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ORIGINAL ARTICLE

Characterization of Desi Ghee Extracted by Different Methods Using Fluorescence Spectroscopy

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Abstract

In the current study, the effect of ghee extraction methods (direct cream DC, milk butter MB and milk skin MS) on its molecular composition has been investigated using Fluorescence spectroscopy. The excitation wavelength of 300 nm was found the best to produce pronounced spectral signatures of beta-carotene, vitamins and conjugated linoleic acid (CLA) in both cow and buffalo ghee types. Principal component analysis (PCA) has been applied on the spectral data to visualize the classification among ghee samples extracted by three methods. Both cow and buffalo ghee contain spectral signatures of vitamin A, E, K, D and CLA which has been verified through plotting loading vectors. The analysis of loading plots has been suggested that for cow ghee, MS extraction method conserve relatively higher concentration of beta carotene while DC and MB methods are a good choice for preserving relatively more concentrations of vitamins D, E and K. Similarly, for buffalo ghee, MS extraction method appear with higher concentration of CLA, whereas DC extraction method looks to preserve relatively higher concentration of vitamin A while MB method retains relatively low concentration of CLA and vitamins as compared to other two methods.

Keywords Cow and buffalo desi ghee · Ghee extraction methods: Direct cream (DC) · Milk butter (MB) · Milk skin (MS) · Fluorescence spectroscopy · Beta-carotene · Vitamins A, E, D, K · Conjugated linoleic acid (CLA) · Principal component analysis (PCA)

Introduction

Desi ghee obtained from buffalo and cow milk by different methods is an important fat rich nutritious dairy product for human health [1]. It is enriched with 62% saturated fatty acids, 29% unsaturated fatty acids, 4% trans fats, fat soluble vitamins [2, 3] and conjugated linoleic acid (CLA) [3]. It is generally obtained by three methods; which includes direct cream (DC), milk skin (MS) and indigenous milk butter (MB) or desi method [4]. Ghee obtained from heat clarification of fresh cream is called DC method and by directly heating the milk skin or lactoderm is called MS extraction method. The heat clarification of butter obtained from milk by applying yogurt culture is called MB or desi method [5]. These extraction methods may affect the nutritional values of ghee including fatty acids, vitamins, colour, quality, flavour and shelf life [4]. In sub-continent, desi or MB method is considered best among others. Therefore, by keeping in mind this perception, the current study was planned to investigate best method for preparing desi ghee or clarified butter so that end users can enjoy valuable benefits.

Different experimental techniques have been employed to explore the molecular composition of ghee. Gas chromatography has been used to study the effects of storage on its chemical composition [4] which, however, involve labourous proceedure. Recently, Raman spectroscopy has been employed to characterize desi ghee along with effect of temperature on its molecular composition [1, 6]. Fluorescence spectroscopy, on the other hand, has proved itself a nondestructive and very successful tool for the analysis of vitamins, carotenoids, milk, edible oils and ghee [7–14]. It has been widely used for the analysis of dairy products and also for the monitoring of their processing mechanism [15–20]. This research article contains characterization of cow and buffalo ghee extracted through DC, MS and MB methods on the basis

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of vitamins, beta-carotene and CLA using Fluorescence spectroscopy.

Materials and Methods

Methods to Obtain Ghee from Milk

Desi ghee samples were prepared separately from cow and buffalo milk through three methods: from direct fresh cream (DC), from milk skin (MS) and milk butter (MB). In total, six types of samples were prepared, out of which three from pure buffalo milk and three from pure cow milk by using above mentioned methods.

The fresh milk samples were obtained from a dairy farm in separate vessels in the personal presence of author to ensure the purity. Three samples of buffalo and cow milk were arranged to extract ghee. First milk samples were prepared by cow's milk which were collected separately from 8 cows whereas buffalo milk from 12 different buffalos. All cow and buffalo milk samples were then mixed separately to exclude any type of discrepancy that might be originated from different animals. Second milk samples were prepared by collecting cow milk from two cows and buffalo milk from two buffalo and third milk samples were prepared by collecting cow milk from three cows and buffalo milk from two buffalo separately. These cow and buffalo milk samples were collected from Mian Channu, the southern region of Punjab province, Pakistan. These final milk samples were used to prepare ghee through following methods.

Using first method, ghee samples were prepared by direct cream (DC) method, in which 250 g of fresh cream samples of buffalo and cow were heated at temperature of 115 °C in an open frying pan with continuous stirring for water evaporation. A K-type thermocouple (Reotemp Instruments, USA) was used to monitor the temperature of molted ghee with an uncertainty of ±2 °C. After stirring for a time period of ~ 20 min, ghee got itself fully separated from the other cream residues due to differences in density of fat and non-fat segments [3]. Larger quantity of fresh cream may take different time for the extraction of ghee.

In milk butter (MB) method, fresh buffalo and cow milk samples were separately boiled and cooled up to temperature of ~ 45 °C, then yogurt culture was applied to buffalo and cow milk separately in metal vessels by mixing one teaspoonful of yogurt (pH 4.6) per liter of milk. These mixtures were then kept at room temperature for a time period of ~ 8 h, which as a result turned in to curd. Then some quantity of cold water was added in curd and mixture was churned with a locally developed churning machine in a clean metal vessel for ~ 7 min to obtain desi butter. This butter of 250 g was then heated at a temperature of 115 °C with continuous stirring for a time period of ~ 10–15 min. As a result, buffalo and cow ghee got fully separated from the rest of butter residues.

In milk skin (MS) method, fresh cow and buffalo milk samples of 3 l each were boiled and then cooled for 6 h by placing at cold place. As a result, a thick layer of milk skin or lactoderm was formed on the milk surface, which was removed separately from buffalo and cow milk surface. It was then heated in an open pan with continuous stirring at temperature of 115 °C for a time period of ~ 20 min. As a result, ghee got fully separated from the rest of cream residues. In all three methods, molten ghee samples were filtered by double-folded muslin cloth and poured in 3 ml cuvettes, which were then placed at 4 °C for further spectroscopic analysis.

In addition, samples of vitamins A (Matrix pharma, Pakistan), vitamin D (SAMI Pharmaceuticals, Pakistan), vitamin E (Merck pharma, Pakistan), vitamin K (Munawar Pharma, Pakistan) and CLA (Nature's Bounty, Inc., USA) were purchased from local markets of Islamabad, Pakistan.

Acquisition and Pre-Processing of Fluorescence Spectra

Ghee containing cuvettes were placed one by one in a spectrofluorometer (FluoroMax-4, Horiba scientific, Jobin Yvon, USA) and spectra were recorded by right angle geometry. Closely lying emission spectra were obtained by fixing the slit width of excitation and emission monochromator at 3 and 2 nm, respectively. Ghee samples of buffalo and cow were excited with different wavelengths from 280 to 410 nm with intervals of 10 nm. The excited wavelength at 300 nm displayed with maximum spectral information and therefore was selected for recording additional 5 emission spectra from each ghee sample to eliminate any instrumental and human error. Similarly, emission spectra of vitamins A, D, E, K and CLA were recorded by using 300 nm wavelength to produce a nice comparison with ghee spectra of both ghee types extracted by three methods.

The spectra of ghee samples were pre-processed in MatLab 2014a (The Mathworks, USA). Savitzky-Golay smoothing method by using 5th order polynomial and 13-point window size has been employed for noise reduction and smoothening the spectra. The smoothed spectra were then vector normalized to produce graphs for a nice comparison between spectra of ghee samples extracted by DC, MS and MB methods and vitamins A, D, E and K along with CLA. As a result, the emission spectra highlighted a number of peaks confirming the presence of different molecular structures in ghee.

Results & Discussion

The emission spectra of buffalo and cow ghee samples recorded by using different exciting wavelengths λ_{ex}: 280–410 nm

with interval of 10 nm is presented in Fig. 1. It shows prominent spectral variations in the emission bands from λ_{em}: 350–750 nm. Figure 1a shows the emission spectra of cow ghee and Fig. 1b of buffalo ghee, where different colours have been used to identify the emission bands depicting similar spectral behaviour. Emission spectra of cow ghee obtained by using excitation wavelength from 280 to 300 nm has been assigned red colour, blue colour for excitation wavelength 310–320 nm, green colour for the excitation range 330–370 nm and magenta colour for excitation wavelength range 380–410 nm, respectively. Prominent emission bands appeared at 380, 440, 475, 525, 552, 620, 635, 660 and 670 nm, of whom molecular assignments have been described in subsequent sections (Figs. 1 and 2).

Figure 1b shows the emission spectra of ghee samples obtained from buffalo milk recorded with λ_{ex}: 280–410 nm with interval of 10 nm, where different colours have been used to identify the emission bands depicting similar spectral behaviour. Red colour has been assigned to emission spectral range which were excited from 280 to 320 nm, green colour has been assigned to emission spectra which were excited from 330 to 370 nm and blue colour has been assigned to emission spectra which were excited from 380 to 410 nm, respectively. Prominent emission bands appeared at 390, 440, 475, 552, 635, 660 and 670 nm of whom origins have been described in subsequent sections.

The emission bands emerging at 635, 660 and 670 nm appeared in both ghee samples have been assigned to isomers of chlorophylls [10, 13, 21] and they do not fluoresce at excitation wavelength below 370 nm. In the following sections, effect of extraction methods on the molecular composition of both cow and buffalo ghee has been explained.

Effect of Extraction Methods on Molecular Composition of Cow Ghee

Figure 2a shows the comparison of emission spectra of cow ghee samples extracted through DC, MB and MS methods with the spectra of vitamin A, K, D, E and CLA, where average of 15 spectra of each ghee type has been displayed. The emission band appearing at 380 nm in cow ghee can be assigned to vitamin E, K and vitamin D which is also in accordance with literature [12]. It is evident that the spectrum of vitamin K fully overlaps the spectra of all three types of cow ghee sample at emission band of 380 nm which consequently evidence its presence. Spectra of vitamin D partially overlaps with the shoulder of emission band at 380 nm and its presence in cow ghee samples can be speculated. Vitamin E overlaps

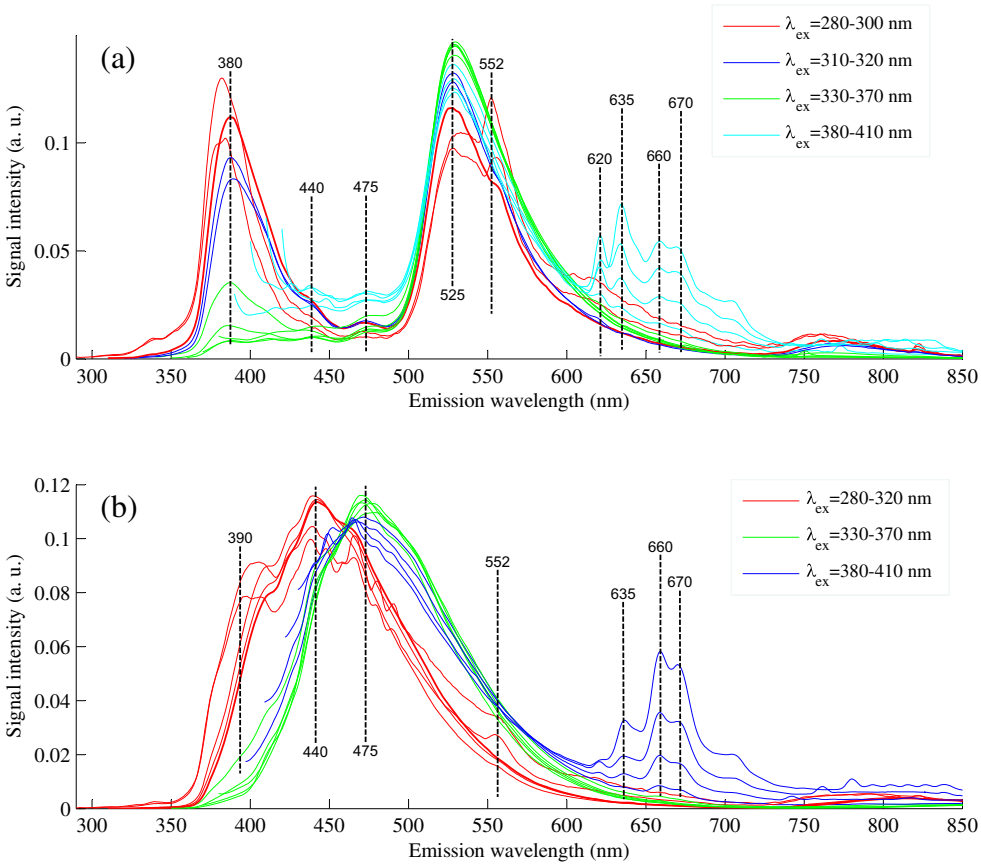

Fig.1 (a) The emission spectra of cow ghee samples excited from 280 to 410 nm with step of 10 nm. (b) The emission spectra of buffalo ghee samples excited from 280 to 410 nm with step of 10 nm

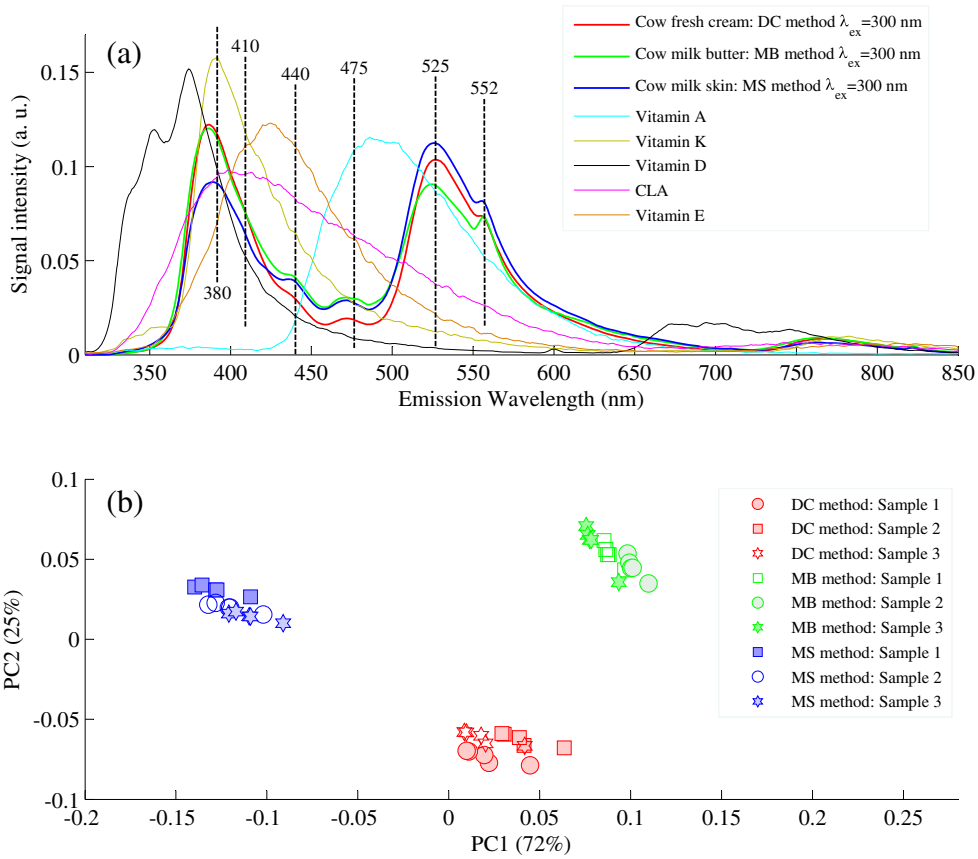

Fig.2 (a) The spectral comparison of the emission spectra of cow ghee samples obtained by direct cream (DC), milk butter (MB) and milk skin (MS) methods with vitamin A, K, D, E and CLA when excited at 300 nm. (b) PCA score plot classification of cow ghee samples extracted by DC, MB and MS methods

with the low intensity emission band at 440 nm and therefore confirms its presence in all three ghee samples extracted through MB, MS and DC extraction methods.

Overall, the spectrum of vitamin K shows relatively strong overlapping with the band at 380 nm as compared to vitamins D and E. It can therefore be inferred that vitamin K has appreciable concentration in all three types of cow ghee samples. Spectrum of CLA evolved as a broad band from 350 to 600 nm and centred roughly at 420 nm, overlaps strongly with a large portion of cow ghee spectra covering emission bands at 380, 440 and 475 nm, ensures its presence in all three types of cow ghee samples, which is already reported [3]. Spectra of vitamin A does not overlap with cow ghee spectra, therefore, it ruled out its presence in cow ghee [3]. The emission band at 525 nm in cow ghee spectra has been assigned to beta-carotene [10, 22] which is a precursor of vitamin-A [23]. The emission band at 552 nm is assigned to oxidized products [22] which might have evolved due to heating of ghee samples during extraction procedure [14].

In order to explore minute spectral variations, principal component analysis (PCA) has been employed on the emission spectra of three sample types to visualize the classification based on dissimilarities either in chemical composition or relative concentration of nutritional ingredients. PCA is an unsupervised chemometric technique, which classifies the samples on the basis of their dissimilarities in the spectral data. The code *pca* in MatLab 2014a (The MathWorks, USA) has been used which calculated principal components (PCs) based on spectral variances. Each principal component explains the variance in data and all PCs are uncorrelated and orthogonal to each other. Therefore, a scatter plot between its PCs produces visual classification in data which clustered in different quadrants on the basis of dissimilarities in data sets.

Figure 2b shows the PCA scatter plot between PC1 (72% variance) and PC2 (25% variance) which displays clustering of cow ghee samples extracted through DC and MB towards the positive side and by MS method towards negative side of PC1 axis. The clustering of three ghee types at different positions in PCA scatter plot depicts that ghee samples extracted by DC, MB and MS methods are slightly different in nutritious values from one another. The clustering of ghee samples extracted through DC and MB method on positive side of PC1 axis shows that they have more similar molecular composition as compared to samples extracted by MS method. From the intensity variation of band at 525 nm, it can be concluded that MS extraction method may retains relatively higher concentration of beta-carotene, second higher by DC and minimum by MB method, respectively.

In order to explain the spectroscopic reasoning of classification in Fig. 2b, loading vectors are produced for three pairs of cow ghee samples extracted through DC & MB, DC & MS and MB & MS methods. The reason is that *pca* code is

designed for producing classification between two types of data sets. However, when it is employed on more than two types of data sets, it very nicely produces the trend of visual classification among them as has been depicted and explained in Fig. 2b. The outcomes of PCA and associated loading vectors for three pairs of cow ghee samples have been depicted in Figs. 3 and 4.

Figure 3a shows comparison of emission spectra between cow ghee samples extracted through DC and MB methods. Prominent spectral variations can be observed at 380, 475 and 525 nm. Figure 3b shows PCA scatter plot with 93% variance in PC1 and 3% variance in PC2. To further illustrate the spectroscopic reasoning of classification shown in Fig. 3b, loading vectors are shown in Fig. 3c, d. Cow ghee samples extracted by DC method clustered on the positive side while ghee samples extracted through MB method clustered towards negative side of PC1 axis of scatter plot which follows from the spectral features associated with the former are loaded positively, while those of the later are loaded negatively.

Evidently, positive loadings can be observed at band of 530 nm and negative loadings at 375 nm (vitamins) which shows that features identified in Fig. 3b are also featured in loadings of PC1 and PC2. Loading positively means that in first type of samples, the relative concentration of corresponding molecules is higher, and negative loading means that the relative concentration of corresponding molecules is lower in second type of samples. This explanation, therefore, suggested that the classification of data groups shown in Fig. 3b is based on the difference in relative concentration of specific molecules in ghee samples extracted through DC and MB methods. There is no separation along PC2 axis in Fig. 3b because there are no clear spectral signatures associated with PC2 in its loading vector.

Figure 4a shows comparison of cow ghee samples extracted through DC and MS methods with prominent spectral variations at emission bands of 380, 440, 475 and 525 nm. Figure 4b shows PCA scatter plot where cow ghee samples extracted by DC method clustered towards positive side and by MS method towards negative side of PC1 axis which follows from the spectral features of former are loaded positively while of the later are loaded negatively as shown in Fig. 4 (c, d). PC1 shows variance of 98% while PC2 only 1% variance, which shows that PC1 explained maximum variance between two data set. In Fig. 4 (c, d), loadings of PC1 shows that emission band at 380 nm loaded positively while at 525 nm loaded negatively depicting the fact that two types of ghee samples extracted by DC and MS methods differ mostly in terms of biomolecules (beta carotene at 510 nm and vitamins at 385 nm) emerging at these two emission band positions. Loading of PC2 are noisy and can thus be neglected.

Another comparison between cow ghee samples extracted through MB and MS methods is shown in Fig. 5a with

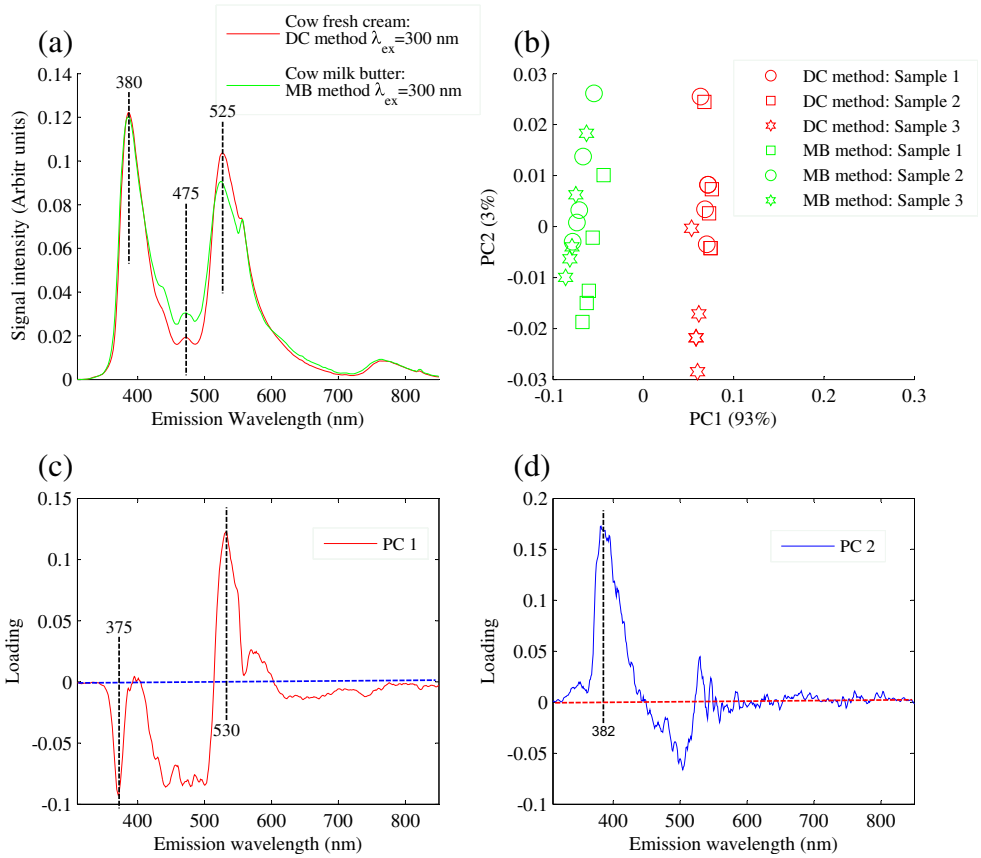

Fig. 3 (a) The spectral comparison of cow ghee samples extracted by DC and MB methods. (b) PCA classification of cow ghee samples extracted by DC and MB methods. (c) Loading vector of PC1. (d) Loading vector of PC2

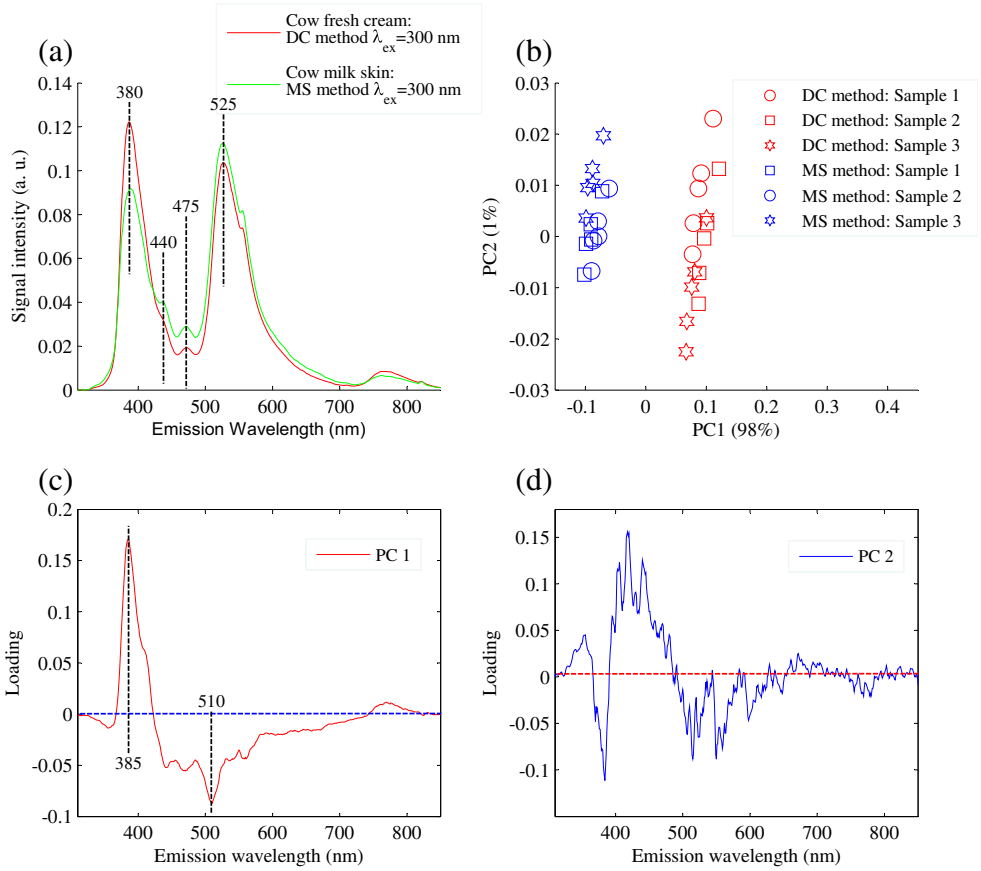

Fig. 4 (a) The spectral comparison of cow ghee samples extracted by DC and MS methods. (b) PCA classification of cow ghee samples extracted by DC and MS methods. (c) Loading vector of PC1. (d) Loading vector of PC2

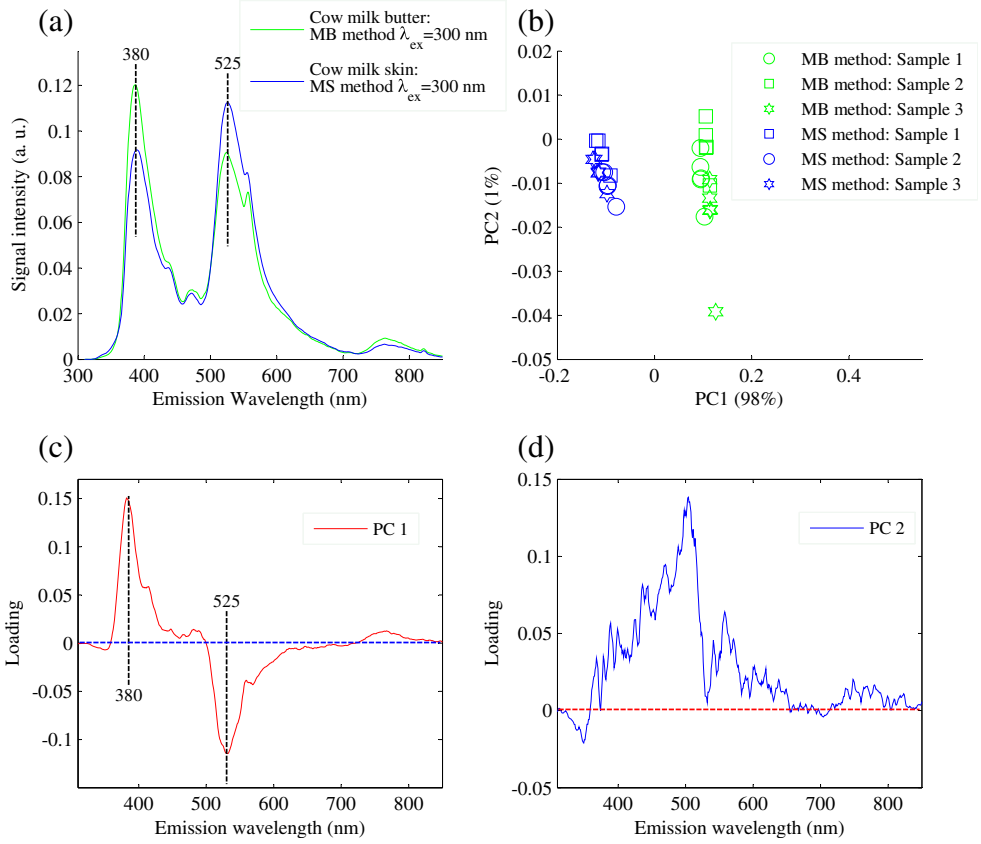

Fig. 5 (a) The spectral comparison of cow ghee samples extracted by MB and MS methods. (b) PCA classification of cow ghee samples extracted by MB and MS methods. (c) Loading vector of PC1. (d) Loading vector of PC2

prominent spectral variations evolving at band positions of 380 and 525 nm and PCA scatter plot between PC1 and PC2 is shown in Fig. 5b. PC1 and PC2 shows variance of 98% and 1% between two data sets. PCA scatter plot completely separates two data set verifying the fact that cow ghee samples extracted by MB and MS methods are different from each other in terms of biomolecules appearing at band positions of 525 and 380 nm. Loadings of PC1 and PC2 are shown in Fig. 5 (c, d) where band at 380 nm loaded positively while at 525 nm loaded negatively. It shows that separation between two ghee samples is based on the difference in relative concentration of biomolecules appearing at emission band positions of 380 and 525 nm induced by MB and MS extraction methods.

It can thus be concluded from above discussion that three extraction methods cast prominent effects on the cow ghee composition. The above qualitative analysis therefore suggested that MS extraction method appears to conserve relatively higher concentration of beta-carotene at 525 nm while DC and MB methods are a good choice for preserving relatively more concentrations of vitamins (D, E and K) at 380 nm. However, regarding CLA, no prominent spectral variations were found in cow ghee samples extracted by three methods.

Effect of Extraction Methods on Molecular Composition of Buffalo Ghee

The comparison of buffalo ghee spectra extracted through DC, MB and MS methods with spectra of vitamin A, K, D, E and CLA have been displayed in Fig. 6a, where average of 5 spectra of each ghee type has been displayed. It shows that the band appearing at 390 nm in buffalo ghee samples may be assigned to vitamin D, K and CLA. It is evident that the spectrum of vitamin D partially overlaps with the shoulder of spectra of all three types of buffalo ghee samples. Spectrum of vitamin K also overlaps partially with the emission band at 390 nm and CLA spectra centred at 410 nm, overlaps strongly with a large portion of buffalo ghee spectra and ensure its presence in all three types of ghee samples. Vitamin E shows a strong overlap with the bands at 390, 410 and 440 nm in all three ghee types and verifies its presence. Vitamin A shows overlapping with the ghee spectra at band position of 470 nm.

The spectral variations shown in Fig. 6a are not much prominent, therefore, chemometric analysis was imperative for the sake of visual classification among buffalo ghee samples obtained by three methods. Principal component analysis (PCA) has been employed on the spectra shown in Fig. 6a and results are shown in Fig. 6b, where PC1 explains 75% variance and PC2 explains 21% variance in the data set. It shows clustering of buffalo ghee samples extracted through DC and MB methods towards the negative side and by MS method towards positive side of PC1 axis. This fact leads to the conclusion that methods DC, MB and MS leaves the ghee samples with slightly different nutritional values. The clustering of ghee samples extracted through DC and MB method on negative side of PC1 axis shows that they have more similar molecular composition as compared to ghee samples extracted by MS method.

The variations in intensity between the emission peaks of buffalo ghee samples show different concentration of the same molecular structures. Therefore, the relative intensity of ghee samples extracted through MS methods looks slightly higher at band positions of 410 nm as compared to DC and MB samples. This can be used to infer that MS method might retain relatively higher concentration of CLA as evidently shown in Fig. 6a.

In order to distinguish the buffalo ghee samples on the basis of DC, MB and MS methods, loading vectors were produced and analysed to find the molecular basis for the separation of data shown in Fig. 6b. The loading vectors for PC1 and PC2 were produced between three pairs of buffalo ghee samples extracted by DC & MB, DC & MS and MB & MS methods, as shown in Figs. 7, 8 and 9. Figure 7a shows comparison of spectra of buffalo ghee samples extracted through DC and MB methods. Minute spectral variations can be observed at 390, 440 and 475 nm. Figure 7b shows PCA scatter plot with 88% variance in PC1 and 5% variance in PC2 where buffalo ghee samples extracted though DC method clustered on the positive side while ghee samples extracted through MB method clustered towards negative side of PC1 axis. The corresponding loading vectors shown in Fig. 7 (c, d) depicted that the spectral features associated with DC samples are loaded positively while those of MB samples are loaded negatively.

Evidently, positive loadings can be observed at fluorescence bands appearing at 390 and 475 nm and negative loadings at 425 nm which shows that features identified in Fig. 7b are also featured in loadings of PC1 and PC2. This explanation suggested that the classification of data in Fig. 7b is based on the difference in relative concentration of molecular composition in both ghee samples extracted through DC and MB methods which mainly appeared at band positions of 390 nm (vitamins D, E and K), at 410 (CLA) and 475 nm (vitamin A). Loadings of PC2 is noisy and can be ignored.

Similarly, Fig. 8a shows comparison of buffalo ghee samples extracted through DC and MS methods with prominent spectral variations at band positions of 410 and 475 nm. Figure 8b shows PCA scatter plot of buffalo ghee samples obtained by DC method clustered towards negative side and by MS method towards positive side of PC1 axis which follows from the spectral features of former are loaded negatively while of the later are loaded positively as shown in Fig. 8 (c, d). PC1 explains 97% variance while PC2 explain 1% variance in data set, which shows that PC1 persist maximum variance between two data set showing that both ghee types

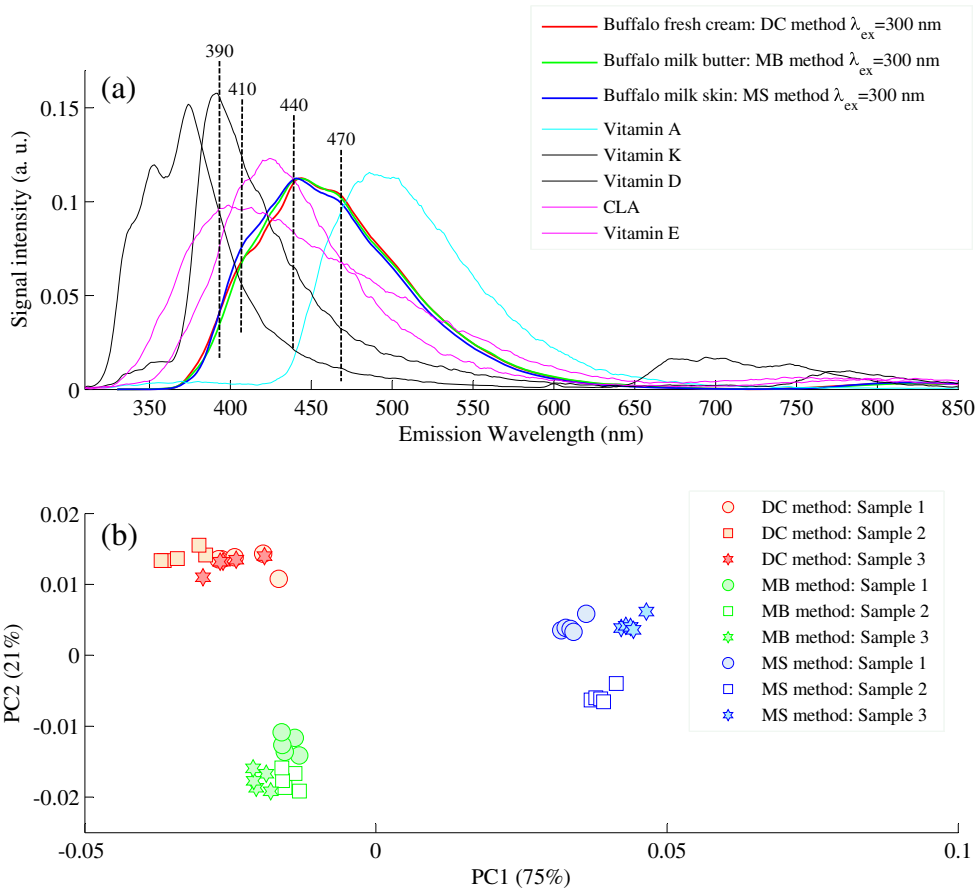

Fig. 6 (a) The spectral comparison of the emission spectra of buffalo ghee samples extracted by direct cream (DC), milk butter (MB) and milk skin (MS) methods with vitamin A, K, D, E and CLA excited at 300 nm. (b) PCA scatter plot classification of buffalo ghee samples extracted by DC, MB and MS methods

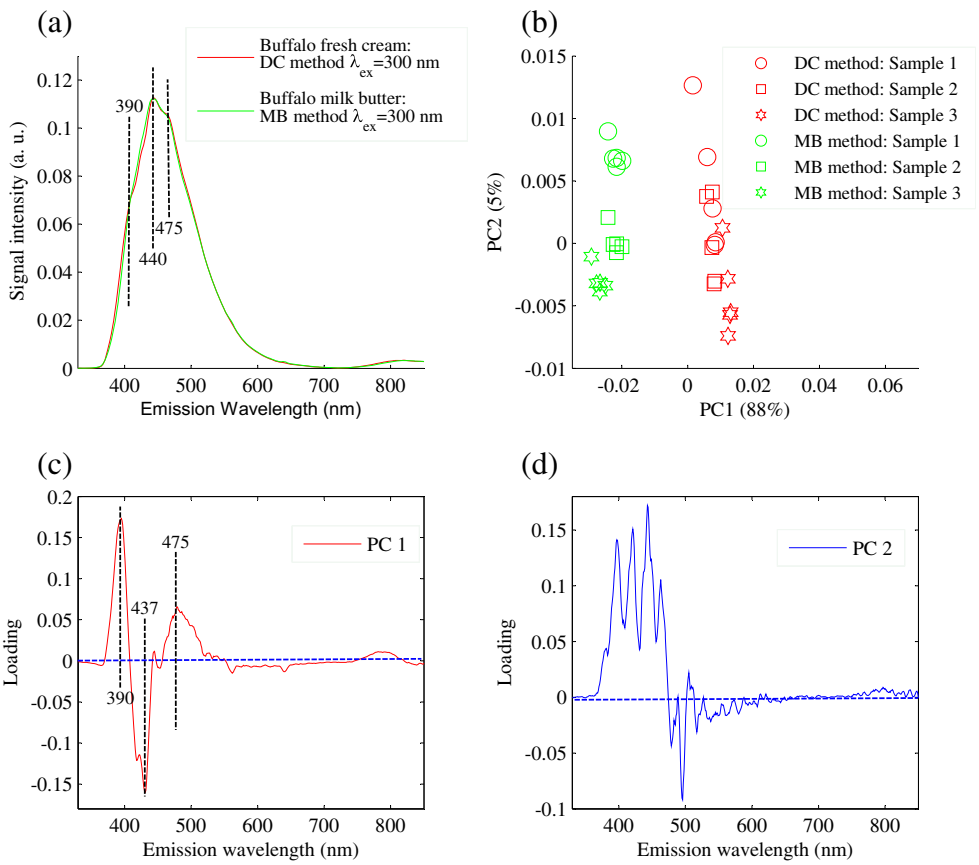

Fig. 7 (a) The spectral comparison of buffalo ghee samples extracted by DC and MB methods. (b) PCA classification of buffalo ghee samples extracted by DC and MB methods. (c) Loading vector of PC1. (d) Loading vector of PC2

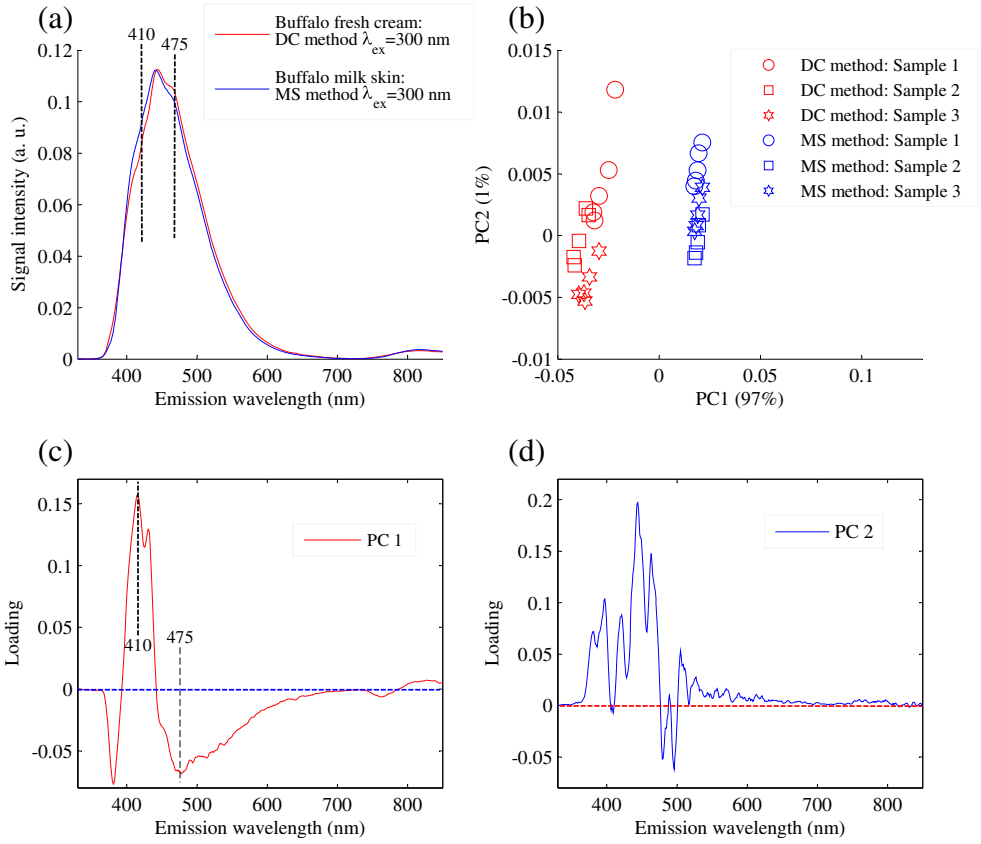

Fig. 8 (a) The spectral comparison of buffalo ghee samples extracted by DC and MS methods. (b) PCA classification of buffalo ghee samples extracted by DC and MS methods. (c) Loading vector of PC1. (d) Loading vector of PC2

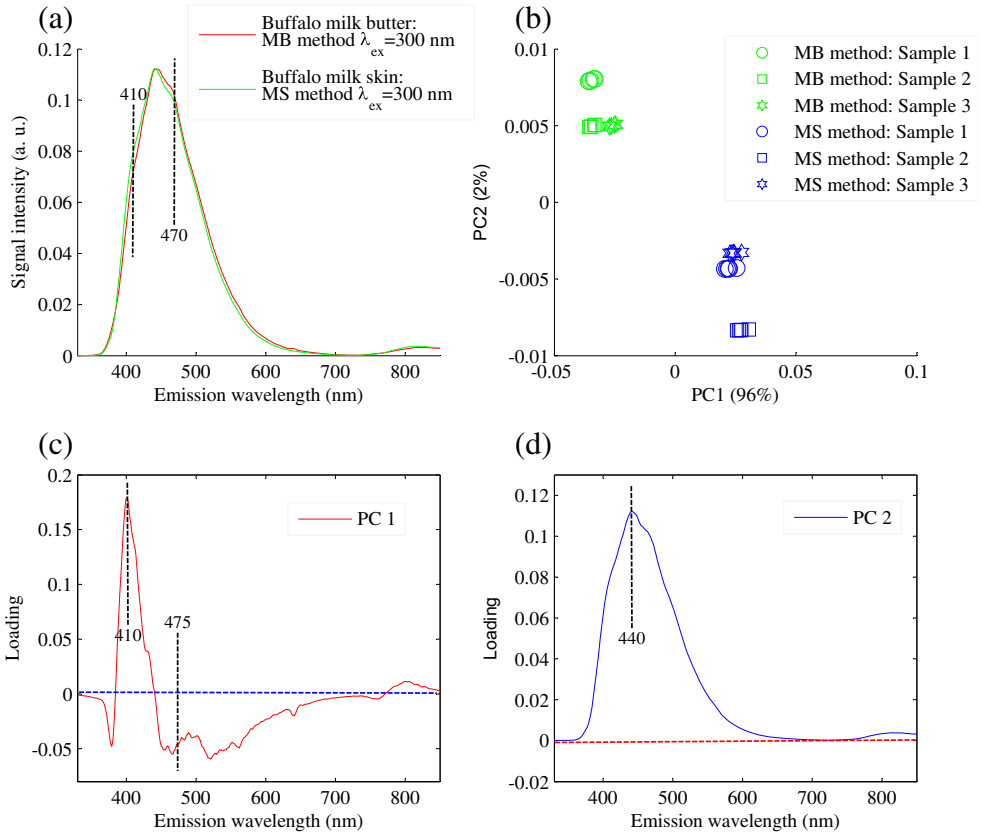

Fig. 9 (a) The spectral comparison of buffalo ghee samples extracted by MB and MS methods. (b) PCA classification of buffalo ghee samples extracted by MB and MS methods. (c) Loading vector of PC1. (d) Loading vector of PC2

are different from each other. Loadings of PC1 shows that band at 410 nm loaded positively while at 475 nm loaded negatively. The loading vectors shows that both ghee types extracted by DC and MS methods differ slightly in relative concentration of biomolecules emerging at these two band positions of 410 nm (CLA) and 475 nm (vitamin A) which also feature in the emission spectra shown in Fig. 8a. There is no separation between buffalo ghee samples extracted by DC and MS methods along PC2 axis because the loading of PC2 is noisy and does not show any spectral signatures which varies along positive and negative of PC2.

In third comparison between buffalo ghee samples extracted through MB and MS methods is shown in Fig. 9a with prominent spectral variations evolving at band positions of 410 and 475 nm. Figure 9b shows PCA scatter plot between PC1 and PC2 for buffalo ghee samples extracted through MB and MS methods. PC1 shows variance of 96% and PC2 2% between two data set. PCA scatter plot completely separates two data set verifying the fact that buffalo ghee samples extracted by MB and MS methods are different from each other. Loadings of PC1 are shown in Fig. 9c where band position at 410 nm loaded positively while at 475 nm loaded negatively. It means that separation between two ghee samples is based on the difference in relative concentration of biomolecules appearing at similar band positions of 410 nm (CLA) and 475 nm (vitamin A) induced by MB and MS extraction methods. Figure 9d shows loadings of PC2 where emission band at 440 nm is loaded positively which verifies the fact that MS extraction method may retains relatively different concentration of CLA appearing around 440 nm as compared to MB method.

Above discussion suggested that the methods MB, DC and MS produce buffalo ghee with varying nutritious values. Overall, MS extraction method can be proposed to appear with higher concentration of vitamins appearing at emission band position of 390 nm and CLA at 410 nm. DC extraction method looks to preserve relatively higher concentration of vitamin A while MB method retains relatively low concentration of CLA and vitamins as compared to other two methods.

Conclusion

The potential of Fluorescence spectroscopy along with chemometrics has been utilized to investigate the effect of ghee extraction methods on the molecular composition of buffalo and cow ghee. It has been found that the extraction methods cast prominent effects on the nutritional values of ghee, which have been verified qualitatively. Results have shown that for cow ghee, MS extraction method retains relatively higher concentration of beta-carotene as compared to DC and MB methods while DC and MB methods can be chosen for preserving relatively more concentrations of vitamins D, E and K. However, no spectral variations were observed for CLA in cow ghee obtained by three methods.

Similarly, for the extraction of buffalo ghee, MS method found to retains relative higher concentration of CLA, DC extraction method preserves relatively higher concentration of vitamin A and MB extraction methods evidenced to preserve relatively low concentration of vitamins and CLA as compared to other methods. As a result, present studies suggested that MB or desi method is not superior among others and every method has some benefits upon others regarding vitamins, beta-carotene and CLA.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Ahmad N, Saleem M, Ahmed M, Mahmood S (2018) Heating effects on Desi ghee using Raman spectroscopy. Appl Spectrosc 72:833–846
2. United States Department of Agricultural (2018) National Nutrient Database for Standard Reference. Baisc Report 01003, Butter oil, anhydrous
3. Sserunjogi ML, Abrahamsen RK, Narvhus J (1998) A review paper : current knowledge of ghee and related products. Int Dairy J 6946: 677–688
4. Andrewes P (2012) Changes in Maillard reaction products in ghee during storage. Food Chem 135:921–928
5. Ganguli NC, Jain MK (1973) Ghee: its chemistry, processing and technology. J Dairy Sci 56:19–25
6. Ahmad N, Saleem M (2018) Raman spectroscopy based characterisation of desi ghee obtained from buffalo milk and cow milk. Int dairy J 1–10
7. Kongbonga YGM (2011) Characterization of vegetable oils by fluorescence spectroscopy. Food Nutr Sci 02:692–699
8. Kulmyrzaev A, Dufour E (2002) Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. Lait 82:725–735
9. Ramanujam N (2000) Fluorescence spectroscopy in vivo. Encycl Anal Chem 20–56
10. Saleem M, Ahmad N, Ali H et al (2017) Investigating temperature effects on extra virgin olive oil using fluorescence spectroscopy. Laser Phys 27:01–10
11. Sikorska E, Khmelinskii IV, Sikorski M et al (2008) Fluorescence spectroscopy in monitoring of extra virgin olive oil during storage. Int J Food Sci Technol 43:52–61
12. Ullah R, Khan S, Ali H et al (2017) Identification of cow and buffalo milk based on Beta carotene and vitamin-a concentration using fluorescence spectroscopy. PLoS One 12:1–10
13. Zandomeneghi M, Carbonaro L, Caffarata C (2005) Fluorescence of vegetable oils: olive oils. J Agric Food Chem 53:759–766

14. Ahmad N, Saleem M (2018) Studying heating effects on desi ghee obtained from buffalo milk using fluorescence spectroscopy. PLoS One 13:e0197340
15. Christensen J, Povlsen VT, Sørensen J (2003) Application of fluorescence spectroscopy and Chemometrics in the evaluation of processed cheese during storage. J Dairy Sci 86:1101–1107
16. Andersen CM, Mortensen G (2008) Fluorescence spectroscopy: a rapid tool for analyzing dairy products. J Agric Food Chem 56:720–729
17. Shaikh S, O'Donnell C (2017) Applications of fluorescence spectroscopy in dairy processing: a review. Curr Opin Food Sci 17:16–24
18. Albani J-R (2012) Fluorescence spectroscopy in food analysis. In: Encyclopedia of Analytical Chemistry. John Wiley & Sons, Ltd, Chichester, UK
19. Ahmad MH, Sahar A, Hitzmann B (2017) Fluorescence spectroscopy for the monitoring of food processes. In: Measurement, Modeling and Automation in Advanced Food Processing. Springer, Cham, pp 121–151
20. Karoui R, Blecker C (2011) Fluorescence spectroscopy measurement for quality assessment of food systems-a review. Food Bioprocess Technol 4:364–386
21. Tena N, García-González DL, Aparicio R (2009) Evaluation of virgin olive oil thermal deterioration by fluorescence spectroscopy. J Agric Food Chem 57:10505–10511
22. Vila FG (2005) Olive oil characterization using excitation-emission fluorescence spectroscopy and three-way methods of analysis, doctoral thesis. Universitat Rovira I Virgili, Spain
23. Olson JA (1989) Provitamin a function of carotenoids: the conversion of β-carotene into vitamin a. J Nutr 119:105–108