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Extended Abstract

Authentication of Vegetable Oils from Lard Adulteration by Sterols Analysis: a case study of olive oil

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Abstract

In this study, the sterols of 5 different vegetable oils (olive, canola, corn, sesame as well as sunflower) and an animal fat (lard) were analyzed by liquid-liquid extraction and gas chromatography. The sterols in real samples were identified by mass spectrometry. Calibration curves were plotted with the correlation coefficients more than 0.99 for each sterol. The detection limits for β-sitosterol, campesterol, and cholesterol were 0.48, 2.38, and 0.44 mg L^{-1}, respectively. The recoveries were obtained in the range of 95-109%, and their relative standard deviations were less than 9% in a day and between three days. The concentration of each sterol in real samples was determined using the purposed method. To detect lard adulteration in vegetable oils, mixtures of extra virgin olive oil and lard in four different ratios were prepared and analyzed. According to the results, at a higher percentage of lard in extra virgin olive oil, cholesterol increased and plant sterols were decreased. The curve plotted via the cholesterol concentration versus the added amount of lard, was linear with the correlation coefficient of more than 0.99. Moreover, this method made the detection of lard adulteration possible in olive oil with 1% detection limit and less than 3% uncertainty. Therefore, the identification of lard adulteration in plant oils (cholestrol-free) can be based on the suggested method.

Keywords: Adulteration of olive oil, β-sitosterol, Campesterol, Cholesterol, Lard

Introduction

The adulteration of valuable vegetable oils with low-price/quality oils has created a serious problem in food supplies (Almeida, Baggio, Mariutti, & Bragagnolo, 2020; Heidari *et al.*, 2020). These additions have the potential to cause serious health problems to consumers (Zhao *et al.*, 2015). Therefore, it is necessary to use advanced methods to supervise food quality and detect adulteration. Many analytical methods have been developed to detect and quantify the specific components of vegetable oils to ensure their authenticity. The main aim of this study was to establish a quantitative validated method using gas chromatography for determination of sterols in extra virgin olive oils.

Materials and methods

β-sitosterol, campesterol, and cholesterol were obtained from Sigma (Darmstadt, Germany). The standards of olive oil and lard were purchased from Supelco (Bellefonte, PA, USA). Potassium hydroxide, ethanol 96%, HPLC-grade methanol and hexane were obtained from Merck (Darmstadt, Germany). Extra Virgin Olive Oil (EVOO), virgine olive oil (VOO), pomace olive oil (POO), purified olive oil (PUO), corn oil (CO), canola oil (CaO), sunflower oil (SFO), and sesame oil (SeO) were purchased from local markets and kept in dark bottles.

An Agilent model 6890 N GC-MS instrument (Santa Clara, CA, USA) equipped HP-5MS capillary column (length=30 m, i.d.=0.25 mm, and film thickness=0.25 μm) and a Young-Lin model 6500 GC-FID instrument equipped with TRB-5 column (length=30 m, i.d.=0.53 mm, and film thickness=1 μm) were used to separate and identify the sterols. Helium and Nitrogen were used as carrier gas for GC-MS and GC-FID instruments, respectively. 1 μL of samples were injected into the gas chromatographs in splitless and following temperature-programmed modes: the initial column temperature of oven was set to 140 °C for 5 min, programmed to increase 240 °C at 20 °C/min, and to increase to 280 °C at 4 °C/min (for 10 min).

To extract the sterols from real samples, 50 mg of each oil sample was saponified with 5.5 mL of 2M alcoholic potassium hydroxide in 75 °C water bath for 30 min. 3 mL hexane and 3 mL water added to the solution. After centrifuging for 10 min at 3000 g, the upper organic layer was collected. The phase separation step was repeated two more times. Then all the collected fractions were combined and evaporated by nitrogen flow to dryness. After that, 1 mL hexane is added and 1 μL is injected into the GC and GC-MS.

Established method was validated for the assesment of linearity, inter- and intra day accuracies and precisions, limit of detection (LOD) and limit of quantification (LOQ).

Results and discussions

1 μL of extracted sample was injected to GC-MS so as to identify analytes in real samples. Mass spectrometry analysis showed that C_{12}-C_{18} fatty acids are the major fatty acids in vegetable oils. For instance, oleic acid in EVOO and VOO, linoleic acid in SFO and myristic acid in lard were found to be the major fatty acid in aformentioned oils. Squalene is one of the hydrocarbons mainly exist in vegetable oils which was detected in trace in lard. Sesamin is the major lignans present in sesame oil. Vegetable oils are the richest source of vitamin E. Sterols such as campesterol, stigmasterol, β-sitosterol and fucosterol can be found in vegetable oils. Lard contains cholesterol while most of plant oils only have cholesterol in trace.

Calibration curves are constructed over the concentration range of 1-3000 mg L^{-1} for β-sitosterol, and cholesterol, and 30-3000 mg L^{-1} for campesterol with correlation coefficient more than 0.99. The LODs were ranged from 0.44 to 2.38 mg L^{-1}.

Under the optimized conditions, the validated method was applied to the determination of sterols in vegteable oils and lard. The findings showed the presence of β-sitosterol and campesterol in all the studied vegetable oils while the concentration ratio of campesterol to β-sitosterol was found to be different. Cholestrol content was evaluated under the limit of detection in SeO and SFO oils. Lard contains choletsrol at much higher level (at least 5 times) than vegetable oils with under the limit of detection β-sitosterol and campesterol.

To investigate the authenticity of EVOO, the mixture of EVOO and lard in four different weight ratio percentages (1, 6, 24, and 70) were analyzed using the validated method. The chromatograms of pure EVOO along with each EVOO/lard weight ratio percentage were depicted in Fig. (1). As can be seen, through increasing the amount of lard, cholestrol level increased while campesterol and β-sitosterol levels decreased. Comparing the concentration

of cholesterol in EVOO and EVOO:lard (1% w/w) using statistical t-test at the 95% confidence level showed a significance difference between the cholesterol levels.

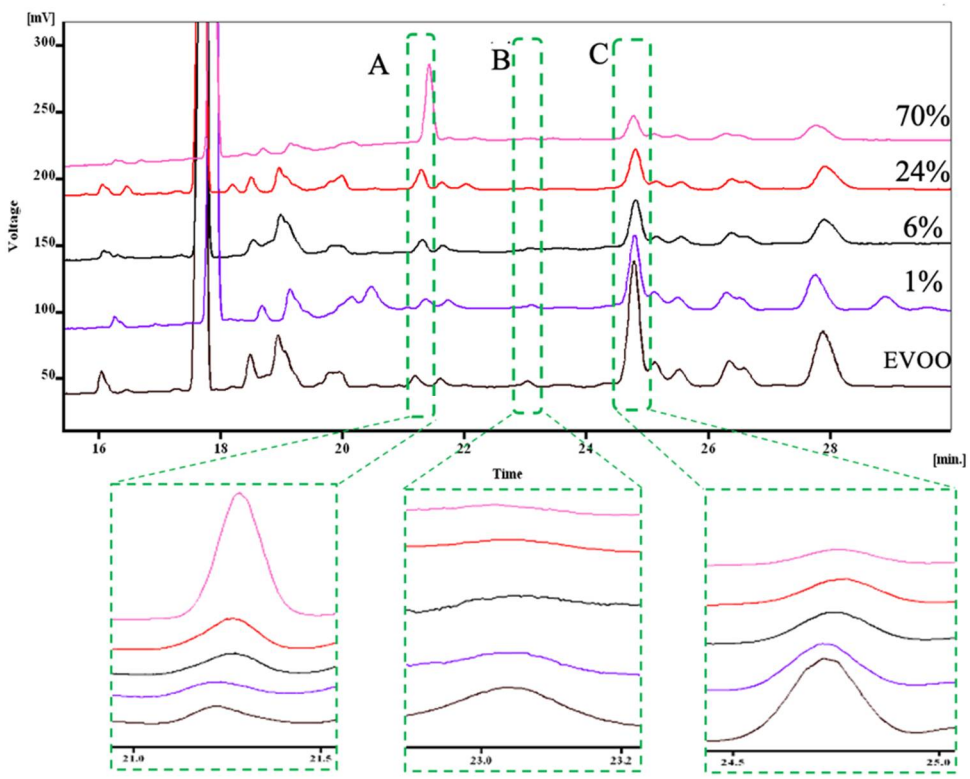

Fig. 1. Chromatograms of pure EVOO and EVOO contaminated with lard in different weight ratio percentages (1, 6, 24, and 70%) under the optimum conditions, A) Cholesterol, B) Campesterol, C) β -sitosterol.

To investigate the predictive ability of the suggested method to quantify the amount of added lard in EVOO, two different EVOO: lard mixtures (2 and 60% w/w) were prepared and analyzed using the developed method. The concentration of cholesterol in each mixture was calculated using the linear eaquation obtained by plotting the concentration of cholesterol versus the added amount of lard in EVOO (1, 6, 24, and 70% w/w). The results are summarized in Table (1). As it can be inferred, the validated method can predict the amoun of added lard in EVOO with errors less than 3%.

Table 1. Linear model for predicting the (%w/w) of lard in extra virgin olive oil

Slope	Intercept	Correlation coefficient	Added lard (% w/w)	Predicted lard (% w/w)	Relative error (%)
157±9	27±3	0.9932	2	2.06± 0.16	3
			60	58.60±0.52	2

*The linear model was constructed using four percentages of lard in EVOO. Each experiment was replicated three times.

**The relative error percentage was calculated by dividing the difference between the predicted value from the added value to added value.

Conclusions

The presented study aims to develop a chromatographic method to detect potential adulteration of vegetable oils by simultanous mesearments of sterols. To this end, the sterols were extracted from different vegetable oils and lard, and the concentration level of β-sitosterol, campesterol, and cholesterol were determined using GC-FID. After analyzing eigth different vegetable oils and lard, it was found that β-sitosterol and campesterol were the the

characteristic components for vegetable oils while the concentration level of cholestrol in lard is much higher than vegetable oils. Therefore, using cholesterol as marker, the developed method could effictevely detect lard in vegetable oils and assess adulteration with errors less than 3%.

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