Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 9, No. 2, 11-20, 2019

Detection of Chemical Properties of Ghee Containing Various Levels of Palm Oil and Beef Tallow on RSM

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Received: 17 February 2019 Accepted: 19 May 2019

ABSTRACT: This study is concerned with the detection of adulterated ghee with various levels of palm oil and beef tallow with the application of particular chemical characteristics of ghee, palm oil and beef tallow namely sterol, and fatty acids profiles by response surface methodology (RSM). Among the parameters investigated, If the fatty acid profile is modeled under the optimized conditions, the detection level for adulteration with palm oil was higher than 8.7% and with beef tallow was 12.84% for ghee samples from different regions. Moreover, if all of the sterols in the adulterated ghee sample with palm oil are assessed and detected, the adulteration detection level for all the three regions will be the minimum added palm oil (<1%). Moreover, Regarding the Raman spectroscopy data showed that the adulteration detection level was between 5.8–16.9%. Therefore, the regression models were a good method for measuring the market acceptance of ghee containing palm oil and beef tallow.

Keywords: *Adulteration, Fatty Acids Profile, Ghee, Raman Spectroscopy, Sterol Composition*.

Introduction

Ghee is a mixture of milk fat, cream or butter that is made by removing water and non-fatty solids. It has a special structure and flavor compounds. Chemically, it contains a combination of triglycerides, mono- and di-glyceride, phospholipids, free fatty acids (FFAs), cholesterol, and other compounds. It is regarded as the most valuable dietary fat for human due to its unique fatty acid profile (Rani *et al.*, 2015). In Iran, the bulk of milk fat is turned into ghee for household and industrial applications. Since milk fat is the most expensive edible fat, it suffers extensive adulteration (Mehta, 2013; Derewiaka *et al.*, 2011). Detection of the level of adulteration is not only important from the consumer's point of view but is a point of interest for policy-makers (Fernandez *et al.*, 2003). This product is adulterated with less valuable fats and oils such as vegetable oils and/or animal fats and tissues (tallow). There are numerous physico-chemical techniques for detecting adulterated oils and fats, each with its own benefits and defects (Deelstra *et al.*, 2014) and some chemical methods are costly and time-consuming. Additionally, there are

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highly precise instruments based on adulteration detection methods (HPLC, GC, etc.), that are again costly and time-consuming and require extensive preparations (Nurrulhidayah *et al.*, 2015). Scientifically, assessing one quality in ghee is not enough to prove the adulteration. Since some compounds are unique to milk and affect its physico-chemical properties, they can be used for adulteration detection (Deelstra *et al.*, 2014; Kirk and Sawyer, 1991; Giuseppe *et al.*, 2001; Borkovcová *et al.*, 2009; Derewiaka *et al.*, 2011).

The use of optical spectroscopy techniques such as Raman spectroscopy and infrared (IR) spectroscopy has shown promising results to replace the previous established techniques. They present a reliable analysis with high speed and operational simplicity. Between Raman and IR spectroscopy techniques, only the former is capable of developing molecular vibration fingerprints. The Raman spectroscopy is capable of analyzing the cis–trans isomerism, classifying oils and fats, predicting the presence of different fatty acids in oils, and finding their degree of unsaturation (El-abassy *et al.*, 2011). The Raman spectroscopy is a high-tech precise method that has been recently used to detect and screen different edible oils, rapidly and eliminating the need for sample preparation with lower costs, that might be employed for spectral detection of ghee. There are signals in Raman spectroscopy method that can be adopted to estimate the parameters of the physical structure and the compounds of a wide variety of lipids. They can also help to detect cis–trans isomerism, unsaturation, double bonds, chain length, free fatty acids, and crystal structure (Beattiea *et al.*, 2004). The most important bands used with respect to lipids include the 1295-1305, 1400-1500, 1640-1665, 1730-1750 cm^{-1} bands. The 1295-1305 cm^{-1} band refers to the bending frequency in the methylene twisting deformation, the 1400-1500 cm^{-1} band is the bending frequency of the methylene scissor deformation, the 1640-1665 cm^{-1} is the stretching frequency of the olefinic C=C group, and the 1730-1750 cm^{-1} belongs to the stretching frequency of the aliphatic (carbonyl C=O) compounds. Therefore, in addition to of fatty acid analysis, the study is concerned with the Raman spectroscopy to detect adulteration of ghee samples, and the effects and interactions of different geographical locations and different adulteration levels (palm oil and beef tallow). The main effects and interactions of different geographical regions and different adulterant levels on the chemical profile of ghee samples (*i.e.* sterol composition, fatty acid profile) and spectral detection of ghee have been considered and studied.

Materials and Methods

- *Preparation of the samples*

Raw milk samples from twenty forage-fed cows were purchased from Javanrud Farms (Kermanshah, Iran), Nahavand Farms (Hamedan, Iran), and Abdanan Farms (Ilam, Iran). The samples were collected from the cattle with similar feeding practices and breed in the morning during summer. The collected milks were stored in a cold room overnight. Raw milk samples from each region were heated and inoculated. The prepared yogurt samples were then fed into a churn, and the fats obtained were filtered and cooled down to 4°C (IDF, 2010).

- *Preparation of adulterated ghee samples*

For the preparation of adulterated ghee samples, pure ghee, palm oil and beef tallow were heated at 60-65°C for 10 min before mixing. The adulterants (palm oil and beef tallow) were added to ghee at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% levels.

- *Preparation of fatty acid methyl esters*

Fatty acid methyl esters were prepared according to ISO 12966-2:2011 (ISO, 2011). 10 to 50 mg of the sample was dissolved in 1

ml of isooctane and methylated with 2 ml of 0.2 M sodium methoxide. The fatty acid methyl esters (FAME) were injected to an ACME 6000M GC equipped with 60 m DM-2330 (Young Lin Instrument Co., Ltd) capillary column (0.25 mm ID × 0.2 µm df, Courtaboeuf Cedax, France) and flame ionization detector (FID) using Helium as the carrier gas. The relative retention time of the obtained results were compared to the standards, and the fatty acids were identified (Munro, 1992).

- *Analysis of the sterol fraction by gas chromatography*

The sterol content of ghee and palm oil were determined according to ISO 12228-1:2014 (ISO, 2014). The sterols were extracted from the samples by isolation of the nonsaponification matter following the separation of sterol fraction from other classes of components present using thin layer chromatography and finally analysis of isolated sterols by gas chromatography (ACME 6000M, Young Lin Instrument Co., Ltd) equipped with flame ionization detector and capillary column (DM-2330 60 m × 0.25 mm ID × 0.2 µm df). The sterols were identified by comparing the relative retention times of the samples with standards,

- *Adulterated ghee preparation and analysis with Raman spectroscopy*

Adulterated ghee samples were held at 55°C in a bain-marie. At this temperature, ghee and vegetable oil samples are liquid. Therefore, the effect of physical changes on the Raman spectrum is eliminated, and only the chemical changes are effective in the method. The ghee samples were then kept at an ambient 15°C temperature and were analyzed by the Raman spectroscopy (Mira M-1, Metrohm, USA) (Beattiea *et al.*, 2004).

- *Statistical design*

Based on the present conditions, a total of 33 experimental runs were conducted on ghee samples to determine the optimal conditions (minimum adulteration detection percent). The effects of different palm oil levels and regions on the dependent variables were studied using regression coefficients. In order to obtain the best fit, only the coefficients of determination or adjusted R^2 ($\bar{R}^2$) greater that 0.8 were analyzed. The regression analysis method in Design Expert v.10 was used to fit the correlation of the dependent variables with ghee samples from different regions and different adulteration levels with palm oil (Montgomery, 2008). The best fitted function among the ghee samples from different regions with different adulteration levels was selected by comparing R^2 of different models (observed, Linear, Logarithmic, Inverse, Quadratic, Cubic, Compound, Power, S, Growth,Fifth , Sixth, 2FI, Exponential, and Logistic). The second-order RSM is as follows:

$$Y= b_0+\sum b_i x_i+\sum b_{ii} x_{ii}^2+\sum b_{ij} x_i x_j$$

Where b_0, b_i, b_j and b_{ij} are the regression coefficients for x-intercept, linear, quadratic and their interactions, respectively, whereas x_i and x_j are the coded independent variables. Table 1 presents the coding for ghee samples from different regions.

Table 1. Study region codes.

Study region/Code	B[1]	B[2]
Ilam (A)	1	0
Kermanshah (B)	0	1
Hamedan (C)	-1	-1

Results and Discussion

- *Statistical analysis*

The effects of different palm oil concentrations and different regions on the chemical properties of the ghee samples have been analyzed using response surface method (RSM). The highest and lowest responses of variables concerning chemical properties of adulterated ghee with palm oil, standard deviation (SD), and the model from

each response are presented in Table 2. Similar data related to adulterated ghee with beef tallow are shown in Table 3. The error distribution of variables was tested using Box-Cox Normality Plot that confirmed the normality of their error distribution.

- Detection of ghee adulteration with palm oil for samples from different regions using fatty acid profile assessment

The fatty acid profile results showed that only butyric acid and linoleic acid were able to detect adulteration levels higher than 20%. The 2FI model was adopted to fit the correlation between the butyric acid and the study variables. The results indicated that the butyric acid content changed in the samples from different regions with different palm oil levels ($p<0.01$). The general equation to analyze the coded factors of butyric acid content is presented in Eq. 1.

$$R1=+0.67-0.67A+0.132B[1]-0.018B[2]-0.13AB[1]+0.18AB[2];\ \bar{R}^2=0.9986$$

Equation (1) - The iodine index of ghee from different regions adulterated with palm oil with coded factors

Table 2. The responses of different variables concerned with chemical properties of ghee containing palm oil

Response	Name	Min	Max	Mean	Std. Dev.	Model
R1	Butyric acid	0	1.59	0.666061	0.445014	2FI
R2	Caproic acid	0	1.43	0.625455	0.415798	2FI
R3	Caprylic acid	0	1.06	0.465455	0.312311	Sixth
R4	Capric acid	0	2.48	1.13394	0.733063	2FI
R5	Decenoic acid	0	0.26	0.105758	0.0942745	RCubic
R6	Lauric acid	0.2	3.05	1.53606	0.862942	2FI
R7	Myrestic acid	1.05	10.56	5.58788	2.93575	2FI
R8	Tetradecenoic acid	0	1.7	0.81303	0.524193	2FI
R9	Decapentanoic acid	0	1.42	0.659697	0.426413	2FI
R10	Palmitic acid	29.95	41.5	36.4918	3.35567	Quadratic
R11	Oleic acid	20.46	40.17	31.2461	5.78652	2FI
R12	Linoleic acid	2.16	10	6.39515	2.33664	2FI
R13	Cholesterol	1.12	99.86	49.6282	31.1684	2FI
R14	Campesterol	0	23.17	11.5979	7.44931	RLinear
R15	Stigmasterol	0	13.87	6.96697	4.45682	RFifth
R16	Beta-sitosterol	0	57.89	28.9748	18.5902	RFifth
R17	Delta-5-avenasterol	0	2.49	1.27909	0.801999	RFifth
R18	Delta-7-stigmasterol	0	0.16	0.0784848	0.0662439	RCubic

Table 3. The responses of different variables concerning physicochemical properties of ghee containing beef tallow

Response	Name	Min	Max	Mean	Std. Dev.	Model
R19	Butyric acid	0	1.59	0.668182	0.444757	2FI
R20	Caproic acid	0	1.43	0.641515	0.40668	Quartic
R21	Caprylic acid	0	1.06	0.46303	0.312875	2FI
R22	Capric acid	0	2.48	1.13727	0.734372	2FI
R23	Decenoic acid	0	0.26	0.106061	0.0947015	RCubic
R24	Lauric acid	0	3.05	1.40121	0.95512	Linear
R25	Myrestic acid	2.67	10.56	6.40333	2.42126	2FI
R26	Tetradecenoic acid	0	1.7	0.814545	0.525393	2FI
R27	Decapentanoic acid	0.67	1.42	0.990606	0.20873	2FI
R28	Palmitic acid	23.46	33.67	27.4582	2.75933	2FI
R29	Oleic acid	20.46	33.45	27.8861	3.66018	2FI

Additionally, Eqs. 2, 3 and 4 show the relations for obtaining the C4:0 content using real factors for adulterated samples from each region with different palm oil contents.

LocIlamR1=+1.59-0.0158p

Equation (2) - The C4:0 content (%) of ghee from A region adulterated with palm oil

lockermanshaR1= +1.297-0.0129p

Equation (3) - The C4:0 content (%) of ghee from B region adulterated with palm oil

lochamedanR1= +1.1145-0.011p

Equation (4) - The C4:0 content (%) of ghee from C region adulterated with palm oil

The optimization results showed that the adulteration detection level using the butyric acid content was 34.15% for Ilam, 22.58% for Kermanshah, and 10.0% for C region.

The 2FI model was adopted to fit the correlation between the linoleic acid and the study variables. The results indicated that linoleic acid content changed in the samples from different regions with different palm oil levels ($p<0.01$). The general equation to analyze the coded factors of linoleic acid content is presented in Eq. 5.

R12=+6.395+3.60A-0.005B[1]+0.32B[2] +0.005AB[1]-0.32AB[2];$\bar{R}^2$=0.9990

Equation (5) - The linoleic acid of ghee from different regions adulterated with palm oil with coded factors

Additionally, Eqs. 6, 7 and 8 show the relations for obtaining C18:2c using real factors for adulterated samples from each region with different palm oil contents.

LocIlamR12= +2.77+0.072p

Equation (6) - The C18:2c content (%) of ghee from A region adulterated with palm oil

lockermanshaR12= +3.43+0.065p

Equation (7) - The C18:2c content (%) of ghee from B region adulterated with palm oil

lochamedanR12= +2.15+0.078p

Equation (8) - The C18:2c content (%) of ghee from C region adulterated with palm oil

The results of the optimization showed that the adulteration detection level using the linoleic acid content (%) was 12.58% for A region, 3.75% for B region, and 18.72% for C region. According to the optimization results, the detection level of adulteration with palm oil using the complete fatty acid profile analysis was 8.54% for A region, 3.75% for B region, and 8.7% for C region. The ghee samples from different regions contained short-chain fatty acids consisting of butyric, caproic, caprylic, capric, and lauric acids, that are not present in palm oil. Moreover, the results showed that palm oil had the highest palmitic acid content. Therefore, the difference in the fatty acid profile can be a solution to detecting adulterated ghee with palm oil. The fatty acids of cow milk are the results of cattle feed and bacterial activities in the rumen of ruminantia. Since the source of ghee is milk, they are transferred into the ghee. The de novo synthesis system in the mammary glands can produce fatty acids C4:0-C14:0 and half of the fatty acid C16:0 from acetate and β-hydroxy. Acetate and butyric acids are produced by fermentation of the digested compounds inside the rumen of ruminantia. Butyric acid converts to beta-hydroxybutyrate while being absorbed from rumen epithelium (Barber *et al.*, 1997; Parodi, 2004). The adulteration detection level was higher than 90% for all the three regions when using palmitic acid. Thus, it is not a suitable criterion for detection of adulterated samples. In fact, palmitic acid is the most common fatty acid in both palm oil and ghee. Unsaturated fatty acids are not produced by the mammary glands of ruminantia, and their amount is dependent on their uptake rate by blood stream and thus to bovine feed. In general, factors like genetics, lactation stage,

feed, diseases (*e.g.* low milk fat syndrome) and environmental conditions can affect the type and amount of fatty acids (Palmquist, 2006).

- Detection of ghee adulteration with beef tallow concerned with the samples from different regions using fatty acid profile assessment

The fatty acid profile results showed that only lauric acid was able to detect adulteration levels higher than 23 %. The linear model was adopted to fit the correlation between the lauric acid and the study variables. The results indicated that the C12:0 changed for samples from different regions with different beef tallow levels ($p<0.01$). The general equation to analyze the coded factors of lauric acid content is as Eq. 9.

$$R24=+1.40-1.44A+0.13B[1]-0.18B[2];\quad \bar{R}^2=0.9471$$

Equation (9) - The lauric acid content (%) of ghee from different regions adulterated with beef tallow with coded factors

Additionally, Eqs. 10, 11 and 12 show the relations for obtaining the C12:0 content using real factors for adulterated samples from each region with different beef tallow contents.

$$LocIlamR24= +2.96-0.03p$$

Equation (10) - The C12:0 content (%) of ghee from A region adulterated with beef tallow

$$lockermanshaR24= +2.66-0.028p$$

Equation (11) - The C12:0 content (%) of ghee from B region adulterated with beef tallow

$$Lochamedan24= +2.88-0.029p$$

Equation (12) - The C12:0 content (%) of ghee from C region adulterated with beef tallow

The optimization results showed that the adulteration detection level using the C12:0 content (%) was 22.97% for A region, 11.51% for B region, and 18.05% for C region. The short-chain fatty acids are not present in beef tallow. Thus, adulteration can be detected by this criterion using the short-chain fatty acid content and the optimization results (according to the ISO recommendations). The results of the analysis of palmitic acid showed that the adulteration detection level for beef tallow is higher than 72% for all regions and it is not a suitable parameter for this purpose. Linoleic and oleic acids did not show a good detection level for adulteration with beef tallow. If the entire fatty acid profile is optimized under the model conditions, the detection level for adulteration with beef tallow will be 12.84% for A region, 8.86 for B region and 2.5% for C region. In another study, Argentinian researchers investigated the adulteration of milk fat with animal body fat. They simulated adulterated milk fat samples with 2, 5, 10 and 15% body fat and concluded that the adulteration detection level error would be lower than 15% if the evaluation is based on the ratio of fatty acids. They used the multiple linear regression to select and validate a model. It was possible to detect adulteration of milk fat with >10% beef tallow and with >5% lard (Rebechi *et al.*, 2015).

- Detection of ghee adulteration with palm oil concerned with samples from different regions using sterol analysis

Given the sterol content of adulterated ghee samples, it was found that cholesterol and β-sitosterol had the best fitting for adulteration detection purposes. Accordingly, the adulteration detection level was below 2% using only these two parameters. The 2FI model was adopted to fit the correlation between the cholesterol and the study variables. The results indicated that the cholesterol content changed in the samples from different regions with different palm oil levels ($p<0.01$). The general equation to

analyze the coded factors of cholesterol percent is shown in Eq. 13.

$$R13=+49.63-48.52A+0.86B[1]-0.5B[2]-0.87AB[1]+0.51AB[2];\ \bar{R}^2=0.9999$$

Equation (13) - The cholesterol content of ghee from different regions adulterated with beef tallow with coded factors

Additionally, Eqs. 14, 15 and 16 show the relations for obtaining the cholesterol content using real factors for adulterated samples from each region with different palm oil contents.

$$LocIlam13=+99.88-0.987p$$

Equation (14) - The cholesterol percent of ghee from A region adulterated with palm oil

$$lockermanshaR13=+97.13-0.960p$$

Equation (15) - The cholesterol content of ghee from B region adulterated with palm oil

$$lochamedanR13=+97.42-0.963p$$

Equation (16) - The cholesterol content of ghee from C region adulterated with palm oil

The optimization results showed that the adulteration detection level using cholesterol content (%) was 2.5% for A region, 1.87% for B region, and 2.5% for C region.

The fifth model was adopted to fit the correlation between the β-sitosterol and the study variables. The results indicated that the β-sitosterol content changed in samples from different regions with different palm oil levels ($p<0.01$). The general equation to analyze the coded factors of β-sitosterol content (R16) is shown in Eq. 17.

$$R16=+28.98+28.94A-0.035B[1]+0.028B[2]-0.007AB[1]-0.006AB[2]+0.032A^2-0.039A^2B[1]+0.038A^2B[2]+0.00008A^3+0.008A^3B[1]+0.005A^3B[2]-0.069A^4+0.074A^4B[1]-0.066A^4B[2];\ \bar{R}^2=0.9999$$

Equation (17) - The β-sitosterol content of ghee from different regions adulterated with beef tallow with coded factors

Additionally, Eqs. 18, 19 and 20 show the relations for obtaining the β-sitosterol content using real factors for adulterated samples from each region with different palm oil contents.

$$LocIlam16=-0.0004+0.579p+0.0000001p^2-0.0000001p^3+0.0000000008p^4$$

Equation (18) - The β-sitosterol content of ghee from A region adulterated with palm oil

$$lockermanshaR16=+0.0006+0.587p-0.00003p^2+0.000004p^3-0.00000002p^4$$

Equation (19) - The β-sitosterol content of ghee from B region adulterated with palm oil

$$lochamedanR16=+0.0009+0.583p-0.00001p^2+0.000002p^3-0.00000001p^4$$

Equation (20) - The β-sitosterol of ghee from C region adulterated with palm oil

The optimization results showed that the adulteration detection level using the β-sitosterol content (%) was 1.66 for A region, 1.65 for B region, and 1.69 for C region. According to the optimization results, the detection level of ghee adulteration with palm oil using sterol profile analysis for all three regions was as high as 1%. The ghee samples from different regions had only one sterol compound (cholesterol) whereas palm oil contains different sterols including β-sitosterol, campesterol, stigmasterol, Δ5-avenasterol, and Δ7-stigmasterol and its cholesterol content is under 1%. Therefore, analysis of the sterol profile of palm oil and ghee might be very useful as one of the best solutions to detect adulterated ghee with this oil. In a study by Farag *et al.*, non-saponified substances were used to detect adulterated ghee with other oils. They suggested that the cholesterol content in ghee were highest in cattle followed by buffalo, lard and margarine.

The highest β-sitosterol content of lipids were also observed in margarine followed by buffalo, cattle and lard (Farag *et al.*, 1982).

- Detection of ghee adulteration with palm oil using Raman spectroscopy

Raman spectroscopy bands can be used to estimate the parameters of the physical structure and the compounds of a wide varieties of lipids. These bands include 295-1305, 1400-1500, 1640-1665, 1730-1750 cm^{-1}. In the detection of adulterated ghee samples with palm oil, only the 1295-1305 cm^{-1} and 1400-1500 cm^{-1} bands had a $\bar{R}^2$ higher than 0.8. The cubic and quadratic models were proposed to fit the relationship between the frequency of 1295-1305 cm^{-1} spectra and the study variables results showed that the region has no significant effect on the frequency of the 1298-1305 cm^{-1} band ($p>0.01$); however, different adulteration levels with palm oil changed the frequency of samples from different regions ($p<0.01$) and $\bar{R}^2$ values was between 0.80 and 0.88. Different regions and palm oil levels had also no significant effect on the 1295-1298 spectrum ($p>0.01$). In order to fit the relationship between the frequency of 1400-1500 cm^{-1} band and the study variables, the fifth, sixth, cubic, 2FI and quadratic models were proposed. The results showed that the model was not significant for the 1400-1414 cm^{-1}band, and different regions had no significant effect on the frequency of the 1414-1426 cm^{-1} band ($p>0.01$). The adulteration level with palm oil changed the frequency in different regions ($p<0.01$), however, they were not analyzed due to low $\bar{R}^2$ (0.39-0.74). Additionally, different regions had no significant effect on the frequency of the 1426-1460 cm^{-1} band ($p>0.01$) although different adulteration level with palm changed the frequency in different regions ($p<0.01$) as the $\bar{R}^2$ ranged between 0.79-0.94. The model was not significant for the 1460-1500 bands ($p>0.01$). Other bands had a $\bar{R}^2$ lower than 0.8. The data optimization results for the 1298-1305 cm^{-1} band showed that the adulteration detection level was between 35.3-53.35% with a coefficient of determination higher than 0.8. The fatty acid profile results revealed that the ghee samples contained more short-chain fatty acids than palm oil. On the contrary, the long-chain fatty acids of palm oil (*e.g.* palmitic acid and stearic acid) were more than ghee. As a result, the difference in fatty acids led to different peaks for a frequency. Generally, the characteristic peaks of ghee include 1650 cm^{-1} (dual bounds stretching frequency), 1440 cm^{-1} (scissor deformation bending frequency), 1265 cm^{-1} (methylene bending frequency at dual bounds site), 1300 cm^{-1} (bending frequency at the twisting plane), and 1747 cm^{-1} (bending frequency of carbonyl group). However, the 1150, 1008, and 1525 cm^{-1} frequencies are related to carotenoids and have at least one peak in ghee (Fox and McSweeney, 1998; Bernstein, 2002; Schulz *et al.*, 2005). The presence of more short-chain fatty acids in ghee than in palm oil makes the peak bending frequency at the methylene twisting plane (1298-1305 cm^{-1}) lower in ghee than in palm oil. The optimization results of the 1426-1460 cm^{-1} band for different palm oil adulteration levels ($\bar{R}^2$ between 0.79 and 0.94) showed that the adulteration detection level for different regions was above 18.25%. This frequency range is highly capable of detecting ghee adulteration with palm oil. The analysis of Raman spectra is dependent on temperature and, if different ghee samples are not analyzed at a similar temperature, data will vary for each range. The 1400-1500 cm^{-1} had a different peak value due to the difference in the melting point of fatty acids (Beattiea *et al.*, 2004). When ghee is in its solid form, the peak in the 1426-1460 cm-1 range for palm oil is higher than ghee's peak. This shows that the more long-chain fatty acids in palm oil increase the peak within the 1426-1460 cm^{-1} band, whereas the short chain fatty acids create weaker peaks due to their lower bending frequency at the scissor plane (Beattiea *et al.*, 2004).

- *Detection of ghee adulteration with beef tallow using Raman spectroscopy*

In the detection of adulterated ghee samples with beef tallow, only the 1400-1500 cm^{-1} bands had a $\bar{R}^2$ higher than 0.8. The cubic and linear models were proposed to fit the relationship between the frequency of the 1400-1500 cm^{-1} band and the study variables Results showed that the region has no significant effect on the frequency of the 1437-1447, 1452-1457, and 1462-1473 cm^{-1} bands ($p>0.01$); however, different adulteration levels with beef tallow changed the frequency of samples from different regions ($p<0.01$). Moreover, $\bar{R}^2$ was between 0.82 and 0.9 for the 1437-1447 cm^{-1} band, between 0.85 and 0.89 for the 1452-1457 cm^{-1} band, and between 0.58 and 0.84 for the 1462-1473 cm^{-1} band. The peak frequency within the 1426-1460 cm^{-1} band was higher for tallow than for ghee. This shows that the more long-chain fatty acids exists in tallow than in ghee that increase the peak frequency within the 1400-1500 cm^{-1} band. The short chain fatty acids create weaker peaks due to their lower bending vibrations at the scissor plane (Beattiea *et al.*, 2004). The higher short-chain fatty acids and lower unsaturated fatty acids are formed in milk and its products due to the ruminal biohydrogenation (Palmquist, 2006).

Conclusion

The ghee production centers in Iran are located mostly in the west of the country. The purity of ghee is a key factor for the consumer acceptance. The gas chromatography instrument was used to analyze the fatty acid and sterol compositions of adulterated ghee with palm oil. The models showed that the adulteration detection levels for all fatty acids and sterols were higher than 9% and 1%, respectively. If the entire fatty acid profile is optimized under the model conditions, the detection level of adulterated ghee with beef tallow is above 13%. Regarding the sterol analysis, the ghee samples from different regions and beef tallow had only one sterol (*i.e.* Cholesterol). Thus, it was impossible to detect adulterated ghee with beef tallow using the sterol profile analysis. According to the results of Raman spectroscopy, only 1298-1305 cm^{-1} and 1426-1460 cm^{-1} bands produced reliable results for detecting adulterated ghee with palm oil. By analyzing these bands, the adulteration detection level was higher than 18.25%. The optimization results for Raman spectroscopy data of ghee samples adulterated with beef tallow showed that only the 1437-1473 cm^{-1} bands had reliable results as compared to the other ranges. Accordingly, the adulteration detection level was between 5.8–16.9%. In general, it can be concluded that the physical and chemical techniques have lower costs but their detection level is not accurate, whereas the expensive instrumental methods offer a high detection accuracy. The Raman spectroscopy technique offers lower costs, shorter processing time, and simpler sample preparation than other methods. Therefore, it might be used as a screening method due to its lower coefficient of determination than the GC method.

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