Comparative appraisal of ghee and common vegetable oils for spectral characteristics in FT-MIR reflectance spectroscopy

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Abstract FT-MIR spectra of ghee (anhydrous milk fat) and common vegetable oils were acquired using HATR in 4000–650 cm⁻¹ region. The differences in absorbance by carbon–hydrogen (C–H) stretch in fatty acid chain at 3.48 μ m and absorbance by carbonyl (C=O) stretch of ester linkage at 5.7 μ m in ghee and that in vegetable oils were studied. The clear differences in the spectra of ghee and that of the vegetable oils were noticed in fingerprint region, which can be very well utilized to develop FT-MIR spectroscopy as a promising tool to detect presence of common vegetable oils mixed in the ghee as an adulterant.

Keywords Ghee · Vegetable oils · FT-MIR · Spectra

Introduction

Ghee is heat clarified form of milk fat. It is the most widely used fat rich milk product in the Indian sub-continent and now also gaining popularity in Australia, Arabian countries, the United States, the United Kingdom, Belgium, New Zealand, Netherlands and many other African and Asian countries (Illingworth et al. 2009; Khoshhesab 2012). Chemically *ghee* is an extremely complex in nature and has variety of different fatty acids. The major variation in composition of fats and oils is the chain length and degree of unsaturation of the component fatty acids (Dupuy et al. 1996; Cabassi et al. 2013). Ghee has both nutritional and therapeutic properties (Nurrulhidayah et al. 2013).

For the characterization of fats and vegetable oils need large amounts of chemicals and solvents. These chemicals are health hazardous and environmental pollution. The use of gas liquid chromatography (GLC) and high pressure liquid chromatography (HPLC) for characterizations of fats and oils are costly, time-consuming, require extensive sample preparation and involve hazardous chemicals as well as solvents (Karoui and Baerdemaeker 2007; Maggio et al. 2010; Capuano and van Ruth 2012). Fourier transform-infrared (FT-IR) spectroscopy has become an effective alternative to the traditional methods of analysis (Javidnia et al. 2013).

The dispersive infrared (IR) spectroscopy is rarely used in food studies due to difficulties in sample preparation and handling as well as difficulties in data acquisition and processing (Guillen and Cabo 1997). Fourier transform (FT) instrumentation provides speedy and accuracy of IR spectroscopy for food studies (Rodriguez-Saona and Allendorf 2011). FT-IR spectrometers provide a dramatic improvement in the signal-to-noise ratio while their multiplexing property allows all frequencies to be detected

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simultaneously, reducing scan time dramatically without loss of resolution. FT-IR spectroscopy is highly desirable for routine analysis of samples in very large number. Some of the essential features of such techniques include high throughput speed, ease of operation, non-destructive (minimal or no sample preparation), no use of hazardous chemicals/solvents and low-cost (Isabel López et al. 2014; Chen et al. 2015). The importance of IR spectroscopy in identification of molecular structures originates from the possibility of assigning certain absorption bands to functional groups. In fats and oils, most of the peaks and shoulders of the spectrum are attributable to specific functional groups (Bendini et al. 2007). In infrared spectrum the region from about 6.9 to 16 μm (1450 to 600 cm^{-1}) is called the fingerprint region, because of the unique patterns found there. Usually, this region is quite complex and often difficult to interpret; however, each organic compound has its own unique absorption pattern (i.e. fingerprint) in this region and thus an IR spectrum be used to identify a compound by matching it with a sample of a known compound. Even two compounds have similar spectra and the bands, they can be distinguished from in the fingerprint region, when their spectra are overlaid.

Middle infrared (MIR) is routinely used for studies of molecules. Mid infrared spectroscopy coupled to multivariate analysis has been successfully applied for quality control and adulterant detection of: meat and meat products, fish, chocolate, honey, wine, cakes, spirit drinks, beer and many other applications. Adulteration in fats and oils using FT-IR spectroscopy and multivariate analysis has been studied in: virgin olive oil, cod liver oil, extra virgin olive oil, hazelnut oil, virgin coconut oil and animal body fats (Quiñones-Islas et al. 2013). Moreover, FT-MIR spectroscopy has great potential for developing rapid and simple methods to assess authenticity and to determine intrinsic quality parameters (fatty acid profile, iodine value, saponification number, peroxide value, free fatty acids content, etc.) of edible fats/oils. Antony et al. (2016, 2017) studied the ghee using FT-MIR. However, it appears from the survey of literature that no attention has been paid so far to characterize and compare the spectra of both ghee and common vegetable oils using this versatile analytical tool.

Materials and methods

Preparation of ghee samples

Samples of ghee were prepared by the direct cream method described by De (2004). Fresh pooled herd milk samples of cow and buffalo were obtained 35 times from animal keepers of villages nearby Anand city, Gujarat state, India.

Milk was separated into cream containing about 35 per cent fat. Cream so obtained was heated in a stainless steel vessel (pan) to remove moisture. Heating was continued and the final temperature was not allowed to exceed 115 °C. The prepared ghee was allowed to settle and filtered through a double folded muslin cloth at 105 °C. The samples were filled into clean and dry beakers, cooled and stored at room temperature for a day or two before acquiring the spectra. From each type of milk (cow and buffalo) 35 samples of ghee were prepared, thus in total 70 samples of ghee were obtained for the study. The samples of cow ghee and buffalo ghee (35 each) prepared in the laboratory were used as authentic pure training samples of ghee.

Samples of vegetable oils

Samples of 11 different vegetable oils were purchased from local market in Anand. From among vegetable oils, those which are most commonly available on commercial scale in the market were considered for the study. These oils were marked (1) Coconut oil, (2) Corn oil, (3) Cottonseed oil, (4) Groundnut oil, (5) Mustard oil, (6) Palm oil, (7) Palm kernel oil, (8) Rice bran oil, (9) Sesame oil, (10) Soybean oil and (11) Sunflower oil.

Infrared spectrophotometer

A combined FT-IR spectrometer (Spectrum 400 FT-IR/NIR spectrophotometer system, PerkinElmer, Inc., 940 Winter Street, Waltham, MA 02451, USA) covering the complete MIR spectral range was used for acquisition of MIR spectra of ghee. The system was equipped with a room temperature infrared source, a sealed and desiccated interferometer, and deuterated-triglycine sulfate (DTGS) and Indium Gallium Arsenic (InGaAs) detectors.

Acquisition of IR spectra

The MIR spectra of ghee samples were acquired in reflection mode using the PerkinElmer Spectrum 400 FT-IR/NIR system. The operating system used was Spectrum 10, Version 4.3.2 from PerkinElmer, USA. White light was used as a source. These spectra were subtracted against the background of an air spectrum.

MIR spectroscopy

To obtain MIR spectra of ghee, the samples were scanned in reflectance mode in the region of 4000–650 cm^{-1} with 32 scans and resolution of 4 cm^{-1} with strong apodization.

MIR reflectance spectroscopy

For MIR spectroscopy in reflection mode, the sampling accessory was an advanced multi-bounce overhead horizontal attenuated total reflectance (HATR), with penetration depth for infrared beam of 50 μm and refractive index of 2.4 at 1000 cm^{-1} . The HATR accessory was built into the sampling station. The accessory comprised transfer optics within a desiccated chamber sealed from the atmosphere by two potassium bromide (KBr) windows. Through these windows the infrared radiation could be directed into the detachable HATR element. The element was a 9-reflections zinc selenide (ZnSe) crystal mounted into a plate with a shallow trough for sample containment. The crystal geometry was a 45° parallelogram with mirrored angled faces. Due to the presence of the KBr windows, the HATR plate could be removed for cleaning without allowing the ingress of water vapor into the spectrometer. The beam splitter was KBr and detector was DTGS.

The measurements were directly carried out by applying samples of melted ghee/vegetable oils (10 ml) on an HATR surface to give total crystal coverage. Immediately after collection of each background, approximately 2 ml of sample (ghee/oil/blend) was uniformly applied to the HATR crystal using a transfer pipette, ensuring that no air bubbles were trapped on the crystal surface.

Examination of spectra

The MIR absorption spectra of ghee in reflectance mode were obtained by scanning the samples in the $4000\text{--}650\text{ cm}^{-1}$ region of the IR spectrum. The FT-MIR spectra of cow and buffalo ghee as well as common vegetable oils were examined.

The overlay spectra of cow ghee, buffalo ghee and each of the selected common oils were acquired separately using MIR reflectance spectroscopy. The spectra were examined for variation in terms of position of peaks and differences in intensity of various peaks. Other technical aspects like speed, convenience and robustness of the spectroscopy were also examined.

Results and discussion

Pattern of FT-MIR spectra of ghee and vegetable oils

For evaluating the ability of FT-MIR reflectance spectroscopy to discriminate between ghee and vegetable oil on basis of differences in their FT-MIR spectra, the overlay spectra of ghee and vegetable oils were acquired by the spectroscopy in reflectance mode. The overlay spectra of

cow ghee and buffalo ghee with selected vegetable oils are presented in Figs. 1, 2, 3 and 4.

From very careful observations of the FT-MIR overlay spectra of ghee and vegetable oil for overall pattern of the spectra, position of different peaks and variation in relative intensity of various peaks. Some differences between spectra of ghee and that of the vegetable oil to varying extent were noticed at $3.2\text{ }\mu\text{m}$ (3100 cm^{-1}), $5.7\text{ }\mu\text{m}$ (1750 cm^{-1}) and in the range $7.1\text{--}7.7\text{ }\mu\text{m}$ ($1400\text{--}1300\text{ cm}^{-1}$), but especially in the peaks at $8.8\text{ }\mu\text{m}$ (1130 cm^{-1}) and $9.2\text{ }\mu\text{m}$ (1080 cm^{-1}) as well as in the characteristic delineation from 8.3 to $11.1\text{ }\mu\text{m}$ (1200 to 900 cm^{-1}). The two compounds can distinguished from in the fingerprint region, when their spectra are overlaid. Hence, the attempt was also made to exploit fingerprint region of FT-MIR spectrum of ghee and vegetable oils. The examination of the overlaid spectra of ghee and vegetable oils in MIR region (Figs. 1, 2, 3, 4, 5) for differences specifically in the fingerprint region, indicated existence of visual differences. Therefore, it can be deduced from the differences in FT-MIR spectra of ghee and that of the vegetable oil may be exploited to development of method for detection and determination of these oils in ghee when mixed as an adulterant. The potential of FT-MIR spectroscopy to detect vegetable oil in ghee, as appeared in the present study is supported by reports appeared in the literature.

Application of MIR spectroscopy for detection adulteration of oils and fats has been suggested by several authors (Binkerd and Harwood 1950; Barlet and Mahon 1958; Lai et al. 1994; Lipp 1995; Dupuy et al. 1996). However, it is very surprising to note that FT-MIR spectra of ghee has not been reported so far in the literature. Recently Pandey and Jariwala (2015) reported a very preliminary work on possibility to detect adulteration in ghee by studying *trans* fatty acids in ghee using IR spectroscopy. Dupuy et al. (1996) studied 17 commercial samples of butter fats and margarines and 15 commercial oil samples by means of FT-IR spectroscopy and principal component analysis (PCA) for their classification in relation to their origin. Cuibus et al. (2015) applied ATR-FT-IR for the discrimination of butter samples adulterated with solid fraction of palm oil. Lai et al. (1994) applied discriminant analysis to FT-IR spectra of extra virgin and refined olive oil has correctly classified both kind of oils, in spite of the high degree of similarity of their spectra. Poiana et al. (2013) assess the potential of ATR-FT-IR spectroscopy for detection of olive oil adulteration by corn oil mixing and concluded that ATR-FT-IR spectroscopy could be successfully used as a rapid, non-destructive technique with a minimum sample preparation for detection of olive oil adulteration.

Fig. 1 FT-HATR-MIR spectra of cow ghee, buffalo ghee and coconut oil

Fig. 2 FT-HATR-MIR spectra of cow ghee, buffalo ghee and cotton seed oil

Fig. 3 FT-HATR-MIR spectra of cow ghee, buffalo ghee and palm oil

Manaf et al. (2007) carried a study out to assess the effectiveness of FT-IR spectroscopy in detecting adulteration of virgin coconut oil with palm kernel olein as a potential adulterant. Javidnia et al. (2013) emphasized on the power of FT-IR spectroscopy for discrimination between different oil samples (especially fat from vegetable oils). Sheibani et al. (2014) used FT-IR spectroscopy to identify and detection the adulteration of pistachio oil

with cheap edible oils of corn, sunflower and soybean. Nurrulhidayah et al. (2014) selected FT-IR spectra at 1072–935 cm^{-1} for quantitative analysis corn oil in sesame oil. Lumaksoa et al. (2015) carried out study to develop a rapid, simple, and accurate analytical method based on FT-MIR spectroscopy technique aided with partial least-squares (PLS) for authenticity determination of avocado oil.

Fig. 4 FT-HATR-MIR spectra of cow ghee, buffalo ghee and soybean oil

Fig. 5 Absorbance at selected peaks in FT-MIR spectra of ghee

Absorbance at selected peaks in FT-MIR spectra of ghee and vegetable oils

The qualitative and quantitative differences in FT-MIR spectra of ghee and that of the vegetable oils were examined. The first MIR milk analyser for fat determination was based on measurement of relative absorbance by carbonyl (C=O) stretch of ester linkage at 5.73 μm (now called Fat A). The absorbance at these selected peaks in FT-MIR spectra of ghee is presented in Fig. 5.

This means that the fat was measured by counting the number of fatty acid chains. However, the fatty acid chain length of milk fat is influenced by species, breeds, feeding pattern and season. This can cause erroneous estimation of fat in terms of large standard deviations of the difference between reference chemistry and instrument predictions. Therefore, measurement of absorbance by carbon–hydrogen (C–H) stretch in fatty acid chain at 3.48 μm (called as

Fat B) was mixed as a second measurement of fat because to compensate for difference in fatty acid chain length (Abd El-Salam et al. 1986). The relative weightage of Fat ‘A’ and Fat ‘B’ was recommended as 73% Fat ‘B’ + 27% Fat ‘A’. The rationale for this approach was that Fat ‘B’ at 3.48 μm measures the CH_2 and CH_3 absorption centres in the fatty acid chains which account on average for 73% of the molecular weight of milk triglycerides while the Fat ‘A’ at 5.7 μm measures the ester linkages joining the fatty acids to the glycerol and carbonyl components which comprise about 27% of milk triglycerides (Biggs and McKenna 1989).

The absorption at 5.7 μm (1733 cm^{-1}), feature to the oils with a high content in saturated fatty acids and short hydrocarbon chain (Alexa et al. 2009; Javidnia et al. 2013). It implies that for a given weight of oil, the oil containing relatively higher amount of saturated and short chain fatty acids give higher absorbance value at 3.48 μm ; compared

to oil containing relatively higher amount of unsaturated and long chain fatty acids. Milk fat and coconut oils are well known for containing saturated short chain fatty acids content. Taking clue from the above resume attempt was made to examine absorbance values of ghee and vegetable oil at 3.48 μm (Fat B) and 5.7 μm (Fat A) and its possible application for detection of vegetable oil in ghee.

The absorbance values of ghee and oils samples at 3.48 and 5.7 μm were recoded from their respective spectrogram and given Table 1. To simplify the variations, difference in the absorbance between Fat B and Fat A was calculated. The deviation in Fat B – Fat A value for a particular oil from that of the ghee also worked out by subtracting the respective of the particular oil from the value of the ghee.

From the examination of absorbance of ghee and vegetable oil at Fat ‘B’ (3.48 μm) and Fat ‘A’ (5.7 μm), it was revealed that the difference do existed in their absorbance values both at 3.48 and 5.7 μm . However, more variation was found in absorbance at 3.48 μm compared to 5.7 μm , reflecting the greater variation in chain length of their constituent fatty acids, compared ester group content. The relative variation in absorbance became more evident and clear when difference in the absorbance between Fat ‘B’ and Fat ‘A’ was calculated and deviation from ghee was worked out.

The variation in difference between Fat ‘B’ and Fat ‘A’ absorbance was similar for cow and buffalo ghee. Except coconut oil and soybean oil, the difference in absorbance between Fat ‘B’ and Fat ‘A’ for vegetable oil was lower than that of the ghee. Such variation for different vegetable oils was ranging from marginal (+ 0.03) to

pronounce (+ 0.35), baring the exception of coconut oil and soybean oil. The deviation was marginal in case of corn oil, cotton seed oil, palm kernel oil, and sunflower oil.

The trend observed from results was as per anticipation, the difference between Fat ‘B’ and Fat ‘A’ was greater in case of ghee and the greatest in case of coconut oil when compared with other oils. Since, the oils having marginal deviation were very common edible oils, the idea of exploring application of difference in absorbance values at Fat ‘B’ (3.48 μm) and Fat ‘A’ (5.7 μm) for detection of vegetable oil in ghee was not taken for further investigation.

Inconvenience in use of FT-HATR-MIR spectroscopy for ghee and oil

There were some problems in application of FT-MIR-HATR spectroscopy in this study. One of the problems was deposition and adsorption ghee and oil on surface of the HATR crystal, creating difficulties of efficient cleaning of the HATR crystal, because the cleaning process was tedious and very time consuming. The traces of residual fatty material left behind even after extensive cleaning, was creating problem of background effect on spectrum on analysis after sizable number of samples. The cleanliness of the sampling accessory creates problem background effect on spectrum after extensive use of the HATR crystal (PerkinElmer 2004). Hence, there is a need of an appropriate sampling assembly which does not get saturated even upon application of samples in large number.

The similar views about difficulties in use of FT-MIR-ATR spectroscopy are also expressed by Aernouts et al.

Table 1 Absorbance of ghee and vegetable oil at specific peaks in MIR

S. No.	Sample	Absorbance at		Difference Fat B – Fat A	Deviation from ghee
		3.48 μm (Fat B)	5.7 μm (Fat A)		
1	Buffalo ghee	1.95 $\pm$ 0.10	1.15 $\pm$ 0.10	0.80	0.00
2	Cow ghee	1.84 $\pm$ 0.10	1.04 $\pm$ 0.10	0.80	0.00
3	Coconut oil	2.22 $\pm$ 0.13	1.13 $\pm$ 0.11	1.09	+ 0.29
4	Corn oil	1.78 $\pm$ 0.09	1.02 $\pm$ 0.08	0.76	– 0.04
5	Cotton seed oil	1.79 $\pm$ 0.09	1.04 $\pm$ 0.09	0.75	– 0.05
6	Groundnut oil	1.76 $\pm$ 0.08	1.10 $\pm$ 0.09	0.66	– 0.14
7	Mustard oil	1.60 $\pm$ 0.06	1.09 $\pm$ 0.05	0.51	– 0.29
8	Palm oil	1.76 $\pm$ 0.09	1.22 $\pm$ 0.08	0.54	– 0.26
9	Palm kernel oil	1.91 $\pm$ 0.06	1.16 $\pm$ 0.07	0.75	– 0.05
10	Rice bran oil	1.61 $\pm$ 0.07	1.08 $\pm$ 0.06	0.53	– 0.27
11	Sesame oil	1.66 $\pm$ 0.06	1.05 $\pm$ 0.05	0.61	– 0.19
12	Soybean oil	1.82 $\pm$ 0.05	0.95 $\pm$ 0.06	0.87	+ 0.07
13	Sunflower oil	1.75 $\pm$ 0.04	0.98 $\pm$ 0.03	0.77	– 0.03

Data presented as mean $\pm$ SD (n = 35)

(2011). They used HATR crystal to acquire MIR spectra of liquid milk samples. The author reported that the HATR crystal was very difficult to clean. Even after cleaning with warm water (50 °C), commercial detergent, ethanol and hexane a fatty layer on top of the crystal was still visible with the naked eye. Moreover, a background scan of an empty ‘clean’ crystal still contained some clear fat absorbance peaks. Consequently, HATR sample presentation was not included in this study. A possible cause for the poor cleaning result is the combination of a lipophilic character and porous surface of the crystal. Consequently, the HATR sample presentation was not included in the further study. Thus, difficulties faced for use of HATR crystal as a sampling device in the present study was very well supported by the views expressed by Aernouts et al. (2011).

Conclusion

FT-MIR spectral pattern of ghee and common vegetable oils were examined using FT-MIR spectroscopy employing HATR. The difference in absorbance values at 3.48 and 5.7 μm for ghee and for vegetable oils were worked out. The differences in absorbance values obtained in many vegetable oils were very near to that of the ghee. In examination of overlaid spectra of pure ghee and vegetable oils in MIR region, differences between spectra of ghee and that of the vegetable oils were clearly evident especially in the fingerprint region. These differences in fingerprint region could be exploited to develop simple, fast and eco-friendly method for detection of the vegetable oils mixed in the ghee as an adulterant. However, an appropriate sampling assembly to be used to avoid saturation of its surface on use for large number of samples.

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