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Using phytosterol as a target compound to identify edible animal fats adulterated with cooked oil

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ABSTRACT

Incidents of edible oil adulteration have increased all over the world, and improving analytical methods that are capable of identifying adulterated oils has become more important. In this study, we developed an analytical method that uses 4 target compounds belong to phytosterol family to identify lard that has been adulterated with cooked oils. For this, we used five kinds of animal fat, including lard, tallow, duck fat, goose fat, and chicken fat, to estimate the matrix effect, and lard samples were used to verify this analytical method. Specifically, samples inspected by sanitation authorities in a 2014 lard adulteration incident in Taiwan were saponified, and phytosterols were extracted and analyzed by a liquid chromatography-tandem mass spectrometer (LC/MS/MS) equipped with an atmospheric pressure chemical ionization (APCI) source. Parameters for sample extraction, including temperature, concentration of alkaline solution, and duration of saponification, were optimized. Our proposed method was then validated for linearity, matrix effect, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). After using our proposed method to analyze 28 lard samples, we determined that the phytosterol contents of inspected lard obtained from companies that had been found guilty in the 2014 lard adulteration incident were ranged from 19.5 to 205.3 μg/g in campesterol and 17.3–408.8 μg/g in β-sitosterol, and were at most 270-fold higher than that of homemade lard, commercial lard, and inspected lard (ranged from 4.5 to 29.6 μg/g in campesterol and 1.5–44.2 μg/g in β-sitosterol) obtained from companies that had not been found guilty in the 2014 adulteration incident. Therefore, our proposed method should be useful to discriminate between adulterated and unadulterated animal fats.

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1. Introduction

Animal fats, such as lard, tallow, goose fat, duck fat, and chicken fat, are widely used in pan-frying, stir-frying, and deep-frying due to a desirable flavor which differs from that of vegetable oils, particularly in Asian countries. In 2014, a Taiwanese trader was discovered to have added cooked oil to lard in order to reduce production costs which has been described in our previous report (Peng et al., 2017). These adulterated lards were mostly used in street foods and night market foods. It caused great economic loss to catering industry, affected public health, and highlighted the importance of analytical methods which can reliably identify adulterated oils. Some such analytical methods have already been established: for example, the triacylglycerol profile of vegetable oils can be determined using high performance liquid chromatography (HPLC) and an evaporative light scattering detector (ELSD) (Cunha & Oliveira, 2006); edible oil mixtures can be simultaneously determined using partial least squares modeling and gas-chromatographic fatty-acid fingerprints (Hajimahmoodi et al., 2005); and low field nuclear magnetic resonance (LF-NMR) can be used to identify commercial vegetable oils that have been adulterated with cooked oil (Zhang, Saleh, & Shen, 2013). However, these methods were established for vegetable oils and lack a target

Abbreviations: APCI, atmospheric pressure chemical ionization; GC, gas chromatography; GC/MS/MS, gas chromatography-tandem mass spectrometry; HPLC, high performance liquid chromatography; ELSD, evaporative light scattering detector; IS, internal standard; LC/MS/MS, liquid chromatography-tandem mass spectrometry; LF-NMR, low field nuclear magnetic resonance; LOD, limit of detection; LOQ, limit of quantitation; MCC, matrix matched calibration curves; MRM, multiple reaction monitoring; S/N ratio, signal to noise ratio; SCC, standard calibration curves.

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compound for effective identification. Since the oil refining process reduces the values of most common quality indexes (Bhattacharya, Sajilata, Tiwari, & Singhal, 2008; Miyagi, Subramanian, & Nakajima, 2003), there are currently no effective methods to discriminate between refined cooked oils and natural edible oils.

Phytosterols are sterols in plants and belong to the triterpene family. As cholesterol stabilizes cell membranes in animals, phytosterols are believed to increase the rigidity of cell membranes in plants (Itzhaki, Borochov, & Mayak, 1990). Among vegetable oils, corn oil and rapeseed oil contain the highest concentrations of free phytosterols (Verleyen et al., 2002). In these plants, the most common phytosterols are campesterol, β-sitosterol, and stigmasterol (Moreau, Whitaker, & Hicks, 2002). Phytosterol content is affected by genetic factors, storage conditions, and process conditions (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000), and can vary in both natural and processed food products. Phytosterols that occur in food consumed by humans are primarily derived from vegetable oils, nuts, seeds, and cereals (Piironen et al., 2000), and humans consume between 160 and 400 mg of phytosterols per day (Ahrens & Boucher, 1978; Cerqueira, Fry, & Connor, 1979).

It was found that, the contents of major phytosterols, including campesterol, β-sitosterol, and stigmasterol are not significantly affected by the oil refining process (Kochhar, 1983). Therefore, these phytosterols could potentially serve as target compounds to identify animal fats that have been adulterated with refined cooked oils. The objectives of this study were to (1) characterize phytosterol contents in homemade lards, commercial lards, and lards that were inspected in the 2014 adulteration incident that occurred in Taiwan, and (2) evaluate the effectiveness of using phytosterol as a target compound to identify adulterated animal fats. To the best of our knowledge, using a target compound to identify adulterated animal fats has not been previously reported in the literature.

2. Materials and methods

2.1. Chemicals and standard solutions

Free phytosterol standards (campesterol, stigmasterol, β-sitosterol, and β-sitostanol) and phytosterol conjugate standard (γ-oryzanol) were purchased from NACALAI (Kyoto, Japan). An isotope-labeled internal standard (IS), cholesterol-2,2,3,4,4,6-d_6, was purchased from Sigma-Aldrich (St. Louis, MO, USA). The molecular formulas and molecular weights of phytosterols and cholesterol-2,2,3,4,4,6-d_6 are shown in Table 1. Methanol, *n*-hexane, and potassium hydroxide were purchased from Merck (Darmstadt, Germany). Absolute alcohol (99.9%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid was purchased from Wako (Osaka, Japan).

All chemicals and solvents used in this study were analytical or HPLC grade. Stock standard solutions and stock internal standard solution (2000 μg/mL) were prepared by dissolving 10 mg of each standard powder in acetone and methanol, respectively, and then adjusting the volumes to 5 mL. Four working standard solutions and a working internal solution were prepared by diluting 0.5 mL of each stock solution to 10 mL through the addition of methanol in order to yield a final concentration of 100 μg/mL. Working standard mixture was prepared by mixing 2 mL of free phytosterol working solutions (100 μg/mL) with each other and adjusting the volumes to 10 mL through the addition of methanol in order to yield a final concentration of 20 μg/mL. After preparation was complete, standard solutions were stored at −18 °C.

2.2. Collection of animal fats

We collected 22 inspected lard samples which were not blended with other oils from 4 companies that had been involved in the 2014 lard adulteration incident in Taiwan. We also purchased 2 commercial lard samples from a supermarket in Taipei City. Finally, 4 homemade lard samples and 4 other animal fat samples were prepared by heating fatty meats purchased from a traditional market in Taipei City on a hot plate, collecting the hot liquid fats, and cooling them to room temperature.

2.3. Sample preparation

The sample preparation procedure employed by this study was modified from that of Mo, Dong, Hurst, and van Breemen (2013). Specifically, for each sample, 20 mg of commercial vegetable oil or animal fat was placed in a 15 mL centrifuge tube and spiked with 5 μL of 100 μg/mL internal standard solution. We then added 2 mL of 1 M ethanolic KOH (56.1 g KOH dissolved in 1 L of absolute alcohol) to the tube and incubated the sample at 70 °C for 10 min with shaking. Following this, the bottom of tube was cooled with running water and the caps were removed. We then added 2 mL of deionized water and 3 mL of *n*-hexane before vortexing the tube for 1 min. After centrifugation at 3000 × g at room temperature for 5 min, the upper organic layer was collected, and the extraction process was repeated one more time. The hexane extracts were combined and evaporated to dryness under a gentle stream of nitrogen. The residue was subsequently dissolved in 2 mL of methanol and filtered through a 0.22 μm PVDF membrane for analysis by liquid chromatography-tandem mass spectrometry (LC/MS/MS).

Table 1
Compounds and LC/MS/MS parameters of phytosterol standards and the isotope-labeled internal standard.

Compound	Molecular formula	Molecular weight	Ion pair	Collision energy (eV)	Cone energy (V)	Retention time (min)
			Precursor ion (*m/z*) > product ion (*m/z*)			
Campesterol	$C_{28}H_{48}O$	400.7	383.4 > 161[a]	26	26	8.36
			383.4 > 147	24		
			383.4 > 149	22		
Stigmasterol	$C_{29}H_{48}O$	412.7	395.4 > 83[a]	24	30	8.63
			395.4 > 147	30		
			395.4 > 161	24		
β-Sitosterol	$C_{29}H_{50}O$	414.7	397.5 > 161[a]	25	26	8.95
			397.5 > 147	26		
			397.5 > 149	24		
β-Sitostanol	$C_{29}H_{52}O$	416.7	399.4 > 163[a]	20	32	9.41
			399.4 > 151	18		
Cholesterol-d_6 (IS)	$C_{27}D_6H_{40}O$	392.7	375 > 152[a]	22	26	7.75
			375 > 109	34		

[a] MRM transitions used for quantitation.

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