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# Detection of Corn Oil in Adulterated Sesame Oil by Chromatography and Carbon Isotope Analysis

**Hye-Young Seo · Jaeho Ha · Dong-Bin Shin · Sung-Lye Shim · Ki-Mi No · Kyong-Su Kim · Kang-Bong Lee · Sang-Bae Han**

**Abstracts** This study was performed to the development of methods for the identification and detection of corn oil in adulterated sesame oil. The fatty acids composition and carbon isotope ratio of authentic sesame oil and adulterated sesame oil with corn oil were studied by using GC-FID and IRMS. The content of palmitic (P), linoleic (L), and linolenic (Ln) acids increased gradually as the mixed rate of corn oil was increased, whereas the content of stearic (S) and oleic (O) acids decreased. On increasing the mixing ratio of corn oil, the stearic acid contents and the $(L \times L)/O$ ratio showed significant differences between authentic and adulterated sesame oils ($p < 0.05$). The $\delta^{13}C‰$ value for authentic sesame oil and corn oil fell into the range of $-27.68$ to $-29.26‰$ and $-16.51$ to $-17.27‰$. The adulterant had more carbon-13 compared with those of authentic sesame oil and the $\delta^{13}C‰$ value was increased linearly ($r^2 = 0.996$). Therefore, the combination of the stearic acid% ($<4.8\%$), $(L \times L)/O$ ratio ($>56.1$) and the carbon isotope ratio ($> -27.0‰$) turned out to be a good tool for detecting the adulteration of sesame oil with the cheaper corn oil, up to the 10% level.

**Keywords** *Sesamum indicum* · Sesame oil · Corn oil · Fatty acid composition · Carbon isotope ratio

H.-Y. Seo · J. Ha · D.-B. Shin
Korea Food Research Institute, Sungnam, Korea

S.-L. Shim · K.-M. No · K.-S. Kim (✉)
Department of Food and Nutrition,
Chosun University, Gwangju, Korea
e-mail: kskim@chosun.ac.kr

K.-B. Lee · S.-B. Han
Korea Food and Drug Administration, Seoul, Korea

## Introduction

Adulteration of oil products is generally motivated by maximizing profit by adding other cheaper or low-quality oils. In Korea, there has been a high incidence of adulteration in sesame oil because sesame oil is more expensive (10 to 20 times) than other vegetable oils and is very popular as a source of food oils having a characteristic flavor. The most frequent adulterations are carried out with corn oil and other oils such as soybean, perilla, and canola oils have also been used. Although in most cases adulteration does not pose a threat to public health, fundamental rights of consumers (right of correct information and buying "value-for-money") are violated by fraudulent malpractice [1]. Thus, continuous vigilance is required to control the adulteration of sesame oil products and to protect the interests of consumers as well as those of industries in general.

Since the biosynthetic pathways of lipids are species specific, the assessment of the identity of foreign oils has been made by the composition of fatty acids and some classic parameters such as the iodine value, saponification value, and refractive index, which is based on a physico-chemical determination of the constituents making up the oils [1, 2]. Determination of the sterols and fatty acid composition complement official methodologies to define the identity of edible vegetable oils [3, 4].

When sesame oil is used to adulterate other vegetable oils, it can be identified by a color reaction (Baudouin test, etc.) or HPLC analysis of characteristic components such as sesamolin, sesamin, and sesamol [5–7]. However, the identification and detection of foreign oils in adulterated sesame oil is not so simple. There have been a few reports about the identification and detection methods of foreign oil in adulterated sesame oil. A lot of the methods use some

instruments such as HPLC or gas chromatograph for analysis of sesamin, sterols, fatty acid composition and NIR spectroscopy have been employed [8–13]. Recently, there have been reports of studies on the detection of adulteration of sesame oil using an electronic nose [14–16]. However, in many cases, these methods are not adequate to detect more elaborated frauds and there is no method which can detect all kinds of foreign oils in adulterated sesame oils. Thus a suitable identification and detection method of foreign oil types is needed.

In this study, we not only tried to detect corn oil adulteration in sesame oil using an analytic protocol comprising two phases with chromatography and carbon isotopic analysis but also tried to predict the adulteration percentage in sesame oil adulterated with corn oil.

## Materials and Methods

### Materials

Sesame seeds with a white coat produced in Korea, Sudan, India, China, and Pakistan (2007 season) were obtained though the Agricultural and Fishery Marketing Corporation, Korea. The authentic sesame oils were obtained from Indian sesame seeds widely consumed in Korea by pressing them with an oil press (NEH-404 K, National Engineering Co., Korea) from pan-roasted (20 min at $200 \pm 5$ °C) seeds in the laboratory. The corn oil for the adulteration test of sesame oil was purchased from a local market.

The sesame oils for the adulteration test were mixed in proportions ranging from 5 to 80% of corn oil in Indian sesame oil; nine groups of samples with different adulteration levels were prepared, namely with 5, 10, 15, 20, 30, 40, 50, 75, and 80% (by volume) adulteration by corn oil. These sesame oils were kept in a cold room (10 °C) before and during analysis.

### Chemicals

The reagents used in the experiments were of analytical grade and obtained from Sigma (St. Louis, MO, USA), Supelco (18918-1AMP, USA) and Fisher Scientific (Normschliff, Geratebau, USA). The standards of triundecanoin and undecanoic acid methyl ester were purchased from Sigma (St. Louis, MO, USA). The standard of fatty acid methyl esters mixture was purchased from Supelco (18918-1AMP, USA). HPLC grade solvents used for methyl esterification of fatty acids were redistilled using a spiral packed double distilling apparatus (Normschliff Gerätebau, Wertheim, Germany) and Milli-Q water that was generated with a water purification system (S.A. 67120; Millipore Co., Molshem, France).

### Analysis of the Fatty Acids Composition by GC-FID

The AOAC official method was modified for the preparation of fatty acid methyl ester (FAME) analysis [17]. Twenty-five milligrams of the sample was placed in a test tube and 1 mL of internal standard (triundecanoin, 1000 μg/mL) and 1.5 mL of methanolic 0.5 N NaOH was added for the saponification, and it was flushed with $N_2$ and vigorously vortexed. The sample was then heated at 100 °C water bath for 5 min, 2 mL of 14% $BF_3$–methanol was added after cooling, it was then flushed with $N_2$, vigorously vortexed and heated in a water bath at 100 °C for 20 min. It was cooled to 30–40 °C and 1 mL of n-hexane was added, then flushed with $N_2$, vigorously vortexed for 30 s, 5 mL of saturated NaCl solution was added and it was flushed with $N_2$ and vigorously vortexed. It was cooled to room temperature and then an aliquot of the supernatant hexane layer was transferred into an amber vial and flushed with $N_2$. These samples were used for gas chromatographic (GC) analysis.

FAME analysis was carried out using a Hewlett-Packard 5890 II Plus (Hewlett Packard, USA) GC equipped with a flame ionization detector (FID). The oven temperature was programmed at 100 °C (isothermal for 1 min) ramped to 200 °C at 25 °C/min (Isothermal for 25 min). The temperatures of the injector and detector were 250 and 260 °C, respectively. The capillary column used was a DB-WAX (30 m × 0.32 mm i.d., 0.25 μm film thickness, J&W, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min, with an injector volume of 1 μL using a 1:20 split ratio.

### Analysis of the Carbon Isotope Ratio by IRMS

The analyses of the carbon in the samples were performed using isotope ratio mass spectrometry (IRMS) (Isochrom-EA, VG ISOTECH, UK). Samples were mixed and 0.15 mg of the sample oils were put into thin capsules. For carbon isotopic analysis, the separated compounds were converted to $CO_2$ by passing the eluting analyte stream through a ceramic oxidation reactor at a gas temperature of 1,030 °C. The carbon dioxide gas standards were injected before and after the species of interest to permit the calculation of $\delta^{13}$C‰ values. Helium was used as carrier gas. They are expressed in the usual per mille notation relative to the Pee Dee Belemnite (PDB) marine carbonate standard.

$$\delta^{13}C = \left[ \frac{(^{13}C/^{12}C)\text{sample}}{(^{13}C/^{12}C)\text{PDB}} - 1 \right] \times 1,000$$

### Statistical Analysis

One-way analysis of variance, ANOVA (Tukey's honest significant difference multiple comparison) was evaluated

using SPSS statistics 12.0 (SPSS Inc., Chicago, USA). $p$ values less than 0.05 were considered statistically significant. All chemical analyses were carried out in triplicate, and the analytical data were used for statistical comparisons.

## Results and Discussion

### Fatty Acid Composition of Authentic Sesame Oil and Corn Oil

The fatty acid composition of the authentic sesame oils and corn oils is shown in Table 1. The major fatty acids in sesame and corn oils were linoleic and oleic acid, and the fatty acid composition of them was in compliance with Codex Alimentarius ranges [18]. The ratio of polyunsaturated to saturated fatty acid (P/S) in sesame and corn oils were similar with 5.15 and 5.09, respectively, however, the composition of each fatty acid was different, except for arachidic acid, which was similar level.

### Detection of Corn Oil Mixed in Sesame Oil by Fatty Acid Composition

Corn oil was added to sesame oil up to final concentrations of 5, 10, 15, 20, 30, 40, 50, 75, and 80% and the fatty acid composition in these samples is shown in Table 1. As increasing the amount of corn oil, the contents of palmitic acid (P), linoleic acid (L) and linolenic acid (Ln) were increased linearly ($r^2 = 0.985$, 0.983, and 0.998, respectively), while the contents of stearic acid (S) and oleic acid (O) were decreased gradually ($r^2 = 0.990$ and 0.970,

respectively) (Figs. 1, 2). Among these patterns of fatty acid composition, the content of stearic acid in adulterated samples with higher than 5% corn oil was significantly different from first group, which is sesame oil ($p < 0.05$). However, content of stearic acid (<4.8%) can give only the clue for additional test, since the content of this fatty acid is low in other vegetable oils [18]. Therefore, to detect corn oil mixed in sesame oil, it should be considered other fatty acids and needs additional method.

The results of the variable ratio (discrimination index) using fatty acid compositions are shown in Table 2. The ratio of (O + L)/(P × Ln) decreased as increasing the mixing ratio of the corn oil, and was significantly different from first group in samples with upper than 15% corn oil.
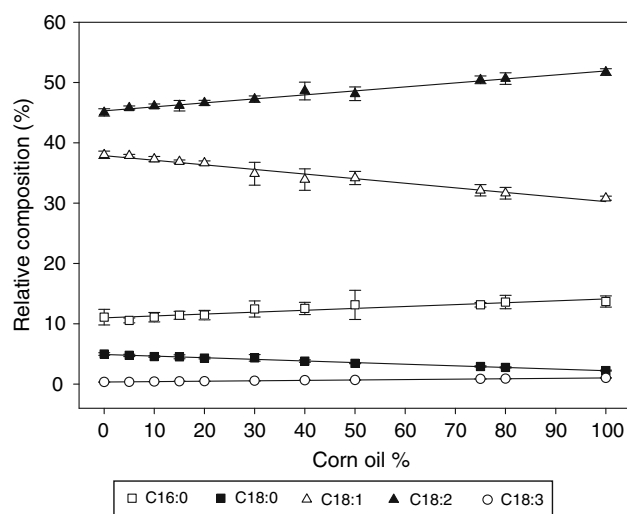


**Fig. 1** Changes of major fatty acid composition in adulterated sesame oils with corn oil

**Table 1** Fatty acid composition of sesame oils mixed with corn oil

| Mixing ratio[a] | Fatty acid composition (%) | | | | | |
|---|---|---|---|---|---|---|
| | $C_{16:0}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ | $C_{20:0}$ |
| 100:0 | $10.29 \pm 0.78$[b] | $5.39 \pm 0.71$ | $39.16 \pm 2.02$ | $44.19 \pm 2.86$ | $0.38 \pm 0.05$ | $0.58 \pm 0.05$ |
| 95:5 | $10.65 \pm 0.41$ | $4.76 \pm 0.20$* | $37.86 \pm 0.23$ | $45.80 \pm 0.32$ | $0.37 \pm 0.00$ | $0.55 \pm 0.04$ |
| 90:10 | $11.10 \pm 0.79$ | $4.57 \pm 0.13$* | $37.30 \pm 0.44$ | $46.09 \pm 0.36$ | $0.41 \pm 0.01$ | $0.53 \pm 0.05$ |
| 85:15 | $11.41 \pm 0.66$ | $4.53 \pm 0.33$* | $36.88 \pm 0.28$ | $46.16 \pm 0.87$ | $0.46 \pm 0.01$ | $0.56 \pm 0.06$ |
| 80:20 | $11.44 \pm 0.78$ | $4.32 \pm 0.13$* | $36.64 \pm 0.37$ | $46.62 \pm 0.42$ | $0.47 \pm 0.02$ | $0.52 \pm 0.05$ |
| 70:30 | $12.45 \pm 1.34$ | $4.34 \pm 0.55$* | $34.88 \pm 1.89$* | $47.24 \pm 0.53$* | $0.54 \pm 0.01$* | $0.55 \pm 0.09$ |
| 60:40 | $12.55 \pm 1.02$ | $3.81 \pm 0.20$* | $33.91 \pm 1.78$* | $48.59 \pm 1.47$* | $0.63 \pm 0.02$* | $0.51 \pm 0.06$ |
| 50:50 | $13.14 \pm 2.42$ | $3.44 \pm 0.06$* | $34.17 \pm 1.10$* | $48.13 \pm 1.12$* | $0.67 \pm 0.02$* | $0.46 \pm 0.13$ |
| 25:75 | $13.14 \pm 0.37$ | $2.92 \pm 0.11$* | $32.13 \pm 0.94$* | $50.45 \pm 0.64$* | $0.85 \pm 0.02$* | $0.50 \pm 0.01$ |
| 20:80 | $13.60 \pm 1.12$* | $2.76 \pm 0.10$* | $31.64 \pm 0.97$* | $50.65 \pm 0.96$* | $0.89 \pm 0.02$* | $0.47 \pm 0.05$ |
| 0:100 | $13.69 \pm 0.93$* | $2.24 \pm 0.07$* | $30.82 \pm 0.34$* | $51.74 \pm 0.56$* | $1.02 \pm 0.01$* | $0.48 \pm 0.04$ |

*Significantly different from the first group ($p < 0.05$)

[a] Sesame oil/corn oil (v/v)

[b] Mean $\pm$ SD ($n = 3$)

Fig. 2 GC chromatograms of fatty acid methyl esters in sesame oil (A) and adulterated sesame oil with 50% corn oil
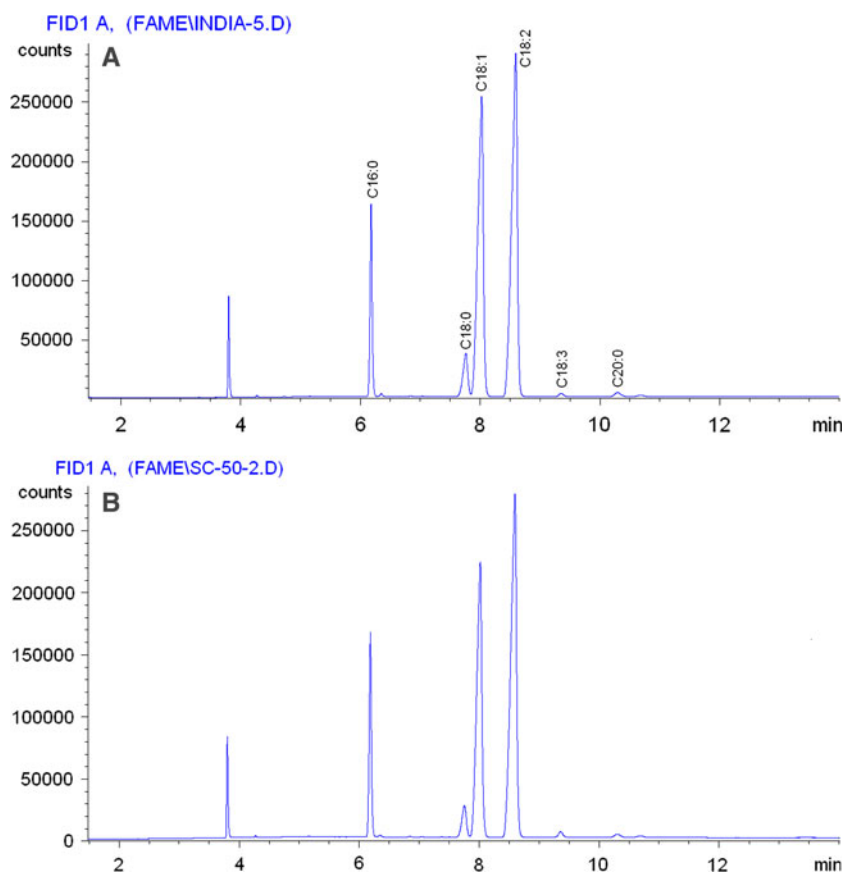


**Table 2** Discrimination index of adulterating sesame oils with corn oil by fatty acid composition

| Mixing ratio[a] | (O + L)/(P × Ln) | (L × L)/O | (P + L + Ln)/(S + O) | (P × L × Ln)/(S + O) |
|---|---|---|---|---|
| 100:0 | 20.37 ± 2.53[b] | 55.01 ± 0.66 | 1.33 ± 0.26 | 4.21 ± 0.55 |
| 95:5 | 21.26 ± 0.95 | 55.72 ± 0.38 | 1.33 ± 0.01 | 4.23 ± 0.15 |
| 90:10 | 18.85 ± 1.21* | 57.08 ± 0.47* | 1.38 ± 0.02 | 4.96 ± 0.29 |
| 85:15 | 15.96 ± 0.75* | 59.15 ± 0.40* | 1.40 ± 0.02 | 5.80 ± 0.12 |
| 80:20 | 15.65 ± 0.90* | 59.37 ± 0.55* | 1.43 ± 0.02* | 6.07 ± 0.28 |
| 70:30 | 12.41 ± 1.64* | 61.93 ± 0.58* | 1.49 ± 0.04* | 7.49 ± 0.71 |
| 60:40 | 10.55 ± 1.03* | 65.65 ± 0.47* | 1.58 ± 0.05* | 9.53 ± 1.03* |
| 50:50 | 9.58 ± 1.72* | 68.46 ± 1.34* | 1.65 ± 0.08* | 11.16 ± 1.78* |
| 25:75 | 7.37 ± 0.32* | 77.32 ± 0.82* | 1.84 ± 0.07* | 16.16 ± 1.18* |
| 20:80 | 6.86 ± 0.59* | 78.96 ± 1.10* | 1.89 ± 0.07* | 17.76 ± 1.51* |
| 0:100 | 5.91 ± 0.39* | 87.19 ± 1.30* | 2.01 ± 0.03* | 21.92 ± 1.22* |

*Significantly different from the first group ($p < 0.05$)

[a] Sesame oil/corn oil (v/v)

[b] Mean ± SD (n = 3)

Whereas the ratio of (L × L)/O, (P + L + Ln)/(S + O), and (P × L × Ln)/(S + O) increased linearly ($r^2 = 0.991$, 0.995, and 0.989, respectively) as the amount of corn oil. Among them, the (L × L)/O ratio was shown good significantly differences between authentic and adulterated sesame oils ($p < 0.05$), and it (>56.1) could be useful for distinguishing sesame oil and corn oil. However, the

percent of corn oil in adulterated sesame oils can be determined with certainty, but when the corn oil was mixed at less than 10% the exact amount could not estimate (Fig. 3).

As a result, the stearic acid content and the ratio of (L × L)/O can be used to detect mixtures of sesame oil with higher than 10% corn oil.
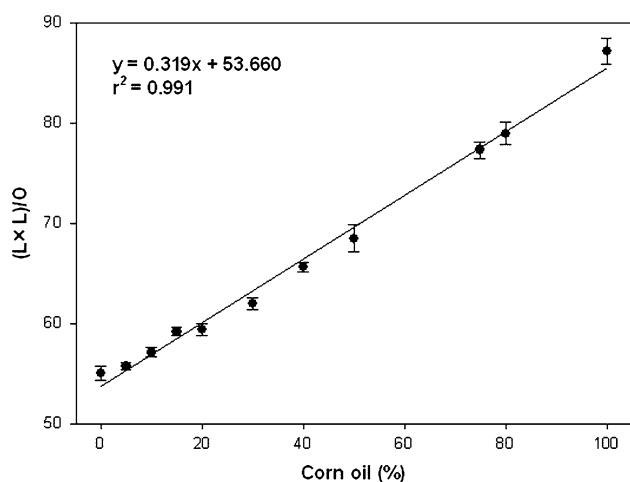
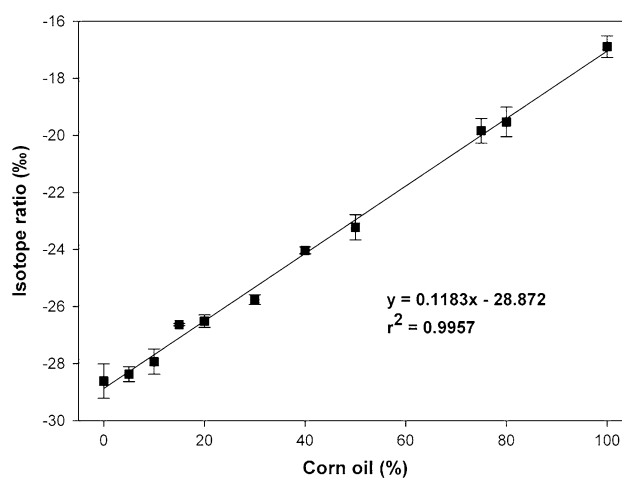Fig. 3 Correlation of (L × L)/O ratio and concentration of corn oil



Fig. 4 Correlation of carbon isotope ratio and concentration of corn oil

**Table 3** The $\delta^{13}C\permil$ value for authentic and adulterated sesame oils

| Samples | $\delta^{13}C\permil$ |
|---|---|
| Sudanese sesame oil | −27.68 ± 0.44 |
| Chinese sesame oil | −28.45 ± 1.01 |
| Pakistan sesame oil | −28.78 ± 0.11 |
| Korean sesame oil | −29.26 ± 0.90 |
| Indian sesame oil (100:0[a]) | −28.61 ± 0.60[b] |
| 95:5 | −28.37 ± 0.26 |
| 90:10 | −27.93 ± 0.44 |
| 85:15 | −26.63 ± 0.04* |
| 80:20 | −26.51 ± 0.22* |
| 70:30 | −25.76 ± 0.17* |
| 60:40 | −24.03 ± 0.12* |
| 50:50 | −23.22 ± 0.44* |
| 25:75 | −19.84 ± 0.43* |
| 20:80 | −19.53 ± 0.52* |
| 0:100 | −16.89 ± 0.38* |

*Significantly different from the first group ($p < 0.05$)

[a] Mixing ratio: Sesame oil/corn oil (v/v)

[b] Mean ± SD ($n = 3$)

## Detection of Corn Oil Mixed in Sesame Oil by Carbon Isotope Ratio

Plants use either the Calvin ($C_3$) or Hatch & Slack ($C_4$) pathway for photosynthetic carbon dioxide fixation, as revealed by differences in both leaf anatomy and $^{13}C/^{12}C$ ratios. Most plants used in the production of vegetable oils, such as rape and groundnut utilize the $C_3$ cycle and a small number of plants such as corn utilize $C_4$ cycle. Therefore, detection is made possible because of significant differences between the $^{13}C/^{12}C$ isotope ratios of corn and other oil types [19, 20].

The $\delta^{13}C\permil$ (delta carbon-13 per mille) value for authentic and adulterated sesame oils were shown in Table 3. The $\delta^{13}C\permil$ value for authentic sesame oil obtained from different countries measured range of −27.68 ∼ −29.26‰. In contrast, that for corn oil was −16.89 ± 0.38‰, and these results agreed with that authentic corn oils typically have global $\delta^{13}C\permil$ values between −14.3 and −16.4 [21]. On increasing the amount of corn oil in adulterated sesame oils, the $\delta^{13}C\permil$ values increased linearly ($r^2 = 0.996$) having a simple equation of $y = 0.1183x − 28.872$ by enriched in carbon-13 (Fig. 4). As well as fatty acid composition, the $\delta^{13}C\permil$ values ($> −27.0\permil$) in adulterated sesame oil with more than 10% corn oil were significantly different from authentic sesame oil. These large differences in the carbon isotope ratio between sesame and corn oil could be used to determine the adulteration of both oils.

Consequently, the detection of corn oil in adulterated sesame oil is made possible by using a combination of the stearic acid% (<4.8%), (L × L)/O ratio (>56.1) and the carbon isotope ratio ($> −27.0\permil$) and an estimation of mixing ratio can be calculated using the equations.

## References

1. Ulberth F, Buchgraber M (2000) Authenticity of fats and oils. Eur J Lipid Sci Technol 102:687–694
2. Aued-Pimentel S, Takemoto E, Antoniassi R, Badolato ESG (2006) Composition of tocopherols in sesame oil: an indicative adulteration. Grasas Aceites 57:205–210
3. Association of Official Analytical Chemists (AOAC) (1995) Official method and recommended practices. Association of Official Analytical Chemists (AOAC), Washington DC

4. International Union of Pure, Applied Chemistry (IUPAC) (1992) Standard methods for the analysis of oils, fats and derivatives. Method 2.403. Blackwell, Oxford

5. Bureau of Indian Standards (BIS) (1976) Methods of sampling and test for oils and fats, IS: 548 (Part-II; Purity tests)

6. Shukla AK, Singh RP (2005) Detection of sesame oil in other oils and fats. J Oleo Sci 54:543–544

7. Kikugawa K, Arai M, Kurechi T (1983) Participation of sesamol in stability of sesame oil. J Am Oil Chem Soc 60:1528–1533

8. Lee CW, Kawk IS (1996) Sesamin and sesamol contents of Korean sesame oils determined by HPLC method and Villavecchia-Suarez color reaction of the oils. Korean J Sani 11:1–8

9. Ro IY, Jeong HS (1980) Studies on the sterol of sesame oil sold in markets and used in restaurants. Korean J Nutr 13:159–166

10. Yamajaki M, Nagao A, Yamamoto H (1993) Estimation of the mixing ratio of a foreign oil in adulterated sesame oil I. Soybean oil. J Jpn Oil Chem Soc 42:605–611

11. Yamajaki M, Nagao A, Yamamoto H (1993) Estimation of the mixing ratio of a foreign oil in adulterated sesame oil II. Rice bran oil or rapeseed oil. J Jpn Oil Chem Soc 43:10–17

12. Kang CH, Park JK, Park JU, Chun SS, Lee SC, Ha JU, Hwang YI. Comparative studies on the fatty acid composition of Korean and Chinese sesame oils and adulterated sesame oils with commercial edible oils. J Korean Soc Food Sci Nutr 31: 17–20

13. Kim YS (1996) NIR analysis for adulteration of the Korean sesame oil. Food Technol 9:87–93

14. Shin JA, Lee KT (2003) The identification of blended sesame oils by electronic nose. Korean J Food Sci Technol 35:648–652

15. Hai Z, Wang J (2006) Electronic nose and data analysis for detection of maize oil adulteration in sesame oil. Sensors and Actuators B 119:449–455

16. Hai Z, Wang J (2006) Detection of adulteration in camellia seed oil and sesame oil using an electronic nose. Eur J Lipid Sci Technol 108:116–124

17. AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington. DC, p 994

18. Codex Alimentarius (FAO/WHO) Codex standard for named vegetable oils. CODEX STAN 210 1999 (revision and amendments: 2003, 2005)

19. Doner LW, Krueger HW, Reesman RH (1980) Isotopic composition of carbon in apple juice. J Agric Food Chem 28:362–364

20. Rossell JB (1991) Purity criteria in edible oil and fats. Fat Sci Technol 93:526–531

21. Leatherhead Food Research Association (1994) Authenticity of vegetable oils and fats. Part XX. Determination of corn oil purity by SCIRA. Leatherhead Food Research Association Research Report