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RESEARCH ARTICLE

Detection of palmolein oil adulteration in milk fat using ATR-FTIR Spectroscopy and Chemometrics

Vivek Sonvanshi[1], Kamal Gandhi[1] (✉), Akshay Ramani[1], Rajan Sharma[1], Raman Seth[1] and Bimlesh Mann[2]

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Abstract: Clarified milk fat (ghee), a household staple in domestic India faces the threat of adulteration due to its economic value. Examination of the purity of milk fat by chemical analysis is complex and time-consuming. The presence of palmolein oil in clarified milk fat was investigated using ATR-FTIR spectroscopy in combination with chemometric techniques. Spectral data within the wavenumber range of 4000-500 cm{ ^{1} were obtained for pure ghee, palmolein oil, and spiked ghee samples at various palmolein oil concentrations (1, 3, 5, 10, 15, and 20%). PCA identified distinct spectral clustering patterns at specific wavenumbers (1167-1137 cm^{-1}) at 5% significance level, effectively separating pure ghee from adulterated samples. The SIMCA method yielded a remarkable 100% classification efficiency for both pure ghee and palmolein oil samples. Additionally, developed PLS and PCR models exhibited strong predictive accuracy, with high R^2 values (0.96), enabling the detection of palmolein oil adulteration in ghee, even at concentrations as low as 1%. This research showcases the potential of ATR-FTIR with chemometrics analysis in ghee adulteration, offering a faster and more accurate routine analysis method.

Keyword: Pure ghee, Palmolein oil, Fatty acid analysis, ATR-FTIR

Introduction

Ghee is a type of clarified butter prepared by heating butter to separate the butterfat from the milk solids, resulting in a clear, golden liquid with a nutty flavor. Its unique flavor and versatility make it popular in Indian cooking. In India, approximately 28% of the milk supply is transformed into ghee (Atbhaiya et al. 2022), and the per capita consumption of ghee in the country stands at around 7 grams per person per day, constituting approximately 27.5% of all dairy product consumption (Gandhi et al. 2023). Ghee plays a vital role in the dairy industry; it serves as a valuable product in milk processing and contributes to the overall sustainability and profitability of dairy operations. Beyond its economic importance, ghee is revered for its health benefits, which is attributed to the better digestibility and anti-cancerous properties due to short chain fatty acids (Atbhaiya et al. 2023). Due to its rich taste and perceived health benefits, ghee has been gaining popularity outside India (Wani et al. 2022). Ghee's chemical composition is quite intricate, as it contains 70% saturated fatty acids and 30% unsaturated fatty acids. It also contains bioactive compounds, including butanoic acid ($C_{4:0}$), conjugated linoleic acid (CLA), *cis* and *trans* palmitoleic acid, and á-linolenic acid (ALA), all of which offer significant health benefits. Studies suggest that butanoic acid ($C_{4:0}$) and cis-9, 12, 15 octadecatrienoic acid ($C_{18:3}$) have potential cancer-preventive properties. CLA is associated with reducing the risk of type 2 diabetes and cardiovascular disease, improving vision, and displaying antithrombotic effects. Furthermore, CLA has been found to enhance immune function, support weight management and the development of lean muscle mass, and inhibit the formation of cancerous cells (Gautam et al. 2022). Ghee contains a significant amount of essential fatty acids and fat-soluble vitamins. It plays a vital role in the preparation of various ayurvedic and umami medicines.

Ghee stands out in the edible fats market due to its exceptional nutritional value and distinctive flavor, making it considerably more expensive than other common fats, often priced at 3-4 times their cost. During the lean season, however, with high demand and limited supply of ghee, unscrupulous traders adulterated ghee with less expensive vegetable oil. This adulteration practice involves replacing milk fat with more economical vegetable oils to increase profits, making it a prevalent issue in India (Atbhaiya et al. 2022). Various foreign fats, including vegetable oils, animal body fat and hydrogenated fats, are frequently used as adulterants (Rani et al. 2015; Upadhyay et al. 2016; Antony et al. 2018; Ramani et al. 2019). Palmolein oil is a very common adulterant used for ghee adulteration in India (Ramani et al. 2018). Palm oil, a natural vegetable oil derived from the mesocarp of palm fruits, comprises 50% saturated fatty acids, 40% monounsaturated fatty

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Dairy Chemistry Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India
ICAR, New Delhi, India

(✉)Kamal Gandhi
Email: kamalgandhindri@gmail.com
Mob No: +91 9729134444

acids, and 10% polyunsaturated fatty acids. Palm oil, a primary edible oil in India, is mainly imported from Malaysia and Indonesia due to its limited domestic production (Tandra et al. 2022). India's palm oil imports account for about 18% of the global total, with a significant volume of 8.4 million metric tonnes. Due to similar physical characteristics and low cost, palmolein oil is the primary source of ghee adulteration. This practice poses significant risks to both consumer health and product authenticity. Detection of palmolein oil adulteration in ghee is a challenging task for the dairy industry. Numerous techniques have been employed to check the authenticity of ghee, *i.e.* physicochemical constants, fatty acid profile, sterol test, and colorimetric tests, but all of these tests have some drawbacks. No quick, simple, and effective tests are available for detecting the presence palmolein oil in ghee. With a rise in fraudulent activities in the dairy sector, more sophisticated instruments, like HPLC and GCMS have been used globally to detect non-dairy substances in dairy products but are quite expensive and time-consuming. Therefore, a rapid, precise, and cost-effective method with minimal sample pre-processing is needed for detecting palmolein oil in ghee.

Fourier transform infrared spectroscopy (FTIR) has emerged as a valuable alternative to traditional examination techniques. Attenuated total reflectance (ATR)-FTIR spectroscopy is very suitable because of its high speed, and simplicity, avoids toxic reagents considered a green analytical tool. Mid-infrared (4000-400 cm^{-1}) spectroscopy is most commonly used because it provides information based on fundamental vibrations and rotational vibration of the functional groups in the sample (Gandhi et al. 2022), on the other hand NIR (Near-infrared spectroscopy) spectra provide information from the complex overtone and high-frequency combinations at the shorter wavelengths (Ozaki & Morisawa, 2021). Attenuated total reflectance Fourier transform mid-infrared spectroscopy (ATR-FT-MIR) is used to analyze samples by examining the chemical composition of their surface by applying principles of total internal reflection (da Silva Bruni et al. 2021). FTIR combined with the chemometrics technique is frequently used for quality monitoring of milk and dairy products (Saji et al. 2024). Recent advancements in FTIR spectroscopy have made the FTIR technique a strong tool for ascertaining the purity of dairy products. In a very short time, this approach can produce accurate outcomes with an identical degree of accuracy and precision as conventional techniques. In spectral quantification, chemometrics approaches are widely utilized.

Chemometric technique applied at wavenumbers in which maximum variation in absorbance peak is found, is the most efficient approach for extracting qualitative and quantitative data from FTIR spectra and eliminating food scams (Mendes et al. 2021). Principal component regression (PCR), partial least squares (PLS), and soft independent modelling of class analogies (SIMCA) are most widely used for this purpose. Using PLS and PCR regression, the relationship between actual and predicted values for both calibration and validation sets can be established. SIMCA is a classification modeling technique wherein samples are independently categorized into their respective classes without dependence on other samples. This approach is versatile, allowing its application across various strategies, ranging from one-class to multi-class classifications (Burmistrova et al. 2021). Numerous literatures are available that indicate the effective use of multivariate analysis along with ATR-FTIR to examine the foods. ATR-FTIR along with multivariate analysis was used to detect pork in beef meatball (Rohman et al. 2010), lard in vegetable oils (Rohman et al. 2011), chicken fat in butter (Nurrulhidayah et al. 2013), cow and buffalo milk adulterated with soy milk (Jaiswal et al. 2015), ghee adulterated with goat and pig body fat in ghee (Upadhyay et al. 2016, 2018), coconut oil in ghee (Gandhi et al. 2022), mineral oil in milk fat (Gandhi et al. 2023), vanaspati ghee (hydrogenated vegetable oil) in milk fat (Sonvanshi et al. 2024) and flunixin residues in milk (Saji et al. 2024). These research findings highlight the efficacy of combining FTIR with chemometric techniques, establishing it as a potent tool for ensuring the quality and authenticity of dairy products.

To identify pamolein oil adulteration in clarified milk fat, this work employed ATR-FTIR spectroscopy in conjunction with chemometric techniques such as principal component analysis (PCA), SIMCA, PLS, and PCR. The effectiveness of PLS and PCR regression methods to detect pamolein oil in clarified milk fat was also studied.

Material and Methods

Sample collection

The ghee sample was prepared using milk obtained from cow and buffalo, housed at the Livestock Research Centre, NDRI, Karnal. Palmolein oil from five reputable brands in Karnal was used to prepare adulterated samples for testing and model development.

Sample preparation

Cow and buffalo ghee were individually prepared using the creamery butter method (De, 2012). A pure mixed ghee (PMG) sample was prepared by blending cow and buffalo ghee in 1:1 ratio. Each type of sample was prepared 15 times and divided into two to have a total of 30 samples. Following the experimental design, pure mixed ghee was then adulterated with 1%, 3%, 5%, 10%, 15%, and 20% w/w palmolein oil (PO) to simulate various levels of adulteration.

Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared and fatty acid analysis by GC as per ISO 15884 (2002) to correlate FT-MIR spectral data of ghee, palmolein oil and adulterated ghee samples with their fatty acid composition.

Spectral acquisition

The samples were analyzed using FTIR (IR-Affinity-01, Shimadzu, Tokyo, Japan) with a diamond crystal cell ATR and integrated IR-Solution software, operating at a resolution of 4 cm^{-1}. The data collection process was conducted at a constant temperature of 40°C for both control and adulterated samples to maintain uniformity. To ensure reliability, each sample was scanned forty times in the mid-infrared region (4000-500 cm^{-1}) against an air background to collect absorption spectra. After each scan, the ATR crystal was carefully cleaned with ethanol and wiped to remove contaminants, using soft tissue paper.

Spectral analysis

The reflectance mode was used to acquire the absorption spectra in the mid-infrared range. The samples included a control sample and a range of pamolein oil spiked ghee samples (1%, 3%, 5%, 10%, 15% and 20% w/w). A comprehensive analysis was performed on the acquired spectra to identify the specific regions where the increased concentration of PO in the adulterated samples had the most notable impact on the intensities and positions of the peaks.

Chemometric analysis

In this study, chemometric analysis was performed using the Unscrambler software (version 10.2; CAMO AS, Trondheim, Norway). The spectral data were partitioned into a training set (calibration set) and a testing set (validation set) to facilitate thorough model assessment. Both sets were balanced, including PMG as control samples and (PO in PMG) as spiked samples, to maintain the integrity of the data. Subsequently, a detailed examination of spectral absorption regions was performed to identify the variables required for PCA, PLS, and PCR modeling. The deliberate selection of these spectral variables served as the basis for a thorough chemometric analysis, leading to the development of predictive models aligned with the research objectives. Specific regions 1167-1137 cm^{-1} were identified through peak analysis in the raw spectra. PCA was employed to explore clustering patterns within these regions, identifying a few outliers within the selected wavenumber range. The importance of choosing a spectral range that accurately represents changes in analyte concentration.

Developing models

For this study, PLS and PCR models were applied to the sample sets, with a focus on the spectral region of 1167-1137 cm^{-1}. The Unscrambler software's cross-validation feature was employed to enhance the robustness of the model. The principal objective was to establish predictive models for accurately quantifying PO content in PMG. Model selection criteria encompassed the optimization of the coefficient of determination (R^2) to achieve the highest precision while concurrently minimizing bias, SEC, and SEP values. This method aligns with the established methodology of previous researchers (Upadhyay et al. 2016, 2018; Jha et al. 2015).

Application of SIMCA to the derived models

A comprehensive model was established through the calibration set, which included both control and spiked samples. Subsequent to this, specific class models were developed to discern the levels of PO added to ghee. The SIMCA method was applied for the classification of each sample based on its PO content. The validation dataset was used to assess class memberships at a 5% significance level. Test samples were then assigned to predefined class models based on their closest resemblance. Efficiency metrics for classification, including true positive, true negative, false positive, and false negative samples, were introduced. True positives represent samples correctly classified within their designated class, true negatives signify accurately rejected samples, false positives involve misclassifying samples into an incorrect class, and false negatives represent samples that do not align with the designated class model (Balan et al. 2020)

Results and discussion

Fatty acid analysis

Milk fat is the most complex of all fats, containing over 400 different types of fatty acids, including short-chain, medium-chain, and long-chain fatty acids (Fox et al. 2015). The fatty acid composition of PMG, PO, and ghee adulterated with different concentrations of PO is shown in Table 1. Palmitic acid was the most prominent fatty acid in PO followed by oleic, stearic, and linoleic acid. Our findings were similar to the earlier results reported by Jeyarani et al. (2005) and Dorni et al. (2018). As the level of spiking increased, the concentration of palmitic acid in PMG also increased. Butyric acid, a characteristic milk fatty acid, was absent in PO, and its level decreased with increased spiking levels. Similarly, short-chain fatty acids like $C_{6:0}$, $C_{8:0}$, and $C_{10:0}$ are absent in PO and exhibit reduced concentrations with increasing spiking. Medium-chain FAs, such as $C_{12:0}$, $C_{14:1}$, and $C_{15:0}$, were also absent in PO and displayed a decrease in concentration with higher spiking levels. Gandhi (2015) reported that pure cow/buffalo ghee exhibited a lower concentration of linoleic acid ($C_{18:2}$) compared to PO, serving as a marker for detecting ghee adulteration up to 5% level. The concentrations of linoleic acid in PMG and PO, 1.22% and 9.78% respectively, were consistent with values reported by Gandhi (2015). Kumar (2015) also employed linoleic acid as a reference FA to identify vegetable oil adulteration in ghee and found that the concentration of oleic acid was significantly higher in PO compared to pure buffalo and cow ghee. In this study, a similar concentration of oleic acid (25.48%) was observed in PMG. Furthermore, they reported that as the concentration of PO increased in ghee, the levels of stearic, linolenic, and arachidic acid significantly decreased, while oleic, linoleic, and palmitic acid concentrations increased.

Examination of FTIR spectra

It can be seen from Fig. 1 that distinct differences were observed in the absorbance peak of both the PMG and PO samples. In the mid-infrared region, 12 absorbance peaks were observed, which was mainly due to the presence of various bonds and functional groups showing stretching and bending movement after striking IR light to the PMG and PO sample. The absorbance peak shown at a wavenumber of 2922.16 cm^{-1} was mainly due to the asymmetric stretching occurring between C-H of functional groups like CH_2 and CH_3. Similarly, the peak shown at the wavenumber of 2852.72 cm^{-1} was due to the symmetric stretching that occurs between C-H of the same functional group. Free fatty acids having carbonyl functional group and stretching occurs in this functional group showing characteristic absorbance peak at a wavenumber of 1743.65 cm^{-1}. Similarly, another peak obtained at a wavenumber of 1465.90 cm^{-1} was due to the bending that occurs between the H-C-H of the CH_2 and CH_3 functional groups. Similarly, the absorbance peak shown at a different wavenumber of 1236.37 cm^{-1} was due to the stretching between C-O in O-C(=O)-CH_2 of ester. Absorbance peak at a wavenumber of 1161.15,1112.93, 1099.43, 966.34, and 721.38 cm^{-1} are due to the stretching between

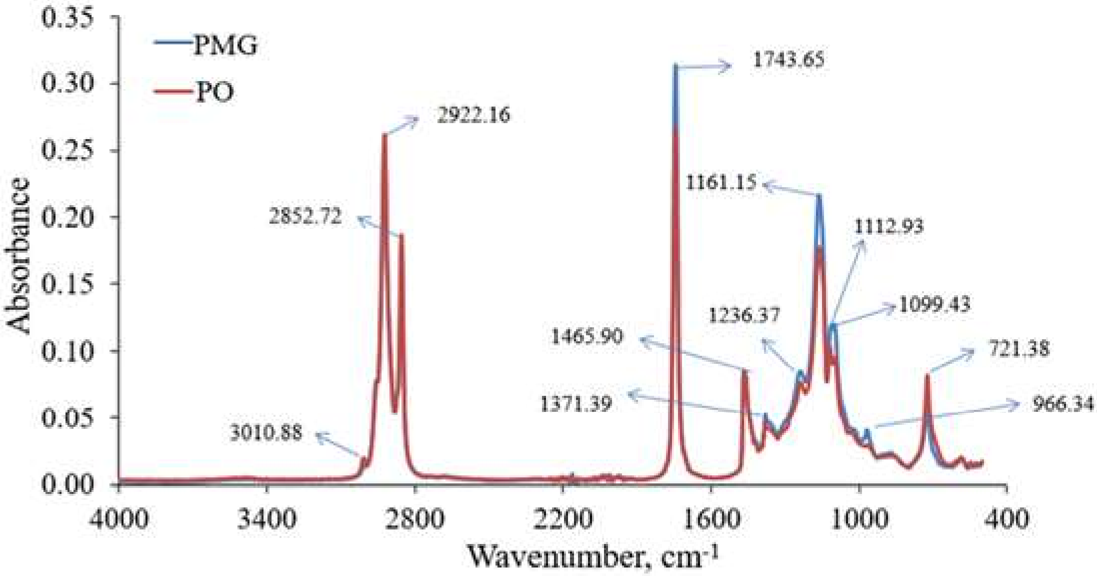

Fig. 1 ATR-FTMIR spectra of pure mixed ghee and palmolein oil

Table 1 Fatty acid compositions of PMG, PO and ghee spiked with different level of PO

FATTY ACIDS	PMG	PO	1% PO	3% PO	5% PO	10% PO	15% PO	20% PO
$C_{4:0}$	3.86 ± 0.518	-	3.84 ± 0.005	3.80 ± 0.006	3.62 ± 0.019	3.40 ± 0.006	3.40 ± 0.002	3.08 ± 0.004
$C_{6:0}$	2.02 ± 0.005	-	1.82 ± 0.059	1.82 ± 0.065	1.82 ± 0.009	1.69 ± 0.007	1.64 ± 0.004	1.46 ± 0.005
$C_{8:0}$	1.18 ± 0.005	-	1.11 ± 0.018	1.09 ± 0.006	1.07± 0.008	0.98 ± 0.010	0.94 ± 0.010	0.87 ± 0.013
$C_{10:0}$	2.33 ± 0.003	-	2.26 ± 0.024	2.20 ± 0.004	2.11 ± 0.023	1.96 ± 0.013	1.88 ± 0.019	1.78 ± 0.001
$C_{12:0}$	3.14 ± 0.003	-	2.92 ± 0.007	2.84 ± 0.004	2.82 ± 0.018	2.57 ± 0.017	2.49 ± 0.019	2.30 ± 0.038
$C_{14:0}$	11.57 ± 0.002	1.61 ± 0.005	11.38 ± 0.039	11.00 ± 0.031	10.77 ± 0.041	10.07 ± 0.043	9.66 ± 0.016	9.11 ± 0.010
$C_{14:1}$	0.80 ± 0.004	-	0.77 ± 0.005	0.77 ± 0.001	0.75 ± 0.012	0.67 ± 0.005	0.65 ± 0.005	0.63 ± 0.004
$C_{15:0}$	1.06 ± 0.004	-	0.99 ± 0.004	0.96 ± 0.013	0.91 ± 0.005	0.89 ± 0.026	0.85 ± 0.008	0.80 ± 0.004
$C_{16:0}$	37.71 ± 0.432	40.51 ± 0.022	38.23 ± 0.021	38.24 ± 0.038	38.61 ± 0.019	38.68 ± 0.094	39.01 ± 0.021	39.05 ± 0.023
$C_{16:1}$	1.55 ± 0.027	-	1.42 ± 0.009	1.41 ± 0.006	1.40 ± 0.002	1.36 ± 0.042	1.24 ± 0.007	1.18 ± 0.004
$C_{17:0}$	0.67 ± 0.00	-	0.67 ± 0.009	0.64 ± 0.012	0.55 ± 0.020	0.54 ± 0.025	0.49 ± 0.012	0.45 ± 0.005
$C_{18:0}$	11.17 ± 0.011	4.82 ± 0.015	11.12 ± 0.013	10.97 ± 0.012	10.94 ± 0.021	10.24 ± 0.002	10.24 ± 0.004	9.69 ± 0.024
$C_{18:1}$	20.48 ± 0.075	42.72 ± 0.091	20.92 ± 0.157	21.57 ± 0.160	21.73 ± 0.045	23.54 ± 0.063	23.93 ± 0.024	25.53 ± 0.013
$C_{18:2}$	1.22 ± 0.046	9.78 ± 0.099	1.34 ± 0.029	1.53 ± 0.029	1.81 ± 0.008	2.36 ± 0.014	2.59 ± 0.016	3.09 ± 0.047
$C_{18:3}$	0.79 ± 0.010	0.19 ± 0.004	0.77 ± 0.004	0.73 ± 0.008	0.70 ± 0.004	0.67 ± 0.005	0.63 ± 0.007	0.61 ± 0.006
$C_{20:0}$	0.37 ± 0.00	0.33 ± 0.00	0.36 ± 0.002	0.35 ± 0.008	0.32 ± 0.005	0.31 ± 0.00	0.30 ± 0.003	0.29 ± 0.008
Total SFA	75.12 ± 0.073	47.29 ± 0.004	74.75 ± 0.120	73.96 ± 0.134	73.58 ± 0.034	71.38 ± 0.024	70.93 ± 0.020	68.93 ± 0.045
Total USFA	24.87 ± 0.072	52.70 ± 0.004	25.24 ± 0.118	26.03 ± 0.133	26.41 ± 0.034	28.61 ± 0.024	29.06 ± 0.021	31.06 ± 0.045

All experiments were conducted in triplicate (n=3). Data were presented as mean ± SD

C-O in HC-O-(C=O) of ester, C-C link of the hydrocarbon chain, bending between C-H in trans double bond and rocking movement that occurs between C-H of CH_2 functional group and cis double bonds, respectively. The responsible bonds, functional groups and various types of vibrational movements were the same as described by Antony et al. (2017). The specific bond and functional group responsible for the characteristic absorbance peak of the sample were presented in Table 2.

The study further revealed that the level of spiking of PO increased from 1 to 15% then maximum variations in absorbance peak occurred in the wavenumber range of 1167-1137 cm^{-1} as shown in Fig. 2 and this region was useful for the detection of PO in PMG. Variations in the concentration of FA in PMG, PO, and spiked samples might be the reason for visual variation in FTIR spectra, in the wavenumber region of 1167-1137 cm^{-1}. GC analysis revealed that the concentration of palmitic, oleic, and linoleic acid was increased as the level of spiking of PO increased. Similarly, stearic, linolenic acid concentration decreased with increased spiking level. Oleic acid was responsible for a distinct absorbance peak at a wavenumber of 3010.88 cm^{-1}. Its concentration was higher in PO as compared to that in PMG so an intense peak was observed in FTIR spectra of PO. Another intense peak was observed at a wavenumber of 1741.72 cm^{-1} which was due to the carbonyl groups of fatty acids present in ghee. Deshwal et al. (2021) reported that a characteristic peak was observed at a wavenumber of 1735 cm^{-1} which was mainly due to the presence of conjugated linoleic acid in ghee.

Chemometrics analysis

Visual changes in absorbance peaks were noted in the wavenumber range of 1167-1137 cm^{-1} as the level of palmolein oil

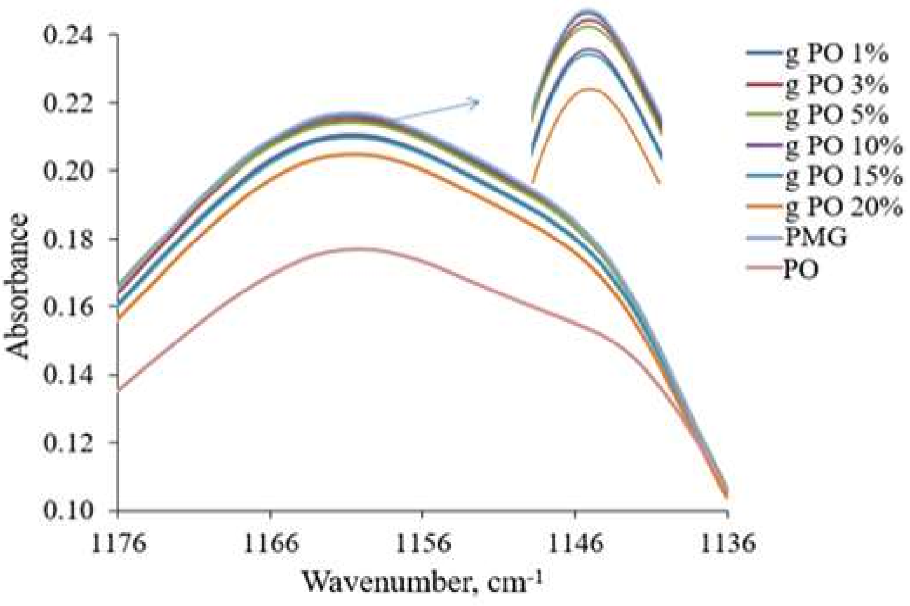

Fig. 2 Spectra of pure mixed ghee and ghee adulterated with different concentrations of palmolein oil at selected 1167-1137 cm{ ^{1} wavenumber range

po- palmolein oil, g 1%po, g 3% po, g 5% po, g 10% po, g 15% po and g 20%po - ghee spiked with 1, 3, 5, 10, 15 and 20% palmolein oil.

Table 2 Wavenumbers, corresponding functional groups and the types of vibrations associated with them

Wavenumber (cm^{-1})	Functional Groups	Type of Vibration/Phenomena
3010.88	C–H asymmetric stretching of CH_2 and CH_3	C–H Stretching
2922.16	C-H symmetric stretching C–H (CH_2)	C-H stretching
2852.72	C–H symmetric stretching of CH_2 and CH_3	C–H Stretching
1743.65	C=H stretching of esters and FFA	C–H Stretching
1465.90	H–C–H bending of CH_2 and CH_3	C–H Bending
1371.39	H–C– H symmetric bending of CH_2	H–C–H Symmetric Bending
1236.37	C–O stretching in O–C(=O)–CH_2 of ester	C–O Stretching
1161.15	C–O stretching in HC–O–(C=O) of ester	C–O Stretching
1112.93	C–O stretching in O–C–C of ester	C–O Stretching
1099.43	C–C links of a hydrocarbon chain	C–C Stretching
966.34	C–H bending of a *trans* double bond	C–H Bending
721.38	C–H rocking of CH_2 and *cis* double bond	C–H Bending

(PO) spiking increased in ghee. This specific region was employed for chemometrics analysis.

Principal Component Analysis (PCA)

PCA was applied in the region where maximum variation in absorbance peak was observed at 1167-1137 cm^{-1}. It was observed that the PO and PMG samples gave separate clusters after applying PCA and as the level of spiking of PO increased clusters shifted toward the PO (Fig. 3). Remarkably, samples spiked with the lowest PO (1%) formed separate clusters distinct from PMG samples. These findings highlight the crucial role of FTIR in accurately detecting PO within PMG. Principal Components 1 and 2 explained cumulative variations of 100% (99% by PC1 and 1% by PC2) in the spectral region 1167-1137 cm^{-1} at a 5% significance level (Fig. 3). These results align with the study conducted by Upadhyay et al. (2018), where PCA effectively detected pig body fat in ghee within the spectral range of 3030-2785 cm{†^{1}, with PC1 and PC2 account for variations of 98% and 2%, respectively. Similarly, the work by Gandhi et al. (2023) reported the successful detection of mineral oil in milk fat using PCA within the specific spectral range (1350-950 and 1800-1600 cm{†^{1}), with PC1 and PC2 explaining variances for both 99% and 1%, respectively.

Partial least square (PLS) and Principal component regression (PCR) Models

The PLS and PCR method was used to generate calibration and validation models in the selected wavenumber region and the impact of different spectral windows on the spectral modelling. PMG and ghee spiked with PO at different levels (120 samples)

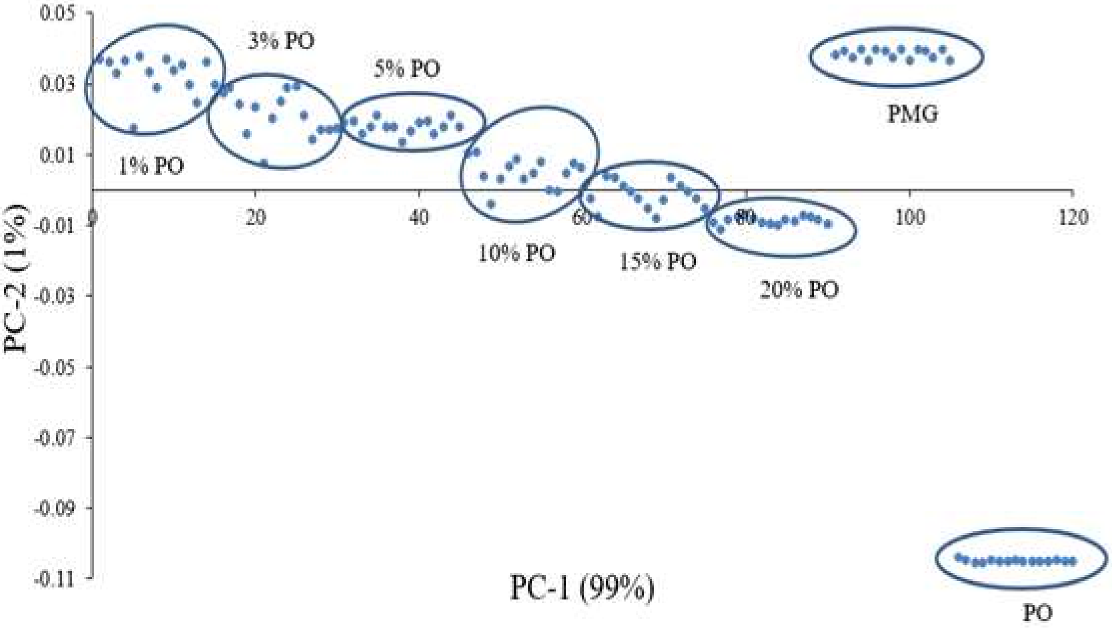

Fig. 3 PCA plot showing the clusters of pure mixed ghee and ghee adulterated with different concentrations of Vanaspati ghee at selected 1167-1137 cm{ ^{1} wavenumber range

po- palmolein oil, g 1%po, g 3% po, g 5% po, g 10% po, g 15% po and g 20%po - ghee spiked with 1, 3, 5, 10, 15 and 20% palmolein oil.

Table 3 Comparison between PLS and PCR regression models applied in the wavenumber region of 1167-1137cm^{-1}

Regression model	Wavenumber range (cm^{-1})	Calibration R^2	Calibration RMSEC	Calibration Bias	Validation R^2	Validation RMSEV	Validation Bias	Difference between RMSEC and RMSEV value
PLS	1167-1137	0.9654	5.8050	0.665	0.9644	5.9410	0.686	0.136
PCR	1167-1137	0.9652	5.8156	0.668	0.9646	5.9174	0.663	0.1018

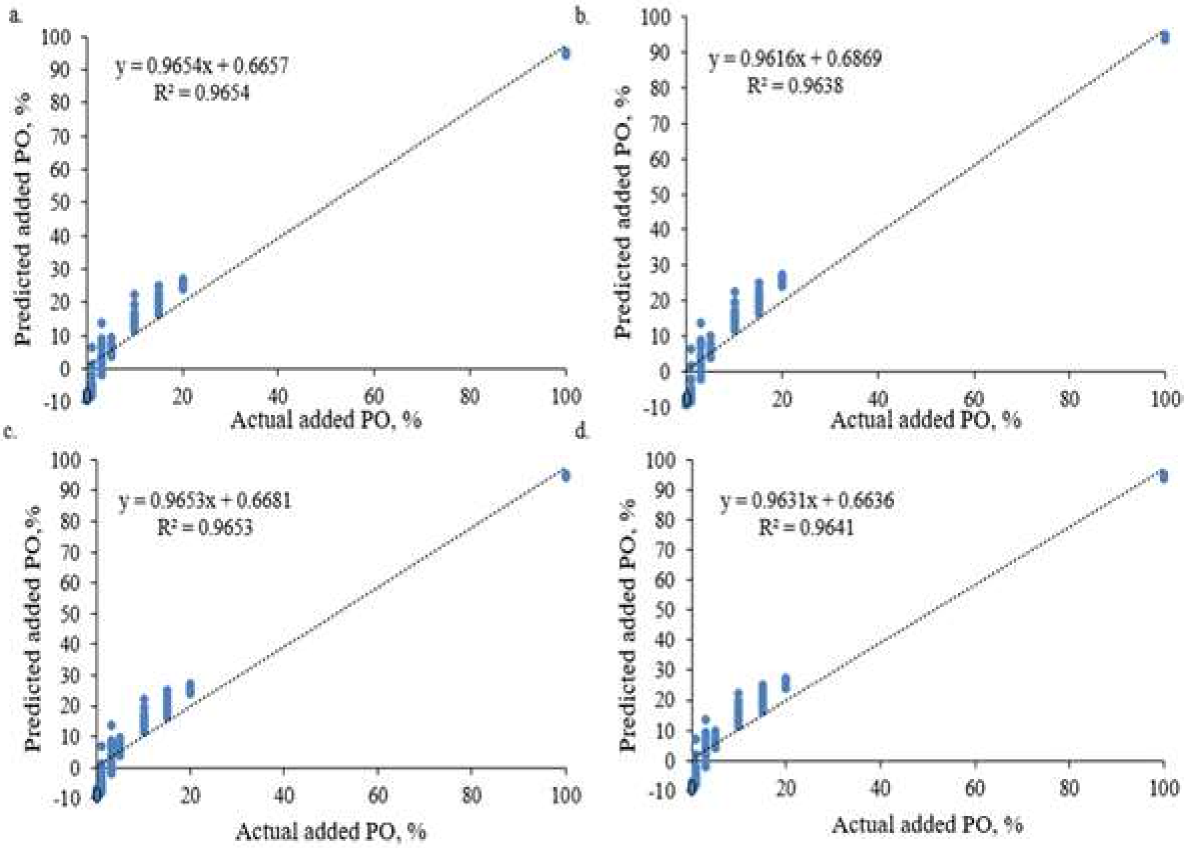

Fig. 4 Relationship between the actual and predicted levels of palmolein oil in ghee using PLS regression *(a) Calibration - Wavenumber Range: 1167-1137 cm{ ^{1} (b) Validation - Wavenumber Range: 1167-1137 cm{ ^{1}*

were used to establish a calibration model using complete cross-validation. The best models were selected based on their higher R^2, and lower RMSEC and RMSEV values. PLS and PCR were applied in the same regions where maximum variations in absorbance peaks were observed *i.e.*, 1167-1137 cm^{-1} and their calibration curves are shown in Fig. 3. It was found that there was a linear relationship between the actual and predicted value of PO because value of coefficient of determination (R2) was near to the one *i.e.*, 0.9654 and 0.9652 for the PLS and PCR models, respectively in the wavenumber region of 1167-1137 cm^{-1}. This suggested that the actual and predicted PO contents in ghee were in agreement. The difference between RMSEC and RMSEV values for developed PLS and PCR models in the specified wavenumber was less, showing that the models were well constructed for detecting the PO in ghee. The difference between both values was about 0.136 and 0.1018 for PLS and PCR models, respectively in the wavenumber region of 1167-1137 cm^{-1}, respectively (Table 3). Earlier researchers employed both PLS and PCR methodologies in their respective studies, revealing the efficiency of the developed models in successfully detecting various adulterants, such as goat body fat and coconut oil, in ghee (Upadhyay et al. 2016; Gandhi et al. 2022).

Classification of samples using SIMCA

The study utilized the soft independent modeling of class analogy (SIMCA) method to evaluate class probabilities for control, PO, and spiked samples. This analysis was performed within the specific spectral range of 1167-1137 cm^{-1}, employing established PLS models. The SIMCA analysis consistently demonstrated precise classification of control and PO, achieving 100% classification accuracy in both spectral regions. Among adulterated ghee samples, those in the region of 1167-1137 cm^{-1} were all correctly classified, except for one sample at 1% level and two samples each at 3% and 10% levels. Table 4 revealed that only five out of 120 samples (4.16%) were misclassified. The classification efficiency for all samples in both regions exceeded 90%, with only five samples (4.16%) misclassified. No spiked sample was classified in PMG and vice versa. These findings underscore the effectiveness of SIMCA in detecting PO with high sensitivity, even at lower levels like 1% in PMG. The PLS model's ability to accurately detect PO adulteration across diverse sample types is supported by the summary in Table 4. These results demonstrate the reliability and accuracy of SIMCA for identifying PO in ghee. The study aligns with Gandhi et al. (2022), who applied the SIMCA approach to detect coconut oil

Table 4 SIMCA applied in the wavenumber region of 1167-1137 cm^{-1}

Wavenumber (cm^{-1})	% PO in PMG	Total no. of sample	Number of selected classes								Misclassified	Classification efficiency (%)
			0	1	3	5	10	15	20	100		
1167-1137	0	15	15	–	–	–	–	–	–	–	0	100
	1	15	–	14	–	–	–	–	–	–	1	93.33
	3	15	–	–	12	–	–	–	–	–	2	86.66
	5	15	–	–	–	15	–	–	–		0	100
	10	15	–	–	–	–	13	–	–	–	2	86.66
	15	15	–	–	–	–	–	15	–	–	0	100
	20	15	–	–	–	–	–	–	15	–	0	100
	100	15	–	–	–	–	–	–	–	15	0	100

adulteration in ghee up to the level of 2%. Previous studies by Upadhyay et al. (2016, 2018) also successfully identified goat body fat at 1% and pig body fat at 3% using the SIMCA approach in ghee.

Conclusion

ATR-FTIR spectroscopy and advanced chemometric techniques were employed to identify and measure the adulteration of pure ghee with palmolein oil. Spectral analysis uncovered unique absorption patterns within the 1167-1137 cm^{-1} spectral ranges for pure ghee, palmolein oil, and ghee samples spiked with palmolein oil. PCA demonstrated distinct clusters, even for ghee samples spiked at a 1% level. The PLS and PCR models in specific spectral regions showed excellent performance, with high accuracy and precision. The addition of the SIMCA method improved sample classification to 100% accuracy. This research highlights the potential of these methods to identify 1% palmolein oil adulteration in ghee. This research not only demonstrates the potential of these methods for adulteration detection but also promotes a safer and environmentally friendly analytical approach, reducing reliance on toxic solvents and contributing to the integrity of dairy products and consumer safety.

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Conflict of Interest

The authors declare no conflict of interest in the presented research work.

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Abbreviation

FT	Fourier transform
ATR	Attenuated total reflectance
FTIR	Fourier transform infrared spectroscopy
SIMCA	Soft-independent modeling of class-analogies
MIR	Mid-infrared
NIR	Near-infrared spectroscopy
PCA	Principal component analysis
PLS	Partial least squares
PCR	Principal component regression
PGM	Pure mixed ghee
PO	Palmolein oil
R^2	Coefficient of determination
SEC	Standard-error of calibration
SEP	Standard-error of prediction
PC	Principal component
RMSEC	Root mean square error of calibration
RMSEV	Root mean square error of validation
FTMIR	Fourier transform mid-infrared spectroscopy

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