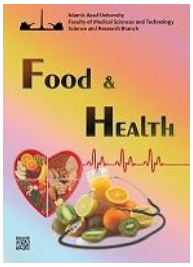

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Comparison of three different methods for detection of corn and sunflower oils in adulterated sesame oil

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ABSTRACT

Edible oils can be misdescribed by substituting one ingredient for a similar, but less expensive or over-declaring a quantitative ingredient. Thus, the identification of raw materials in edible oils is important for authentication. In This study, three methods (saponification value, sterol and fatty acids analysis) were used to compare for fraud detection in edible oils. Tests used to assess the quality of sesame oil and oils obtained from mixing sesame oil with sunflower and corn oils. The results showed that campesterol, Δ7-avenasterol, Δ5-avenasterol and Δ7-stigmastenol values can be used as detectors for corn oil mixed with sesame oil, even at a concentration of 5%. Also, in the detection of sesame oil fraud, measuring the fatty acids include oleic, linolenic, linoleic and behenic is more suitable even at a concentration of 5% of sunflower oil adding. In general, the best method for detecting of fraud at all concentrations of added sunflower oil was the saponification value method, while fatty acid composition and desmethylsterols composition was suitable for both corn and sunflower oils mixing.

1. Introduction

Sesame seed is one of the most nutritious seeds and dried whole sesame seeds are rich in calories (573 kcal) and are composed of 5% water, 23% carbohydrates, 12% dietary fiber, 50% fat and 18% protein (1). Sesame also contains phenols, plant sterols, phospholipids and vitamins, especially vitamin E and B group vitamins and minerals, such as calcium, magnesium, zinc, copper, and phosphorus (2). Sesame seed oil contains a mixture of unsaturated and composed of the following fatty acids: linoleic acid (41% of total), oleic acid (39%), palmitic acid (8%), stearic acid (5%) and others in small amounts (3). Because of its high nutritional value, health benefits, spicy flavor and a good taste, the sesame seeds are called oilseeds queen (4). Sesame oil was often adulterated because of its high quality and price than other vegetable oils. The most frequent adulterations in sesame oil are carried out with corn, sunflower, and other low-price oils. 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 (5). For example, food authenticity and traceability become very important by helping consumers in making informed choices about the food they buy and eat (6). Thus, one of the risks that are gaining attention from industry, governments, and standards-setting organizations is the fraud conducted for economic gain by food producers, manufacturers, processors, distributors, or retailers (1). Today, adulterations are more sophisticated. Therefore, it is necessary to use advanced and suitable methods to detect adulteration. Generally, the use of physical properties such as refractive index, viscosity, melting point, saponification and iodine value are not more practical for the detection of adulteration. These properties are well arranged in adulterated VOFs (vegetable oils and fats) to mask the adulteration (7). Edible oils and fats consist of major and minor components. Major components in edible oils and fats are triacylglycerols (TAG) and minor components include sterols, carotenoids, tocopherols, chlorophylls and other minor compounds. Among VOFs, some have particular components which are

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absent in other ones (2, 8-10). To detect the adulteration of edible oils and fats, it is possible to use both major and minor components as a detection tool. Since each oil and fat may have especial components at a known level, their presence and amounts should be considered as a detection tool. For example, phytosterols (i.e. campesterol, stigmasterol, and sitosterol) are present in small amounts in butter, if there is a high level of phytosterols in butter, it can be concluded that the butter has been admixed with VOFs (11-13). In this regard, we investigate the fraud mixing of sunflower and corn oil in sesame oil by measuring the sterols, fatty acids and the number of soap deals.

2. Materials and methods

Sesame oil was obtained from Saman Konjed Company, Iran. Sunflower and corn oil were obtained from Behshahr Industry, Iran. The experiments consisted of pure sesame oil (control), mixed sesame oil with the different amount of sunflower oil (5, 10 and 15%), mixed sesame oil with the different amount of corn oil (5, 10 and 15%) that analyzed by gas chromatography in triplicate.

2.1. Determination of sterol composition by GC

In this method, α-cholestanol as an internal standard was added to a sample of oil with a solution of alcoholic potash saponification. The sample oils (5 g), containing 2.0 ml of ISS, was saponified with 50 ml of KOH/ethanol (0.5 mol/L) solution and heated for 1 h. The UM (unsaponifiable matter) was extracted three times with 60 ml of n-hexane in a separator funnel. After separation of non-saponifiable material, the analysis of sterols was done by an Agilent GC 6890N gas chromatograph equipped with a selective detector 5975B inert and a split-splitless injector, in splitless mode (Agilent, Palo Alto, CA, USA). The injection volume was 1μL using the solvent-flush technique. Oven temperature was 255˚C until it reached 285°C and was finally stopped 20 min into this temperature. The temperature site of injection was 300˚C (14).

2.2. Determination of fatty acid composition by GC

In order for lipid-bound fatty acids to be analyzed by gas chromatography (GC), first the fat is hydrolyzed into glycerol and fatty acids and then fatty acid converted to the corresponding fatty acid methyl esters (FAMEs) (15). All samples were injected into GC–FID for separation and quantification of the FAMEs. The analysis was carried out using a BPX-70 fused silica capillary column. The run was under an optimized temperature program as follows: initial column temperature 150˚C and remain for 10 min, programmed to increase at a rate of 10˚C min up to 180˚C. This temperature was maintained for 50 min. Helium was used as the carrier gas at a flow rate of 1 ml min−1 with a split ratio of 30:1 (16, 17).

2.3. Determination of saponification value

Saponification value is a measure of the average molecular weight (or chain length) of all the fatty acids present. The fatty acid composition obtained by gas chromatography was used to measure the saponification number of samples based on the AOCS standard cd-3-25 (18).

2.4. Statistical analysis

The analysis of data was performed using the SPSS ver.20 software and a probability value <0.05 was considered to indicate statistical significance. For comparison, Duncan's multiple range test was done at the 5% level. Charts were drawn using Microsoft Excel 2013 software.

3. Results and discussion

The compositions and amounts of desmethylsterols of sesame oil, sunflower, and corn oils that determined by gas chromatography are presented in Table 1. As noticed in the table, the percentage of β-sitosterol and campesterol in sesame oil and corn oil was higher than other sterols, whereas β-sitosterol and Δ7-stigmastenol in sunflower were highest, respectively. The results of this study are consistent with other studies (19). Table 2 shows desmethylsterols values in sesame oil alone and in mixing with different percentage of sunflower oil. According to the results, a decrease in β-sitosterol, campesterol and stigmastenol were noticed in sesame oil and sunflower oil mixture due to its low percentage in sunflower oil. Δ7-stigmastenol and Δ7-avenasterol increased in different mixtures of virgin sesame oil and sunflower oil. Table 3 and Fig. 1 shows that the mixing of corn oil at various concentrations r educed the amount of stigmasterol, Δ5-avenasterol, Δ7-stigmastenol, and Δ7-avenasterol, while an increase of campesterol of sesame oil was observed after mixing with corn oil. The same results reported by Youseff et al. (20), Aparicio et al. (21) and Jabeur et al. (22). Analyzing the data from Table 3, for the amount of campesterol, it was observed a significant increase in the mixing of corn oil and sesame oil to 15 percent.

Table 1. Desmethylsterols composition in sesame, sunflower and corn oils (expressed as percentage of total desmethylsterols).

Desmethylsterol	Campesterol	Stigmasterol	β-Sitosterol	Δ^5-avenasterol	Δ^7-stigmastenol	Δ^7-avenasterol	Others
Sesame oil (*Sesamum indicum* L.)	20.28	7.90	60.61	7.24	0.78	1.06	2.13
Sunflower oil (*Helianthus annuus*)	8.13	0.23	57	0.2	15.21	4.02	15.18
Corn oil (*Zea mays*)	20.51	6.28	64.6	2.89	0.24	0.46	5.02

Table 2: Composition of desmethylsterols in pure sesame oil and mixing of sesame oil and sunflower oil.

Sterol	Pure sesame oil (Control)	Sesame + sunflower oils 5%	Sesame + sunflower oils 10%	Sesame + sunflower oils 15%
Campesterol	20.28±0.27[a]	19.62±0.19[b]	19.45±0.29[b]	18.50±0.09[c]
Stigmasterol	7.90±0.06[a]	7.86±0.16[a]	7.18±0.10[b]	7.17±0.33[b]
β-Sitosterol	60.61±0.02 [a]	60.18±0.87 [a]	60.06±0.22[a]	58.44±0.93[b]
Δ5-24- stigmastadienol	0.75±0.1[b]	0.66±0.10[b]	0.89±0.11[a]	0.50±0.06[c]
Δ5-avenasterol	7.24±0.10[a]	7.20±0.38[b]	7.19±0.38[b]	7.19±0.04[b]
Δ7-stigmastenol	0.78±0.01[a]	1.01±0.06[b]	1.49±0.03[c]	1.74±0.05[d]
Δ7-avenasterol	1.06±0.09[a]	1.44±0.04[b]	1.83±0.06[c]	1.87±0.01[c]
Others	1.30	2.34	3.20	3.72

** Value followed by the same letter is not significantly different at the 0.05 level.*

Therefore, it's possible the use of campesterol to measure fraud detection when 15 or more percent of corn oil is added in pure sesame oil. Even though stigmasterol and β-sitosterol content's variation cannot be used to detect sesame oil adulteration because their content to be less than campesterol without a specific value (23, 24). Desmethylsterols as one of the important non-soap ingredients are used to detect fraud in seed or fruit oil (25).

Table 3. Composition of desmethylsterols in pure sesame oil and mixing of sesame oil and corn oil

Sterol	Pure sesame oil (Control)	Sesame + corn oils 5%	Sesame + corn oils 10%	Sesame + corn oils 15%
Campesterol	20.28±0.27[a]	20.35±0.11[a]	20.60±0.47[a]	21.03±0.19[b]
Stigmasterol	7.90±0.06[a]	7.84±0.03[ab]	7.67±0.07[b]	7.62±0.04[ab]
β-Sitosterol	60.61±0.02[a]	61.61±0.52[a]	62.20±0.13[a]	62.41±0.52[a]
Δ5-24- stigmastadienol	7.24±0.10[a]	7.17±0.10[b]	6.02±0.01[c]	5.87±0.41[d]
Δ5-avenasterol	0.75±0.1[a]	0.88±0.07[b]	0.91±0.01[b]	1.00±0.12[b]
Δ7-stigmastenol	0.78±0.01[a]	0.76±0.06[ab]	0.74±0.04[ab]	0.70±0.07[b]
Δ7-avenasterol	1.06±0.09[a]	0.97±0.12[b]	0.90±0.10[bc]	0.75±0.09[c]
Others	1.30	1.55	1.98	2.26

** Value followed by the same letter is not significantly different at the 0.05 level.*

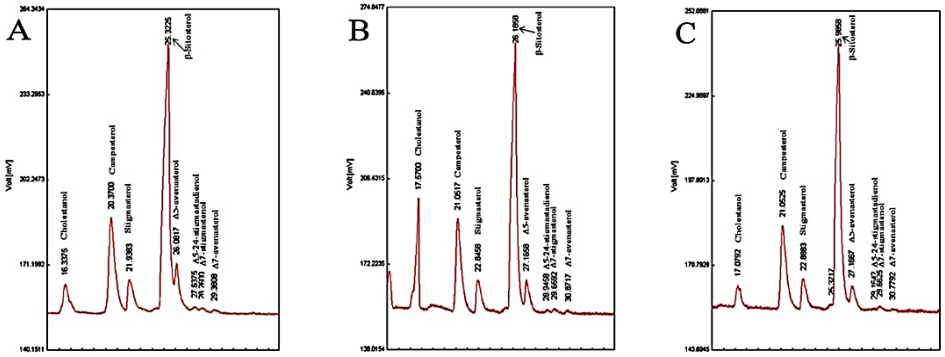

Fig. 1. GC-FID desmethylsterols chromatogram of an authentic. A: sesame oil 95% + corn oil 5%, B: sesame oil 90% + corn oil 10%, and C: sesame oil 85% + corn oil 15%.

4-Desmethylsterols have been used to detect olive oil adulteration with vegetable oils at levels as low as 5% (24). The same results were also obtained in this experiment. Δ7-stigmastenol and campesterol have been used to detect olive oil adulteration with sunflower and soybean oil (24). Different types of olive oil (virgin, refined and solvent-extracted) can be classified by using some 4-desmethylsterols (stigmasterol, clerosterol, Δ5-avenasterol, Δ7-stigmasterol, and Δ7-avenasterol) as a differentiating factor (26, 27). In this study, campesterol, Δ7-avenasterol, Δ5-avenasterol and Δ7-stigmastenol values were used as detectors for corn oil mixed with sesame oil, even at a concentration of 5% (except campesterol; 15%).

3.1. Determination of fatty acid composition

Fig. 2 chromatogram and Table 4 shows the amount of fatty acids in sesame oil, sunflower oil, and corn oil. According to the results of gas chromatography, the greatest amount of fatty acids found in sesame oil contains linoleic acid (43.21%) followed by oleic acid (37.93%), palmitic acid (11.03%) and stearic acid (5.08%), respectively. According to the results of sunflower oil, the amounts of myristic acid (C14: 0), linoleic acid (C18: 2), behenic acid (C22: 0) and lignoceric acid (C24: 0) were higher than the amount of these acids in sesame oil (Table 4).

Fig. 3 chromatogram and Table 5 shows the fatty acid composition of sunflower oil mixed with sesame oil in different concentrations. The decrease in oleic acid (C18: 1), linoleic acid (C18: 2), and linolenic acid (C18: 3) polyunsaturated fatty acids, as well as C20: 0, behenic acid

(C22: 0), lignoceric acid (C24: 0) saturated fatty acids at all concentrations of sesame oil in mixing with sunflower oil, was seen. Palmitic acid (C16: 0) and palmitoleic acid (C16: 1) values in concentrations of 10 and 15% of sunflower oil decreased significantly compared to the control sample.

Table 4. Compares the fatty acid composition of sesame oil, corn and sunflower oil used in this study with the standard values.

Fatty acid	Sesame oil	Standard (sesame)	Sunflower oil	Standard (Sunflower)	Corn oil	Standard (Corn)
C 14: 0	0.08	0.1>	0.09	0-0.2	0.05	0.3>
C 16: 0	11.03	7.9-12	7.28	5-8	11.50	8.6-16.5
C 16: 1	0.19	0.01-0.2	0.13	0.5>	0.09	0.5>
C 18: 0	5.08	4.8-6.7	4.07	2.5-7	2.20	0.33>
C 18: 1	37.93	35.9-43	24.64	13-40	31.49	20-42.2
C 18: 2	43.21	39.1-47.9	61.19	40-74	52.39	34-65.6
C 18: 3	0.47	0.3-0.5	0.30	2>	0.71	2>
C 20: 0	0.70	0.3-0.7	0.31	0.5>	0.56	0.3-1
C 20: 1	0.21	0.3>	0.16	0.5>	0.22	0.2-0.6
C 22: 0	0.20	1.1>	0.71	0.5-1	0.09	0.5>
C 24: 0	0.10	0.3>	0.25	0.2-0.3	0.19	0.5>
Other	0.32	-	0.67	-	0.32	-
Total	99.67	-	99.32	-	99.67	-

** Value followed by the same letter is not significantly different at the 0.05 level.*

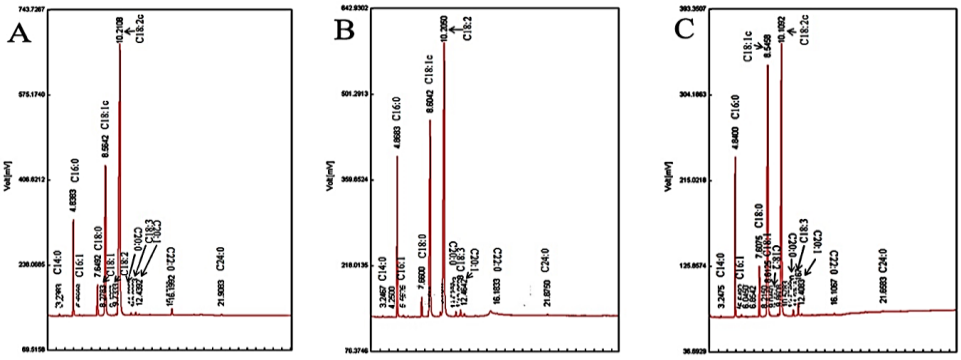

Fig. 2. Chromatogram of fatty acids obtained from A: sesame oil, B: sunflower oil, and C: corn oil.

Also, adding this oil in sesame oil caused a slight decrease in the stearic acid (C18: 0) in concentrations of 15% in comparison with pure sesame oil. In general, in the detection of sesame oil fraud, measuring the fatty acids include oleic, linolenic, linoleic and behenic is more suitable even at a concentration of 5% of sunflower oil adding. Changes in the types of fatty acids and its location in the triacylglycerol molecule can affect the quality and nutritional value of oil (28). Fig. 4 chromatogram and Table 6 shows the fatty acid composition of sesame oil in mixing with different concentrations of corn oil. The effect of adding corn oil was reducing myristic, palmitoleic and linolenic acids compared to the control values. With the addition of 10 and 15% corn oil to sesame oil, the concentration of stearic acid and oleic acid was decreased. Also, by mixing corn oil, linoleic acid was increased in all concentrations and lignoceric acid was increased at a concentration of 15% compared to the control.

Table 5. The fatty acid mixture of sesame and sunflower oils.

Fatty acid	Pure sesame oil (Control)	Sesame + Sunflower oils 5%	Sesame + Sunflower oils 10%	Sesame + Sunflower oils 15%
C 14: 0	0.08±0.001^{a}	0.08±0.001^{a}	0.08±0.002^{a}	0.08±0.005^{a}
C 16: 0	11.37±0.03^{b}	10.57±0.05^{b}	9.97±0.03^{c}	9.87±0.04^{c}
C 16: 1	0.19±0.001^{a}	0.19±0.002^{a}	0.18±0.003^{b}	0.18±0.001^{b}
C 18: 0	5.08±0.04^{a}	4.99±0.07^{a}	4.95±0.09^{a}	4.91±0.07^{b}
C 18: 1	37.93±0.03^{a}	37.29±0.01^{b}	36.83±0.30^{c}	35.92±0.07^{d}
C 18: 2	43.21±0.15^{d}	44.31±0.05^{c}	45.35±0.25^{b}	46.14±0.09^{a}
C 18: 3	0.47±0.01^{a}	0.39±0.01^{b}	0.34±0.01^{c}	0.26±0.01^{d}
C 20: 0	0.70±0.02^{a}	0.65±0.01^{b}	0.64±0.01^{b}	0.61±0.02^{c}
C 20: 1	0.21±0.001^{a}	0.18±0.001^{b}	0.18±0.001^{b}	0.18±0.001^{b}
C 22: 0	0.20±0.001^{d}	0.22±0.001^{c}	0.25±0.001^{b}	0.27±0.001^{a}
C 24: 0	0.10±0.001^{b}	0.11±0.001^{b}	0.12±0.001^{a}	0.13±0.001^{a}

** Different Latin letters in each row indicate significant differences at a confidence level of 95 percent.*

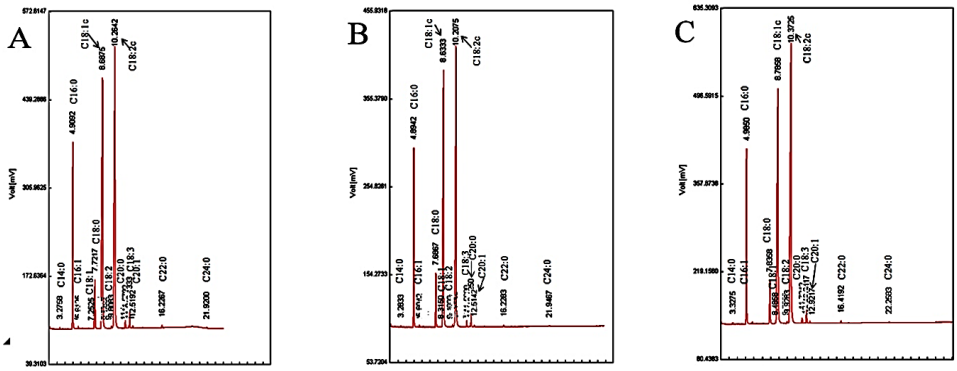

Fig. 3. Chromatogram of fatty acids obtained from A: sesame 95% and sunflower 5% oils B: sesame 90% and sunflower 10% oils C: sesame 85% and sunflower 15% oils.

Table 6. The fatty acid mixture of sesame and corn oils.

Fatty acid	Pure sesame oil (control)	Sesame + Corn oils 5%	Sesame + Corn oils 10%	Sesame + Corn oils 15%
C 14: 0	0.08±0.001[a]	0.07±0.05[b]	0.06±0.002[c]	0.05±0.005[c]
C 16: 0	11.37±0.03[b]	11.25±0.05[c]	11.55±0.13[a]	11.24±0.06[c]
C 16: 1	0.19±0.001[a]	0.19±0.002[a]	0.17±0.003[b]	0.15±0.004[c]
C 18: 0	5.08±0.04[a]	4.85±0.14[b]	4.73±0.14[b]	4.56±0.04[c]
C 18: 1	37.93±0.03[a]	37.41±0.16[b]	37.19±0.01[b]	36.97±0.02[c]
C 18: 2	43.21±0.15[d]	43.91±0.15[c]	44.27±0.05[b]	44.78±0.08[a]
C 18: 3	0.47±0.01[a]	0.44±0.05[a]	0.37±0.01[b]	0.35±0.01[c]
C 20: 0	0.70±0.02[a]	0.67±0.01[b]	0.66±0.02[b]	0.65±0.01[b]
C 20: 1	0.21±0.001[a]	0.18±0.001[b]	0.18±0.001[b]	0.18±0.001[b]
C 22: 0	0.20±0.001[a]	0.20±0.001[a]	0.19±0.001[a]	0.19±0.001[a]
C 24: 0	0.10±0.001[b]	0.11±0.001[b]	0.11±0.001[b]	0.12±0.001[a]

** Different Latin letters in each row indicate significant differences at a confidence level of 95 percent.*

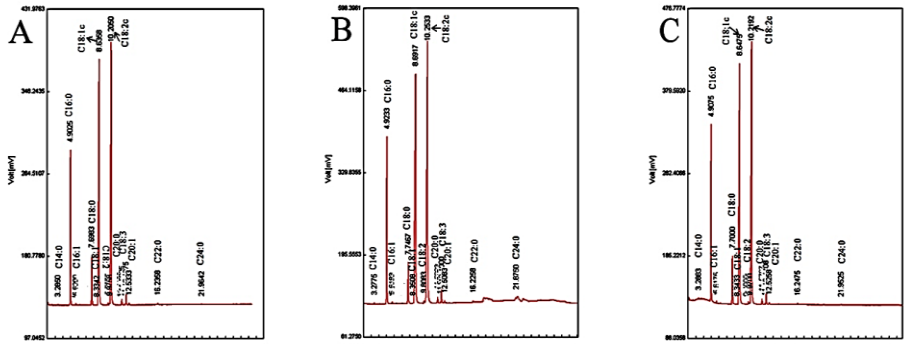

Fig. 4. Chromatogram of fatty acids obtained from A: sesame oil 95% and corn oil 5% B: sesame oil 90% and corn oil 10% C: sesame oil 85% and corn oil 15%.

Eicosenoic acid (C20: 1) and arachidic acid values had a slight decrease (Table 5). The composition of fatty acids has traditionally been used in the food industry as an indicator of purity (29). According to Hassanien and Abdel-Razek (30), mixing oils without any chemical and biological process changes the fatty acid composition and physicochemical properties of oils. This was consistent with the results of the present results. Fatty acid composition is useful for detection of adulteration of some oils such as olive oil with the following vegetable oils, including soybean, walnut, canola, rapeseed, peanut, and mustard, even at the level of adulteration below 5% (31). In this research, the fraud determination of sunflower and corn oils in sesame oil can be investigated by the fatty acid composition.

3.2. Saponification

Another unique property of edible oils that are used to detect and distinguish oils from each other, is saponification. This number is usually 175-250 for edible oils and represents the fatty acids structure attached to the glycerides. It is inversely related to the chain length fatty acids. In this study, the saponification number of sesame oil, corn oil, and sunflower oil were 192.82, 193.02 and193.83, respectively. These results

were in consistent with Codex ranges of saponification value of sesame oil (188-195) sunflower oil (188-194), and corn oil (189-196) (18). The same results were obtained by Rudnik et al. (32). According to Fig. 5, the addition of sunflower oil to sesame oil caused a significant increase in the amount of saponification in all proportions, so that the highest saponification was 15% sunflower oil (194.28). As shown in Fig. 5, saponification mixing of 5 and 10% sunflower oil to sesame oil was not significant in relation to each other, hence mixing of 5 to 10% is not easily identifiable, but the incorporation of 15 % is easily identifiable.

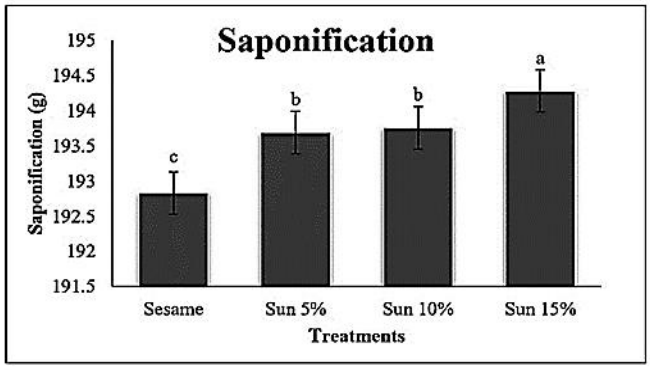

Fig. 5. Saponification values of sunflower oil mixed with the sesame oil in different concentrations (Different Latin letters on each column represents significant differences at a confidence level of 95 %).

The addition of 5% corn oil did not cause a significant change in the saponification value (Fig. 6). Therefore, if 5% corn oil is added to sesame oil, saponification method cannot be used to detect the fraud, but the saponification will be significantly higher at 10% or more.

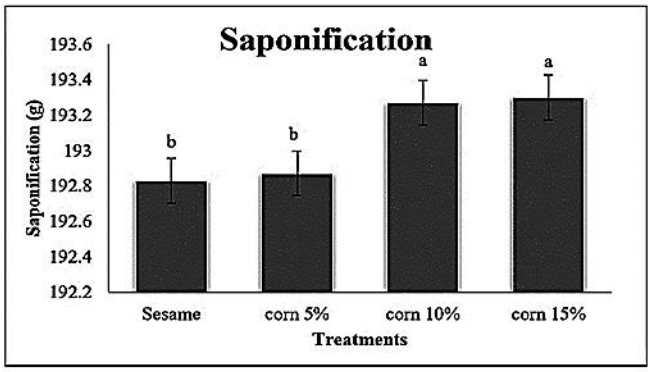

Fig. 6. Saponification values of corn oil mixed with the sesame oil in different concentrations (Different Latin letters on each column represents significant differences at a confidence level of 95 %).

Finally, saponification is reached to the maximum amount at 15% of corn oil adding (193.88) (Fig. 6). Obviously, the saponification value method cannot be useful in identification of sesame fraud in mixing with less than 10% corn oil. Generally, the use of physical properties like refractive index, viscosity, melting point, saponification and iodine value are no more practical for the detection of adulteration and modern methods (chromatography) are recommended. This recommendation has also been mentioned in other studies (2, 8, 9).

4. Conclusion

Fraud in sesame oil because of its high price occur frequently. In this experiment, three methods were used for detection of fraud and results showed that chromatography method was superior to saponification in mixing of corn oil to sesame oil, while fraud detection even at 5% sunflower oil mixing to sesame oil was identified by saponification method. This finding is due to the high saponification value of sunflower oil than corn and sesame oils. The contents of campesterol, Δ5-avenasterol, Δ7-stigmastenol and Δ7-avenasterol even at a concentration of 5% of mixing corn or sunflower oils, has been identified appropriate to identify adulteration. The fatty acids include oleic acid, linoleic, linolenic, arachidic, eicosenoic and behenic acids can be used for fraud detection the sunflower oil mixing to sesame oil, also in corn oil mixing, myristic, palmitic, stearic, oleic, linoleic, arachidic and eicosenoic acids were suitable options for fraud detection. Overall, the results of this study showed that saponification value, the fatty acids, and desmethylsterols compositions are useful for detection of adulteration in both corn and sunflower oils mixing.

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