

Qualitative analysis of desi ghee, edible oils, and spreads using Raman spectroscopy

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Keeping in view the importance of dietary fats in modulating disease risk, a study was planned to compare edible oils, spreads, and desi ghee based on fatty acid composition through Raman spectroscopy. The double bonds in unsaturated oils tend to react more with oxygen causing oxidative stress in living cells; therefore, the excessive use of processed vegetable oils may pose risk for human health. In the spectral analysis, Raman peaks at 1063 and 1127 cm^{-1} represent out-of-phase and in-phase aliphatic C–C stretch for saturated fatty acids. The peak at 1300 cm^{-1} , labeled for alkane, decreases with increase in the double bond contents (unsaturation). Further, the Raman peak at 1655 cm^{-1} showed a monotonic increase as a function of unsaturation. The double bond contents in the Raman spectra from 1650–1657 cm^{-1} represent unsaturated fatty acids that changes during the synthesis of spreads and banaspati ghee. Desi ghee, extracted from cow and buffalo milk, showed distinctive Raman peaks at 1650 and 1655 cm^{-1} , which originates because of isomers of conjugated linoleic acid. These Raman shifts differentiated desi ghee from other artificially produced banaspati ghee, spreads, and oils. Conjugated linoleic acid has proved to be anti-carcinogenic, anti-inflammatory, and anti-allergic properties; therefore, the limited use of desi ghee may reduce the risk of cardiac diseases. Principal component analysis has been applied on the Raman spectra that clearly differentiated desi ghee, mono-unsaturated extra virgin olive oil, and extra virgin olive oil spread from other oils, oil mixtures, spreads, and ghee. In addition, principal component analysis has been blindly applied successfully on 13 unknown samples to classify them with reference to the known ghee sample. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: Raman micro-spectroscopy; desi ghee; edible oils; fatty acids; principal components analysis (PCA)

Introduction

The relationship between food and health has been proven for the maintenance of life, but the concept has now been changed from maintenance to better health quality. Among all the diet ingredients, fats become more important for both health specialists and the public. Fats can be classified into saturated fatty acids (SFA) with no double bonds in aliphatic chain and unsaturated fatty acids (USFA). USFA are either monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA), which are based on the presence of one or more double bonds between carbon atoms. USFA are normally in *cis* form (*cis*-isomer) in vegetable oils where hydrogen atoms are on the same side of double bonds. In *trans* configuration, the hydrogen atoms are on the opposite side of the carbon chain and adopt the linear configuration like SFA.^[1] PUFA include soya bean oil, corn oil, sunflower oil, safflower oil, and cottonseed oil, margarines made from PUFA oils, walnuts, and seafood. Olive oil, canola oil, peanut oil, almonds, olives, peanuts, hazelnuts, cashew nuts, pistachio nuts, and avocados are rich in MUFA.^[2]

Saturated fatty acids are known to increase blood cholesterol and thus increase the risk of heart diseases.^[3] These saturated fats usually taken from animal sources are in the form of butter, traditional clarified butter, or desi ghee. Desi ghee is a type of liquid butter made from the cow/buffalo milk through adding yogurt culture. It is originated in ancient Hindustan (presently Pakistan, India, and Bangladesh) and is commonly used in Asian countries. The smoke point of desi ghee is 400–500 °F, which is higher than regular butter (325–375 °F),^[4] and is therefore preferred in cooking applications. Like any clarified butter, ghee is composed of 62% saturated fats.

Desi ghee is a rich source of conjugated linoleic acid (CLA), which is a mixture of linoleic acid isomers, containing two conjugated double bonds at different carbons in the fatty acid chain like (*cis*-9, *cis*-12, $\text{C}_{18:2}$)^[5] and (*cis*-9, *trans*-11, $\text{C}_{18:2}$) accounts for 75–90% of total CLA isomers.^[6] Another form of ghee is vegetable or banaspati ghee, which is partially hydrogenated vegetable oil and has been used instead of desi ghee because of its low price. Banaspati ghee contains *trans* fats, known to increase the risk of coronary heart disease even more than saturated fats and nearly 50% deaths have been reported to cause in the past 20 years.^[7]

Unlike traditional fats (butter, desi ghee, lard, and olive oil), the vegetable oils are extracted from chemical processing of various seeds and are cheap substitution of traditional fats. The dietary fats can induce changes in fatty acid composition of cell membrane, which in turns causes physiological and biochemical changes. Cell membranes are sensitive to the PUFA ratio of omega-3 to omega-

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Abbreviations: CVD, cardiovascular diseases; CLA, conjugated linoleic acid; USFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EVOO, extra virgin olive oil; PCA, principal component analysis.

6,^[8] and edible oils are excessively rich in omega-6 PUFA that disturb the required ratio. Therefore, the continuous consumption of edible oils may cause potential risk to human health.

In the local market, variety of oils, oil mixtures, desi ghee, and banaspati brands are available for cooking. In order to ensure their quality, the brands in market must be characterized through reliable, efficient, fast, and economical technique for better health care. Existing chemical methods for the characterization of edible oils are gas chromatography and nuclear magnetic resonance, which are costly, time consuming, and require rigorous sample preparation.^[9–11] During the last decade, Fourier transform infrared spectroscopy has been reported for the analysis of edible oils.^[12–15] However, because of strong absorption band of water and other long chain molecules in the infrared region, the spectroscopic signatures of the ingredients of interest are buried inside the spectra. Raman spectroscopy, on the other hand, is an advantageous technique that is based on the scattering of light photons from the vibrational modes of molecules rather than absorption. It is not affected by the strong absorption band of long chain molecules and water; however, it suffers from fluorescence background that is removed with the polynomial fitting or experimental techniques.^[16,17] Raman spectroscopy complements the infrared spectroscopy; some molecular structures are IR active, some are Raman active, and some are both.

Raman spectroscopy proved itself a powerful, label free and nondestructive fingerprint tool for the characterization of the body fluids and tissues for disease recognition.^[18–21] Raman micro-spectroscopy has been used for the identification of microorganism.^[22] It is now being considered a suitable detection probe with lab-on-chip to produce an efficient clinical diagnostic tool.^[23–25] Recently, it has been reported for the prediction of dengue virus infection and plasmodium viral infection in human sera/plasma.^[26,27] Raman spectroscopy along with Fourier transform infrared has been utilized for the comparative study of different edible oils and fats.^[28] It has also been utilized for the analysis of olive oil adulteration and its authentication,^[14,29,30] fatty acid contents of vegetable oils,^[31] components of the fats and adipose tissues of bovine.^[32]

The present study is intended to validate the already identified Raman peaks in edible oils, desi ghee, and spreads for their comparison and moreover, to develop an efficient, quick, reliable, and economical technique for the characterization of different brands available in the market based on fatty acid composition. Further, desi ghee is characterized to reveal its ingredients for comparative study with artificially processed oils, ghee, and spreads. Principal component analysis (PCA) has been applied to differentiate ghee, oils, and spreads depending upon their Raman spectral signatures.

Materials and methods

Samples used in the present study were taken from the local market, which include five edible oils; imported extra virgin olive oil (EVOO), local brands of sunflower oil, canola oil, cooking oil I (blend of olive oil, canola and sunflower oils), cooking oil II (blend of canola, soya bean, and sunflower oils), five spreads that include local brand margarine, imported sunflower spread, imported EVOO spread, imported rapeseed spread, imported blend of canola and sunflower oils spread. Five different types of ghees included desi ghee of buffalo (homemade), local brand desi ghee of cow, local brand banaspati I, II, and III (*trans* fats free).

Raman spectra were recorded directly from the sample by placing a small quantity on the aluminum substrate using Raman

system (PeakSeeker PRO-785 coupled with microscope RSM-785, Agiltron, USA) having a spectral resolution of 10 cm^{-1} . It employed diode laser emitting at 785 nm to excite the sample for Raman scattering. From each sample, 20 spectra were recorded from different positions by shining a laser power of 40 mW for the acquisition time of 10 s. Raman spectra were obtained from 300 and 1800 cm^{-1} spectral range, which were pre-processed in MATLAB (Mathworks, release 2009) and established protocols^[33] for the identification of molecular bonds and functional groups.

Data pre-processing included smoothing, baseline correction, vector normalization, and substrate removal. All the spectra, including substrate backgrounds, were vector normalized and smoothed using a Savitzky–Golay smoothing method (order 5, 13-point window). A rubber band correction for baseline removal for all the spectra was carried out, and the substrate spectra were subtracted from each spectrum.^[34] Same protocols^[33] were used for applying PCA on all the Raman spectra for clustering of individual species based on their Raman spectral finger prints.

Results and discussions

In this study, Raman spectroscopy has been applied as a probe for the classification of different oils and fat sources. In Figs 1–3, Raman spectra ranging from 800 to 1800 cm^{-1} showed various Raman signatures representing vibrational mode in the aliphatic chain of oils, spreads, margarine, and ghee (banaspati and desi ghee) as summarized in Table 1.

Figures 1–3 show the mean spectra of individual ghees, margarine/spreads, and oils, respectively, depicting the characteristics bands of saturated and unsaturated fats. In each figure, the significant Raman spectral features are labeled for differentiating molecular signatures. The Raman features associated with the saturation are contributed by peaks at 1296 cm^{-1} representing in-phase methylene twist (CH_2) and 1437 cm^{-1} for scissoring mode of methylene (CH_2), whereas in USFA, these peaks showed a slight shift of 1300 and 1439 cm^{-1} for in-phase methylene twist and scissoring mode of methylene, respectively.

The positive contributors for unsaturation are peaks at 1079, 1266, and 1655 cm^{-1} as shown in the spectra of oils and few spreads (S2–S5). The peak intensity at 1655 cm^{-1} is specific to $\text{C}=\text{C}$ bonds and reflects the degree of USFA. The CH_2 scissoring

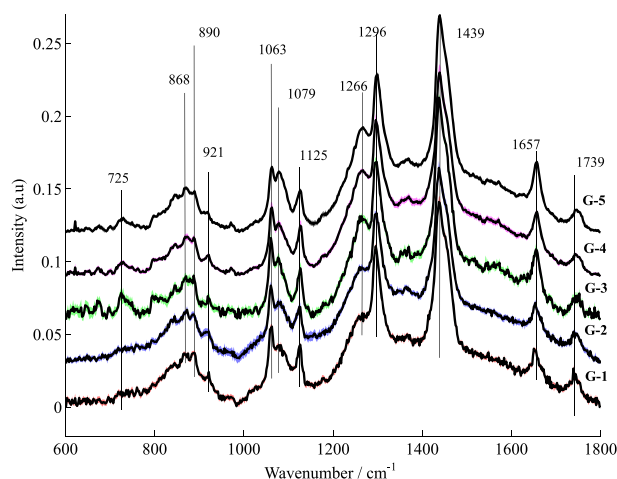


Figure 1. Mean Raman spectra of all ghees from G1 to G5. Different market brands are labeled from G1 to G5 to hide their actual identity.

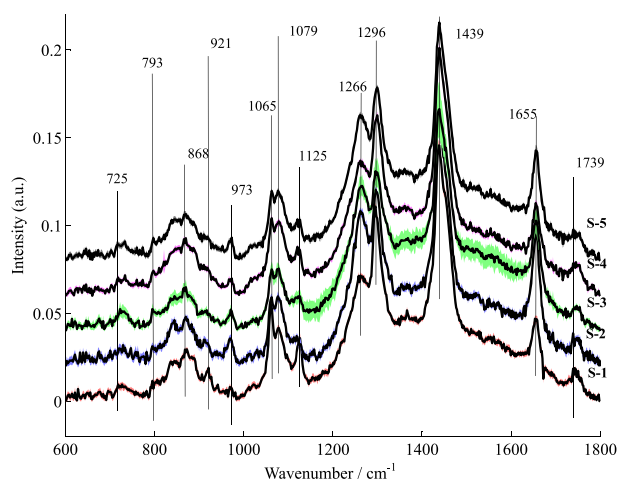


Figure 2. Mean Raman spectra of all margarine/spreads from S1 to S5. Different market brands are labeled from S1 to S5 to hide their actual identity.

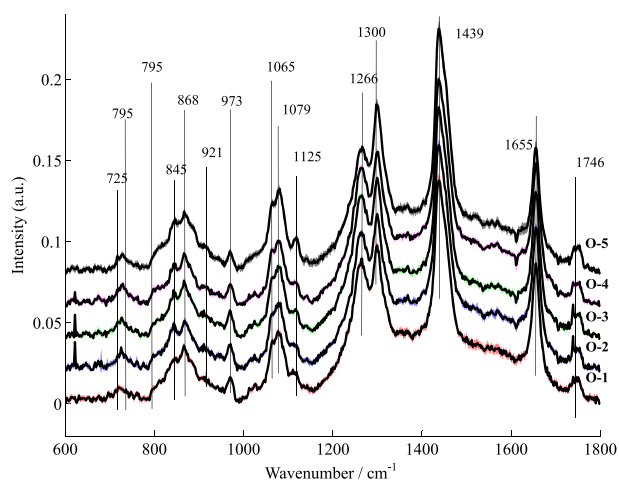


Figure 3. Mean Raman spectra of all oils from O1 to O5. Different market brands are labeled from O1 to O5 to hide their actual identity.

region ($1400\text{--}1500\text{ cm}^{-1}$) related with the packing of fatty acids chain shows broad peak at 1439 cm^{-1} in all ghees and margarines/spreads as compared with oils.

In the previous studies, the major differences among the Raman bands of edible oils and fat sources lie in the range of $800\text{--}3013\text{ cm}^{-1}$.^[31,35–39] In Figs. 1 and 2, desi ghee (G1 and G2), banaspati ghee (G3, G4, and G5), and margarine (S1) clearly depicted characteristics Raman peaks at 1065 and 1127 cm^{-1} , representing three SFA (lauric C12:0, palmitic C16:0, and stearic C18:0) for C–C out-of-phase and in-phase solid fats, respectively.^[40] The earlier studies on the adipose tissue of veal, beef, lamb, and pork^[32] differentiated solid and liquid phase fatty acids methyl ester (FAME) with the bands stretch at $1000\text{--}1200\text{ cm}^{-1}$. The difference of liquid and solid phase FAME is obvious in all figures (Figs 1–3), but in Fig. 2, spreads samples from S2 to S5 are derived from plant oils and are converted to solid state but showed band pattern like liquid phase FAME. Whereas margarine S1 (local brand) that is claimed to be manufactured from plant origin depicted the deviated pattern from liquid phase at this region. The intensity of band for S1 at 1065 cm^{-1} in Fig. 2 is stronger than other spreads as S1 is a margarine, although claimed to be originated from vegetable oils but contains high amount of SFA.

In the previous studies, the spectral range represents C=C stretch vibrations in the range of $1650\text{--}1665\text{ cm}^{-1}$, $2700\text{--}3000\text{ cm}^{-1}$ represents C–H stretch, and $3005\text{--}3020\text{ cm}^{-1}$ represents =C–H vibrations, which have been used to distinguish SFA, MUFA, and PUFA, respectively.^[41] The C–H stretch represents saturated C–C chains with intensity variation and variety of strong bands. On the other hand, the C=C stretch band that represents unsaturated fats was found more informative with Raman shift from 1654 to 1657 cm^{-1} and in agreement with the already reported ones while moving from MUFA to PUFA.^[41] Figure 3 shows the C=C stretch band with a Raman peak at 1655 cm^{-1} in vegetable oils rich in MUFA. These include cooking oil brand I (O1) containing canola, canola oil (O3), and EVOO (O5). Whereas cooking oil brand II (O4) showed a Raman band at 1657 cm^{-1} that represents PUFA, although this brand is a blend of oils (olive and canola) rich in MUFA thus raising the question mark on the quality of the brand. The previous work on adipose tissue of pork reported a slight increase in the peak at 1659 cm^{-1} that differentiated the omega-3 (α -linolenic acid) from omega-6 (γ -linolenic acid) fatty acid.^[39] The distinctive peaks at 1650 and 1654 cm^{-1} in the spectra of desi ghee made from buffalo and cow milk is assigned for CLA that has been found as an antioxidant and anti-atherogenic.^[5] As the Raman spectroscopy is sensitive to the structural changes in the molecules, so Raman shift and intensity of spectral bands modify as a result of modification in molecular structures. The Raman shifts of C=C band for *trans*-9-18:1 (1669 cm^{-1}) and *cis*-9-18:1 (1655 cm^{-1}) isomers in the CLA are shifted to lower wave numbers for *trans*-9, *trans*-11-18:2 isomer at 1658 cm^{-1} , and *cis*-9, *cis*-11-18:2 isomer at 1644 cm^{-1} because of conjugation.^[6]

The Raman spectrum from 800 to 920 cm^{-1} was reported for complex broad liquid phase bands.^[38] In Figs 1–3, the various minor peaks ranging from 844 to 920 cm^{-1} in oils and spreads are shifted to higher wave numbers in all ghee types. The peak at 968 cm^{-1} is labeled for *trans* RHC = CHR^[39]; however, in our case, Raman peak appeared at 973 cm^{-1} , which is pronounced in all oils and spreads while suppressed in margarine and ghee (S1, G1, G2, and G3). Similarly, the minor peaks in the Raman spectra labeled for carbonyl stretch C=O that are observed in the range of 1730 to 1750 cm^{-1} show the complex pattern and not clearly differentiated among oils, ghee, and spreads.

In order to differentiate all the categories based on their individual Raman spectral signatures, PCA has been applied to spectral data of oils, ghee, and spreads. Figure 4 depicts the PCA scatter plot, which shows the clustering up of EVOO from the other vegetable oils. Similarly, both desi ghees are clustered up separately from the artificially produced banaspati ghee, spreads, and oils. The major variations are explained by the first principal component (PC1) among oils, ghee, and spread as 45.3% and remaining variation is explained by PC2 as 20.2%. The average spectra of all the oils, ghee, and spreads depicted the differences in shape and intensity of Raman spectra, but the edible oils share almost similar Raman finger prints, whereas the EVOO and EVOO spreads are separated out from these clusters as shown in Fig. 4 of PCA scatter plot. Similarly, all banaspati ghee, margarine, and rapeseed spread mixed up in the PCA plot.

Figure 5 represents the PCA loadings, which represent biochemical finger prints of the ghees, oils, and margarine/spreads. PCA differentiated all the species from each other based on the loading vectors. The significant loadings vectors are labeled as 846 , 862 , 973 , 1079 , 1266 , 1439 , and 1655 cm^{-1} with positive direction, whereas 890 , 1065 , 1125 , and 1298 cm^{-1} with negative signs. The loading vectors with positive signs are responsible for clustering

Table 1. Raman signatures representing vibrational mode in the aliphatic chain of oils, spreads, margarine, and ghee

Sample	Code	Ingredients as mentioned by company	Ingredients as mentioned in literature	ν (C1–C2), CH ₃ rk, ν (C–O) Complex broad table in liquid, 800–920	C–C bending out of plane	Out-of-phase C–C stretch	In-phase C–C stretch	Symmetric rock in <i>cis</i> -double bond δ (=C–H)	In-phase methylene twist (CH ₂)	Scissoring mode of methylene δ (CH ₂)	<i>cis</i> -double bond stretching for unsaturation ν (C=C)	C=O stretch of ester group
Cooking oil I	O1	Blend of canola, sunflower and soybean oils	Linoleic acid, MUFA	845* 868	971	1079	–	1266	1300	1439	1655	1739*
Sunflower	O2	Sunflower	60% linoleic acid	845, 868	971	1079	–	1266	1300	1439	1657	1739
Canola	O3	Canola seeds	MUFA	845, 868	971	1079	1125*	1266	1300	1439	1655	1739
Cooking oil II	O4	Blend of canola, sunflower and olive oils	MUFA + PUFA	845, 868	971	1079	1125*	1266	1300	1439	1657	1739*
Extra virgin olive oil	O5	Cold press of olives	MUFA	845* 868	971	1079	1125	1266	1300	1439	1655	1742*
Margarine	S1	Vegetable oils	PUFA	868	–	1063	1127	1266*	1296	1439	1655	1739*
Sunflower spread	S2	Sunflower oil		868	973	1065, 1079	–	1266	1296	1437	1655	1739
Canola, sunflower spread	S3	Vegetable Oils 65% (containing 52% canola and sunflower oils)	Canola oil – 63% oleic acid (MUFA), 21% linoleic acid, 9% from ALA (omega-3 fatty acid), and 7% SFA.	866, 868	973	1065, 1079	1125*	1266	1296	1437	1655	1742*
Rapeseed oil spread	S4	64% butter with rapeseed oil		866, 868	973	1065, 1079	1123, 1125	1266	1300	1439	1655	1742*
Extra virgin olive spread	S5	From extra virgin olive oil	MUFA	868	973*	1065, 1079	1123, 1125	1266	1300	1439	1655	1742*
Desi ghee (buffalo, home made)	G1	Saturated fat	Butter fat 99.8%	868, 870, 890	–	1063	1127	–	1296	1437	1650	1739
Desi ghee (cow)	G2	Saturated fat	Butter fat 99.8%	870, 890	–	1063	1127	–	1296	1437	1654	1739*
Hydrogenated oil brand I	G3	Blend of canola, sunflower and soybean oils	Saturated fats	870, 890	–	1063	1127	1266*	1296	1437	1655	1739*
Hydrogenated oil brand II	G4	Blend of palm, cottonseed, sunflower and soybean oils	Saturated fats	870, 890*	–	1063	1127	1266*	1296	1437	1655	1739*
Hydrogenated oil brand III	G5	unknown	Saturated fats	870, 890*	–	1063	1127	1266*	1296	1437	1655	1739*

* Weak signals.

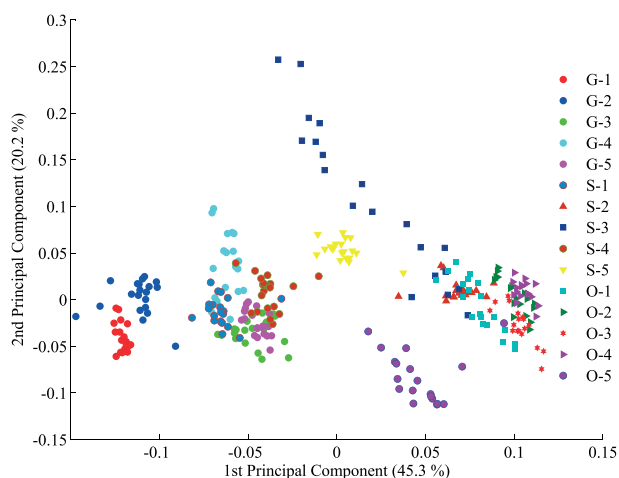


Figure 4. PCA scatter plot for all ghees from G1 to G5, all margarine/spreads from S1 to S5 and all oils from O1 to O5.

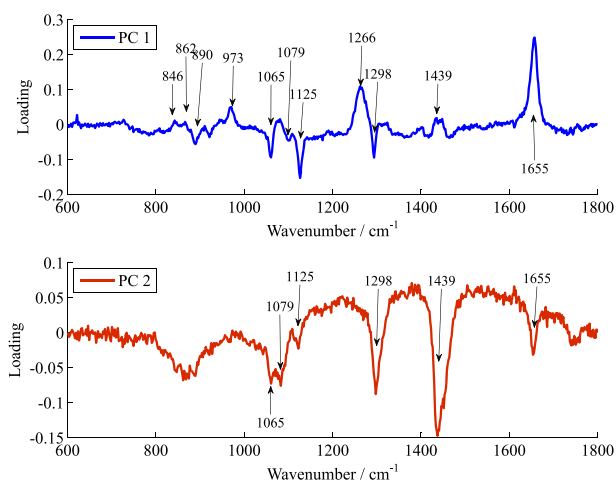


Figure 5. PCA loadings for all ghee including G1 to G5, all margarine/spreads from S1 to S5 and all oils from O1 to O5.

of different categories in positive places in PCA plot, and negative loading features cluster the categories in negative places.

In addition, 13 samples were collected from dairies and market, and their Raman spectra were recorded to validate our results. PCA was applied on all these Raman spectra that is shown in Fig. 6. A nice presentation of the classification with a known ghee sample with homemade ghee (G1) was shown. Later on, these samples were compared with the known samples and found all correctly classified.

Predicted samples from 1 to 7 were desi ghee which were clustered near homemade desi ghee, from 8 to 10 were olive oils (extra virgin) of three different brands and from 11 to 13 were three spreads and clustered together. Figure 6 suggests a nice direction of Raman spectroscopy that can be practically applied to validate the quality edible oils, spreads, and desi ghee.

Vegetable oils are the rich sources of omega-6, an essential fatty acid. Several studies suggested that excessive intake of omega-6 PUFA and a very high omega-6/omega-3 ratio stimulate the pathogenesis of diseases like cancer, cardiovascular disease (CVD), and inflammatory and autoimmune diseases. Therefore, there should be a balance in omega-3 and omega-6 fatty acids consumption. A ratio of omega-6/omega-3 fatty acids (2:1–4:1) or even lower is

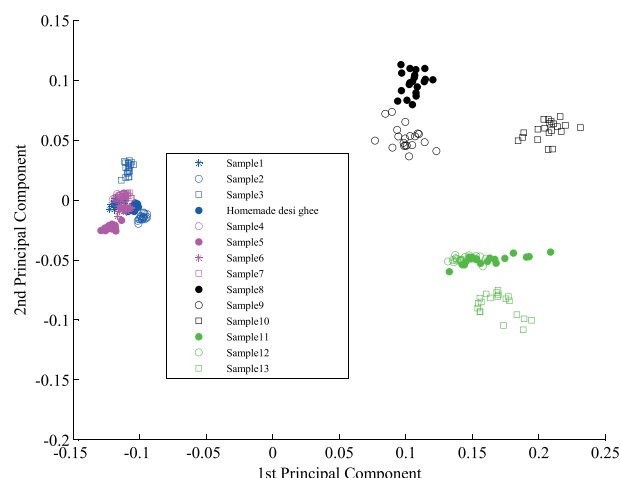


Figure 6. PCA scatter plot for unknown desi ghee (1–7), unknown olive oils (8–10) and unknown spreads (11–13) along with homemade desi ghee (4).

recommended to reduce the risk of many chronic diseases.^[42–44] In Pakistan, banaspati ghee is the major source of *trans* fatty acid in diet and it is 14.2–34.3% in its consumers, whereas hard margarines contain 1.6–23.1%.^[6] It has been an established fact that *trans* fatty acids cause CVD.^[7] Recently, food producers are using interesterification for the hardening of unsaturated oils, in which the oils are blended with naturally occurring highly saturated oils such as palm oil; as a result, the interesterified product is claimed *trans*-fat free. The long-term effect of ingesting interesterified fats is still unknown, and research is on the way.^[45] Desi ghee has been in use for many years in Pakistan and India; if it is consumed less than 10% of total calories, it can help to lower CVD especially with the consumption of other fats from plant oils.^[44] It may help to increase the level of omega-3 that lower ratio of omega-6/omega-3 which is essential for the human body.

Conclusion

In the present studies, results showed that Raman spectroscopy along with chemometric techniques can potentially be utilized for the characterization of edible oils/ghee and spread available in markets for their efficient and reliable compositional analysis and authentication. Mono-unsaturated EVOO and EVOO spread are separated out from rest of the edible oils and ghee, which are indicative of its specific ingredients that are essential for better health quality. In addition, the distinction of desi ghee (cow and buffalo) from rest of the artificially processed oils spreads and banaspati ghee reveals its purity in specific ingredients that has hidden benefits for human. Outcomes of present studies may open new research insights. Further research in this direction is in progress in our group.

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