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Short communication: Rapid detection of milk fat adulteration with vegetable oil by fluorescence spectroscopy

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ABSTRACT

This study assessed the potential application of fluorescence spectroscopy in detecting adulteration of milk fat with vegetable oil and characterizing the samples according to the source of the fat. Pure butterfat was adulterated with different vegetable oils at various concentrations (0, 5, 10, 15, 20, 30, and 40%). Nonfat and reduced-fat milk were also adulterated with vegetable oils to simulate full-fat milk (3.2%). The 2- and 3-dimensional front-face fluorescence spectroscopy and gas chromatography were used to obtain the fluorescence spectra and fatty acid profile, respectively. Principal component analysis and 3-way partial least squares regression analysis were applied to analyze the data. The pure and adulterated samples were discriminated based on the total concentration of saturated fatty acids and unsaturated fatty acids, and also on the 3 major fluorophores: tryptophan, tocopherols, and riboflavin. Fluorescence spectroscopy was able to detect up to 5% of adulteration of vegetable oil into the butterfat. The saturated fatty acids showed higher predictability than the unsaturated fatty acids (R^2 = 0.73–0.92 vs. 0.20–0.65, respectively). The study demonstrated the high potential of fluorescence spectroscopy to rapidly detect adulteration of milk fat with vegetable oil, and discriminate commercial butter and milk according to the source of the fat.
Key words: detection, milk fat adulteration, vegetable oil, fluorescence spectroscopy

Short Communication

Milk fat is an important food component and plays a significant role in the economics, nutrition, and physical and chemical properties of milk and milk-derived products; it is also a good source of fat-soluble vitamins and essential FA (Kumar et al., 2010). Due to increased demand, milk fat has been a target of the fraudulent practices, such as replacement with cheaper or more readily available non-milk fats of plant and animal origin. Replacement of milk fat with fat from other sources is not only an economic fraud, but may also pose a risk to human health.

Fatty acid composition of milk fat has long been used as criteria to detect adulteration with vegetable oil, mainly because milk fat is characteristic of short-chain FA (Molkentin and Precht, 1998), whereas vegetable oils have medium- to long-chain FA. However, FA composition is influenced by several factors, such as nutrition and genetics. Several methods have been developed to date for detection of milk fat adulteration, some of which are laborious and expensive, thereby limiting the application on a regular basis. Conversely, spectroscopic methods yield information on the components of a mixture in one spectrum, and usually without the need for derivatization (Knothe and Kenar, 2004). Although fluorescence spectroscopy has been applied in several studies, the use of multidimensional fluorescence spectroscopy in dairy products has not been widely explored, and very limited information is available in this area. Therefore, the objective of the study was to evaluate the potential application of 2- and 3-dimensional (**3D**) front-face fluorescence spectroscopy, combined with chemometric tools, to detect adulteration of vegetable oil in milk fat and characterize the samples according to the source of adulterant oil and the level of adulteration.

Commercial butter, full-fat (3.2%), reduced-fat (1.2%), and nonfat milks, as well as vegetable oils (sunflower, canola, maize, and rice bran) were obtained from the local supermarket. The reagents (methanol, hexane, sodium hydroxide, boron triflouride, sodium chloride, and anhydrous sodium acetate) were obtained from Sigma-Aldrich (Shanghai, China). First, the butter was melted at 60°C, centrifuged at 11,180 × *g* for 10 min at 20°C, and filtered through a filter paper. The filtered butterfat was adulterated with canola and sunflower oils at various concentrations (0, 5, 10, 15, 20, 30, and 40%). The butterfat was also adulterated

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Table 1. Saturated fatty acid composition (%) of commercial and adulterated milks

Sample[1]	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
CW1	0.73 ± 0.03^{c}	1.12 ± 0.03^{d}	0.86 ± 0.03^{de}	2.30 ± 0.03^{d}	2.84 ± 0.03^{f}	10.70 ± 0.03^{j}	33.36 ± 0.04^{l}	12.09 ± 0.04^{i}	0.14 ± 0.01^{ab}
CW2	0.56 ± 0.03^{b}	1.06 ± 0.03^{cd}	0.91 ± 0.03^{e}	2.60 ± 0.03^{d}	3.20 ± 0.03^{g}	10.84 ± 0.03^{k}	30.53 ± 0.03^{i}	13.32 ± 0.03^{l}	0.16 ± 0.03^{ab}
CW3	0.47 ± 0.03^{bc}	1.00 ± 0.01^{c}	0.75 ± 0.02^{d}	2.02 ± 0.03^{cd}	2.70 ± 0.02^{e}	9.68 ± 0.03^{h}	31.97 ± 0.03^{j}	12.65 ± 0.04^{j}	0.21 ± 0.03^{abcd}
CW4	0.80 ± 0.03^{d}	1.14 ± 0.03^{d}	0.83 ± 0.02^{de}	2.55 ± 0.02^{d}	2.72 ± 0.02^{e}	10.43 ± 0.03^{i}	32.54 ± 0.04^{k}	12.77 ± 0.03^{k}	0.13 ± 0.03^{a}
SFc	0.47 ± 0.02^{ab}	0.74 ± 0.04^{b}	0.48 ± 0.04^{bc}	1.21 ± 0.01^{b}	1.49 ± 0.01^{c}	5.90 ± 0.02^{e}	22.28 ± 0.03^{e}	9.61 ± 0.03^{f}	0.28 ± 0.01^{cd}
SFf	0.44 ± 0.03^{ab}	0.63 ± 0.03^{a}	0.49 ± 0.03^{bc}	1.28 ± 0.01^{b}	1.57 ± 0.01^{c}	6.20 ± 0.02^{f}	22.74 ± 0.02^{f}	10.47 ± 0.03^{h}	0.17 ± 0.01^{ab}
SFm	0.50 ± 0.03^{bc}	0.80 ± 0.04^{b}	0.57 ± 0.03^{c}	1.45 ± 0.02^{bc}	1.78 ± 0.03^{d}	6.94 ± 0.02^{g}	26.45 ± 0.02^{h}	10.23 ± 0.03^{g}	0.19 ± 0.01^{abc}
SFr	0.40 ± 0.03^{a}	0.61 ± 0.02^{a}	0.44 ± 0.02^{b}	1.11 ± 0.01^{b}	1.35 ± 0.02^{b}	5.44 ± 0.02^{d}	25.41 ± 0.03^{g}	8.23 ± 0.02^{e}	0.25 ± 0.01^{bcd}
RFc	ND[2]	ND	0.07 ± 0.01^{a}	0.14 ± 0.01^{a}	0.23 ± 0.01^{a}	0.60 ± 0.01^{a}	6.39 ± 0.02^{a}	2.73 ± 0.02^{c}	0.49 ± 0.02^{e}
RFf	ND	ND	0.07 ± 0.01^{a}	0.14 ± 0.01^{a}	0.18 ± 0.01^{a}	0.63 ± 0.01^{a}	7.69 ± 0.03^{b}	5.05 ± 0.02^{d}	0.29 ± 0.01^{cd}
RFm	ND	ND	ND	0.16 ± 0.01^{a}	0.24 ± 0.01^{a}	0.76 ± 0.04^{b}	14.00 ± 0.03^{c}	2.49 ± 0.02^{b}	0.30 ± 0.01^{d}
RFr	ND	ND	0.08 ± 0.01^{a}	0.17 ± 0.01^{a}	0.23 ± 0.01^{a}	0.88 ± 0.03^{c}	18.16 ± 0.03^{d}	2.08 ± 0.03^{a}	0.43 ± 0.02^{e}

$^{a-i}$Values within a column with different letters are different ($P < 0.05$).

[1]The uppercase letters in the sample names represent the type of milk (CW = commercial whole milk; RF = replaced fat; SF = supplemented fat), whereas the numbers and the lowercase letters represent the various manufacturers and adulterant oils, respectively (1–4 = manufacturers; c = canola; f = sunflower; m = maize; r = rice bran).

[2]ND = not detected.

with rice bran and maize oils for comparison purposes. The adulterated butterfat samples were prepared in 2 groups; one was used for gas chromatographic analysis, whereas the other was used for fluorescence measurement (dissolved in n-hexane before measurement). Next, the 2 types of adulterated milks were prepared to simulate full-fat milk (3.2%), and the samples consisted of the complete replacement and partial supplementation of milk fat with vegetable oil. The samples were homogenized (Nano homogenize machine, AH basic model, ATS Engineering Inc., Shanghai, China) to ensure thorough mixing of milk and oil. The commercial milk from different manufacturers were obtained and used as control. For FA analysis, milk fat separation was carried out following the rapid method developed by Feng et al., (2004) with minor modifications. Briefly, 30 mL of milk samples were tempered to 20°C and centrifuged (Multifuge X1R Centrifuge, Heraeus, Trenton, NJ) at 12,555 × *g* for 30 min. The fat layer was transferred to micro tubes and allowed to stand for 30 min at room temperature before being centrifuged at 14,737 × *g* for 20 min at the same temperature. The top layer was taken for analysis.

The milk fat and vegetable oil mixtures were converted to FA methyl esters using sodium hydroxide. A portion of 0.10 g butterfat or vegetable oil was added into test tubes with caps. Two milliliters of 0.5 mol/L NaOH (in methanol) was added to the mixture, tightly capped, and placed in a water bath for 30 min at 60°C. Two milliliters of 25% boron trifluoride solution (in methanol) was added to the mixture and kept in a water-bath for 20 min at 60°C. After cooling to room temperature, 2 mL of n-hexane was added and stirred. Two milliliters of saturated NaCl solution was added, and the top organic part was transferred into a dry test tube after centrifugation 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 the sample tubes for subsequent chromatographic analysis. The samples were prepared in quadruplicates and 2 replicates were mixed together to form duplicate samples used for analysis.

Gas chromatography was used to determine the FA composition of the samples. Analysis was carried out on Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) equipped with flame ionization detector and a 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 hydrogen 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, the column temperature was programmed as follows: 120°C in the beginning for 3 min, increased to 190°C