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Assessment of authenticity of sesame oil by modified Villavecchia Test and HPLC-ELSD analysis of triacylglycerol profile

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ABSTRACT

Sesame oil is a nutritive and high-value edible oil. Concern about its adulteration has gained much attention. Here, we used a simple, modified colorimetric Villavecchia test and triacylglycerol (TAG) profile analysis to assess the authenticity of sesame oil. Sesame oil samples with 100-fold dilution and negative Villavecchia test results were blended sesame oil. Meanwhile, we developed a promising and feasible high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) analysis to compare the TAG profiles of sesame, soybean and canola oil. We found unique TAG species not existing in authentic sesame oil but occurring in soybean oil (dilinoleoyl linolenoyl glycerol (LLnL), oleoyl linolenoyl linoleoyl glycerol (OLnL) and palmitoyl linolenoyl linoleoyl glycerol (PLnL)) and canola oil (OLnL and dioleoyl linolenoyl glycerol (OLnO)), which could be used to judge the authenticity of sesame oil. Both first-order and quadratic regression curves for ELSD were feasible for determining the blending ratio of adulterated oil; however, the quadratic regression curve was recommended for quantification at a lower blending ratio because the first-order calibration curve would lead to underestimation of 0 to 20% adulteration and the corresponding intercept would be substantially negative.

1. Introduction

Sesame seed oil is traditionally used in cooking and provides distinct flavor in Asian cooking. In the past 2 decades, sesame oil has been considered a popular health food because it contains a considerable amount of bioactive phytochemicals such as phytosterols and lignans. Numerous studies have shown that sesame lignans have multiple functions, including antioxidation, antihypertention, hypocholesteremia, anticancer, and immunoregulation (Hirose et al., 1991; Landete, 2012; Shyu & Hwang, 2002; Hedelin, Lof, Andersson, Adlercreutz, & Weiderpass, 2011). Therefore, the authenticity of marketed sesame oil products has gained much attention because sesame oil is more expensive than other vegetable oils, produced from staple crops such as soybean and rapeseed. The adulteration of high-price oils with other cheap oils is motivated by maximizing profits in industry (Cserhati, Forgacs, Deyl, & Miksik, 2005). Adulterated oil may not affect human health, but it may influence consumer rights and legal compliance. Therefore, the authenticity of oil products must be confirmed and methods developed to judge the authenticity of sesame oil.

The composition of edible oil is complicated, and the content of each component varies by nature. Many researchers have developed the assessment and identification of foreign oils by oil properties, such as the composition of fatty acids, iodine value and saponification value (Aued-Pimentel, Takemoto, Antoniassi, & Badolato, 2006; Kamm, Dionisi, Hischenhuber, & Engel, 2001; Ulberth & Buchgraber, 2000). A few other approaches such as sensory evaluation (Aued-Pimentel et al., 2006; Ravi, Prakash, & Bhat, 2005; Zheng & Jun, 2006), Fourier transform infra-red spectra (Noh, Jeong, Min, Park, & Kim, 2004; Rohman & Man, 2010, 2011) and carbon isotope ratio mass (Seo et al., 2010) are limited by low sensitivity and resolution, processing conditions, or application to only particular oils.

The American Oil Chemists' Society (AOCS) has recommended the Villavecchia test (Official Method Cb 2–40) for detecting sesame oil in animal and vegetable fats and oils (Official and Tentative Methods of the AOCS, 2009). The test is based on a chromogenic reaction of a pink-reddish complex generated by incubating tested oil with conc. HCl acid and furfural. Unique lignans in sesame oil, namely sesamolin and sesamol, are responsible for the reaction (Haslam & Haworth, 1955). Although the lignan content in sesame oil may vary by seed variety and may change during the production of sesame oil (Lee, Su, Lee, & Lin, 2013), sesamin and sesamolin as well as a small amount of sesamol are the predominant lignans in sesame oil. Wu (2007) analyzed the lignan contents of 14 brands of commercial sesame oils in Taiwan and found that the contents of sesamolin and sesamol ranged from 0.83-3.37 and 0.16-0.48 mg/g oil, respectively, and the content of sesamol was negatively correlated with that of

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sesamolin. Sesamol is generated from the degradation of sesamolin during the roasting of sesame seeds (Lee, Jeung, Park, Lee, & Lee, 2010; Wu, 2007). We previously showed that the process of roasting sesame seeds affected lignan content in the resulting oil and influenced the chromogenicity of the oil in the Villavecchia test (Lee et al., 2013). However, little information is available on how to judge the authenticity of sesame oil by the Villavecchia test. Furthermore, edible oils are mainly composed of TAGs. The TAG profile of different kind of oil is not alike and the variation of TAG composition is greater than of fatty acid (Ulberth & Buchgraber, 2000).

In the present study, we examined a series of diluted oil samples with the modified Villavecchia test to indirectly estimate whether sesame oil was blended with other oil. The determination was based on the minimal level of sesamolin that may occur in authentic sesame oils from unroasted seeds. Moreover, we developed a promising chromatographic system with HPLC-ELSD for determining TAG species of sesame, soybean, and canola oil and their blended samples. Some unique TAG species existing in soybean or canola but not sesame oil were considered indicators of the adulteration of sesame oil with soybean or canola oil. Our modified Villavecchia test and HPLC-ELSD system for measuring TAG composition may be feasible for estimating the authenticity of sesame oil and identifying the adulteration of sesame oil.

2. Materials and Methods

2.1. Materials

We prepared 12 authentic sesame oils (samples E1-E12) from 12 varieties of sesame seeds obtained from Flavor Full Foods Inc. (Taipei) and originating from India, Pakistan, Sri Lanka, Myanmar, Nigeria and Ethiopia. To explore the influence of roasted sesame seeds on the lignan content in oils and the effect in the Villavecchia test, sesame seeds were roasted with an automatic roasting machine at 180 and 230 °C for 5 min before extracting oil. Soybean oils (samples S1-S6) were prepared from soybean seeds from Taiwanese cultivars Tainan No. 4 and No. 1, organic soybeans from the United States and Australia, and genetically modified soybean from China and the United States purchased from a local supermarket in Taiwan. Authentic sesame and soybean oils were obtained by extraction from crushed seeds with 20-fold ethyl acetate (*v/w*); the solvent was removed under reduced pressure to obtain the oils. Moreover, 16 different commercial brands of oils, including 5 soybean oils (S7-S11), 4 canola oils (C1-C4) and 7 sesame oils (Product A-Product G) were purchased from local grocery stores in Taipei.

Sesamin and sesamolin were prepared from sesame oil as described (Reshma et al., 2010) and purified by semi-preparative HPLC. Sesamol and furfural were from Sigma Aldrich (St. Louis, MO, USA). Methanol, ethanol, n-hexane, ethyl acetate, 2-propanol, acetonitrile and hydrochloric acid were from Merck (Darmstadt, Germany). The color-forming reagent for the Villavecchia test was 2 mL well-mixed furfural and 100 mL of 95% ethanol. The chemical standards of TAGs were from Supelco Analytical (Bellefonte, PA, USA). All chemicals were of analytical grade and obtained from a local supplier in Taiwan.

2.2. Chromogenic analysis of oil samples with the modified Villavecchia test

An amount of 1 g of each oil sample was dissolved with an equal amount (w/v) of solvent consisting of n-hexane and ethyl acetate at a 3:1 (v/v) ratio, and then underwent a series of dilutions for use in the Villavecchia test. The Villavecchia test was adapted from the AOCS Cb 2–40 (Official and Tentative Methods of the AOCS, 2009). Briefly, 1 mL sample was mixed with 1 mL hydrochloric acid, and then 0.01 mL color-forming reagent was added. The solutions were mixed by strong shaking, and then incubated for 15 s for separating 2 immiscible liquid phases. The lower aqueous layer was collected and centrifuged. The resulting aqueous solution was measured by absorbance at 520 nm with a UV/VIS spectrophotometer (Hitachi, Tokyo). The contents of sesamin, sesamolin and sesamol in sesame oil were determined by use of a Thermo HPLC system consisting of a ConstraMetric 3200 pump and a SpectroMonitor 3200 UV/VIS detector. HPLC involved a C18 column (Atlantis, 4.6 × 250 mm, 5 μm; Waters Co., Milford, MA) with an isocratic 70% aqueous-methanol mobile-phase flow rate 1.0 mL/min. Each sample was diluted with the solvent n-hexane and ethyl acetate at a 3:1 (v/v) ratio and was injected at a 20 μL volume, with detection wavelength 290 nm. The contents of sesamin, sesamolin and sesamol were determined by corresponding calibration curves prepared by a serial measurement of the standards.

2.3. HPLC-ELSD analysis of TAG species in oil samples

Before TAG analysis of oil samples, the potent polar constituents of samples were removed by use of a J. T. Baker silica solid-phase extraction cartridge (Phillipsburg, NJ, USA). In brief, an amount of 0.1 g oil sample was suspended in 10 mL n-hexane and then loaded slowly onto the cartridge, which was conditioned previously by 2 column volumes of n-hexane. Then, the cartridge was eluted with an extra 2 volumes of n-hexane. The non-adsorbed and n-hexane-eluted fractions were combined, and the solvent was removed under reduced pressure to obtain oil for TAG analysis.

To optimize the HPLC conditions for TAG analysis, such as composition of the mobile phase and detector settings, oil samples were analyzed under a reverse-phase HPLC system (P1000, Thermo Fisher Scientific, Barrington, IL, USA) equipped with an ELSD (Alltech 2000, Deerfield, IL, USA) and a C-30 analytical column (Develosil C30-UG-5, 4.6 × 250 mm, 5 μm; Nomura Chemical Co., Anada-cho Seto, Japan). The initial conditions of separation for accessing the composition of the mobile phase were as described (Park, Chang, & Lee, 2010), but 2-propanol was used to replace chloroform to develop a new HPLC condition for TAG analysis based on the cost and their similar polarity index (Miller, 2009). We evaluated reproducibility and compared TAG profiles of soybean oil with previous known results to validate the HPLC condition in this work. We analyzed TAG composition

Table 1
Effect of roasting conditions on sesamin, sesamolin and sesamol contents of sesame oils and color reactions of sesame oils with the Villavecchia test (+, positive result; −, negative result).

Sample	Lignan content (mg/g oil)			Villavecchia test (referred as the absorbance at 520 nm)		
	Sesamol	Sesamin	Sesamolin	50×	100×	200×
Unroasted 1	0	3.59 ± 0.17	0.81. ± 0.02	0.15 ± 0.02/+	0.07 ± 0.00/+	0.04 ± 0.00/−
Unroasted 2	0	13.89 ± 0.35	6.65 ± 0.11	0.79 ± 0.05/+	0.45 ± 0.01/+	0.22 ± 0.01/+
Unroasted 3	0	5.62 ± 0.37	2.61 ± 0.08	0.38 ± 0.03/+	0.28 ± 0.01/+	0.17 ± 0.00/+
Roasted at 180°C	0	7.75 ± 0.31	3.34 ± 0.07	0.51 ± 0.02/+	0.40 ± 0.02/+	0.23 ± 0.01/+
Roasted at 230°C	0.37 ± 0.02	7.98 ± 0.44	2.38 ± 0.14	0.91 ± 0.04/+	0.76 ± 0.02/+	0.37 ± 0.02/+

Roasted sesame oils (180 °C and 230 °C) were prepared from the same variety of seeds with Unroasted 3.
Data are mean ± SD of triplicate determinations.
Blank ± 3 SD (detection limit) = 0.05.