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Characterization of fatty acid profile by FFFS

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Abstract The purpose of the study was to assess potential application of front face fluorescence spectroscopy as a rapid and non-destructive technique to discriminate between fats of animal and plant origin based on their fatty acid profiles, and to predict concentration of fatty acids from fluorescence spectra. Vitamin E emission spectra (300–500 nm) of butterfat and vegetable oil samples were recorded with excitation wavelength set at 295 nm. Fatty acid composition of the samples was determined by gas chromatography. Principal component analysis and partial least squares regression analysis were applied to the gas chromatography and fluorescence spectroscopy data. The butter-fats and vegetable oils were discriminated based on the total saturated and unsaturated fatty acids respectively. Tocopherols and tocotrienols accounted for the variability among various oils. A good prediction model was established with $R^2 = 0.745$–0.992 for saturated fatty acids. The unsaturated fatty acids were characterized by low coefficients of determination ($R^2 < 0.339$). The fatty acid profiles predicted from fluorescence spectra did not show significant difference to those determined by gas chromatography used as references. A good association was established between the two data tables. The study demonstrated great potential of front face fluorescence spectroscopy to rapidly discriminate between fats of animal and plant origin, and predict their saturated fatty acids composition, which could in turn be used for detection of milk fat adulteration with vegetable oil.

Keywords Characterization · Prediction · Butterfat · Vegetable oil · Fatty acid profile · Fluorescence spectroscopy

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Introduction

Fatty acid composition of bovine milk and vegetable oils influences technological application of fats and oils and also presents some potential benefits for human health. Plant oils contain many components, such as triacyl- and diacyl-glycerols (TAGs and DAGs) of various saturated and unsaturated fatty acids (UFA) as main components, and tocopherols, phytosterols, phospholipids, free fatty acids, waxes as minor ingredients [1]. Therefore, the composition of oils is a characteristic of the plant species. A number of methods have been developed to date to quantify fatty acid composition of fats and oils, most of which are laborious and expensive, which hamper the implementation of such techniques in monitoring programs at large scale. Chromatographic methods are among the most commonly used methods for determination of fatty acid profiles for animal fats, vegetable oils and their derivatives. However, these methods call for long sample pre-treatment procedures such as saponification and esterification, whereby the fatty acids are first converted to methyl esters.

Spectroscopic methods on the other hand, yield information on the components of a mixture in one spectrum,

and usually without the need for derivatization [2]. The near-infrared reflectance spectroscopy (NIR) has been applied to determine oil content and fatty acid composition in intact seeds of perilla [3]. The fatty acid composition of vegetable oils has also been determined by nuclear magnetic resonance (NMR) spectroscopy [2]. Fluorescence spectroscopy has been used to characterize vegetable oils [4–6]. However, the possibility of using fluorescence spectroscopy in the determination of fatty acid composition has not been explored, hence the unavailability of information in this area. Therefore, the objective of the study was to assess the potential application of fluorescence spectroscopy to rapidly characterize fats of animal and plant origin, and to estimate their fatty acid composition based on vitamin E emission spectra. The performance of fluorescence spectroscopy was also evaluated against the well-established gas chromatography.

Materials and methods

The study was performed on nine and ten commercial butter (identified as BF-A to BF-I) and vegetable oil samples (sunflower seed, maize, canola, rice-bran, peanut, soybean, virgin olive, tea/camellia, sesame and blended oil) respectively. Five butter-fat (BF-A to BF-E) and six vegetable oil samples (sunflower, maize, virgin olive, camellia, rice bran and soybean) were used as calibration samples. Four butterfat (BF-F to BF-I) and vegetable oil samples (blended oil, canola, peanut and sesame) were used as validation samples. Methanol, hexane, sodium hydroxide, boron triflouride, sodium chloride and anhydrous sodium acetate were obtained from Sigma-Aldrich (Shanghai, China). Water used throughout the experiment was purified by Milli-Q system (Millipore, Bedford, MA).

Sample preparation (Gas chromatography)

Firstly, butter was melted at 60 °C, centrifuged at 11,180×*g* for 10 min at 20 °C and filtered through a filter paper. The butterfat and vegetable oil were converted to fatty acids methyl esters using sodium hydroxide following a method described by Araujo et al. [7] with minor modifications. Portions of 0.10 g butterfat and vegetable oil samples were separately added into test tubes with caps. 2 mL of 0.5 mol/L NaOH (in methanol) was added to the mixture, tightly capped and placed in the water-bath for 30 min at 60 °C. 2 mL of 25 % Boron triflouride (BF3) solution (in methanol) was added to the mixture and kept in water-bath for further 20 min. After cooling to room temperature, 2 mL of *n*-hexane was added and stirred. 2 mL of saturated NaCl solution was also added. The top organic part was transferred into dry test tubes after centrifugation (Heraeus Multifuge X1R Centrifuge, Trenton, NJ, USA) for 10 min at 1,789×*g*. Anhydrous sodium acetate was added to the solution to remove the residual water, and the top layer of the solution was transferred into sample tubes for subsequent chromatography analysis. The samples were prepared in quadruplicates and two replicates were mixed together to form duplicate samples used for analysis.

Gas chromatography conditions

Fatty acid analysis was carried out on Shimazu GC-2010 gas chromatograph (Kyoto, Japan) equipped with flame ionization detector and CP-WAX column (30 m × 0.32 mm; 0.25 μm film thickness). The injector and detector temperatures were both set at 250 °C. The carrier gas was nitrogen at the column flow rate of 3 mL/min, the fuel gas was helium at the flow rate of 47 mL/min, and the oxidant gas was air at the flow rate of 400 mL/min. To optimize the conditions, column temperature was programmed as follows: 120 °C in the beginning for 3 min and increased to 190 °C at the rate of 10 °C/min, and hiked further to 220 °C at the rate of 2 °C/min and then maintained for 15 min. A sample of 0.8 μL was injected with the split ratio of 1:8. Fatty acids were identified by comparing their retention times with those of the authentic samples and standards kept in a library of references within the system, as well as data from literature [8]. The results were expressed as w/w (%) of the total fatty acids. The samples were analyzed in duplicates.

Fluorescence spectroscopy

Before measurements, small discs of solid butter (25 mm diameter, 10 mm thick) were taken from the center using the core sampler and mounted between two quartz slides. The samples of neat vegetable oil were placed in a quartz cuvette. Fluorescence spectra were recorded at room temperature from F-7000 FL spectrophotometer (Hitachi High Technology, Tokyo, Japan) mounted with a variable angle front-surface accessory. Two-dimensional scans were performed with incidence angle of excitation set at 56° to ensure that the reflected light, scattered radiation and depolarization phenomenon were minimized. Excitation and emission slits were both set at 5 nm. Emission spectra of vitamin E (300–500 nm) were recorded on the samples with excitation wavelength set at 295 nm. The samples were analyzed in duplicates with three scans performed on each replicate.

Data processing and analysis

Before application of multivariate analytical tools, multiple scans for each replicate were averaged and normalized. Multivariate partial least squares (PLS2) regression

Table 1 Saturated fatty acid composition (%) of butterfat and vegetable oil as determined by gas chromatography

Sample	Butyric C4:0	Caproic C6:0	Caprylic C8:0	Capric C10:0	Lauric C12:0	Myristic C14:0	Palmitic C16:0	Stearic C18:0	Arachidic C20:0
BF-A	1.15 ± 0.03^{d}	1.22 ± 0.04^{c}	0.84 ± 0.14^{a}	2.56 ± 0.08^{c}	4.45 ± 0.07^{d}	11.55 ± 0.12^{d}	30.02 ± 0.09^{k}	11.75 ± 0.17^{k}	nd
BF-B	1.10 ± 0.01^{d}	1.18 ± 0.07^{bc}	0.91 ± 0.12^{a}	2.82 ± 0.06^{d}	4.49 ± 0.07^{d}	12.04 ± 0.08^{e}	29.26 ± 0.13^{j}	11.58 ± 0.12^{k}	nd
BF-C	0.77 ± 0.10^{ab}	1.07 ± 0.06^{abc}	0.76 ± 0.04^{a}	2.27 ± 0.04^{b}	3.13 ± 0.05^{bc}	11.54 ± 0.10^{d}	34.51 ± 0.10^{m}	9.14 ± 0.11^{h}	nd
BF-D	1.02 ± 0.06^{cd}	1.11 ± 0.06^{abc}	0.75 ± 0.12^{a}	2.35 ± 0.08^{bc}	3.21 ± 0.07^{c}	11.97 ± 0.09^{e}	36.11 ± 0.10^{n}	9.16 ± 0.10^{h}	nd
BF-E	0.97 ± 0.08^{bcd}	1.01 ± 0.05^{a}	0.81 ± 0.05^{a}	2.26 ± 0.12^{ab}	3.07 ± 0.06^{bc}	11.55 ± 0.11^{d}	29.54 ± 0.07^{j}	10.12 ± 0.10^{i}	nd
BF-F	0.98 ± 0.04^{bcd}	1.02 ± 0.04^{ab}	0.57 ± 0.12^{a}	2.02 ± 0.06^{a}	2.68 ± 0.11^{a}	10.85 ± 0.11^{b}	28.62 ± 0.17^{i}	10.55 ± 0.11^{j}	nd
BF-G	0.74 ± 0.14^{a}	1.01 ± 0.06^{a}	0.75 ± 0.09^{a}	2.25 ± 0.10^{ab}	3.03 ± 0.06^{bc}	11.17 ± 0.05^{c}	33.57 ± 0.13^{l}	10.32 ± 0.10^{ij}	nd
BF-H	0.93 ± 0.06^{abcd}	1.09 ± 0.05^{abc}	0.76 ± 0.12^{a}	2.34 ± 0.10^{bc}	3.11 ± 0.07^{bc}	11.23 ± 0.10^{c}	33.80 ± 0.15^{l}	10.36 ± 0.15^{ij}	nd
BF-I	0.86 ± 0.12^{abc}	1.03 ± 0.06^{ab}	0.72 ± 0.15^{a}	2.34 ± 0.12^{bc}	2.99 ± 0.03^{b}	10.64 ± 0.16^{b}	30.26 ± 0.13^{k}	12.36 ± 0.13^{l}	nd
Blended oil	nd	nd	nd	nd	nd	0.05 ± 0.03^{a}	6.95 ± 0.23^{c}	3.64 ± 0.25^{c}	0.38 ± 0.08^{bc}
Sunflower	nd	nd	nd	nd	nd	0.06 ± 0.02^{a}	5.71 ± 0.13^{b}	4.02 ± 0.05^{e}	0.53 ± 0.01^{c}
Maize oil	nd	nd	nd	nd	nd	0.03 ± 0.02^{a}	12.40 ± 0.08^{g}	1.72 ± 0.10^{ab}	0.25 ± 0.06^{abc}
Olive oil	nd	nd	nd	nd	nd	0.01 ± 0.00^{a}	10.15 ± 0.10^{f}	2.82 ± 0.12^{c}	0.37 ± 0.11^{abc}
Peanut oil	nd	nd	nd	nd	nd	0.03 ± 0.01^{a}	10.97 ± 0.24^{g}	3.93 ± 0.14^{de}	0.33 ± 0.10^{abc}
Rice bran oil	nd	nd	nd	nd	nd	0.17 ± 0.02^{a}	16.86 ± 0.10^{h}	1.37 ± 0.09^{a}	1.59 ± 0.12^{d}
Sesame oil	nd	nd	nd	nd	nd	0.01 ± 0.00^{a}	8.81 ± 0.13^{e}	4.76 ± 0.11^{f}	0.53 ± 0.10^{c}
Camellia oil	nd	nd	nd	nd	nd	0.04 ± 0.01^{a}	7.86 ± 0.11^{d}	1.98 ± 0.08^{b}	0.48 ± 0.06^{c}
Soybean oil	nd	nd	nd	nd	nd	0.06 ± 0.02^{a}	10.20 ± 0.14^{f}	4.31 ± 0.11^{ef}	0.06 ± 0.03^{ab}
Canola oil	nd	nd	nd	nd	nd	0.11 ± 0.01^{a}	4.29 ± 0.07^{a}	1.82 ± 0.11^{b}	0.33 ± 0.12^{abc}

Values within a column with different letters are significantly different ($p < 0.05$)

nd not detected, *BF* butterfat, *A–I* different butterfat brands

Table 2 Unsaturated fatty acid composition (%) of butterfat and vegetable oil as determined by gas chromatography

Sample	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Gadoleic C20:1
Butterfat				
BF-A	25.40 ± 0.09^{de}	1.26 ± 0.08^{a}	0.55 ± 0.09^{cde}	nd
BF-B	19.27 ± 0.13^{a}	1.36 ± 0.09^{a}	0.63 ± 0.08^{de}	nd
BF-C	25.12 ± 0.11^{d}	2.62 ± 0.10^{d}	0.40 ± 0.06^{bcde}	nd
BF-D	23.16 ± 0.10^{b}	1.72 ± 0.14^{b}	0.57 ± 0.09^{cde}	nd
BF-E	27.21 ± 0.08^{h}	1.24 ± 0.09^{a}	0.65 ± 0.07^{de}	nd
BF-F	28.56 ± 0.12^{i}	1.34 ± 0.10^{a}	0.66 ± 0.12^{de}	nd
BF-G	26.19 ± 0.07^{f}	2.18 ± 0.11^{c}	0.39 ± 0.07^{bcd}	nd
BF-H	25.35 ± 0.12^{de}	2.15 ± 0.11^{c}	0.35 ± 0.16^{abc}	nd
BF-I	26.56 ± 0.18^{g}	3.65 ± 0.19^{e}	0.38 ± 0.10^{bcd}	nd
Veg. oil				
Blended oil	33.18 ± 0.13^{k}	50.42 ± 0.17^{l}	3.51 ± 0.12^{g}	0.42 ± 0.10^{a}
Sunflower oil	25.59 ± 0.13^{e}	62.39 ± 0.14^{o}	0.10 ± 0.00^{a}	1.54 ± 0.02^{b}
Maize oil	31.81 ± 0.13^{j}	52.08 ± 0.06^{m}	0.67 ± 0.11^{e}	0.25 ± 0.10^{a}
Olive oil	80.09 ± 0.07^{p}	5.03 ± 0.03^{f}	0.57 ± 0.13^{cde}	0.36 ± 0.05^{a}
Peanut oil	41.86 ± 0.08^{n}	36.65 ± 0.08^{i}	0.10 ± 0.03^{a}	0.23 ± 0.11^{a}
Rice bran oil	39.98 ± 0.14^{m}	38.68 ± 0.09^{j}	1.05 ± 0.02^{f}	0.82 ± 0.11^{a}
Sesame oil	38.91 ± 0.13^{l}	46.49 ± 0.14^{k}	0.27 ± 0.09^{ab}	0.51 ± 0.12^{a}
Camellia oil	80.14 ± 0.09^{p}	8.82 ± 0.13^{g}	0.23 ± 0.06^{ab}	0.14 ± 0.05^{a}
Soybean oil	23.56 ± 0.09^{c}	53.29 ± 0.13^{n}	6.97 ± 0.14^{h}	0.47 ± 0.10^{a}
Canola oil	59.94 ± 0.16^{o}	20.43 ± 0.13^{h}	7.83 ± 0.12^{i}	0.26 ± 0.10^{a}

Values within a column with different letters are significantly different ($p < 0.05$)

nd not detected, *BF* butterfat, *A–I* different butterfat brands

analysis was applied to the normalized vitamin E spectra and the fatty acid data profile with internal cross validation performed on each analysis. The established model was used to predict fatty acid concentration of validation samples, and the predicted values were compared to the reference values determined by gas chromatography. In order to assess discriminatory power of the fluorescence spectroscopy against the well-established methods, principal component analysis (PCA) was applied to the fatty acid data profile of the samples determined by gas chromatography, and the resulting score plots were compared to those obtained in PLS2 regression analysis. The statistical analysis was carried out using Unscrambler v9.7 (CAMO Software, Domlur, India) and SPSS 16.0 (SPSS Inc., Chicago, USA). Tukey's test was used at $p < 0.05$ to determine the mean differences. Paired samples t test was applied to the measured and predicted fatty acid data.

Results and discussions

Fatty acid composition

Fatty acid profile of butterfat and vegetable oil samples is shown in Tables 1 and 2. The vegetable oil samples did not show the presence of short-chain fatty acids (C4:0–C12:0), but displayed high concentration of long-chain UFA. Butterfat samples were high in saturated fatty acids (SFA). Milk fat contains significantly higher concentrations of short-chain and medium-chain fatty acids and relatively lower concentrations of UFA compared to other dietary sources of vegetable and animal fat [9, 10]. The fatty acid composition of butter samples used in this study was consistent with fatty acid profiles of butter samples reported by Derewiaka et al. [11]. The most important fatty acid of bovine milk fat from quantitative point of view is palmitic acid which accounts for approximately 30 % by weight of the total fatty acids, while myristic and stearic acids make up 11 and 12 % by weight respectively [12].

Among vegetable oil samples, rice bran had the highest amount of palmitic acid, while sunflower had the lowest. The predominant UFA were oleic and linoleic with olive and camellia oil samples showing exceptionally high concentration of oleic acid (≥80 %) relative to others which ranged between 23 and 60 %. The two oil samples also displayed the lowest concentration of linoleic acid while sunflower had the highest concentration. Sunflower oil is a good source of essential linoleic acid and very low in palmitic acid which is believed to increase the low density lipoprotein-cholesterol in blood [13]. Soybean and canola oil samples were found to have high concentration of linolenic acid (6.97 and 7.83 % respectively) relative to other oil samples. Soybean has been reported to be generally having linolenic acid content of 8–10 % [14, 15]. A number of factors might be attributed to the differences in concentrations obtained in this study and others such as environmental conditions, time of harvest, stability after harvest, refining procedure, commercial hydrogenation procedures [16], and/or variations between laboratories.

Principal component analysis of fatty acid profiles

Figure 1 shows the PCA similarity map and the factor loadings corresponding to fatty acid profiles of butterfat and vegetable oil samples. The samples were discriminated according to the first two principal components (PCs) which accounted for 99 % of total data variability (Fig. 1a). According to PC1 (63 %), butterfat and vegetable oil samples were clearly discriminated from each other with negative and positive scores respectively. This was attributed mainly to the high content of saturated and UFA in butterfat and vegetable oil respectively as seen in Tables 1 and 2. Among the vegetable oils, those with high positive PC2 scores (36 %) and those with high negative

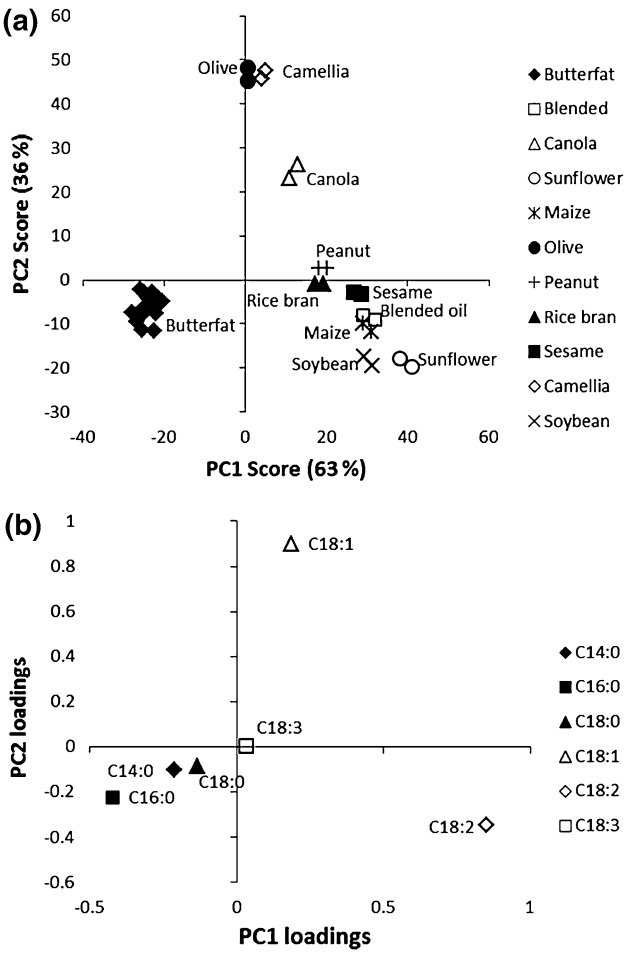

Fig. 1 PCA similarity map **a** and matrix loadings plot **b** for fatty acid profiles of butterfat and vegetable oil samples

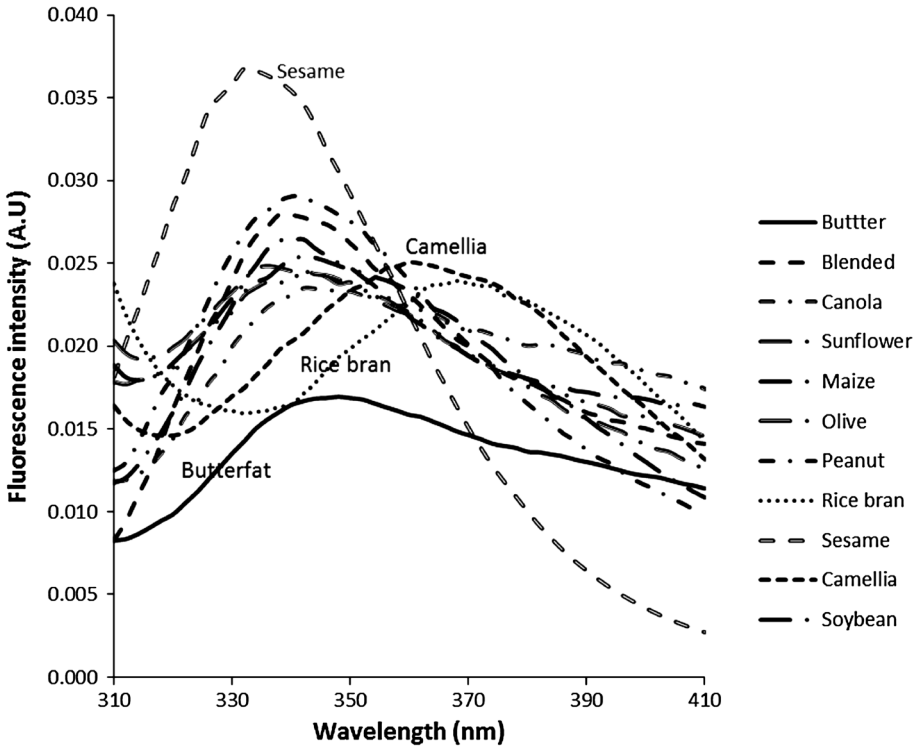

Fig. 2 Vitamin E emission spectra for butterfat and vegetable oils recorded after excitation at 295 nm

scores correlated well to oleic and linoleic acids respectively (Fig. 1b).

Vitamin E emission spectra

Figure 2 shows normalized vitamin E emission spectra for the representative butterfat and various vegetable oil samples. All the butterfat samples displayed similar spectra with emission maxima at around 345 nm, therefore one representative spectrum is shown to enable easy viewing of the figure. Among the vegetable oils, sesame displayed exceptionally high fluorescence intensity at 332 nm. Majority of the samples showed maxima at around 340 nm, with peanut and canola oils displaying highest and lowest intensity at this wavelength. The difference in intensity might be related to various external factors associated with oil samples [16], and to the variations in the content of vitamin E compounds for different vegetable oils [17]. The butterfat spectra on the other hand displayed exceptionally low intensity relative to the oils probably because vitamin E is higher in vegetable oil than butterfat. Therefore, the variations in fluorescence intensity between the butterfat and vegetable oil, and among the oil samples might be attributed to vitamin E content. Camellia and rice bran spectra displayed emission maxima at 360 and 370 nm respectively, which may be ascribed to the concentration of various vitamin E compounds including both tocopherols and tocotrienols in different forms [18–20]. Tocopherols are known to produce intense peaks at 315–330 nm [4–6]. Non-esterified tocopherols and tocotrienols exhibit strong native fluorescence at 295/330 nm especially α-tocopherols [5]. The fluorescence maxima of other tocopherols are at slightly longer wavelengths in accordance with their absorbance spectra.

Partial least squares regression (PLSR) analysis

Figure 3 shows score and factor loadings plots from the PLSR analysis of normalized vitamin E spectra and the fatty acid data profile of butterfat and vegetable oil samples. The analysis was focused only on these six fatty acids which were detectable in both butterfat and vegetable oil, excluding short-chain and long-chain fatty acids: myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3).

Fluorescence spectra of the samples were discriminated according to the first three PCs which accounted for 96 % of total data variability. Considering PC1 (65 %) and PC2 (21 %), butterfat spectra were clearly discriminated from those of vegetable oils which clustered towards the center with positive PC2 scores while the butterfat spectra were characterized by the negative scores for both PC1 and PC2 (Fig. 3a). Sesame was discriminated from other oils with positive and negative PC1 and PC2 scores respectively. The fatty acid matrix loadings showed that linoleic acid was highly negatively correlated to the SFA especially palmitic (Fig. 3b). The plots in Fig. 3a, b are similar to those in Fig. 1, which shows the great potential of

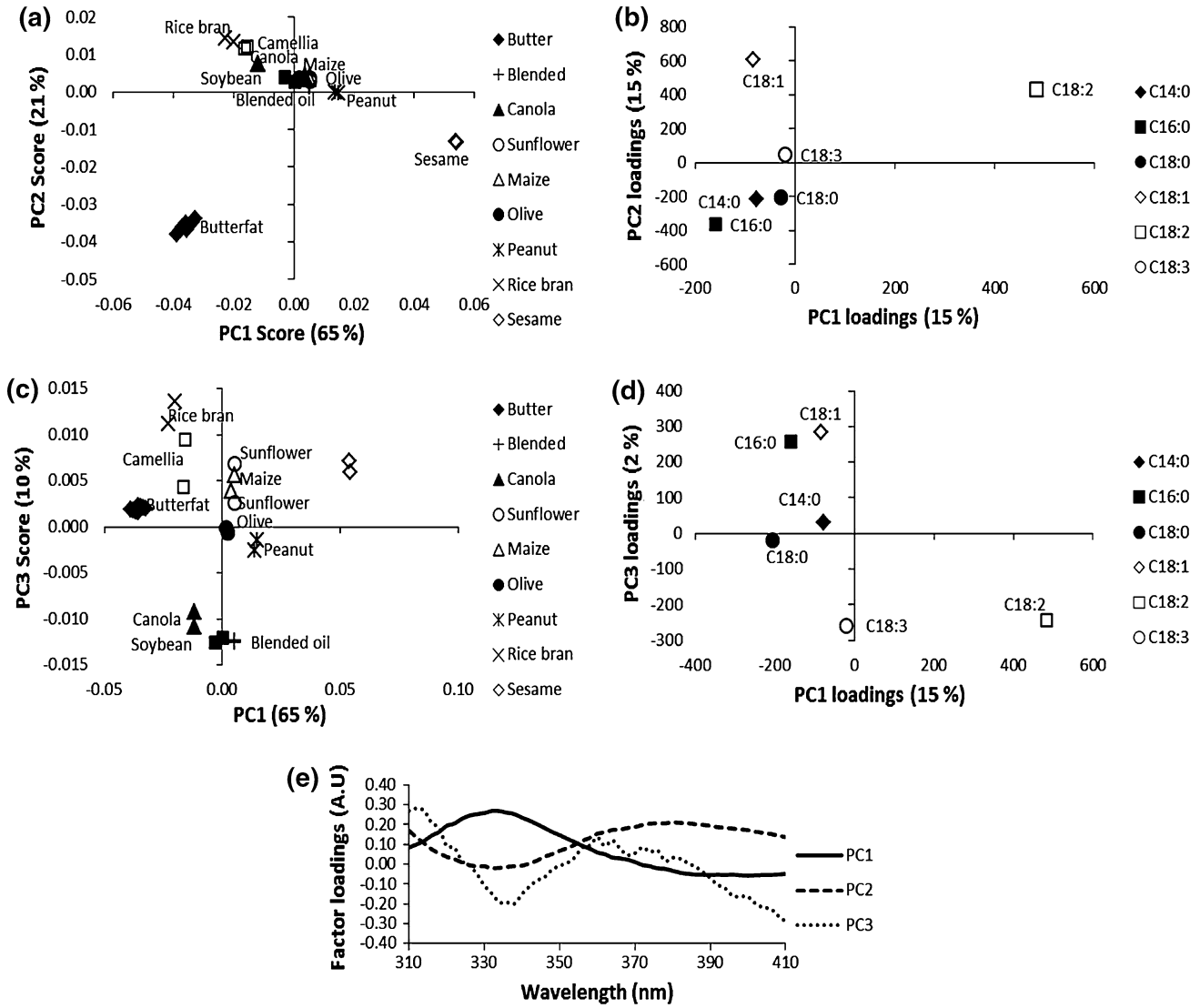

Fig. 3 Multivariate partial least squares regression analysis similarity maps (**a** and **c**), and factor loadings corresponding to fatty acid profiles (**b** and **d**) and fluorescence (**e**) for vitamin E emission spectra and fatty acid profiles of butterfat and vegetable oil samples as determined by gas chromatography

fluorescence spectroscopy to rapidly discriminate between butterfat and vegetable oil samples. The spectra for canola, soybean and blended oil samples were displayed with low negative PC3 (10 %) scores (Fig. 3c), and this correlated to linolenic acid (Fig. 3d). This was ascribed to high concentration of linolenic acid in these oil samples as indicated in Table 2.

The sample patterns displayed on score plots (Fig. 3a, c) also corresponded to the spectral factor loadings (Fig. 3e). The spectral loadings corresponding to PC1 displayed an opposition between a positive peak at 332 nm and negative band at around 386 nm, and the opposite was true for the spectral loadings corresponding to PC2. This was attributed to the variations in the tocopherols content in the samples as shown in normalized spectra (Fig. 2). The spectral loadings corresponding to PC3 was somehow noisy and not easy to interpret, however, an opposition between a negative peak at 334 nm and small positive peaks beyond 360 nm were recognized, and this might be describing the variations in the spectra of the rice bran and camellia which were different from others as shown in Fig. 2.

Prediction of fatty acid profiles

The potential of the established model to be used in the prediction of fatty acids concentration was evaluated by comparing coefficients of determination (R^2) corresponding to the six fatty acids. SFA showed higher prediction potential ($R^2 = 0.992$, 0.745 and 0.945 for C14:0, C16:0 and C18:0, respectively) than the UFA which were

Table 3 Predicted and measured fatty acid concentrations of butterfat and vegetable oil

Samples	Predicted C14:0	Measured C14:0	Predicted C16:0	Measured C16:0	Predicted C18:0	Measured C18:0	Predicted C18:1	Measured C18:1	Predicted C18:2	Measured C18:2	Predicted C18:3	Measured C18:3
Butterfat												
BF-F	12.02	11.61	31.86	32.27	12.21	12.35	25.16	25.03	0.16	1.20	0.63	0.42
BF-G	12.02	11.51	31.33	32.84	11.97	11.92	25.69	25.31	1.07	1.32	0.65	0.58
BF-H	11.44	11.58	30.83	32.31	11.75	12.13	26.19	25.71	1.92	1.33	0.67	0.74
BF-I	9.312	11.82	27.00	31.66	10.05	11.59	30.03	25.47	8.45	1.26	0.83	0.60
Veg. oil												
Blended oil	0.24	0.04	10.72	6.82	2.83	3.54	46.34	33.12	36.20	50.33	1.51	3.46
Canola	0.80	0.12	11.73	4.24	3.27	1.76	45.33	59.83	34.49	20.34	1.47	7.75
Peanut	−0.20	0.03	9.92	10.84	2.47	3.86	47.14	41.83	37.57	36.61	1.54	0.09
Sesame	−1.30	0.01	7.95	8.75	1.60	4.71	49.11	38.92	40.92	46.43	1.63	0.24

BF butterfat, *F–I* different butterfat brands, *Measured fatty acids* by gas chromatography, *Predicted fatty acids* by fluorescence spectroscopy

Table 4 Paired samples correlations for predicted and measured fatty acids

FA pairs[a]	N	Correlation	Sig. ($p < 0.05$)
C14:0	8	0.984	0.000
C16:0	8	0.970	0.000
C18:0	8	0.960	0.000
C18:1	8	0.746	0.034
C18:2	8	0.926	0.001
C18:3	8	0.387	0.343

N number of samples

[a] Pairs = Predicted and measured fatty acids

Table 5 Paired samples test for predicted and measured fatty acids

FA pairs[a]	Paired differences			*t*	df	Sig. (2-tailed)
	Mean	SD	SEM			
C14:0	−0.299	1.088	0.385	−0.776	7	0.463
C16:0	0.201	3.757	1.328	0.151	7	0.884
C18:0	−0.714	1.355	0.479	−1.490	7	0.180
C18:1	2.471	8.361	2.956	0.836	7	0.431
C18:2	0.245	8.314	2.939	0.083	7	0.936
C18:3	−0.619	2.516	0.889	−0.696	7	0.509

SD std. deviation, *SEM* std. error mean. Sig. ($p < 0.05$)

[a] Pairs = Predicted-measured fatty acids

characterized by low R^2 values ($R^2 = 0.171$, 0.339 and 0.077 for C18:1, C18:2 and C18:3, respectively). The fluorescence spectra for validation (four butter-fats and vegetable oils) samples were used to validate the model by predicting their fatty acid concentrations and comparing them to the reference values determined by gas chromatography (Table 3). For butter-fats, SFA were better predicted than UFA probably due to the fact that milk fat contains significantly higher concentrations of short-chain and medium-chain SFA and relatively lower concentrations of UFA [9, 10]. Significant correlation was found between the measured and predicted fatty acids, with the exception of linolenic acid (Table 4). The paired samples test for the measured and predicted fatty acids established that there were no significant differences ($p > 0.05$) between the means of measured and predicted fatty acids as shown in Table 5, which indicates a good association between data obtained from gas chromatography and fluorescence spectroscopy.

It was therefore established in the present study that fluorescence spectroscopy in combination with chemometric tools has great potential to rapidly discriminate between butterfat and different vegetable oil samples. This enabled it to be used for detection of butterfat adulteration with vegetable oil and characterize commercial milk of different composition and origin [21, 22]. The fatty acid profiles of fats and oils have also been successfully predicted from their fluorescence spectra. Arising from the effects of geographical origin and processing conditions, it is therefore appealing to us to carry out a further study using more samples of oil and butterfat in order to also validate the results obtained in the current preliminary study.

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