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Research Article

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Detection of adulteration in ghee from markets of Ahmedabad by FTIR spectroscopy

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ABSTRACT

Fourier Transform Infrared Spectroscopy is one of the most modern techniques used in the field of analytical chemistry for the identification of the constituents of the substance by determination of the functional groups. In Forensic Science the IR spectroscopy is very helpful in the identification of the substances by knowing their constituents. Along with the determination of active components of ghee, the trans fatty acids by FT-IR spectroscopy, the adulteration in ghee can also be detected by studying the spectrogram.

Keywords: FT-IR, Ghee, adulteration, Forensic

INTRODUCTION

Food adulteration is one of the major problems faced by the consumers of India. Since times immemorial Ghee has been always used in the Indian foods. It has been noticed and has become a common practice to adulterate ghee with cheaper vegetable and animal body fats. Hydrogenated vegetable fats, popularly known as vanaspati ghee in this country, is the most common adulterant used to adulterate ghee apart from paraffin and wax similar looking like substances.

Adulteration of ghee has lead to serious threats to human health as well as enormous economic losses to the food industry. Considering the diversity of adulterants possibly mixed in ghee, such as vanaspati, lard, wax, paraffin and so forth, a rapid, widely available, high-throughput, cost-effective method is needed for detecting each of the components in ghee at once. The literature review shows that more recently; Rama- Murthy et. al (1) reported a thin layer chromatographic method for detecting ghee adulteration with vegetable oils and fats. Detection of animal body fats in ghee is more difficult. An opacity method based on differential melting point ranges for common animal body fats (43 to 50^0 C) and ghee (30 to 44^0 C) has been developed by Singhal et. al (2). It is claimed to detect 5% animal body fats in ghee. However, this test is useless for cotton tract ghee which has a melting point near that of animal body fats. The methylene blue reduction test developed by these authors (3) overcame this difficulty because only cotton tract ghee decolorizes methylene blue. FTIR has hardly been used for the detection of adulteration of ghee therefore in this paper, Fourier Transform Infrared spectroscopy (FTIR) was used to detect the presence of adulteration and trans fat in ghee. This non-destructive method can be used for a correct discrimination on whether the ghee is adulterated with deleterious substances and it provides a new simple and cost-effective alternative to test the components of ghee. Infrared (IR) spectroscopy is an old analytical technique that has been widely utilized as a routine tool by the fats and oils industry for the detection of Trans fatty acids [2]. IR deals with the infrared region of electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. Because of similar application and instrumentation, IR spectrum is usually divided into three sub-regions: Near IR region:

14000-4000 cm^{-1}. It can excite overtone or harmonic vibrations. Mid-IR region: 4000-400 cm^{-1}. It is used to study fundamental vibrations and associated rotational-vibrational structure. Far-IR region: 400-10 cm^{-1} lying adjacent to microwave is used to study rotational spectroscopy.

As with all spectroscopic techniques, it can be used to study qualitative and quantitative analysis of chemicals. T*rans* fat labeling and other regulatory actions in many countries prompted the food industry worldwide to accelerate food reformulation to reduce the amount of total *trans* fat in food products [4]. Currently there is no unanimous consent among countries as to which *trans* FAs are to be included in the definition of total *trans* FA content [4]. Based on a strict chemical definition this would include all FAs having one or more double bonds in the *trans* configuration, irrespective of whether it is industrially produced during partial hydrogenation, naturally produced in ruminants, or as a result of food processing. This position was recently taken by Australia and New Zealand, and appears to imply that human milk, ruminant meats and dairy fats would be considered "unhealthy" [5]. Denmark and Switzerland have chosen to limit *trans* fats to industrially produced fats, while considering all ruminant derived *trans* FAs as "healthy" [4]. The USA, Canada, the EU and Codex Alimentarius have also attempted to differentiate between the origins of the *trans* FAs by excluding from the definition of total *trans* FA content those that contain conjugated *trans* double bonds [4]. However, this definition presumes that all conjugated FAs are "healthy", and all FAs containing isolated *trans* double bonds are not.

EXPERIMENTAL SECTION

Fifteen (15) Ghee samples about 50 g each were collected from the local dairy of Ahmedabad and were preserved at room temperature. Just before the analysis the ghee samples were melted and stored in the glass container and a small amount was then put onto a sample holder. FTIR spectra were scanned using a FTIR spectrometer Bruker with a resolution of 4 cm−1, in the 600–4000 cm−1 region. The samples were placed in contact with attenuated total reflectance (ATR) element (ZnSe crystal) at controlled ambient temperature (20^0 C). The instrument was calibrated before the analysis of the ghee samples. The small amount of ghee from each sample was taken in a sequence and was analysed at the rate of 60 scans/minute. Between the analysis of every ghee samples, the sample holder of the instrument was cleaned with methanol using tissue paper three to four times in order to avoid the contamination of one sample by another. The spectrogram obtained after the analysis was saved and studied for the determination of the functional group.

RESULTS AND DISCUSSION

The analysis of 15 ghee samples were carried out and the spectrogram was analysed.

The analyses focused on the measurement of the FTIR spectra of ghee in the 4000-600 cm–1 spectral region. The characteristic infrared spectra of ghee are shown in Figure 1. Strong absorptions were observed at 700cm^{-1}, respectively, corresponding to (CH_2) stretching vibration and 1379 C–H s stretching vibrations. At 1741 cm^{-1}, another strong band was present, which is reported to be associated with –C=O stretching vibrations of acids and esters .This band and the next at 1529 cm^{-1} arising from N–H bending vibration are most likely associated with the amide I and amide II of proteins. In the last part of the spectra (1300-1000 cm^{-1}), stretching vibrations of the C–H bond of ketones. The band at 966 cm^{-1}, associated with –HC=CH out-of-plane deformation vibrations, has been previously reported as a marker band for the determination of trans-fats has not being found in the ghee samples.

Among all the 15 ghee samples sample 1, 2, 3, and 7 has shown the presence of ester at 1742 cm^{-1} , whereas sample 8-15 have shown the presence of ester at 1741cm^{-1}. Alkenes were found to be present in sample 1-4 with strong stretching vibrations at 1651cm^{-1}. Strong stretching vibrations were observed at 1464cm^{-1} for alkanes in sample 1-4 and 9-15. Only one sample no.5 has shown wave no at 964 cm-1 for anhydride related to trans fatty acid. Most of the samples have shown stretching at 722cm-1 for alkanes. Amines and amides have shown stretching at 1585-1548cm^{-1} and 1529cm^{-1}. Ketones have shown stretching at 1145$cm^{-1.}$

SAMPLE NO.	WAVE NUMBER	RANGE	MOLECULAR MOTION	FUNCTIONAL GROUP
1.	1742	1750-1735	−C=O	Ester
	1651	1690-1630	−C=C−(isolated)	Alkenes
	1464	~1464	-CH_2-	Alkanes
	1171	1210-1160	C-C(O)-C and all others	Ester
	722	~722	-CH_2-[4 or more]	Alkanes
2.	1742	1750-1735	−C=O	Ester
	1651	1690-1630	−C=C−(isolated)	Alkenes
	1464	~1464	-CH_2-	Alkanes
	722	~722	-CH_2-[4 or more]	Alkanes
3.	1742	1750-1735	−C=O	Ester
	1651	1690-1630	−C=C−(isolated)	Alkenes
	1464	~1464	-CH_2-	Alkanes
	1171	1210-1160	C-C(O)-C and all others	Ester
	1238	1260-1230	C-C(O)-C[Acetates]	Ester
4.	1741	1750-1735	−C=O	Ester
	1651	1690-1630	−C=C−(isolated)	Alkenes
	1464	~1464	-CH_2-	Alkanes
	1171	1210-1160	C-C(O)-C and all others	Ester
	1240	1210-1160	C-C(O)-C and all others	Ester
5.	1740	1750-1735	−C=O	Ester
	1173	1210-1160	C-C(O)-C and all others	Ester
	964	1300-900	-C-O	Anhydride
	722	~722	-CH_2-[4 or more]	Alkanes
6.	1741	1750-1735	−C=O	Ester
	1171	1210-1160	C-C(O)-C and all others	Ester
	722	~722	-CH_2-[4 or more]	Alkanes
7.	1742	1750-1735	−C=O	Ester
	1171	1210-1160	C-C(O)-C and all others	Ester
	1240	1210-1160	C-C(O)-C and all others	Ester
8.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones
	722	~722	-CH_2-[4 or more]	Alkanes
9.	1741	1750-1735	−C=O	Ester
	1651	1690-1630	−C=C− (isolated)	Alkenes
	1464	~1464	-CH_2-	Alkanes
	1171	1210-1160	C-C(O)-C and all others	Ester
	722	~722	-CH_2-[4 or more]	Alkanes
10.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones
	722	~722	-CH_2-[4 or more]	Alkanes
11.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones
	722	~722	-CH_2-[4 or more]	Alkanes
12.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones

	722	~722	-CH_2-[4 or more]	Alkanes
13.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones
	722	~722	-CH_2-[4 or more]	Alkanes
14.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones
	722	~722	-CH_2-[4 or more]	Alkanes
15.	1741	1750-1735	−C=O	Ester
	1585	1640-1500	-N-H	Amines
	1548	1640-1500	-N-H	Amines
	1529	1570-1515	-N-H[1°]	Amides
	1464	~1464	-CH_2-	Alkanes
	1379	1430-1290	C-H in plane bend	Alkenes
	1145	1300-1100	-C-H	Ketones
	722	~722	-CH_2- [4 or more]	Alkanes

Fig-1: The FT-IR spectrogram of Sample-9

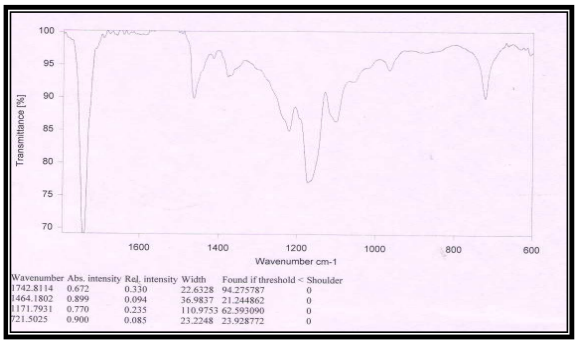
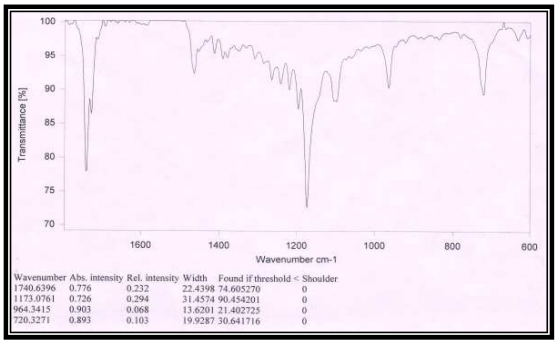


Fig-2:The FT –IR spectrogram of Sample-10



CONCLUSION

From the present study it is observed that the ghee contains high degree of unsaturation due to presence of alkene compound. From the spectrogram it can be concluded that the ghee has methyl ester as its major constituents and along with the organic compounds containing carboxylic, ketonic and amino groups. In the present study none of the samples out of 15 were found to be adulterated with lard or animal beef tissue and only one sample has shown the presence of trans fatty acid. Still extensive work is required for quantitative work which could be done by GC apart from FTIR. The constituents of ghee help in study of adulteration if present in a sample because the adulterants used for this purpose are fat soluble. The IR spectroscopy would be very useful for the forensic analyst to test the presence of adulterant, trans fatty acids if any and the composition of the ghee samples.

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