Scientific Research and Essays Vol. 5(20), pp. 3193-3200, 18 October, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2010 Academic Journals

Full Length Research Paper

Evaluation of trans fatty acids contents in commercial brands of ghee available at Indian markets

Deepti Mahapatra[1], Debashree Sethi[1] and R. Balaji Raja[2]*

[1]Department of Biotechnology, SRM University, Kattankulathur, Tamilnadu, India.
[2]Department of Biotechnology, National Institute of Technology, Raipur, Chhattisgarh, India.

Accepted 15 September, 2010

Ghee has a considerable amount of trans fatty acids found in the form of alkanes, alkenes and alkynes. Trans fatty acids are quite harmful to general health of an individual especially the CVS (Cardio Vascular System). Accumulation of trans fatty acids in blood vessels and other tissues/organs of the body can have fatal effect especially in obese population. Four commercial brands of ghee found in Indian markets were chosen and their trans fatty acid content was determined by FTIR (Fourier Trans Infra Red) spectroscopy. The trans fatty acids were found in all the four brands with GRB having the highest concentration of it. Sakthi brand was observed to have moderate content of trans fatty acids. Aachi and RKG brands were having the least concentration of trans fatty acids. These results obtained from the present study would be aiding to an increase in consumer awareness about presence of trans fatty acids in food items and better appraisal by the companies and thus improving the current scenario.

Key words: Ghee, trans fatty acids, FTIR, consumer awareness, cardiovascular system, public health, Indian market, food items.

INTRODUCTION

Ghee, also known as clarified butter in anglophone countries, is made by simmering unsalted butter in a cooking vessel until all water has boiled off and the milk solids, or protein, have settled to the bottom (Basu et al., 1962). The cooked and clarified butter is then spooned off to avoid disturbing the milk solids on the bottom of the pan. Unlike butter, ghee can be stored for extended periods without refrigeration, provided it is kept in an airtight container to prevent oxidation and remains moisture-free (Dastur 1955). Texture, colour or taste of ghee depends on the source of the milk from which the butter was made and the extent of boiling (Machado et al., 2010).

Traditional ghee is produced from the milk of buffalo indigenous to the regions of India and Pakistan, but it can also be made from any other milk-producing animal. The process begins with the standard butter created through the churning of milk fats, solids and water. This butter still contains a significant amount of moisture, which must be boiled off to create a clarified butter (Gaba and Jain, 1972). Ghee is considered a saturated fat, since it is derived from animals. Nevertheless, some studies suggest that it is healthier overall than traditional Western fats such as lard and margarine. Ghee uses a natural process to maintain stability without refrigeration, unlike the hydrogenated and partially-hydrogenated vegetable oils used in Western cooking (Ray and Patel, 1949). Authentic ghee can be made at home by using traditional methods, but it can be time-consuming and a bit tricky without an experienced guide. Prepared ghee can be found in the Ethnic Foods section of well-stocked grocery stores and at many Asian markets (Mozaffarian et al., 2010).

Trans fat is the common name for unsaturated fat with trans-isomer fatty acid(s). Trans fats may be monounsaturated or polyunsaturated but never saturated. Unsaturated fat is a fat molecule containing one or more double bonds between the carbon atoms. Since the carbons are double-bonded to each other, there are fewer bonds connected to hydrogen, so there are fewer hydrogen atoms, hence "unsaturated". Cis and trans are

*Corresponding author. E-mail: balajiraja_vlr@yahoo.com. Tel: +91-09942574378.

terms that refer to the arrangement of chains of carbon atoms across the double bond. In the cis arrangement, the chains are on the same side of the double bond, resulting in a kink. In the trans arrangement, the chains are on opposite sides of the double bond and the chain is straight (Micha et al., 2010).

Each fat molecule has three hydrocarbon chains. The process of hydrogenation adds hydrogen atoms to cis-unsaturated fats, eliminating double bonds and making them into partially or completely saturated fats. These more-completely saturated fats have a higher melting point, which makes them more attractive for baking and the saturation extends their shelf-life (Srinivasan and Anantakrishnan, 1964). However, partial hydrogenation converts a part of cis-isomers into trans-unsaturated fats instead of hydrogenating them completely. Complete hydrogenation converts the fat into a saturated "hard" fat. Alternatively, hard fats can be softened by cutting with cis fats such as vegetable oil, or by transesterification with cis fats into fats with cis unsaturated and saturated hydrocarbon chains.

Trans fats occur also naturally, although to a limited extent: vaccenyl and conjugated linoleyl (CLA) containing trans fats occur naturally in trace amounts in meat and dairy products from ruminants, although the latter also constitutes a cis fat. Trans fats occur also naturally, although to a limited extent: vaccenyl and conjugated linoleyl (CLA) containing trans fats occur naturally in trace amounts in meat and dairy products from ruminants, although the latter also constitutes a cis fat.

Unlike other dietary fats, trans fats are not essential and they do not promote good health (Ramamurthy et al., 1968). The consumption of trans fats increases the risk of coronary heart disease by raising levels of "bad" LDL cholesterol and lowering levels of "good" HDL cholesterol. Health authorities worldwide recommend that consumption of trans fat be reduced to trace amounts. Trans fats from partially hydrogenated oils are more harmful than naturally occurring oils (Anderson et al., 1961; Gerberding, 2009).

Trans fats raise your bad (LDL) cholesterol levels and lower your good (HDL) cholesterol levels. Eating trans fats increases your risk of developing heart disease and stroke. It is also associated with a higher risk of developing type 2 diabetes. Before 1990, very little was known about how trans fat can harm your health. In the 1990s, research began identifying the adverse health effects of trans fats. Small amounts of trans fats occur naturally in some meat and dairy products, including beef, lamb and butterfat. It is not clear; though, whether these naturally occurring trans fats have the same bad effects on cholesterol levels as trans fats that have been industrially manufactured (Vergroesen, 1972). The American Heart Association recommends limiting the amount of trans fats you eat to less than 1% of your total daily calories. That means if you need 2,000 calories a day, no more than 20 of those calories should come from trans fats. That is less than 2 g of trans fats a day (Vergroesen and Gottenbos, 1975). Given the amount of naturally occurring trans fats you probably eat every day, this leaves virtually no room at all for industrially manufactured trans fats (Mensink and Katan, 1990; Lock et al., 2009).

FTIR spectroscopy can give routine, easy and rapid measurements which leads to ambiguous information about the component composition. Specially a precise wavelength scale of the Fourier method is helpful here. A relatively good spatial resolution is important. The study was undertaken to determine the trans fatty acid content in the commercial brands of ghee available in the Indian market and to give an awareness to the general public about their implications in their health.

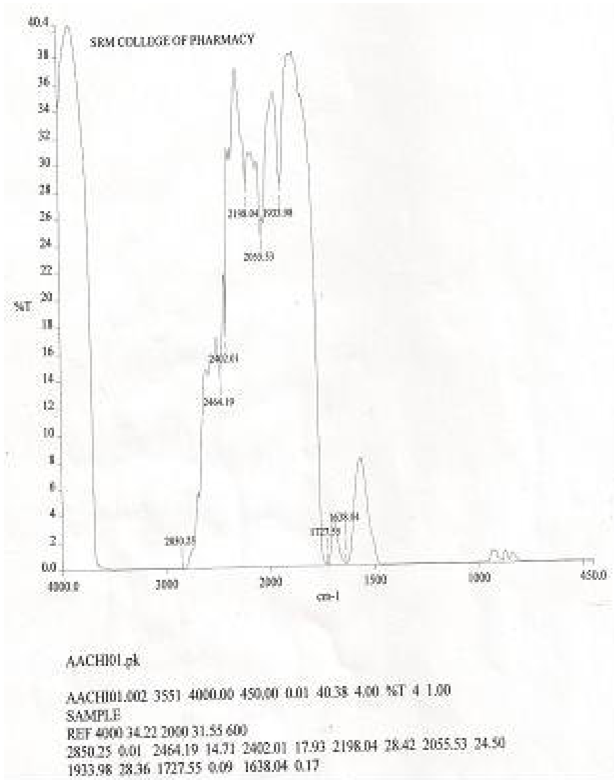
MATERIALS AND METHODS

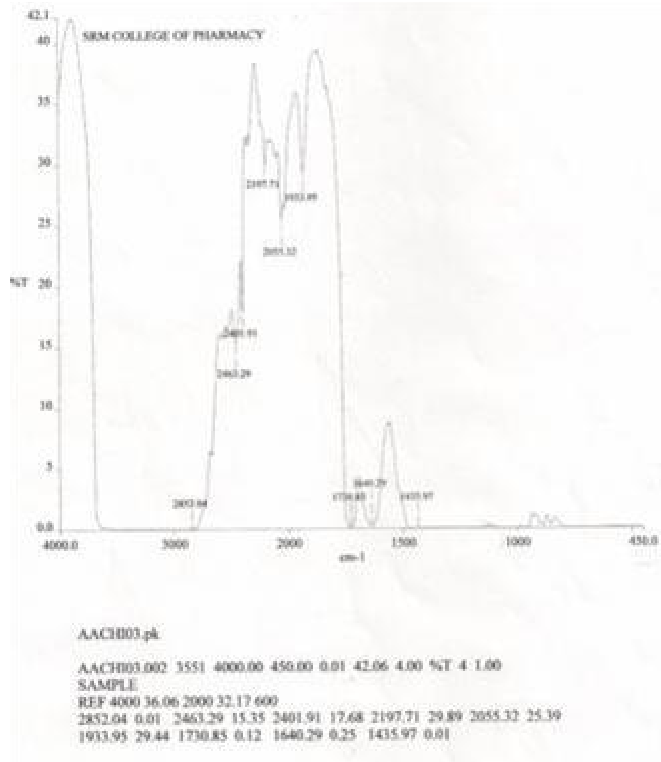
Food items were selected for analysis, based on data from previous studies which reported that major sources of TFA could be found in bakery products, fast food and frozen food, packaged snacks and fats and oils. The food items in the above categories were selected if vegetable oil was a listed ingredient and if the fat content of the food exceeded 5%. The four brands of ghee were purchased from retail outlets in Chennai, Tamilnadu, India. Ghee items were stored at an appropriate temperature. Triplicate amounts of the food item (typically 5 g with the specific weight recorded) were extracted with 2:1 chloroform-methanol solution containing Lycopene as an antioxidant (Sies and Stahl, 1995).

Extraction of lycopene from tomato

Obtain a tomato paste sample by crushing fresh tomatoes bought from local market. Mortar and pestle were used for the above mentioned process. About 1 g of tomato paste was weighed into a 15 mL screw-cap centrifuge tube. The solid material was extracted by shaking the capped tube with 4 mL of a 50% (by volume) mixture of acetone and low-boiling petroleum ether until the solid residue looks dry and fluffy. Then a flat-bladed microspatula was used to run and crush it against the sides of the tube. The shaking and crushing steps were repeated several times. The extract was then seperated by centrifugation or with a filter-tip pipet and transfered it to a second 15 mL centrifuge tube. Then the extraction of the solid residue with another 4 mL portion of 50% acetone/petroleum ether was effected and combined with the extracts in the second centrifuge tube. The combined extracts were then washed with 5 mL of saturated sodium chloride solution, followed by 5 mL of 10% aqueous potassium carbonate and another 5 mL portion of saturated NaCl solution. Lycopene-containing organic layer was dried with anhydrous magnesium sulphate and collected it in a 5 mL conical vial and concentrate it to a volume of 0.1 – 0.2 mL by evaporating most of the solvent under vacuum or in a stream of dry nitrogen without heating (Etminan et al., 2004).

The solution of extracts were extracted on the aluminum oxide column by first removing a first pink compound with hexane. The extract was set aside for UV-VIS spectroscopic analysis. Lycopene was eluted with a mixture of 10% acetone-in-hexanes and the orange band of lycopene as well as a slower moving yellow-orange band was separated (Giovannucci et al., 2002). The solvent of both fractions in a stream of nitrogen was removed and the weight of the dry lycopene fraction was obtained. This lycopene sample was used for further studies. Quantification of TFA in triplicate samples





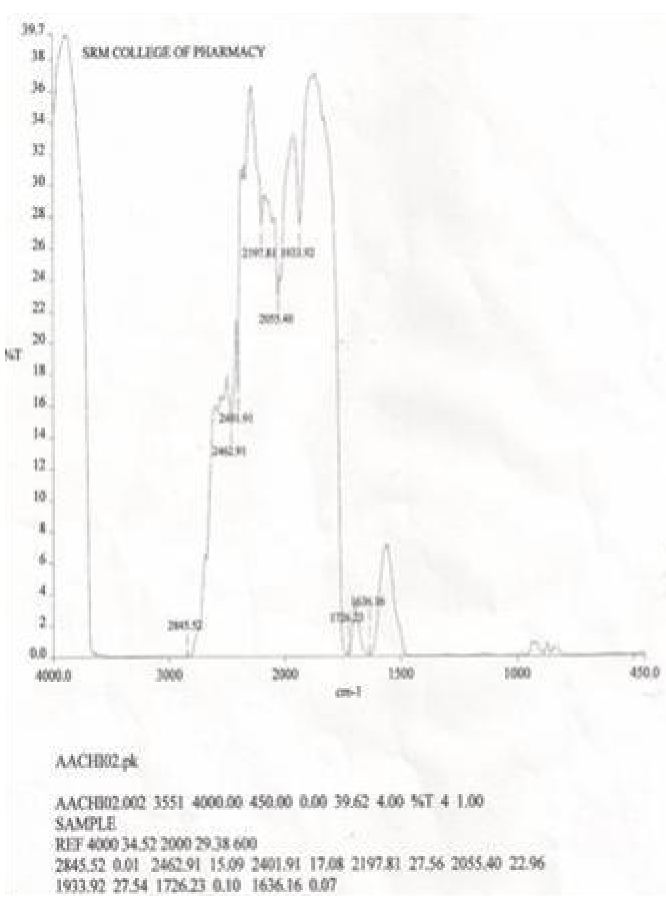


Figure 1. FTIR Tranmittance graph of Aachi brand of ghee (Triplicate samples).

of all the 4 ghee brands were analysed using FTIR (Perkin elmer, USA, Spectrum 100 model).

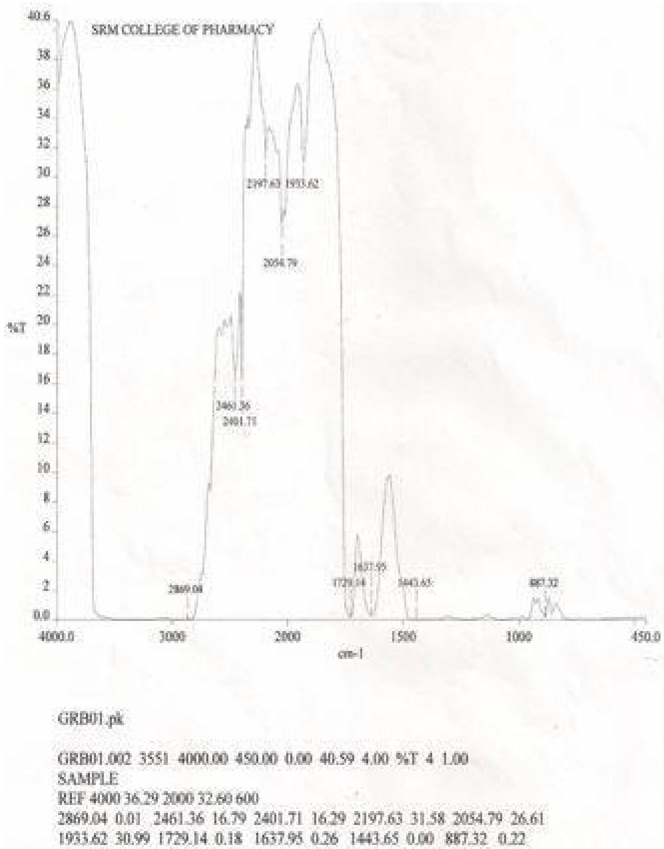
RESULTS AND DISCUSSION

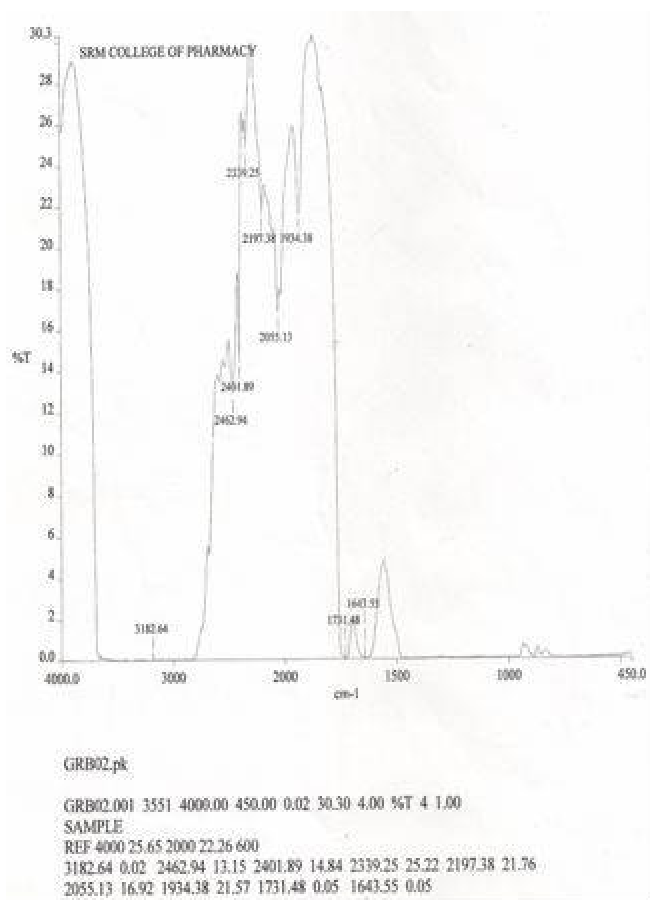
The Trans fatty acid content found in the commercial brand 'Aachi' was found to be least in comparison with other 3 brands taken up for study. Aachi brand had 9 characteristic peaks with only 2 peaks (1570 cm^{-1}, 1866 cm^{-1}) corresponding to the standard trans fatty acid peak (Figure 1).

The Trans fat acid content found in the commercial brand 'GRB' was found to be the highest in comparison with other 3 brands taken up for study. GRB brand had 9 characteristic peaks with 4 peaks (1100 , 1906 , 2334, 3028 cm^{-1}) corresponding to the standard trans fatty acid peak (Figure 2).

The Trans fat acid content found in the commercial brand 'RKG' was found to be least in comparison with other 3 brands taken up for study. RKG brand had 8 characteristic peaks with only 2 peaks (1637 and 1934 cm^{-1}) corresponding to the standard trans fatty acid peak (Figure 3).

The Trans fat acid content found in the commercial brand 'Sakthi' was found to be having moderate amount of trans fat acid content. Sakthi brand had 9 characteristic peaks with 3 peaks (1834, 1946, 3434 cm^{-1})





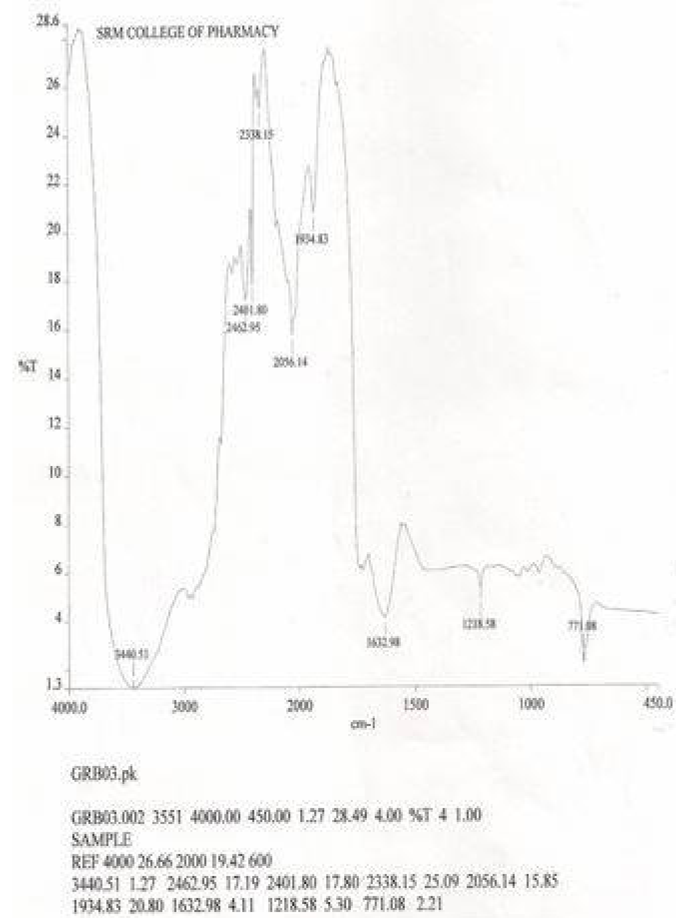


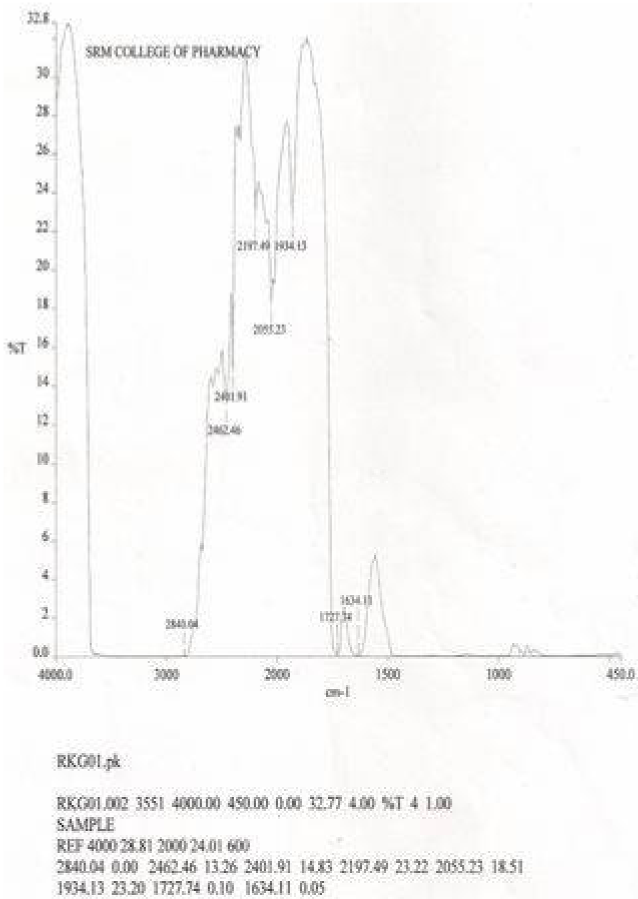
Figure 2. FTIR Tranmittance graph of GRB brand of ghee (Triplicate samples).

corresponding to the standard trans fatty acid peak (Figure 4).

Out of the 4 different commercial ghee brands chosen for the study, high content of trans fat was found in GRB, moderate amount was found in RKG and Least amount was found in Aachi, Sakthi (Tables 1 - 4). The trans fat acid contents of the food items are not mentioned in their back wrapper as far as Indian markets are concerned. As it has been clearly established that trans fatty acids increase the CVS (Cardio Vascular System) problems, it becomes very important to indicate the trans fatty acid content in all packaged items. In a growing economy like India, the relevance of health consciousness and consumer awareness are need of the hour. Separate marking of the amount of Trans fat acid found in each and every potential food item will be beneficial for the consumers.

ACKNOWLEDGEMENTS

We would like to thank the management of SRM University for providing necessary support to carry out



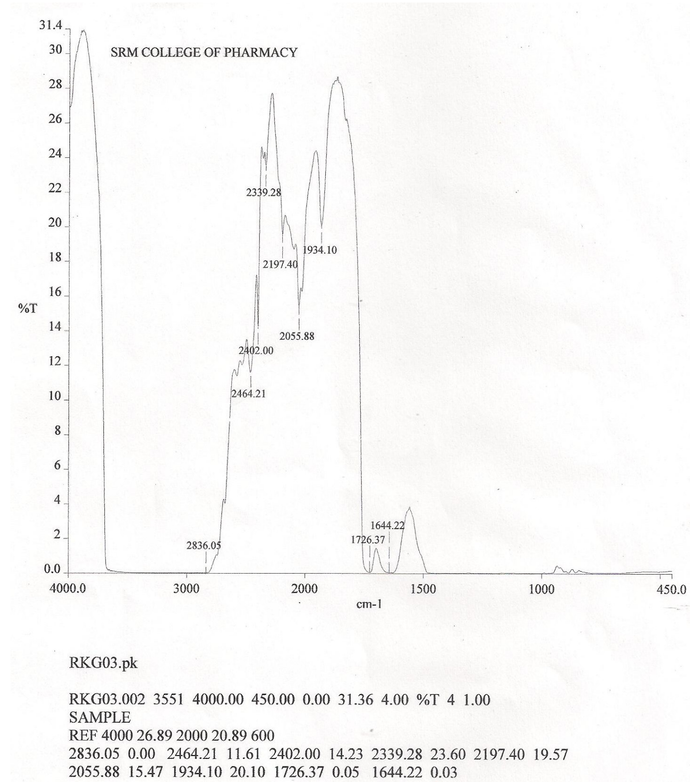
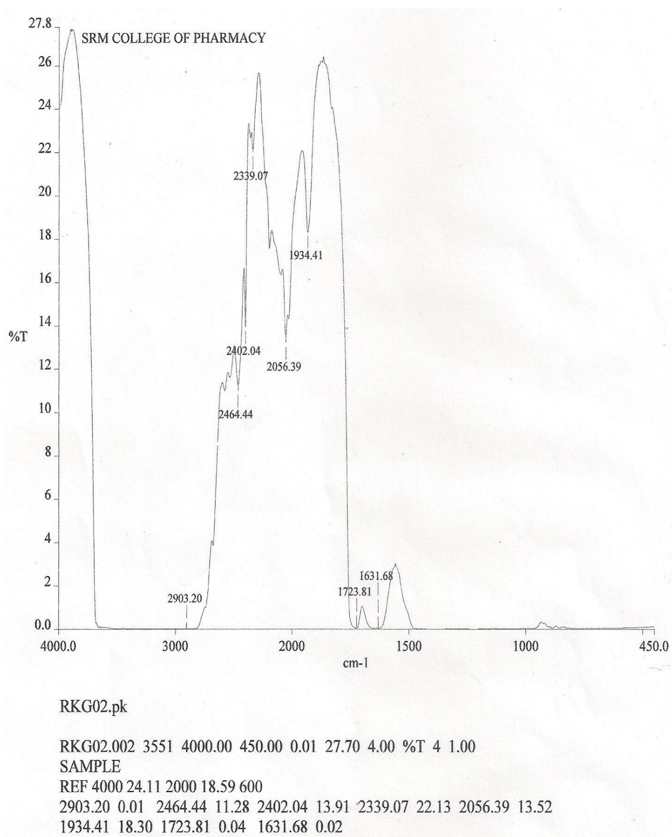
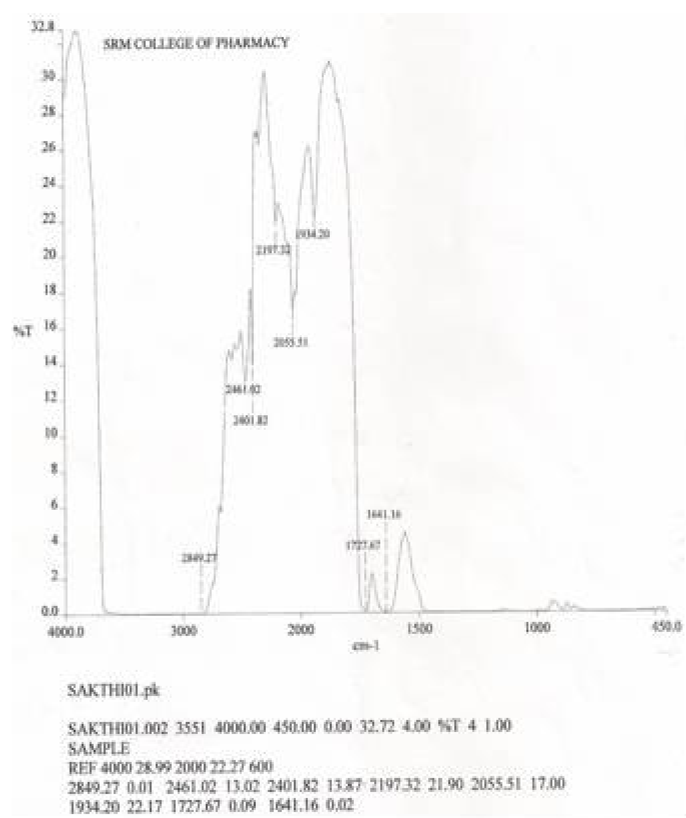
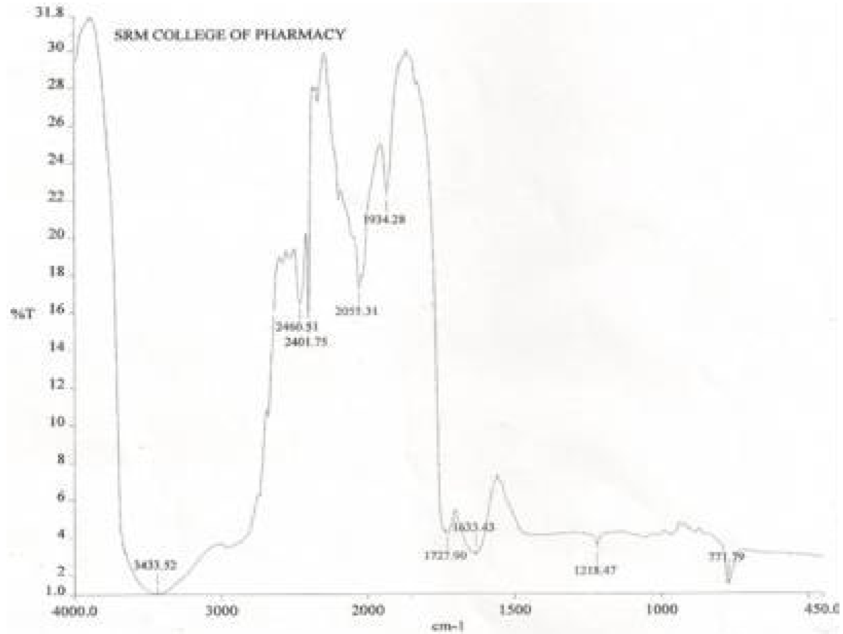


Figure 3. FTIR Tranmittance graph of RKG brand of ghee (Triplicate samples).

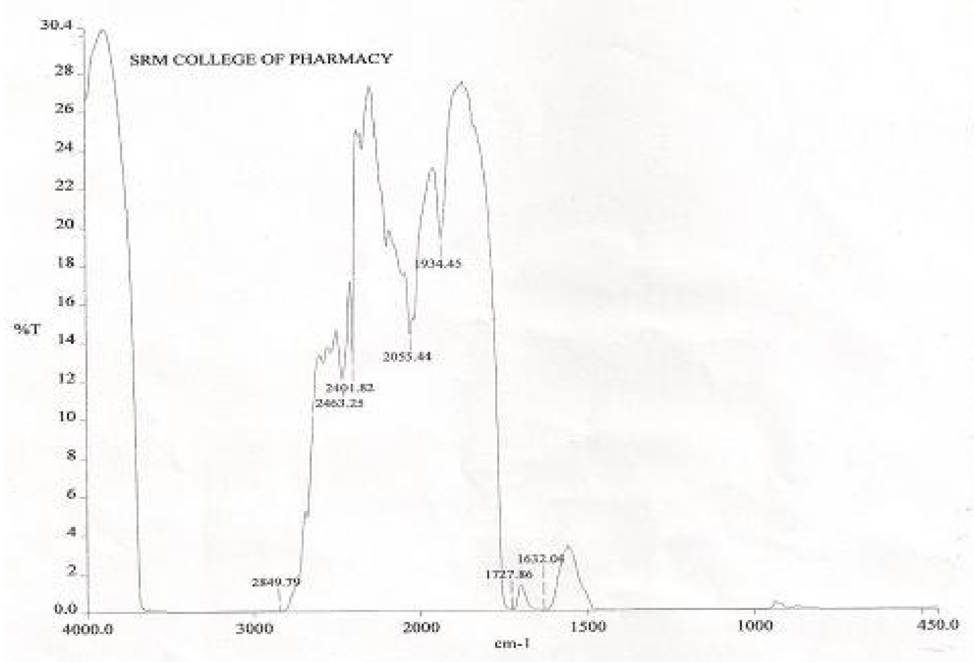






SAKTHI02.pk

SAKTHI02.002 3551 4000.00 450.00 0.97 31.76 4.00 %T 4 1.00
SAMPLE
REF 4000 29.46 2000 21.41 600
3433.52 0.97 2460.51 16.44 2401.75 15.91 2055.31 17.29 1934.28 22.45
1727.90 4.16 1633.43 3.06 1218.47 3.60 771.79 1.51



SAKTHI03.pk

SAKTHI03.002 3551 4000.00 450.00 0.01 30.30 4.00 %T 4 1.00
SAMPLE
REF 4000 26.67 2000 19.36 600
2849.79 0.01 2463.25 12.13 2401.82 13.43 2055.44 14.37 1934.45 19.51
1727.86 0.05 1632.04 0.02

Figure 4. FTIR Tranmittance graph of Sakthi brand of ghee (Triplicate samples).

Table 1. FTIR analysis of Brand 1 “AACHI”

Absorbance peak value (cm^{-1})	Transmittance peak value (cm^{-1})	Functional Group	Intensities	Shape	Formula
2430	1570	Alkynes	Strong	Broad	$\square$C≡C
2302	1698	Nitriles	Strong	Broad	C≡N
2134	1866	Alkynes	Strong	Sharp	C≡C
1985	2015	Phenyl Ring Substitution Overtones	Strong	Sharp	C-H
1849	2150	Phenyl Ring Substitution Overtones	Strong	Sharp	C-H
1666	2334	Phenyl Ring Substitution Overtones	Medium	Sharp	C-H
1557	2443	Nitro Compounds	Medium	Sharp	NO_2
1280	2720	Nitro Compounds	Medium	Sharp	NO_2
1148	2852	Esters	Medium	Broad	C-O

Table 2. FTIR analysis of Brand 2 “GRB”.

Absorbance peak value (cm^{-1})	Transmittance peak value (cm^{-1})	Functional Group	Intensities	Shape	Formula
2900	1100	Alkanes	Strong	Sharp	C-H
2535	1465	Carboxylic acids	Strong	Broad	O-H
2265	1735	Nitriles	Strong	Broad	C≡N
2094	1906	Alkynes	Strong	Sharp	C≡C
1938	2062	Phenyl ring substitution overtones	Strong	Sharp	C-H
1756	2244	Esters	Medium	Sharp	C=O
1666	2334	Alkenes	Medium	Sharp	C=C
1557	2443	Aromatic Rings	Medium	Sharp	C=C
972	3028	Alkenes	Medium	Sharp	C-H

Table 3. FTIR analysis of Brand 3 “RKG”

Absorbance peak value (cm^{-1})	Transmittance peak value (cm^{-1})	Functional Group	Intensities	Shape	Formula
2363	1637	Alkynes	Strong	Broad	C-H
2274	1934	Alkynes	Strong	Broad	C≡C
1944	2056	Phenyl ring substitution overtones	Strong	Sharp	C-H
1756	2244	Phenyl ring substitution overtones	Strong	Sharp	C-H
1619	2381	Phenyl ring substitution overtones	Medium	Sharp	C-H
1557	2443	Aromatic Rings	Medium	Sharp	C=C
1264	2736	Esters	Medium	Sharp	C-O
1164	2836	Esters	Medium	Broad	C-O

Table 4. FTIR analysis of Brand 4 “Sakthi”.

Absorbance peak value (cm^{-1})	Transmittance peak value (cm^{-1})	Functional group	Intensities	Shape	Formula
2652	1648	Carboxylic acids	Strong	Broad	O-H
2442	1558	Nitriles	Strong	Broad	N-H
2166	1834	Alkynes	Strong	Sharp	C≡C
2054	1946	Alkynes	Strong	Sharp	C≡C
1822	2178	Phenyl ring substitution Overtones	Strong	Sharp	C-H

Table 4. Contd.

1674	2326	Esters	Medium	Sharp	C-O
1300	2700	Esters	Medium	Sharp	C-O
1539	2461	Aromatic Rings	Medium	Sharp	C=C
566	3434	Alkenes	Weak	Broad	C-H

this study. Our gratitude goes to Dean and staff, SRM school of pharmacy for their help in FTIR analysis.

REFERENCES

Anderson JT, Grande F, Keys A (1961). Hydrogenated fats in the diet and lipids in the serum of man. J. Nutr., 75: 388-394.

Basu KP, Paul TM, Shroff NB, Rahman MA (1962). Composition of milk and ghee. ICAR Report Series 8, New Delhi, India.

Dastur NN (1955). Standards for ghee, Indian Dairyman, 1: 267.

Etminan M, Takkouche B, Caamano-Isorna F (2004). The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiol. Biomark. Prev., 13: 340-345.

Gaba KL, Jain MK (1972). A note on the flavor changes in ghee on storage: their sensory and chemical assessment. Indian J. Ani. Sci., 4: 56-60.

Gerberding JL (2009). Safer Fats for Healthier Hearts: The Case for Eliminating Dietary Artificial Trans Fat Intake. Ann Int. Med.,151: 137-138.

Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC (2002). A prospective study of tomato products, lycopene, and prostate cancer risk. J. Natl. Cancer Inst., 94: 391-398.

Lock K, Stuckler D, Charlesworth K, McKee M (2009). Potential causes and health effects of rising global food prices. BMJ, 339: b2403-b2403.

Machado RM, Stefano JT, Oliveira CPMS, Mello ES, Ferreira FD, Nunes VS, de Lima VMR, Quintao ECR, Catanozi S, Nakandakare ER, Lottenberg AMP (2010). Intake of trans Fatty Acids Causes Nonalcoholic Steatohepatitis and Reduces Adipose Tissue Fat Content. J. Nutr., 140: 1127-1132.

Mensink RP, Katan MB (1990). Effect of dietary trans fatty acids on highdensity and lowdensity lipoprotein cholesterol levels in healthy subjectsN. . Engl. J. Med., 323: 439-445.

Micha R, King IB, Lemaitre RN, Rimm EB, Sacks F, Song X, Siscovick DS, Mozaffarian D (2010). Food sources of individual plasma phospholipid trans fatty acid isomers: the Cardiovascular Health Study. Am. J. Clin. Nutr., 91: 883-893.

Mozaffarian D, Jacobson MF, Greenstein JS (2010). Food Reformulations to Reduce Trans Fatty Acids. NEJM, 362: 2037-2039.

Ray SC, Patel BM (1949). Studies on cotton seed feeding to milch animals. Part II. The quality of ghee from buffalo fed on cotton seed in combination with dry or green fodder. Indian J. Dairy Sci., 2: 30.

Ramamurthy MK, Narayanan KM, Bhalerao VR (1968). Effect of phospholipids on the keeping quality of ghee. Indian J. Dairy Sci., 21: 62.

Sies H, Stahl W (1995). Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am. J. Clin. Nutr., 62(suppl.): 1315S-1321S.

Srinivasan MR, Anantakrishnan CP (1964). Milk products of India. ICAR, New Delhi, India.

Vergroesen AJ (1972). Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. Proc. Nutr. SOC., 31: 323-329.

Vergroesen AJ, Gottenbos JJ (1975). The role of fats in human nutrition: an introduction. In the Role of Fats in Human Nutrition. A. J. Vergroesen, editor. Academic Press, New York, NY., pp. 1-41.