UTTAR PRADESH JOURNAL OF ZOOLOGY

43(4): 59-66, 2022
ISSN: 0256-971X (P)

FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR ANALYSIS OF DAIRY GHEE ADULTERATED WITH MINERAL OIL

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i42932

Editor(s):
(1) Dr. Juan Carlos Troiano, University of Buenos Aires, Argentina.
Reviewers:
(1) Anil Kumar, Shoolini University, India.
(2) Gamal Saad Elhadidy, Agriculture Research Center, Egypt.

Received: 20 January 2022
Accepted: 26 March 2022
Published: 27 March 2022

Original Research Article

ABSTRACT

Concept of food integrity to health is a hot topic with research ongoing globally to reduce adulteration in food. In an effort to follow up this cause, present research focused on detection of adulterated lower quality fats with genuine milk fats (ghee) is a key concern. Ghee is a pure clarified butter fat, widely used milk product in Indian sub-continent. Because of its satisfactory flavor and appetizing aroma, excessive dietary value, unique nature and its health benefits intend for its adulteration has usually been an extreme concern. The available methods to detect adulteration of mineral oil are time consuming and laborious. In order to get rid of these in discrepancies, the present paper deals with a promising, fast and effective method to detect the adulteration in ghee circulating in the market. Attenuated total reflectance – Fourier transform infrared spectroscopy (ATR- FTIR) method for identifying mineral oil even at 0.1% was established. The results reveal that six peaks are common, especially 721.38cm-1 (C-Cl) and 1137.17cm-1 (C-H stretching) peaks area percentage decreases and intensity increases with the concentration of adulteration. From these results, FTIR techniques play a potential role and serves as a robust method for detection of mineral oil adulteration in dairy ghee.

Keywords: FTIR; ghee; mineral oil; adulterant; functional groups.

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1. INTRODUCTION

The dairy ghee occupies a conspicuous place in the Indian culture diet because of its exhaust nutritional content, excellent aroma with high calorific value in which the presence of fat-soluble vitamins, and essential fatty acids [1]. Ghee fulfils a primary and vital function of four fat-soluble vitamins namely A, D, E, K and essential fatty acids like linolenic acid and arachidonic acid, and has rich and pleasant sensory properties [2], [3]. It has various beneficial actions on human well-being, such as anticancer, antidiabetic, anti-cholesterolemic, antimicrobial, antioxidant, anti-atherogenic, and anti-adipogenic properties due to the enrichment of conjugated linoleic acid and beta carotene activities [4]. India is the leading milk producer in the world, around 146.3 million metric tons (MMT) generated during the year 2014-2015 [5]. Ghee is the second principal dairy product in India prepared from milk [6]. As per the FSSR (Foods standard and safety regulations) "Ghee" means the pure milk fat, which clarified from the milk protein and other substances. Apart from the fat without any colouring matter or preservative accepted as pure ghee. However, ghee supplies fall short of demand in the lean season. To contribute and extend the available supplies, producers or the ghee sellers in the market, tend to adulterate [7] ghee. Using inexpensive oils and fat like vegetable oils, animal frame fat, hydrogenated fat, and on occasion even the non-fit for human consumption mineral oils, wax, and paraffin particularly for the duration of lean season to earn extra money. Recently, the hassle of adulteration has assumed a totally extreme measurement and communal. To come upon and manifesting those issues, numerous styles of studies supported the identity of adulterants [6,8-12]..

Mineral oil is liquid by-product occurred from the crude oil which is composed of hydrocarbon molecules especially alkenes (paraffin), cyclic alkenes (naphthene) and aromatics. It is essential to make the gasoline and petroleum products. The varieties containing alkylated benzene moieties from non-aromatic oils, MOSH (mineral oil saturated hydrocarbons) and MOAH mineral oil aromatic hydrocarbons). N-alkanes occurring naturally in terpenes, which have odd-numbered carbons (C21-C35) such as squalene, styrene or carotenoids. Usually for food packaging polyolefin oligomeric saturated hydrocarbons (POSH) (polyethylene or polypropylene) are added to the total saturated hydrocarbons of mineral oils. Applications of mineral oil on the topical administration can also utilized depend on the grade but oral administration is hazardous to health [13].

Authentication and characterization of edible milk fat spiked with inedible mineral oil analysis using routine chemical methods are highly laborious and time-consuming [14]. Because mineral oil having the physical property of inert flavour and taste. It is also miscible with other oils, fats, and dairy products [15]. According to a survey of the literature, systematically no one explores detection of mineral oil in milk fat using the non-destructive technique. Still, there is a lacuna; no approaches are successful for detecting the mineral oil adulterant in a single robust method. Over the past few years, Fourier transforms infrared (FTIR) spectroscopy has materialized as a fast food analytical tool with minimum sample preparation, necessary and successfully to detect adulteration of inedible products [16] such as hazelnut oil adulteration in olive oil [17], virgin coconut oil with paraffin oil [18]. Cold associated pressed sesame oil spiked with hazelnut, canola, and sunflower oil [19]. This disperse method can identify chemical bonds by using different elements that absorb light at different frequencies [20]. The light measured using an infrared spectrometer, which produces the material output of an infrared spectrum that is absorbed and transmitted.

Chemical based detection of Saponification value is reversal to molecular weight and high saponification value will show an increase in the quantity of lower chain fatty acids or decrease in higher chain fatty acids. However, the method can be used for detecting the presence of mineral oils, such as liquid paraffin in milk fat as they are not acted upon by alkali and such a heterogeneous solution on saponification as a base for Holde's test [21].

To the best of our knowledge, there are no previous reports providing information about the detection of mineral oil constituents in ghee using the FTIR spectroscopy method. Therefore, the present study aimed to detect the mineral oil adulteration in the ghee using FTIR spectroscopy and analyzed functional group variations for better understanding the detection limit of mineral oil.

2. MATERIALS AND METHODS

2.1 Sample Collection

Raw milk was collected from the umbalacherry cattle breed in the region with latitude and longitude of 7006°N, 79.0843°E, Thirukanurpatti, Tamil nadu, India. Cow ghee was prepared using the milk skin method [8] and preserved under 4°C for future purposes. The mineral oil was purchased from a local market, it has a prolonged shelf life, once hold on in

an airtight containers are often preserved at room temperature for years.

2.2 Methods of Ghee Preparation

Economically and traditionally acceptable milk skin methods been chosen for the preparation of clarified milk fat [22]. Ghee was prepared by collecting the nutrient-based yogurt and stored in frozen condition. Then churning with ice-cold water been programmed for once a week. Then the churned butter washed to remove the impurities, remaining protein-casein, and water by mechanical pressing. Thus, butter melted at 60°C for half an hour until the aromas and the oil float. The brown residue will be leftover. After filtration, clarified fat packed in a glass bottle for further use.

2.3 Adulteration Preparation

The pure dairy ghee mixed with mineral oil at 0.1% and 0.2% levels and heated up to 40°C for 10 min. After the preparation of adulterant ghee, the samples thoroughly mixed and kept in the refrigerator for further analysis.

2.4 FTIR Measurements

The FTIR spectra of samples of pure milk ghee, mineral oil and adulteration ghee recorded with an FTIR (Shimadzu IR tracer-100). The FTIR spectra collected in the 4000-400 cm^{-1} region with a coding of 45 scans and a resolution of 4 cm^{-1}. A new background with reference spectrum recorded after each scan. Spectra recorded in triplicate as transmission values at each data point. The range of mineral oil and pure milk ghee samples compared to the Shimadzu library.

3. RESULTS AND DISCUSSION

The visual examination of pure and adulterated ghee shows that there are no changes observed at trace amounts of adulteration figure1. Some analytical techniques needed to establish the adulteration. Hence, the typical FTIR spectrum of dairy ghee [23], mineral oil, and adulterant ghee samples was examined in transmittance mode and are given in table2 and figure2. The spectrum observation denoted that the total number of peaks and their positions. The dairy ghee and mineral oil were almost similar peak values at 721.38, 1377.17, 1463.97, 2852.72, 2922.16, and 2953.02 cm^{-1}. Peak Intensity and area may vary depending on the concentration of adulteration, which is visible at 721.38 to 2953.02 cm^{-1}. Intensity for dairy ghee is increasing gradually from 84.63, 85.16, 87.42, and 92.97 because of 0.1% and 0.2% of mineral oil. Peak area distance in dairy ghee with adulteration shows 772.562, 659.529, 514.324 mineral oil 227.736 respectively. From these results, the peak at 721.38 and 1377.17 cm^{-1} are showing unique characters between the dairy ghee and mineral oil and showing the differences to detect the adulterant ghee. Similarly, various researchers have worked to detect the presence of adulterants. The adulterants that are present as hydrogenated fats in butter, foreign fat in dairy products, lard in chocolate formulations. Likewise, cheaper vegetable fats in butter, margarine in butter, adulteration of butter by margarine in bakery products. Also with chicken fat in butter and lard as an adulterant in butter using FTIR spectroscopy [24], [25]. The consignment of various bonds along with their functional groups responsible for absorption by dairy ghee and adulterant ghee given in Table 1. The corresponding peak 721.38 cm^{-1} responsible for Halogen group (C-Cl) and O-H bend peak 1377.17cm^{-1} that denote phenol group in which `the area percentage were reduced in adulterant ghee at the 0.1 and 0.2 percent of mineral oil (Table 2). In lipids, the bond that participates in IR uptake is the CH bond present in the CH2 groups in the fatty acyl chain and its terminal CH3 group, as well as the CH and CH2 bonds found in the glycerine unit are present stands for the O-H phenol group. The C-H stretching of the cis double bonds in the unsaturated fatty acids produced absorption at a lower wavenumber than in the saturated portions of the fatty acid molecules samples reported for edible fats [26].

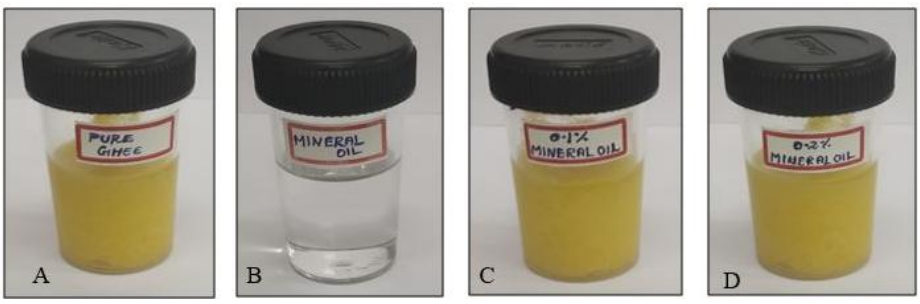

Fig. 1. Representation of A)pure ghee B) mineral oil C) Adulterated ghee with 0.1% mineral oil D) Adulterated ghee 0.2% mineral oil

The spectrum of pure dairy ghee and mineral oil analysed and compared with the Shimadzu library and its pharmaceutical compounds given in Tables 3 and 4. The examination of dairy ghee shown the presence of Cefaclor, L- Tryptophan, Promethazine hydrochloride, Levodopa, Ciprofloxacin hydrochloride, Furosemide, Erythromycin, Sulfamethoxypyridazine, Phenacetin, Cefalotin sodium, Artesunate, Cefodizime Sodium, Imipramine Hcl, Hydralazine hydrochloride, Penicillin v potassium, Cyproheptadine hydrochloride, Cephalexin and Piracetam functional groups which are identified to have remedial activity against various human illness. However, the Benzil and Etizolam toxic functional groups identified in mineral oils. Thus, the FTIR spectroscopy method may be useful in the detection of dairy ghee adulterated with mineral oil and subsequently may lead to the identification of toxins in the edible food materials even at trace amounts of adulteration. From these results, The peaks at 721.38 cm^{-1} (C-Cl; Halogen group) and 1377.17 cm^{-1} (O-H bend; Phenol or tertiary alcohol) are highly recommended for FTIR based detection of mineral oil adulteration as low as 0.2% level. FT-IR spectroscopy is an accurate and rapid method to differentiate between pure dairy ghee and mineral oil.

Table 1. Functional group organization of ghee and mineral oil adulterated ghee

Peak list	Ghee	Mineral Oil	Wave number (cm-1)	Functional group assignment	Chemical constituents
1	582.50	--	600-500	C-I stretch	Aliphatic iodo
2	721.38	721.38	500-738	C-Cl	Halogen compound (Chlorocompound)
3	966.34	--	650-1000	PO3 stretch	Phosphate ion, alkene bends
4	1099.43	--	1100-1000	C-O stretch	Ether
5	1112.93	--	1020-1250	C-N stretch	Aliphatic amines
6	1159.22	--	1000-1200	C-O stretch	Ester
7	1236.37	--	1200-1280	C-H stretch	Aromatics
8	1298.09	--	1000-1320	C-O stretch	Alcohol, carboxylic acids, esters, ethers
9	1377.17	1377.17	1410-1310	O-H bend	Phenol or tertiary alcohol
10	1417.68	--	1600-1400	C=C_C, Aromatic ring	Aromatic
11	1463.97	1463.97	1440-1470	C-H stretch	Bending vibration of CH2 and CH3 aliphatic groups, nitrosamines
12	1750	--	1740-1725	C=O stretch	Aldehyde
13	2852.72	2852.72	2850-2815	C-H stretch	Methoxy methyl ether
14	2922.16	2922.16	2935–2915	Asymmetric stretching of –CH (CH2) vibration,	Lipids, protein
15	2954.95	2954.95	2500-3300	C-H	Carboxylic acid

Table 2. Peak area and intensity of ghee and adulterated mineral oil

Peak values	Ghee		Mineral Oil		0.1% Mineral Oil		0.2% Mineral Oil	
	Intensity (a.u.)	Area (%)	Intensity (a.u.)	Area (%)	Intensity (a.u.)	Area (%)	Intensity (a.u.)	Area (%)
721.38	84.63	772.562	92.97	227.736	85.16	659.529	87.42	514.324
1377.17	88.12	618.905	86.1	389.845	87.99	610.462	88.09	519.346
1463.97	81.47	687.39	78.32	746.235	81.34	698.168	81.17	661.377
2852.72	64.22	1094.961	62.37	1494.323	64.04	1111.097	63.92	1158.648
2922.16	53.38	1937.157	50.6	2204.729	53.22	1925.609	52.82	1977.685
2953.02	83.04	388.435	73.57	768.429	82.73	431.742	80.85	449.139

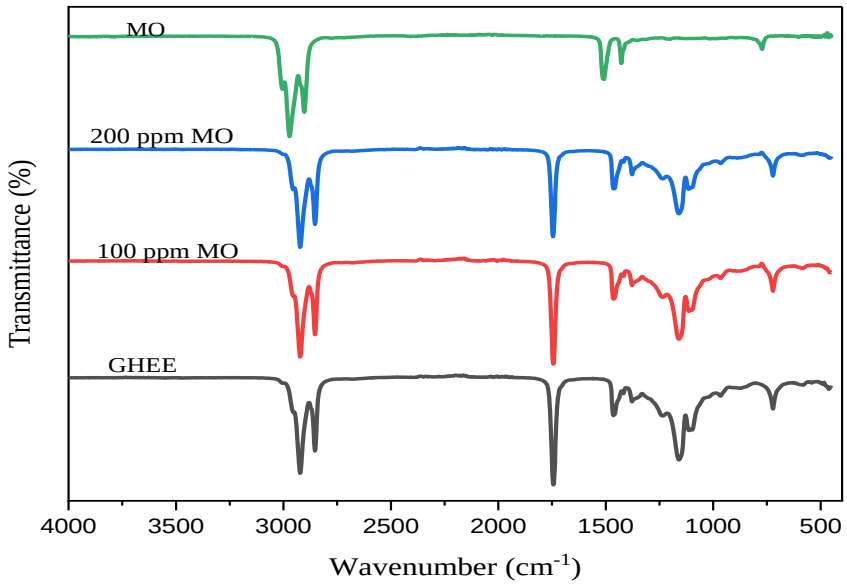

Fig. 2. FTIR characterisation of A)pure ghee B) mineral oil C) Adulterated ghee with 0.1% mineral oil D) Adulterated ghee 0.2% mineral oil

Table 3.Chemical and pharmaceutical compounds in ghee

S.No	**Name**	**Molecular formula**	**Molecular weight**	**Library search**	**Score**
1	Cefaclor	C15H14ClN3O4S	367.81	149 - IRs Pharmaceuticals	536
2	L- Tryptophan	C11H12N2O2	204.22	28 - IRs Pharmaceuticals	522
3	Promethazine hydrochloride	C17H21ClN2S	320.8793	2 - IRs Pharmaceuticals	490
4	Levodopa	C9H11NO4	197.19	33 - IRs Pharmaceuticals	485
5	Ciprofloxacin hydrochloride	C17H18FN3O3.HCl	367.8	75 - IRs Pharmaceuticals	482
6	Furosemide	C12H11ClN2O5S	330.7423	48 - IRs Pharmaceuticals	469
7	Erythromycin	C37H67NO13	733.9352	56 - IRs Pharmaceuticals	468
8	Ephedrine	C10H15NO	165.2346	57 - IRs Pharmaceuticals	465
9	Lanatoside C	C49H76O20	985.13	34 - IRs Pharmaceuticals	458
10	Sulfamethoxypyridazine	C11H12N4O3S	280.3	117 - IRs Pharmaceuticals	455
11	Phenacetin	C10H13NO2	179.21	13 - IRs Pharmaceuticals	455
12	Cefalotin sodium	C16H15N2NaO6S2	418.42	147 - IRs Pharmaceuticals	450
13	Artesunate	C19H28O8	384.42	96 - IRs Pharmaceuticals	450
14	Cefodizime Sodium	C20H20N6Na2O7S4	630.66	143 - IRs Pharmaceuticals	447
15	Imipamine hcl	C19H25ClN2	438.31	37 - IRs Pharmaceuticals	446
16	Hydralazine hydrochloride	C8H9ClN4	196.6389	41 - IRs Pharmaceuticals	445
17	Penicillin v potassium	C16H17KN2O5S	388.4707	14 - IRs Pharmaceuticals	443
18	Cyproheptadine hydrochloride	C21H21N.HCl	323.89	70 - IRs Pharmaceuticals	442
19	Cephalexin	C16H17N3O4S	347.388	83 - IRs Pharmaceuticals	441
20	Piracetam	C6H10N2O2	142.15	8 - IRs Pharmaceuticals	441

Table 4.Chemical and pharmaceutical compounds in Mineral Oil

S.No	Name	Molecular formula	Molecular weight	Library search	Score
1	Carumonam Sodium	C12H12N6Na2O10S2	510.37	150 - IRs Pharmaceuticals	479
2	Hyoscine hydrobromide	C17H21NO4.HBr.3H2O	384.2691	38 - IRs Pharmaceuticals	467
3	Peplomycin Sulfate	C61H88N18O21S2.H2SO4	1571.67	171 - IRs Pharmaceuticals	450
4	Bromocriptinemesylate	C32H40BrN5O5.CH3SO3H	750.7	88 - IRs Pharmaceuticals	443
5	Hydralazine hydrochloride	C8H9ClN4	196.6389	41 - IRs Pharmaceuticals	443
6	Epirubicin hydrochloride	C27H29NO11.HCl	580	124 - IRs Pharmaceuticals	439
7	Oxacillin sodium	C19H19N3O5S	401.43	16 - IRs Pharmaceuticals	437
8	Cefbuperazone Sodium	C22H28N9NaO9S2	649.63	145 - IRs Pharmaceuticals	430
9	Benzil	C14H10O2	210.23	92 - IRs Pharmaceuticals	430
10	Actinomycin D	C62H86N12O16	1255.42	156 - IRs Pharmaceuticals	429
11	Phenethicillin potassium	C17H19KN2O5S	402.5	12 - IRs Pharmaceuticals	429
12	Ranitidine Hydrochloride	C13H22N4O3S.HCl	350.87	164 - IRs Pharmaceuticals	424
13	Pimaricin	C33H47NO13	665.73	169 - IRs Pharmaceuticals	422
14	Nafcillin sodium	C21H21N2NaO5S.H2O	454.47	22 - IRs Pharmaceuticals	422
15	CefroximeAxetil	C20H22N4O10S	510.47	135 - IRs Pharmaceuticals	418
16	Sulfamethoxy-pyridazine	C11H12N4O3S	280.3	117 - IRs Pharmaceuticals	416
17	Dexamethasone acetate	C24H31FO6	434.5037	68 - IRs Pharmaceuticals	416
18	Potassium Clavulanate	C8H8KNO5	237.25	167 - IRs Pharmaceuticals	413
19	Etizolam	C17H15ClN4S	342.85	123 - IRs Pharmaceuticals	409
20	Benzyl penicilline Potassium	C16H17KN2O4S	372.48	153 -IRs Pharmaceuticals	406

4. CONCLUSION

The FTIR spectrum of dairy ghee and mineral oil qualitatively analyzed and differences were found in the specific functional groups of the adulterant ghee samples. These variations of ghee functional group properties can easily identified in order to develop a method for characterizing pure milk ghee. The finding suggested that the FTIR spectroscopy-based method serves as an efficient and convenient analytical tool for the detection and identification of inedible mineral oil at levels mixed in the dairy ghee. The developed method will serve as a helpful way of detecting adulteration in dairy industry.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. C. Anandharamakrishnan, Director, NIFTEM - Thanjavur for providing the facility to carry out the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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