



Original research

A chromatographic method for detection of palm oil in butter

Javad Feizy^a, Moslem Jahani^{b,*}

^a Department of Food Safety and Quality Control, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

^b Department of Food Chemistry, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

ABSTRACT

Replacement with the cheaper animal fats or vegetable oils is conventional in milk through direct incorporation or homogenization of skimmed milk with less expensive foreign fats. The main objective of the present study was to evaluate the capability of a simple, fast, and reliable method for the detection of added palm oil in butter. The butter samples were mixed with palm oil (0.5-50 wt%) followed by gas chromatography analysis of fatty acids and sterols. The results confirmed the potential of discrimination based on a significant increase in the content of unsaturated fatty acids (C18:1 and C18:2) and a decrease in saturated fatty acids (mainly C10:0, C12:0, and C14:0). The differences are detectable for at least 10.0 wt% of the palm oil. Also, significant variations were observed in the cholesterol and sitosterol as marker sterols in the butter and palm oil, respectively. The study demonstrates the high potential of the procedure to rapidly detect and discriminate between butter-palm oil blends among pure samples.

Keywords: Food adulteration, Milk-fat, Saturated fatty acids, Sitosterol, Cholesterol

Received 25 April 2020; Revised 2 June 2020; Accepted 8 June 2020

Copyright © 2020. This is an open-access article distributed under the terms of the Creative Commons Attribution-4.0 International License which permits Share, copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, even commercially.

1. Introduction

Higher nutritional value, sensory qualities, and potential health benefits lead to more interest in natural edible oils such as milk fat (Jabeur et al., 2016). Only pasteurized cream produced from cow milk is sold as butter, and its quality is greatly influenced by the content of peptides, amino acids, and free fatty acids (FFA) (Kim et al., 2015; Tomaszewska-Gras, 2012; Upadhyay et al., 2016). However, mixing with foreign low price/quality fats is a considerable challenge concerned by consumers and human health organizations. Butter adulterants can be classified into vegetable oils and animal body fats. Palm oil (PO), coconut, sunflower, soya bean, and rapeseed oils are common in the former case, which can be detected by chromatographic techniques. But, time-consuming and complicated analytical approaches are needed to identify the latter case (Kumar et al., 2015).

Classical analyses of acidity, peroxide, and iodine values are conventional methods in the characterization of oils (Dankowska et al., 2014). Generally, they are expensive and time-consuming methods with less accurate results. Recognition can be made based on specific compounds as markers. Additionally, the profile of fatty acids (FAs), triacylglycerols (TAGs), sterols, or other chemicals

can be compared to distinguish between different products (Li et al., 2011).

Instrumental methods of ultraviolet and visible (UV-Vis) (Jiang et al., 2015), infrared (Upadhyay et al., 2016), Raman (Uysal et al., 2013), fluorescence (Dankowska et al., 2014), and nuclear magnetic resonance (NMR) spectroscopy (Zhang et al., 2013) are developed in the authentication of oil samples. Non-spectroscopy methods such as sensors (Marina et al., 2010), thermal analysis (Tomaszewska-Gras, 2016), and hyphenated chromatographic techniques such as HPLC-MS and GC-MS (Bajoub et al., 2014; Fasciotti & Pereira Netto, 2010) are also employed.

The unique profile of FAs and TAGs can be the basis of fraud detection in oils (Indelicato et al., 2017). The composition of FAs can be determined through gas chromatography analysis. Butyric acid (C4:0), a saturated fatty acid (SFA), is absent in vegetable oils and is accepted as an indicator FA for milk fat (Tomaszewska-Gras, 2016). The vegetable oils are characterized by a considerable amount of *trans*-fatty acids (TFAs) that formed from linoleic and alpha-linolenic acids. In the milk fat, *trans*-11 18:1 exists as the main isomer (45-50% of total *trans* isomers), but in the vegetable oils, *trans*-9 and *trans*-10 18:1 are the major ones. In the milk fat, *trans*-18:1 acid has a low content (4 to 6 g/100 g of FA), while its quantity can vary from 10 to 60 g/100 g of FA in the vegetable oils (Destailats et al., 2006).

*Corresponding author.

E-mail address: m.jahani@rifst.ac.ir (M. Jahani).

<https://doi.org/10.22059/jfabe.2020.76393>

The official European Community proposed the GC analysis to determine the composition of TAGs and milk fat purity. The method can detect the addition of at least 5.0 wt% of another fat of different origin (Dankowska et al., 2014; Derewiaka et al., 2011). Tocochromanols or plant sterols can also be measured by gas chromatography to recognize foreign vegetable oils in butter (Gornas et al., 2014). Infrared spectroscopy is also an emerging strategy to verify the authenticity of edible oils due to its simplicity, rapidity, and ease of sample preparation (Rohman & Man, 2010). The application of multivariate statistical methods can significantly facilitate the evaluation of analytical results that contained a high number of variables (Alexa et al., 2009; Zhu et al., 2017). Thermo-analytical methods such as differential scanning calorimetry (DSC) are also advantageous for the authentication analysis of oils. DSC is a relatively simple, fast, and conventional technique that the specific composition of FAs and TAGs will appear in the melting or crystallization curves (Tomaszewska-Gras, 2012, 2016).

The oil palm considers as the most efficient oil-producing plant, and PO is widely used in the biofuel, soap, detergent, and food industries. Regarding low-trans FAs, food manufacturers utilize PO as an alternative to the hydrogenated vegetable oils (Lin, 2011). Due to little polyunsaturated fatty acids (PUFAs), palm oil is oxidatively stable. The crude PO is rich in minor components such as carotenoids, phospholipids, triterpene alcohols, aliphatic alcohols, and aliphatic hydrocarbons. Otherwise, carotenes, tocopherols, tocotrienols, sterols, and squalene are significant components of interest. Carotenes and tocopherols are antioxidants and stabilize the oil against oxidation (Ariffin et al., 2014; Tres et al., 2013). Due to little polyunsaturated fatty acids (PUFAs), palm oil is oxidatively stable. The application of a simple and fast analytical method to detect palm oil in butter samples was interested in the present study. The composition of the FAs and phytosterols in the mixed (or blended) samples was determined using GC-FID. This study has the advantage of a fast and easy

procedure that is applicable in most food analytical laboratories and even in food factories. The short time of analysis, minimal steps of sample preparation, and ease of data manipulation under computer control represent a further benefit of GC-FID analysis.

2. Material and Methods

2.1. Chemical reagents and samples

All chemicals were of analytical grade and used as received without any purification. The organic solvents such as n-hexane, n-heptane, methanol, ethanol, and chloroform were purchased from Merck Chemical Co. (Darmstadt, Germany). The standard materials for cholesterol, brassicasterol, stigmaterol, campesterol, sitosterol, and a fatty acid methyl esters (FAMES) Mix standard were obtained from Sigma-Aldrich (St. Louis, Mo., USA). Sodium hydroxide, potassium hydroxide, sodium chloride, and other chemicals were also provided by the Merck Chemical Co. Butter and palm oil samples were collected from the local dairy manufacturers. All samples were stored under refrigeration conditions until the analysis. A set of butter samples (n=5) was melted at 50°C and blended to make a butter-fat stock. Palm oil samples (n=3) similarly mixed to prepare palm oil blend.

2.2. Sample preparation and analysis

The stock blends (butter and palm oil) were heated and melted in a water bath (50°C) to a clear liquid phase. The mixed samples prepared by adding PO (ranging from 0.5 to 50 wt%) to the butter fat stock sample. All the samples stored under refrigeration until analysis.

Table 1. The fatty acids composition of the crude and mixed samples*.

| FAs | Fatty acids content (wt%) | | | | | | | | | |
|------------------------------------|---------------------------|-------------------------|-------------------------|--------------------------|--|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Crude samples | | | | Mixed samples (Added percentage of PO to butter (wt%)) | | | | | |
| | PO | Butter | 0.5 | 1 | 1.5 | 2 | 2.5 | 10 | 20 | 50 |
| Butyric (C4:0) | 0 | 1.11±0.02 ^{AB} | 1.10±0.02 ^{AB} | 1.11±0.01 ^A | 1.09±0.01 ^{AB} | 1.08±0.005 ^{AB} | 1.06±0.02 ^B | 0.89±0.01 ^C | 0.72±0.01 ^D | 0.41±0.01 ^E |
| Caproic (C6:0) | 0 | 1.25±0.02 ^{AB} | 1.27±0.02 ^A | 1.29±0.02 ^A | 1.29±0.02 ^A | 1.27±0.03 ^{AB} | 1.21±0.02 ^B | 1.12±0.02 ^C | 0.96±0.02 ^D | 0.72±0.01 ^E |
| Caprylic (C8:0) | 0.01±0.001 | 1.14±0.02 ^A | 1.16±0.01 ^A | 1.17±0.02 ^A | 1.15±0.02 ^A | 1.14±0.04 ^A | 1.13±0.01 ^A | 1.05±0.02 ^B | 0.86±0.02 ^C | 0.54±0.01 ^D |
| Capric (C10:0) | 0.01±0.001 | 3.01±0.02 ^A | 3.05±0.03 ^A | 3.08±0.03 ^A | 3.07±0.04 ^A | 3.00±0.09 ^A | 2.68±0.02 ^B | 2.42±0.03 ^C | 2.21±0.03 ^D | 1.48±0.02 ^E |
| Lauric (C12:0) | 0.19±0.01 | 3.75±0.05 ^A | 3.77±0.02 ^A | 3.72±0.06 ^A | 3.71±0.04 ^A | 3.63±0.04 ^{AB} | 3.54±0.05 ^B | 3.25±0.06 ^C | 2.91±0.05 ^D | 2.10±0.04 ^E |
| Myristic (C14:0) | 1.19±0.02 | 12.98±0.21 ^A | 13.02±0.03 ^A | 12.68±0.05 ^A | 12.73±0.14 ^A | 12.84±0.05 ^A | 12.69±0.08 ^A | 11.78±0.21 ^B | 10.11±0.21 ^C | 7.72±0.11 ^D |
| Myristoleic (C14:1) | 0.05±0.001 | 1.35±0.02 ^A | 1.37±0.02 ^A | 1.29±0.02 ^A | 1.30±0.04 ^A | 1.31±0.04 ^A | 1.31±0.05 ^A | 1.19±0.03 ^B | 0.95±0.03 ^C | 0.65±0.02 ^D |
| Pentadecanoic (C15:0) | 0 | 1.07±0.02 ^{AB} | 1.09±0.01 ^{AB} | 1.08±0.03 ^{AB} | 1.11±0.01 ^{AB} | 1.12±0.03 ^A | 1.04±0.05 ^B | 0.87±0.02 ^C | 0.77±0.02 ^D | 0.53±0.01 ^E |
| Palmitic (C16:0) | 43.51±0.05 | 34.48±0.39 ^C | 34.50±0.7 ^C | 33.95±0.67 ^C | 34.68±0.73 ^C | 34.29±0.43 ^C | 34.55±0.07 | 36.35±0.50 ^B | 37.38±0.50 ^B | 39.62±0.70 ^A |
| Palmitoleic (C16:1) | 0.16±0.01 | 1.33±0.04 ^A | 1.36±0.01 ^A | 1.29±0.02 ^A | 1.31±0.02 ^A | 1.34±0.02 ^A | 1.21±0.02 ^B | 1.14±0.03 ^C | 0.98±0.04 ^D | 0.71±0.03 ^E |
| Margaric (C17:0) | 0.09±0.001 | 0.55±0.02 ^{AB} | 0.54±0.02 ^{AB} | 0.53±0.02 ^{ABC} | 0.57±0.01 ^A | 0.52±0.02 ^{BC} | 0.52±0.01 ^{BC} | 0.49±0.01 ^C | 0.42±0.01 ^D | 0.43±0.02 ^D |
| Stearic (C18:0) | 3.08±0.03 | 11.38±0.08 ^A | 11.47±0.08 ^A | 11.36±0.11 ^A | 11.24±0.1 ^A | 11.35±0.13 ^A | 10.97±0.25 ^A | 10.35±0.07 ^B | 9.22±0.17 ^C | 7.06±0.12 ^D |
| Octadecenoic (C18:1 <i>trans</i>) | 0 | 1.22±0.03 ^A | 1.26±0.02 ^A | 1.21±0.03 ^A | 1.24±0.04 ^A | 1.29±0.03 ^A | 1.21±0.04 ^A | 1.06±0.05 ^B | 0.81±0.02 ^C | 0.62±0.03 ^D |
| Oleic (C18:1 <i>cis</i>) | 45.83±0.28 | 21.64±0.26 ^D | 21.64±0.26 ^D | 20.51±0.54 ^D | 21.45±0.43 ^D | 21.90±0.53 ^D | 21.46±0.37 ^D | 24.57±0.57 ^C | 28.76±0.58 ^B | 33.85±0.75 ^A |
| Linoleic (C18:2 <i>cis</i>) | 5.68±0.19 | 1.45±0.05 ^D | 1.41±0.05 ^D | 1.39±0.06 ^D | 1.51±0.04 ^D | 1.49±0.04 ^D | 1.45±0.04 ^D | 1.75±0.06 ^C | 2.18±0.03 ^B | 3.23±0.05 ^A |
| Saturated FAs (SFAs) | 48.08 | 70.72 | | | | | 69.39 | 68.57 | 65.56 | 60.61 |
| Unsaturated FAs (UFAs) | 51.72 | 26.99 | | | | | 26.64 | 29.71 | 33.68 | 38.79 |

* Results are expressed in g/100 g sample. All the values are mean ± SD (n=3); SD standard deviation. The values with different superscripts in the same row are significantly different (p ≤ 0.05). PO: palm oil.

2.2.1. Analysis of the fatty acids

A standard procedure was applied to convert FAs to the corresponding methyl esters (ISO12966-4, 2015; Jabeur et al., 2016). The protocol includes vigorous shaking of the oil sample solution (0.1 g oil in 2.0 mL n-heptane) with 0.2 mL of methanolic potassium hydroxide (2.0 mol L⁻¹) at 40-50°C for 15 min. The supernatant was collected and subjected to the GC analysis.

The FAMES were separated and quantified using an Agilent 7890A GC instrument (Palo Alto, CA, USA) equipped with a flame ionization detector (FID), split/ splitless injector and a BPX-70 fused silica column (120 m, 0.25 mm i.d. and 0.25 µm film thickness). BPX-70 (SGE Analytical science Co. Australia) is a highly polar capillary column coated with a 70% Cyanopropyl Polysilphenylene-Siloxane phase and a custom design for separation of FAMES. Gas chromatography conditions were as follows: separation was carried out under an isothermal condition with a column temperature of 198°C (Shirasawa et al., 2007). The injector and detector were kept at 250°C and 300°C, respectively. Ultrapure nitrogen was used as the carrier (1 mL min⁻¹) and make-up gas (15 mL min⁻¹). The sample injections were carried out in triplicates with an injection volume of 1 µL and a split ratio of 1:10. The data collection and calculations were conducted on Chemstation software. Differences between samples were determined using one-way ANOVA and Tukey's means test (p ≤ 0.05) on Minitab 16.

2.2.2. Analysis of the sterols

Butter samples were saponified for the analysis of phytosterols. Initially, the samples (2.0 g) were mixed with 50.0 mL ethanolic potassium hydroxide solution (2.32 mol L⁻¹) under sonication. The solution was then refluxed for one hour, cooled to room temperature, and transferred to a separatory funnel. Then, the unsaponifiable content was extracted three times by distilled water (50.0 mL) and n-hexane (100.0 mL). After shaking for 20 min, the supernatant was separated. All unsaponifiable fractions were finally collected and taken to dryness with a rotary evaporator under vacuum and at room temperature. Chloroform (1.0 mL) was added to the dried mass, and the solution injected to GC (Chen et al., 2015). The GC analysis of the sterols performed on an HP-5 capillary column (30 m, 0.32 mm i.d. and 0.25 µm film thickness) with an injection volume of 1 µL and a split ratio 1:10. Helium was used as carrier gas (1 mL min⁻¹) with a total run time of 40 min and the equilibration time of 0.5 min. The column oven, injection port, and detector temperatures were 260, 300, and 310°C, respectively.

3. Results and Discussion

3.1. Fatty acids composition of the pure and mixed samples

Analysis of TAGs, FAs, sterols, and tocopherols typically used for the detection of the impure butter samples. The composition of FAs was previously studied as a purity indicator of the edible oils. But, data interpretation is difficult because the climate and geographical conditions can significantly change the content of the FAs. However, it can specify a proper direction for additional tests (Kala et al., 2016). The FAs compositions of the pure and mixed butter samples are presented in Table 1.

Generally, PO identifies as a saturated fat even though it contains equal proportions of the SFAs (mainly palmitic acid, 44-45%) and UFAs (primarily oleic acid, 39-40%) along with linoleic acid (10-11%) and a trace amount of linolenic acid. The low level of linoleic acid (C18:2) and the virtual absence of linolenic acid (C18:3) makes it relatively stable to the oxidative reactions (Ariffin et al., 2014; Lin, 2011; Tres et al., 2013). Since saturated fats have linked to cardiovascular disease, PO and many PO fractions identify as unhealthy fats. The analysis of FAs in raw PO shows C18:1cis (45.83 wt%) and C16:0 (43.51 wt%) are the predominant FAs (Table 1) followed by C18:2cis (5.68 wt%) and C18:0 (3.08 wt%). A literature review showed the fatty acid composition of C18:1cis (34-55 wt%), C16:0 (30-50 wt%), C18:2cis (5-13 wt%), and C18:0 (3-10 wt%) in PO that confirms our results (Montoya et al., 2014).

In previous studies, C16:0 (17.2-39.1 wt%), C14:0 (7.1-15.1 wt%), and C18:0 (5.9-14.8 wt%) were reported as the main SFAs and C18:1cis (18.8-33.9 wt%) as the main UFAs in the milk fat (Markiewicz-Keszycka et al., 2013). In the pure butter, C16:0 (34.48 wt%), C18:1cis (21.64 wt%), C14:0 (12.98 wt%), and C18:0 (11.38 wt%) are predominant fatty acids which are in a good agreement with the previous reports (Table 1). Moreover, 1.11 wt% of butyric acid (C4:0) was detected in butter, as the specific FAs in milk fat. The milk fat contains 1.1-1.8 wt% of butyric acid, with anti-carcinogenic properties. It differs seasonally and the lowest amount observed in the winter (Upadhyay et al., 2016). A standard sample should contain 1.0-2.4 wt% of C18:2 and 0.25-1.1 wt% of C18:3, as the principal polyunsaturated FAs in milk fat. The results also revealed 1.45 wt% and 0.25 wt% for the linoleic and linolenic acids, respectively. The total amounts of the SFAs and UFAs in different samples are listed in Table 1. Palm oil has equal quantities of the saturated and unsaturated FAs but, butter has a much higher portion of the SFAs. Additionally, butter shows a higher amount of saturated FAs than PO.

Table 2. The sterols composition of the crude and mixed samples*.

| Sterols | Sterols content (wt%) | | | | | | |
|----------------|-----------------------|------------|--|-----------|------------|------------|------------|
| | Crude sample | | Mixed samples (Amount of PO added to the butter (wt%)) | | | | |
| | PO | Butter | 5 | 10 | 15 | 25 | 35 |
| Cholesterol | 2.42±0.01 | 96.30±1.05 | 93.55±0.04 | 90.34±0.1 | 85.32±0.09 | 75.61±0.11 | 66.19±0.21 |
| Brassicasterol | 1.72±0.03 | - | 0.15±0.06 | 0.20±0.01 | 0.35±0.01 | 0.54±0.01 | 0.73±0.01 |
| Campesterol | 23.71±0.12 | - | 0.62±0.02 | 1.33±0.03 | 2.45±0.04 | 4.53±0.06 | 6.28±0.04 |
| Stigmasterol | 12.31±0.18 | - | 0.24±0.01 | 0.95±0.01 | 1.25±0.05 | 2.67±0.07 | 3.23±0.07 |
| Sitosterol | 59.83±0.47 | - | 1.68±0.09 | 3.22±0.1 | 6.82±0.1 | 12.83±0.09 | 18.52±0.1 |

* Results are expressed in g/100 g sample. All the values are mean ± SD (n=3); SD standard deviation. The values with different superscripts in the same row are significantly different (p ≤ 0.05). PO: palm oil.

Table 3. Comparison of proposed method with other reported methods to detect fraud in butter.

| No. | Method | Adulterant | Bio-markers | Characteristics and limitations | Ref. |
|-----|---------------------------|---|------------------------------|---|---------------------------|
| 1 | Fluorescence spectroscopy | - palm oil - Coconut oil | - | - Multiple linear regression models were used to calculate the level of adulteration - The lowest detection limit of adulteration was 5.5 %wt - Spectrofluorometer is not a common instrument in food laboratories. | (Dankowska et al., 2014) |
| 2 | GC-FID | partially hydrogenated vegetable oils | TAGs | - The separation of milk fat TAGs was achieved using a short apolar open tubular capillary column in less than 4 min. - The relative increase of trans-9 18:1 was an indicator of the adulteration of milk fat | (Destailats et al., 2006) |
| 3 | DSC | Palm oil | - | - With an increase of PO in butter (0-35%) the melting temperature increased by approx. 2 °C. - DSC technique is applicable for the quantitative detection of PO in butter within the range of concentrations from 2 to 35%, based on the parameters of peak area and peak height. - DSC is not a common instrument in food laboratories. | (Tomaszewska-Gras, 2016) |
| 4 | GC-FID | - Soybean oil - corn oil - lard - tallow | - TAGs - FAs - Sterols | - These bio-markers enabled the detection of as low as 10% adulteration - A running time of 67 min (for FAs) - Oleic acid (C18:1) and linoleic acid (C18:2) were suggested as biomarkers for FA analysis | (Kim et al., 2015) |
| 5 | GC-FID | - Palm oil | - FAs - Sterols | - These bio-markers enabled the detection of as low as 10% adulteration - A running time of 40 min (for FAs) - Oleic acid (C18:1) and linoleic acid (C18:2) were suggested as biomarkers for FA analysis | This work |

A considerable reduction in the amount of all FAs (particularly for C10:0, C12:0, and C14:0) recognized in the samples and the differences are meaningful at least in 10 wt% of palm oil. However, the addition of PO leads to a significant rise in the oleic, linoleic, and unsaturated fatty acids, mainly due to the high content of UFAs in vegetable oils. The low variations in the percentages of C17:0, C18:3, and C20:0 can be a result of their close values in butter and PO samples (Table 1). Generally, in the butter samples containing higher quantities of PO, the saturated FAs were decreased while the unsaturated FAs increased. Consequently, the detection of mixed samples is possible based on significant changes of FAs but in PO levels higher than 2.5 wt%.

3.2. Variation in the composition of sterols

Sterols, a significant portion of unsaponifiable matter, are found in almost all fats and oils. The profile of sterols is used as an indicator of vegetable oils authenticity and mixing with other cheaper ones (Soha et al., 2015). The results of the analysis of sterols in different samples are summarized in Table 2.

Cholesterol is the predominant sterol in animal fats (at least 95 wt%), but vegetable oils mainly contain phytosterols including β -sitosterol, campesterol, stigmasterol, avenasterol, and brassicasterol (Derewiaka et al., 2011). The results represent a continuous decrease in cholesterol of the mixed samples (Table 2). Due to their health benefits in reducing blood cholesterol or preventing colon cancer development, phytosterols are employed in some of the functional foods (Jiang et al., 2019). The previous studies also report 23.3 wt% of campesterol, 11.7 wt% of stigmasterol, and 46.7 wt% of sitosterol that are in good agreement with our results

(Normen et al., 2007). The sitosterol (59.83 wt%) was the primary sterol in the PO and followed by campesterol (23.71 wt%) and stigmasterol (12.31 wt%). On the other hand, an increasing trend was observed in the content of phytosterols in the mixed samples. The presence of β -sitosterol in butter, as a typical vegetable sterol, can approve fraud with the palm oil.

4. Conclusion

The development of new and reliable techniques for the authentication of edible oils continues apace along with increasing consumer awareness of its safety. In this study, the potential of GC-FID was evaluated for the assessment of possible adulterations of butter by palm oil. The results have shown that butter and palm oil exhibit significant differences in the profile of FAs and sterols. In fact, it seems a decision based on the variation in the content of some FAs or sterols is possible. The results confirmed a significant increase in the quantity of C18:1 and C18:2 FAs, and these variations can be detected for at least 10.0 wt% of PO. The total SFAs were increased, along with a decrease in UFAs in the mixed samples. Variation in the quantity of cholesterol and the presence of phytosterols in mixed samples could be the basis for the detection of fake samples. The content of the selected sterols widely accepted as one of the most important markers for the detection of adulterated oils.

The new method was compared with other similar studies (Table 3). The developed method has the advantages of simplicity and low cost relative to the complicated methods. By considering all of the results obtained, it may be concluded that GC-FID is suitable for the assessment of the adulteration of butter with palm

oil and it can recommend for authentication evaluations. However, the final confirmation of the suitability of the method requires further studies for the purpose of the quantitative evaluation of adulteration.

Acknowledgment

The authors would like to acknowledge the central laboratory and instrumental laboratory of the research institute of food science and technology (Razavi Khorasan, Mashhad, Iran), which supports the gas chromatography analysis.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Alexa, E., Dragomirescu, Pop, A., Jianu, G., Dragos, C., & Dan. (2009). The use of FT-IR spectroscopy in the identification of vegetable oils adulteration. *Journal of Food Agriculture and Environment*, 7.
- Ariffin, A. A., Ghazali, H. M., & Kavousi, P. (2014). Validation of a HPLC method for determination of hydroxymethylfurfural in crude palm oil. *Food Chemistry*, 154, 102-107.
- Bajoub, A., Carrasco-Pancorbo, A., Ouazzani, N., & Fernández-Gutiérrez, A. (2014). UHPLC-MS in Virgin Olive Oil Analysis (pp. 213-242).
- Chen, Y.-Z., Kao, S.-Y., Jian, H.-C., Yu, Y.-M., Li, J.-Y., Wang, W.-H., & Tsai, C.-W. (2015). Determination of cholesterol and four phytosterols in foods without derivatization by gas chromatography-tandem mass spectrometry. *Journal of Food and Drug Analysis*, 23(4), 636-644.
- Dankowska, A., Małecka, M., & Kowalewski, W. (2014). Application of synchronous fluorescence spectroscopy with multivariate data analysis for determination of butter adulteration. *International Journal of Food Science & Technology*, 49(12), 2628-2634.
- Derezińska, D., Sosinska, E., Obiedziński, M., Krogulec, A., & Czaplicki, S. (2011). Determination of the adulteration of butter. *European Journal of Lipid Science and Technology*, 113(8), 1005-1011.
- Destailhats, F., de Wispelaere, M., Joffre, F., Golay, P.-A., Hug, B., Giuffrida, F., . . . Dionisi, F. (2006). Authenticity of milk fat by fast analysis of triacylglycerols: Application to the detection of partially hydrogenated vegetable oils. *Journal of Chromatography A*, 1131(1-2), 227-234.
- Fasciotti, M., & Pereira Netto, A. D. (2010). Optimization and application of methods of triacylglycerol evaluation for characterization of olive oil adulteration by soybean oil with HPLC-APCI-MS-MS. *Talanta*, 81(3), 1116-1125.
- Gornas, P., Siger, A., Czubinski, J., Dwiecki, K., Seglina, D., & Nogala-Kalucka, M. (2014). An alternative RP-HPLC method for the separation and determination of tocopherol and tocotrienol homologues as butter authenticity markers: A comparative study between two European countries. *European Journal of Lipid Science and Technology*, 116(7), 895-903.
- Indelicato, S., Bongiorno, D., Pitonzo, R., Di Stefano, V., Calabrese, V., Indelicato, S., & Avellone, G. (2017). Triacylglycerols (TAGs) in edible oils: Determination, characterization, quantitation, chemometric approach and evaluation of adulterations. *Journal of Chromatography A*, 1515, 1-16.
- ISO12966-4. (2015). Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters -Part 4: Determination by capillary gas chromatography (1st edition) (pp. 21).
- Jabeur, H., Zribi, A., & Bouaziz, M. (2016). Extra-virgin olive oil and cheap vegetable oils: Distinction and detection of adulteration as determined by gc and chemometrics. *Food Analytical Methods*, 9(3), 712-723.
- Jiang, L., Zhao, X., Xu, J., Li, C., Yu, Y., Wang, W., & Zhu, L. (2019). The Protective Effect of Dietary Phytosterols on Cancer Risk: A Systematic Meta-Analysis. *Journal of Oncology*, 2019, 7479518.
- Jiang, L., Zheng, H., & Lu, H. (2015). Application of UV spectrometry and chemometric models for detecting olive oil-vegetable oil blends adulteration. *Journal of Food Science and Technology*, 52(1), 479-485.
- Kala, A. L. A., Sabeena, K., & Havanur, P. P. (2016). Determination of triacyl glycerol and sterol components of fat to authenticate ghee based sweets. *Journal of Food Science and Technology*, 53(4), 2144-2147.
- Kim, J.-M., Kim, H.-J., & Park, J.-M. (2015). Determination of milk fat adulteration with vegetable oils and animal fats by gas chromatographic analysis. *Journal of Food Science*, 80(9), C1945-C1951.
- Kumar, A., Upadhyay, N., Padghan, P., Gandhi, K., Lal, D., & Sharma, V. (2015). Detection of Vegetable Oil and Animal Depot Fat Adulteration in Anhydrous Milk Fat (Ghee) using Fatty Acid Composition. *MOJ Food Processing & Technology*, 01.
- Li, C., Yao, Y., Zhao, G., Cheng, W., Liu, H., Liu, C., . . . Wang, S. (2011). Comparison and Analysis of Fatty Acids, Sterols, and Tocopherols in Eight Vegetable Oils. *Journal of Agricultural and Food Chemistry*, 59(23), 12493-12498.
- Lin, S. W. (2011). *Palm Oil Vegetable oils in food technology* (pp. 25-58): Wiley-Blackwell.
- Marina, A. M., Che Man, Y. B., & Amin, I. (2010). Use of the SAW sensor electronic nose for detecting the adulteration of virgin coconut oil with RBD palm kernel olein. *Journal of the American Oil Chemists' Society*, 87(3), 263-270.
- Markiewicz-Keszycska, M., Czyżak-Runowska, G., Lipińska, P., & Wójtowski, J. (2013). Fatty acid profile of milk - A review. *Bulletin-Veterinary Institute in Pulawy*, 57, 135.
- Montoya, C., Cochard, B., Flori, A., Cros, D., Lopes, R., Cuellar, T., . . . Billotte, N. (2014). Genetic architecture of palm oil fatty acid composition in cultivated oil palm (*Elaeis guineensis* Jacq.) compared to its wild relative *E. oleifera* (H.B.K) Cortés. *PLoS one*, 9(5), e95412-e95412.
- Norsten, L., Ellegerd, L., Brants, H., Dutta, P., & Andersson, H. (2007). A phytosterol database: Fatty foods consumed in Sweden and the Netherlands. *Journal of Food Composition and Analysis*, 20(3-4), 193-201.
- Rohman, A., & Man, Y. B. C. (2010). Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. *Food Research International*, 43(3), 886-892.
- Shirasawa, S., Sasaki, A., Saida, Y., & Satoh, C. (2007). A rapid method for trans-fatty acid determination using a single capillary GC. *J Oleo Sci*, 56(2), 53-58.
- Soha, S., Mortazavian, A. M., Piravi-Vanak, Z., Mohammadifar, M. A., Sahafar, H., & Nanvazadeh, S. (2015). Adequacy of the Measurement Capability of Fatty Acid Compositions and Sterol Profiles to Determine Authenticity of Milk Fat Through Formulation of Adulterated Butter. *Recent Pat Food Nutr Agric*, 7(2), 134-140.
- Tomaszewska-Gras, J. (2012). Detection of butter adulteration with water using differential scanning calorimetry. *Journal of Thermal Analysis and Calorimetry*, 108(2), 433-438.
- Tomaszewska-Gras, J. (2016). Rapid quantitative determination of butter adulteration with palm oil using the DSC technique. *Food Control*, 60, 629-635.
- Tres, A., Ruiz-Samblas, C., van der Veer, G., & van Ruth, S. M. (2013). Geographical provenance of palm oil by fatty acid and volatile compound fingerprinting techniques. *Food Chemistry*, 137(1-4), 142-150.
- Upadhyay, N., Jaiswal, P., & Jha, S. N. (2016). Detection of goat body fat adulteration in pure ghee using ATR-FTIR spectroscopy coupled with chemometric strategy. *Journal of Food Science and Technology*, 53(10), 3752-3760.
- Uysal, R. S., Boyaci, I. H., Genis, H. E., & Tamer, U. (2013). Determination of butter adulteration with margarine using Raman spectroscopy. *Food Chemistry*, 141(4), 4397-4403.

- Zhang, Q., Saleh, A. S. M., & Shen, Q. (2013). Discrimination of edible vegetable oil adulteration with used frying oil by low field nuclear magnetic resonance. *Food and Bioprocess Technology*, 6(9), 2562-2570.
- Zhu, W., Wang, X., & Chen, L. (2017). Rapid detection of peanut oil adulteration using low-field nuclear magnetic resonance and chemometrics. *Food Chemistry*, 216, 268-274.