GC–MS) at IIT Guwahati Biotech Park. Across treatments, 34 volatile compounds were identified, including fatty acids (11), alcohols (1), hydrocarbons (4), ketones (2), terpenoids (2), organic acids and vitamins (5), and other bioactive compounds (9). Samples of T_2 (yoghurt culture + bael extract) and T_4 (*Lc. lactis* ssp. *lactis*biovar*diacetylactis* + bael extract) exhibited superior sensory attributes and oxidative stability, preserving key volatile compounds throughout storage. These samples also contained unique bioactive flavour molecules - thymol, citronellol, ascorbic acid, β-sitosterol, and phytol - that were absent in market ghee, suggesting that bael enrichment combined with functional starter cultures significantly improved both flavour and shelf-life of traditional *desi* cow ghee.

Keywords: *Desi* ghee, GC-MS, volatile compounds, flavour profile

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Introduction

Ghee is characterized by its pleasant, nutty, and mildly caramelized flavour, which is due to a complex interplay of raw materials, processing conditions, and microbial activity. Factors such as the source of raw material (butter, cream, or curd), method of preparation (traditional or *desi*, direct cream, creamery butter, pre-stratification, or continuous process), clarification temperature, holding time, and duration of storage significantly influence the final flavour profile (Bhide, 2014). A wide spectrum of compounds including alcohols, aldehydes, carboxylic acids, fatty acids, ketones, and lactones contribute to ghee's characteristic aroma and taste (Su et al. 2024). These compounds are primarily generated during heating through Maillard-type reactions between proteins and lactose, degradation of protein-lactose complexes, lipid oxidation, and decomposition of free fatty acids (FFAs) (Wadodkar et al. 2002; Newton et al. 2012). Microbial fermentation further enhances the unique flavour and texture of ghee (Feng et al. 2021). During fermentation of milk or cream, three major metabolic pathways namely, glycolysis of lactose or glucose to lactic acid, lipolysis of glycerides to FFAs, and proteolysis of casein releasing amino acids yield diverse aromatic compounds. The accumulation of these metabolites contours the sensory characteristics of ghee, with shorter fermentation producing a fruity aroma, whereas prolonged fermentation imparts buttery and cheesy notes (Liang et al. 2025).

Commercial ghee, typically prepared from unripened cream, lacks the characteristic flavour of traditionally produced ghee made from ripened raw materials such as *dahi* (curd), ripened butter, or cream. Flavour development can be enhanced by fermenting these raw materials with selected starter cultures, as the flavour compounds generated during fermentation are carried forward into the final product during clarification (Yadav and Srinivasan, 1987). Thermophilic starter cultures such as *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus* are widely used in yoghurt fermentation - the former facilitates controlled proteolysis of casein, while the latter efficiently ferments lactose. In contrast, the mesophilic *Lc. lactis* ssp. *lactis* biovar *diacetylactis* produces key volatile compounds such as diacetyl and acetoin, which contribute desirable buttery flavour notes to dairy products.

Considering the flavour-enhancing roles of *Lb. delbrueckii* ssp. *bulgaricus*, *Str. thermophilus*, and *Lc. lactis* ssp. *lactis*biovar*diacetylactis*, the present study was designed to evaluate their combined effects on the flavour quality and shelf-life of cow's milk ghee. To further enrich the flavour and oxidative stability, ripened pulp extract of *Aeglemarmelos* (bael), known for its golden-yellow hue and distinctive aroma, was incorporated. Bael pulp contains natural antioxidant compounds including coumarins (marmelosin, scoparone, marmesin, psoralen), phenolic acids and flavonoids (kaempferol, chlorogenic, ellagic, ferulic, gallic, protocatechuic acids, quercetin), alkaloids (aegelenine, marmeline, aegelin, marmesiline), and ascorbic acid (Rajan et al. 2011; Murthy and Bapat, 2019). Ghee samples were prepared from cow's milk fermented with these starter cultures, with or without. the addition of ripened bael pulp extract, and were periodically analyzed for flavour compounds and compared with market ghee samples.

Materials and Methods

Procurement of materials

Cow's milk was procured from the Livestock Farm, College of Veterinary Science, Assam Agricultural University, Khanapara Campus, Guwahati, Assam, and standardized to 4.5% fat. *Aegle marmelos* (bael) fruits were sourced from the local market, and the pulp extract was prepared following the method described by Roy and Saran (2011). Freeze-dried starter cultures of *Lc. lactis* ssp. *Lactis* biovar *diacetylactis* (NCDC 60) and yoghurt culture comprising *Lb.delbrueckii* ssp. *bulgaricus* and *Str. thermophilus* (NCDC 144) were obtained from the ICAR–National Dairy Research Institute, Karnal, Haryana. Both cultures were activated in reconstituted and sterilized skim milk (12.5%, w/v). The NCDC 60 culture was incubated at 30°C, and the NCDC 144 culture at 42°C, until curd formation. Prior to use, active cultures (18h) were prepared by inoculating the stock cultures @ 3% (v/v) into sterilized skim milk (12.5%, w/v).

Preparation of Ghee

Ghee was prepared by following the traditional method. The cow's milk was first boiled, cooled to room temperature, and then divided equally into four portions corresponding to the four treatment groups: T_1, T_2, T_3, and T_4. Samples of T_1 and T_3 were prepared using starter cultures @ 3% (v/v), while T_2 and T_4 were prepared using starter cultures along with *Aeglemarmelos* (bael) pulp extract @ 1% (v/v), as illustrated in the flow chart (Fig. 1). The prepared ghee samples were packed in glass bottles,

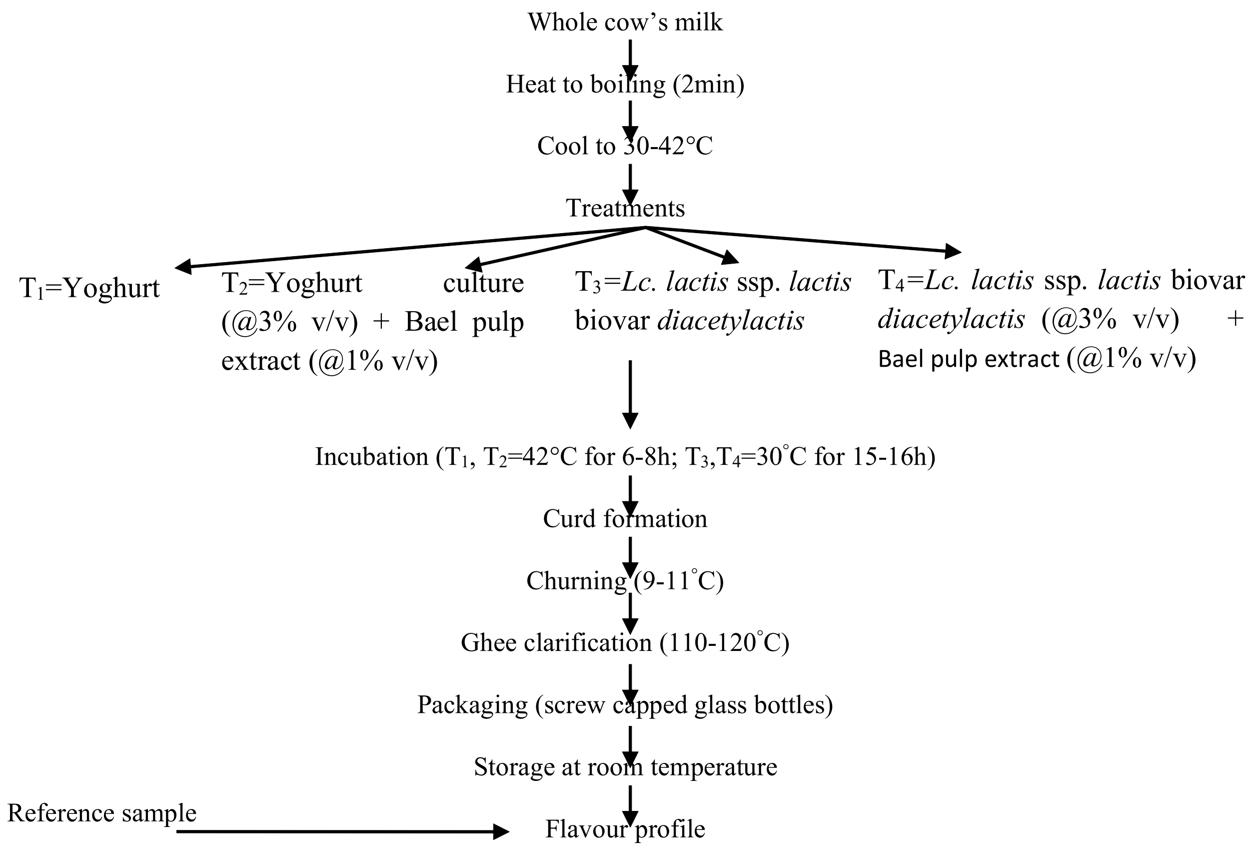


Fig. 1 Flow Chart of Ghee Preparation

covered with aluminum foil, and stored at ambient summer temperature (21-30°C) for subsequent analysis. A commercially available branded ghee sample was used as the reference.

Flavour Compounds of Ghee

For GC-MS analysis the ghee samples were prepared by the liquid-liquid extraction method (Arya et al. 2020). The flavour compounds were analyzed by GC-MS in the Indian Institute of Technology, Guwahati Biotech Park, Assam, using PerkinElmer (USA) with GC-MS model Clarus 680 GC and Clarus 600 MS equipped with a liquid auto-sampler and with the aid of Turbo mass version 5.4.2 software. The peaks for flavour compounds were identified using software NIST-2008 (National Institute of Standards and Technology) mass spectral library. Only qualitative identification of the flavour compounds present in the ghee samples was performed, and these were compared with known flavour constituents reported for *Aeglemarmelos* (bael) fruit.

Table: 1 Effects of starter cultures and bael pulp extract on flavour compounds of cow'smilk ghee

Sl. No.	Flavour Compounds	MW	T_1 0D	T_1 6M	T_2 0D	T_2 6M	T_3 0D	T_3 6M	T_4 0D	T_4 6M	Reference 0D	Reference 6M
A.	Fatty Acids:											
1.	Acetic Acid	60	+	+	+	+	+	+	+	-	-	-
2.	Decanoic / Capric Acid	172	+	+	+	+	+	+	+	+	-	-
3.	Docosanoic / Behenic acid	340	+	+	+	+	+	+	+	+	-	-
4.	Formic Acid	46	+	+	+	+	+	+	+	+	+	+
5.	Hexadecanoic/ Palmitic acid	256	+	+	+	+	+	+	+	+	-	-
6.	Hexanoic Acid	116	+	-	+	+	+	-	+	+	+	-
7.	Myristic Acid	228	+	-	+	+	+	-	+	+	+	-
8.	Propionic Acid	74	+	+	+	+	+	+	+	+	+	+
9.	Stearic Acid	284	+	-	+	+	+	-	+	+	+	-
10.	Oleic Acid	282	+	-	+	+	+	-	+	+	+	-
11.	Succinic Acid	118	+	+	+	+	+	+	+	+	-	-
B.	Alcohols:											
12.	Heptanol	116	+	+	+	+	-	-	-	-	-	-
C.	Hydrocarbons:											
13.	Dodecane	170	+	+	+	+	-	-	-	-	-	-
14.	Heptadecene	240	+	+	+	+	-	-	-	-	-	-
15.	Nonane	128	+	+	+	+	-	-	-	-	-	-
16.	Tetradecane	198	+	+	+	+	-	-	-	-	-	-
D.	Ketones:											
17.	Dodecanone	170	+	+	+	+	+	+	+	+	-	-
18.	Octanone	128	+	+	+	+	+	+	+	+	-	-
E.	Terpenoids:											
19.	Thymol	150	-	-	+	+	-	-	+	+	-	-
20.	Citronellol	156	-	-	+	+	-	-	+	+	-	-
F.	Organic Acids and Vitamins:											
21.	Ascorbic Acid	176	-	-	+	+	-	-	+	+	-	-
22.	Fumaric Acid	383	-	-	+	+	-	-	+	+	-	-
23.	Oxalic Acid	90	-	-	+	+	-	-	+	+	-	-
24.	Vitamin K1	450	-	-	+	+	-	-	+	+	-	-
G.	Others:											
25.	Decylsulfone	346	-	-	+	+	-	-	+	+	-	-
26.	Eicosane	408	-	-	+	+	-	-	+	+	-	-
27.	Hexadecane	352	+	+	+	+	+	+	+	+	-	-
28.	Nonadecane	310	-	-	+	+	-	-	+	+	-	-
29.	Nonadecane	324	-	-	+	+	-	-	+	+	-	-
30.	Octadecane	366	-	-	+	+	-	-	+	+	-	-
31.	Phytol	296	-	-	+	+	-	-	+	+	-	-
32.	Sulfurous Acid	376	-	-	+	+	-	-	+	+	-	-
33.	Tetracontane	594	-	-	+	+	-	-	+	+	-	-
34.	β-Sitosterol	414	-	-	+	+	-	-	+	+	-	-

*MW=Molecular weight, D=day, M=Month

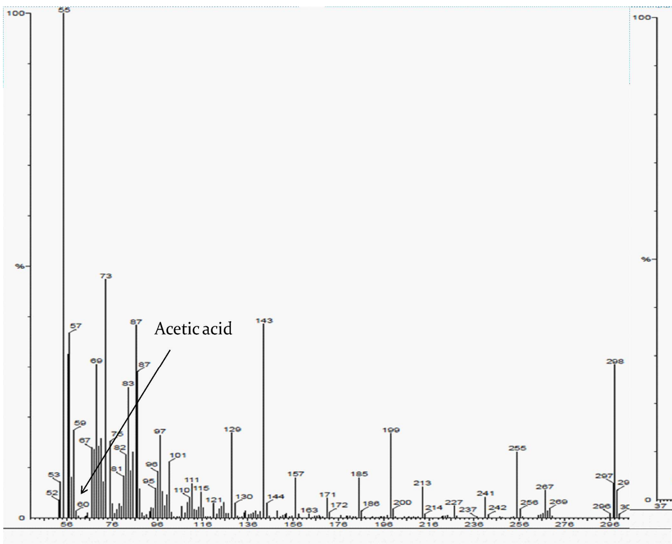
Preparation of fatty acid methyl esters (FAME) from the ghee samples and their subsequent GC–MS analysis were conducted according to the procedure outlined by FSSAI (2016).

Results and Discussion

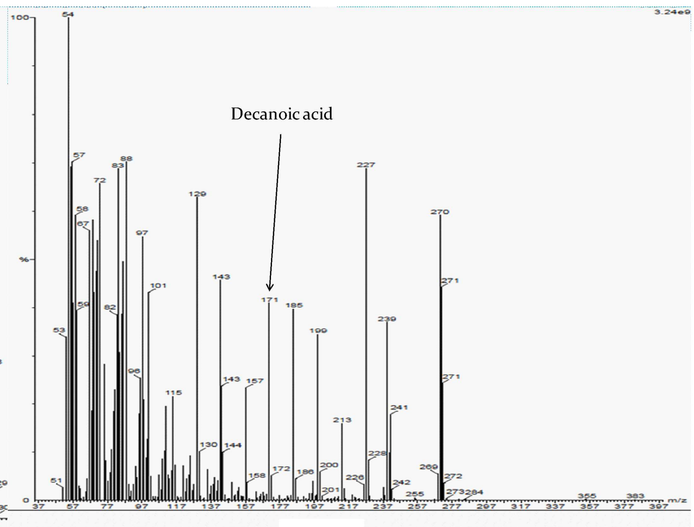
A total of 34 volatile flavour compounds identified by GC-MS analysis on the basis of their molecular weight are presented in Table I, and the corresponding chromatograms are presented in Figures 2a to 2f.

Fatty Acids

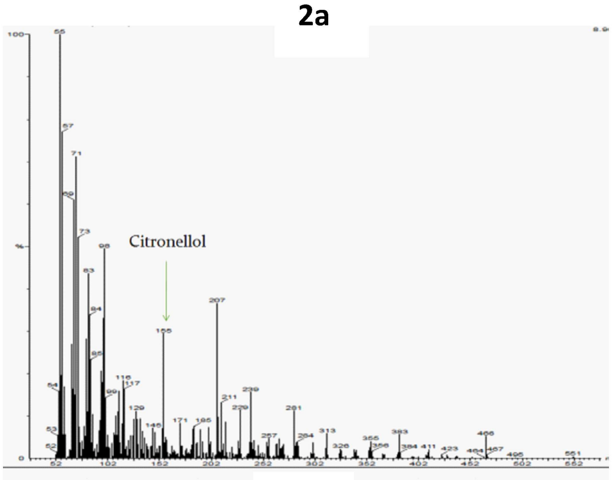
In the present study, the fatty acids detected in the ghee samples included acetic, decanoic (capric), docosanoic, formic, hexadecanoic, hexanoic, myristic, propionic, palmitic, stearic, oleic, and succinic acids (Table 1). The presence of myristic, palmitic, stearic, and oleic acids in ghee has also been reported by Kumar et al. (2024). Among these, acetic, decanoic, docosanoic, hexadecanoic and succinic acids were found in samples of T_1, T_2, T_3 and T_4 both at 0d and after 6months of storage at ambient temperature and might be the metabolic end products of the starter cultures employed and the *bael* pulp extract. Acetic, decanoic, docosenoic, hexadecenoic and succinic acids were not found in the reference sample and hexanoic, myristic, stearic and oleic acids were found only on 0d and could not be detected after storage for 6months, which might be attributed to their degradation due to oxidation (Wang and Schulz, 1989), while the ghee samples of the treatment groups could retain these compounds till 6months of storage which might be due to the antioxidant properties of both the starter cultures (Feng and Wang, 2020) and that of the *bael* pulp extract (Meeran et al. 2017; Hazra et al. 2020). Formic and propionic acid were found in the ghee samples of all the treatment groups including the reference sample on 0d and after 6months of storage at room temperature. Formic acid has previously been



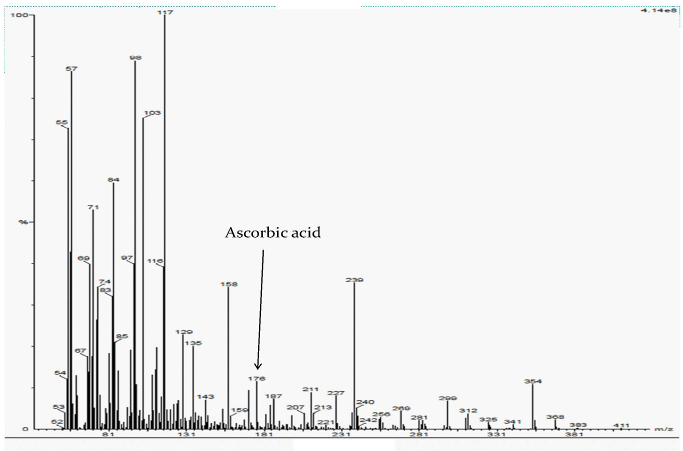
2a



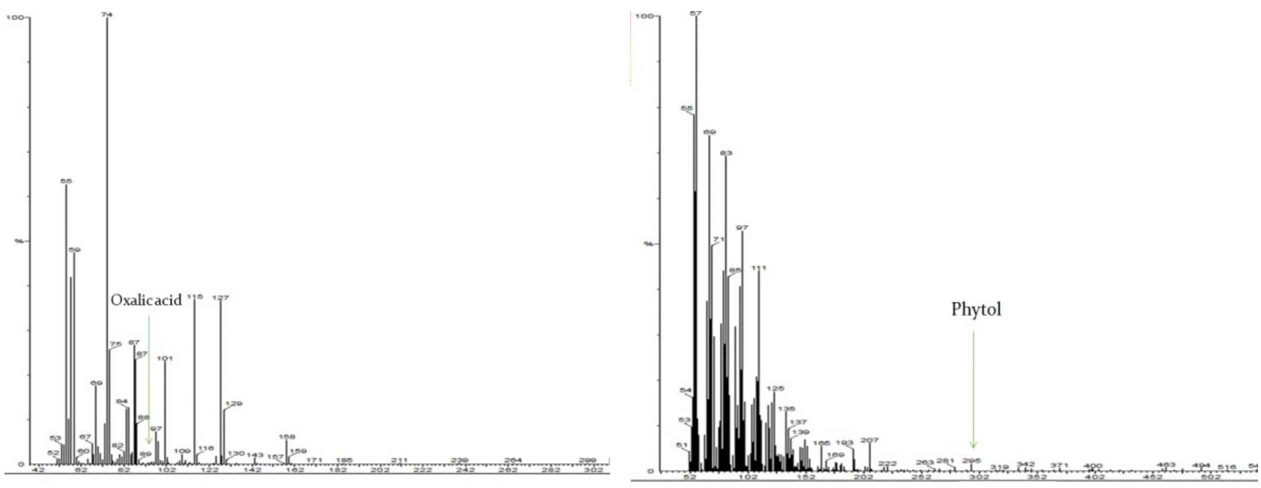
2b



2c



2d



2e **2f**

Fig. 2 (a-f) Chromograms of the volatile compounds based on their molecular weight

reported as a flavour compound in yoghurt (Komaru et al. 2021), while propionic acid has been identified as a characteristic flavour constituent of ghee (Edris, 2014).

Free fatty acids are key contributors to the flavour profile of fermented milk products.

These are produced either through hydrolysis of fat during heat processing or *via* lipolysis catalyzed by microbial lipases, both of which markedly influence the flavour characteristics of ghee (Yadav and Srinivasan, 1985; Sserunjogi et al. 1998). According to Erfani et al. (2020), decanoic and hexanoic acids are specifically associated with the development of cheesy and rancid odours in ghee.

Volatile compounds reported in *Aeglemarmelos* (bael) fruit include oleic, hexadecanoic, stearic, tetradecanoic, docosanoic, and myristic acids (Ahmad et al. 2021; Sarkar et al. 2020; Tagad et al. 2018; Vardhini et al. 2018). In the present study, all these compounds were detected in the T_2 and T_4 samples, suggesting their origin from the incorporated bael pulp extract (Table 1). Their presence may also be attributed to the combined action of metabolites produced by the starter cultures and the inherent fatty acids present in the bael pulp extract

Alcohol

Consistent with the findings of Wadodkar et al. (2002), heptanol was the only alcohol detected in ghee samples prepared from curd inoculated with yoghurt cultures. Heptanol was found only in T_1 and T_2 samples on 0d and after 6months of storage at ambient temperature. The yoghurt cultures were found to produce heptanol, which serves as a flavour precursor. Dan et al. (2019) also reported the production of heptanol by *Lb. delbrueckii* ssp. *bulgaricus* during milk fermentation. Similarly, Kondybayev et al. (2018) identified heptanol as a volatile metabolic product of lactic acid bacteria (LAB) in fermented milk, attributing its formation to the degradation of unsaturated hydroperoxides *via* alkoxy radical intermediates. Additionally, aliphatic alcohols such as heptanol may arise from microbial enzymatic activity or through lipid oxidation processes (Erfani et al. 2020).

Hydrocarbons

In conformity with the findings of Erfani et al. (2020), dodecane, heptadecene, nonane, and tetradecane were the four hydrocarbons detected in T_1 and T_2 ghee samples at both day 0 and after 6 months of storage. These compounds were absent in T_3, T_4, and the reference samples. Zhang et al. (2020) found these compounds in yoghurt fermented with *Str. thermophilus*. Hydrocarbons are generally derived from oxidation of unsaturated fatty acids which in turn contribute to the flavour development during fermentation of milk (Erfani et al. 2020; Kondybayev et al. 2018). Erfani et al. (2020) also reported that dodecane and tetradecane imparted a peppery and pungent odour in milk.

Ketones

Dodecanone and octanone were the only two ketones detected in the ghee samples across all treatment groups at both 0/ days and after 6/ months of storage and were absent in the reference Dodecanone contributes a typical nutty flavour to the ghee and is formed either during heat processing of ghee or by microbial fermentation (Erfani et al. 2020). Octanone has also been reported in fermented ghee (Wadodkar et al. 2002) and milk (Thierry et al.

2016). In general, ketones are generated *via* the breakdown of β-ketoacids through thermal processes or enzymatic activity (Erfani et al. 2020). Interestingly, in the present study, diacetyl, an important ghee flavour compound, was not detected in any ghee samples. This absence may be due to the incubation temperature used: milk samples inoculated with *Lc. lactis* ssp. *lactis* var. *diacetylactis* were incubated at 30°C, which promotes acid production (both total and volatile acids), whereas incubation at 22°C favours diacetyl and acetaldehyde formation (Yadav and Srinivasan, 1985).

Terpenoids

Terpenoids, specifically thymol and citronellol, were detected only in ghee samples prepared with bael pulp extract, at both storage periods. These compounds likely originated from the bael pulp itself (Meena et al. 2022; Pathirana et al. 2020), as they were absent in T_1, T_3, and the reference samples, confirming their source as the added bael pulp extract in T_2 and T_4. Thymol is a terpenoid derivative of p-cymene, a volatile compound found in bael fruit, leaves, and bark (Meena et al. 2022; Pathirana et al. 2020), and is known for its pleasant aromatic odour (O'Connell, 2019). Citronellol, the other terpenoid detected in T_2 and T_4 ghee samples, aligns with previous reports (Jha and Prasad, 2011).

Organic Acids and Vitamins

Oxalic and fumaric acids were the only organic acids identified in the ghee samples containing bael pulp extract, both on 0d and after 6 months of storage. Succinic and acetic acids were detected in all the treatment groups, whereas these acids were absent in the reference sample, suggesting that their presence resulted from microbial fermentation in milk. Only oxalic and fumaric acids were detected in ghee samples containing bael pulp extract, both at 0/ day and after 6/ months of storage. Succinic and acetic acids were present in samples of all the treatment groups but absent in the reference sample, indicating that their formation likely resulted from microbial fermentation of the milk. The detection of these organic acids in the ghee samples is consistent with the findings of previous studies (Hazra et al. 2020; Meena et al. 2022). Organic acids contribute not only to the flavour profile but also enhance its nutritive value and stability (Walker and Famiani, 2018). Moreover, the composition of organic acids in fermented milk products is influenced by the specific LAB cultures employed during fermentation (Özcelik et al. 2016). Interestingly, Vitamin C (ascorbic acid), typically absent in milk, was detected in ghee samples containing bael pulp which was in consistence with Hazra et al. (2020); ascorbic acid functions as a potent antioxidant. Additionally, Vitamin K_1 was also detected in these ghee samples.

Others

Eicosane, decylsulfone, tetracosane, nonadecane, sulfurous acid, β-sitosterol were detected exclusively in ghee samples with bael pulp extract. Others reported these compounds to be present in different parts of *bael* tree including the fruit (Pathirana et al. 2020; Meena et al. 2022). The other volatile compounds, namely, octadecane, hexadecane, tetracontane, phytol found in T_2 and T_4 were reported to be present in *bael* fruit pulp (Tagad et al. 2018; Vardhini et al. 2018). Hexadecane was detected in samples of all the treatment groups, suggesting its contributions from both the starter cultures and bael pulp extract (Ilicic et al. 2012; Tagad et al. 2018; Vardhini et al. 2018). None of these compounds were detected in the reference sample.

The characteristic intense flavour of the ghee prepared traditionally from ripened milk is ascribed to the metabolic activity of the background microbiota on various constituents of milk like the lactose, glucose, and citrate which get incorporated in the final product during clarification process. The precise mechanism, however, remains unclear. It is believed that during the clarification process, water soluble flavour compounds, which have a higher boiling point than that of the water, remain in the fat phase contributing to the flavour of ghee (Sserunjogi et al. 1998). A direct correlation exists between the flavour compounds and acid content of fermented milk/ dahi/ yoghurt (Yadav and Srinivasan, 1987). In contrast, Sserunjogi et al. (1998) observed loss of almost half of the volatile compounds of butter/ dahi/ cream during the clarification process due to their volatility.

Conclusion

GC-MS analysis confirmed the presence of a range of volatile flavour compounds and FFAs in cow's milk ghee. These compounds were either metabolic by-products of the inoculated starter cultures or derived from the added bael pulp extract. They significantly contributed to the flavour profile and oxidative stability of the ghee. Notably, ghee samples incorporated with bael pulp extract and specific starter cultures retained key flavour compounds even after 6 months of storage at ambient temperature, unlike the reference market ghee. These findings indicate that the combined use of selected LAB and bael pulp can enhance both the flavour quality and shelf-life of traditionally prepared ghee.

Acknowledgements

The authors are thankful to the authorities of the Assam Agricultural University, Khanapara Campus, Guwahati, Assam for providing necessary facilities to carry out the research work. The help of Dr. Jakir Hussain in carrying out the statistical analysis of the research data is duly acknowledged.

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