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Composition of tocopherols in sesame seed oil: an indicative of adulteration

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RESUMEN

Composición de tocoferoles en aceite de semilla de sésamo: indicativo de adulteración

Este trabajo examina la importancia de los tocoferoles en la detección de la adulteración del aceite de sésamo comercializado en Brasil. Se analizaron cinco muestras a las que se le determinaron su composición en ácidos grasos, esteroles y tocoferoles. Una de las muestras se reveló puro aceite de semilla de sésamo; en otra, todos los parámetros estaban en desacuerdo. En las demás, el perfil de ácidos grasos caracterizaba el aceite de sésamo, sin embargo los tocoferoles y esteroles permanecieron en desacuerdo. Los resultados indican adulteración con otros aceites vegetales como soja, aceites láuricos y maíz.

PALABRAS-CLAVE: Aceite de semilla de sésamo - Adulteración - Composición de tocoferoles.

SUMMARY

Composition of tocopherols in sesame seed oil: an indicative of adulteration

The objective of this research was to verify how the composition of tocopherols can help to reveal adulteration in samples of sesame seed oils commercialized in Brazil. Five samples have been analyzed. One sample presented the composition of fatty acids, tocopherols and desmethylsterols of authentic sesame oil. Another one presented only non complying parameters. Three other samples showed the fatty acid composition of pure sesame oil, but the tocopherol and desmethylsterol profiles did not comply with those for sesame seed oil. The results indicate that samples could be adulterated by other vegetable oils like soybean, lauric and corn oils.

KEY-WORDS: *Adulteration - Sesame seed oil - Tocopherol composition.*

1. INTRODUCTION

The diversity of edible oils and fats has been growing all over the world. The search for new oleaginous seeds and the contributions of technological and biotechnological processes have promoted improvements in quantity and quality of fats and seeds but can alter the composition of the original food nutrients (Euro alert, 2000). Food authenticity is an important topic in both a commercial and a health point of view. Nowadays consumers are more demanding and conscious about their rights and the benefits food nutrients should provide to human health. These subjects have generated more studies to increase the knowledge of chemistry, composition and structure of oils and fats and therefore, a better characterization of them. The determination of the triacylglycerols and the minority components of the unsaponifiable fraction of vegetable oils, applying more sensitive and precise analytical techniques has aided in determining the quality and authenticity of those products (Aparicio and Aparicio-Ruiz, 2000; Aparicio, 2003; Cert *et al.*, 2000)

In Brazil there has been a high incidence of adulteration in vegetable oils, mainly in olive oil and other imported oils of high commercial value, and sesame oil is included in this category (Antoniassi *et al.*, 1988; Aued-Pimentel *et al.*, 1993; Aued-Pimentel *et al.*, 2002; Badolato *et al.*, 1981). The assessment of the identity of vegetable oils has been made by the composition of fatty acids and some classic parameters such as the iodine value and refractive index, and in many cases these are not enough to detect more elaborated frauds in vegetable oils. Determination of the desmethylsterol and fatty acid composition comprehend official methodologies to define the identity of edible vegetable oils (AOAC 1995; IUPAC 2.403, 1992). Antoniassi *et al.* (1998), to evaluate both quality and identity of olive oil samples marketed in Brazil, verifying that only the desmethylsterol composition provides the distinction and definition of identity and quality of the samples.

Several authors have pointed out the composition of the tocopherols and tocotrienols in vegetable oils as a good parameter to aid in the identification and differentiation of them (Aparicio and Aparicio-Ruiz, 2000). Gutfinger and Letan (1974) detected, through the determination of the tocopherol composition, the addition of soybean oil in olive oil samples. Soybean oil is rich in delta and gamma tocopherols while olive oil presents significant amounts of alpha-tocopherol. Dionisi *et al.* (1995) used HPLC with amperometric detector to determine the tocopherol and tocotrienol composition in vegetable oils and detected small quantities (1 to

2%) of palm and grape seed oils in olive oil. Mariani *et al.* (1999) employed tocopherol composition to investigate the addition of hazelnut oil in olive oil samples.

Tocopherols are natural antioxidants that inhibit oil oxidation. Tocopherols act as biological kidnappers of free radicals and could prevent diseases, besides possessing an important nutritional function for human beings as a source of Vitamin E (Brigelius-Flohe *et al.*, 2002; Monahan *et al.*, 1993). The objective of this paper is to determine the composition of tocopherols in sesame oils commercialized in Brazil and verify if this can indicate frauds or alterations in those vegetable oils, along with fatty acid and desmethylsterol composition.

2. MATERIAL AND METHODS

2.1. Samples, standards and reagents

Five (5) samples of sesame seed oil commercialized in Brazil have been analyzed.

Alpha, beta, gamma and delta high purity tocopherol standards were purchased from Merck. A mixture of fatty acid methyl ester standards from C_4 to C_{24} with certificate of composition was purchased from Supelco Park (Bellefonte, PA, USA). Desmethylsterol standards were purchased from Sigma Chemical (Saint Louis, USA). Methanol, n-hexane, isopropanol (HPLC grade) were obtained from EM Science (USA). All other solvents and chemicals had reagent grade.

2.2. Methods

2.2.1. Composition of tocopherols

Tocopherols were separated and quantified by HPLC, according to AOCS Ce 8-89 methodology. Oil was dissolved in n-hexane and submitted directly to HPLC analysis. A system of liquid chromatography from Shimadzu was used, composed by the modules: bomb LC-10AD; Rheodyne Injector with a 20 mL loop, fluorescence detector (RF-10AXL) with wavelengths of excitation at 290 nm and of emission at 330 nm; communication module CBM-10-AD and software Class LC10-AD for data storing and total control of the system.

The components were separated in a normal phase chromatographic column SI 60, 5 µm, 250 x 4.6 mm id purchased from SGE Analytical Products. The mobile phase was 1.2 mL/min flow n-hexane/isopropanol (99.5:0.5 V/V). The solvents used had HPLC grade. The mobile phase was filtered through 0.45 µm membrane and degassed with helium gas for 15 minutes in a degasser on line with the chromatograph (Model DGU-14 - Shimadzu). Oils were diluted in n-hexane to obtain a concentration of about 8 mg/mL and filtrated through a 0.45µm membrane and injected into the chromatograph. Samples were analyzed three times.

Tocopherols were quantified by external standard with alpha, beta, gamma and delta tocopherols, high purity standards (Merck). Standards' purity was monitored by measures of $E^{1\%}_{1cm}$ in methanol in an HP 8453 spectrophotometer.

Tocopherols were identified by co-injection of tocopherol standards by comparison of the retention times and through the addition of standards in the samples. Quantification was performed by plotting calibration curves from tocopherol standards and comparing the peak area of the correspondent peaks in samples.

2.2.2. Composition of fatty acids

Fatty acid methyl esters were prepared by an acid catalyzed procedure. About 25 mg of oil sample was weighed into a transmethylation flask and 15 mL of a methanol solution with 2% H_2SO_4 and 3 mL of n-hexane were added. The sample was refluxed for 45 minutes. A saturated solution of NaCl was added to separate the phases (Instituto Adolfo Lutz, 2004; Badolato and Almeida, 1977). The upper phase (1µL) was analyzed in a GC-17A Shimadzu model gas chromatograph equipped with a flame ionization detector. The compounds were separated in a 50 m CP-Sil 88 capillary fused silica column, 0.25 cm internal diameter and 0.20 mm film thickness. Operation conditions were as follows: oven temperature 80 to 220°C (5°C/min); injector temperature, 230°C; detector temperature, 240°C; carrier gas: hydrogen; gas linear velocity 40 mL/min; ratio of sample division, 1:50. Fatty acid methyl esters were identified by co-injection of the standards. Quantification was performed by area normalization.

2.2.3. Composition of desmethylsterols

The unsaponifiable matter of oils was separated following AOCS method Ca-6b-53, 1997. Thin layer chromatography was used to fractionate the unsaponifiable matter in accordance to IUPAC method 2.403. The desmethylsterol fraction was submitted to gas chromatography. The components were separated by capillary fused column of methylsilicone (HP-1) of 25 mm length, 0.32 mm internal diameter and film thickness of 0.17 µm. The analysis was made with the oven temperature at 260°C to 290°C (3°C/min); injector temperature, 300°C; detector temperature, 300°C. The components were identified by co-injection of standards and by comparison of sesame and sunflower oils obtained from seeds and authentic olive oil analyzed at the same time. Quantification was performed by area normalization.

3. RESULTS AND DISCUSSION

Tables 1, 2 and 3 present, respectively, the composition of tocopherols, fatty acids and desmethylsterols obtained in commercial sesame

Table 1
Composition of tocopherols in vegetable oils samples (mg/100 g of oil)

Tocopherols	Alpha	Beta	Gamma	Delta
Sample A * **Commercial sesame seed oil**	2.4± 0.1	0.28±0.04	42 ± 1	0.71±0.04
Sample B* **Commercial sesame seed oil**	36 ± 2	0.80±0.04	16 ± 3	0.17±0.05
Sample C* **Commercial sesame seed oil**	0.24± 0.05	0.38±0.09	57±4	0.91±0.06
Sample D* **Commercial sesame seed oil**	19 ± 2	0.58 ± 0.06	38 ± 3	13 ± 2
Sample E* **Commercial sesame seed oil**	7.4 ± 0.1	0.8±0.2	25 ± 1	0.79±0.03
Sunflower **	40.3 –93.5	ND –4.5	ND-3.4	ND-0.7
Sesame **	ND- 0.33	ND	52.1-98.3	0.4 -2.1
Soybean**	0.9 -35.2	ND-3.6	8.9 - 230.7	15.4-93.2
Corn**	2.3 -57.3	ND-35.6	26.8-246.8	2.3-7.5

*Mean and standard deviation of three determinations.
** Codex Stan 210, 1999.
ND – Non detectable

oil samples, codified as A, B, C, D and E. Tables 1, 2, 4 and 5 present reference values for tocopherols, fatty acids and sterols from literature.

All samples presented percentages of some fatty acids different from values referred by Codex Alimentarius (1999), but samples B and E are in compliance with AOCS ranges (Firestone, 1999). The fatty acid composition of 721 from different samples of sesame seed oil obtained on a worldwide basis by Yermanos *et al.* (1972) was presented in Table 2 and these values differed markedly from the ranges of AOCS (Firestone, 1999) and Codex Alimentarius (1999). The fatty acid profiles of samples A, B and E are in compliance with reported values by Yermanos *et al.* (1972). The fatty acid profile of sample C was in compliance with that range, except for palmitic acid, which was just a little below.

At the same time, considerable differences were observed among the relative proportions of desmethylsterols of sesame seed oil from the literature (Table 4). The ranges reported by Codex Alimentarius (1999) and AOCS (Firestone, 1999) are not comparable with profiles of desmethylsterols of sesame (*Sesamun indicum*) from Sudan (Kamal Eldin *et al.*, 1992, 1994b) and samples of sesame oil from Central Africa, Egypt, Nigeria, Sudan and Mexico reported by Bocca *et al.* (1988). The high percentage of Δ^7-stigmastenol and Δ^7-avenasterol reported by Codex and AOCS are common to sunflower (*Helianthus annuus* L.) and safflower oils (*Carthamus tinctorius* L.).

Table 2
Fatty acid composition of sesame seed oil samples (expressed as percentage of total fatty acids)*

Fatty acid samples	C8:0	C10:0	C12: 0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2 cis	C18:2 trans	C18:3 cis	C20:0	C20:1	C22:0	C24:0
Sample A	-	-	-	-	9.97	0.11	0.08	6.09	34.93	46.96	0.59	0.31	0.56	0.16	0.10	0.11
Sample B	-	-	-	-	9.77	-	0.09	4.55	38.13	46.23	-	0.39	0.44	0.17	0.07	0.15
Sample C	-	-	-	-	7.38	0.13	0.12	4.18	39.68	47.19	0.16	0.31	0.34	0.16	0.29	0.10
Sample D	038	0.33	5.10	1.77	15.00	-	0.10	1.90	28.59	42.49	-	3.49	0.28	0.22	0.16	0.23
Sample E					9.14	0.14	0.07	5.32	42.78	40.87		0.51	0.55	0.26	0.12	0.22
Codex Stan 210 (1999)				ND-0.1	7.9-12.0	0.1-0.2	ND-0.2	4.8-6.1	35.9-42.3	41.5-47.9	-	0.3-0.4	0.3-0.6	ND-0.3	ND-0.3	ND-0.3
Firestone (1999)				ND-0.1	7.9-12.0		ND-0.2	4.4-6.1	33.5-44.1	40.3-50.8	-	0.3-0.5	0.3-0.7	ND-0.3	ND-0.3	ND-0.3
Yermanos *et al* (1972)					8.3-10.9			3.4-6.0	32.7-53.9	39.3-59.0						

*Mean of two determinations.
ND – Non detectable, defined as ≤ 0.05%

Table 3
Composition of desmethylsterols in sesame seed oil samples (expressed as percentage (%) of total desmethylsterols)

Desmethysterol	Sample A	Sample B	Sample C	Sample D	Sample E
Campesterol	15.83	13.22	18.57	14.94	21.42
Stigmasterol	6.40	6.43	6.41	12.36	5.39
β-sitosterol	57.58	64.28	59.15	54.30	57.23
Δ^5-avenasterol	17.22	5.77	12.36	6.47	6.14
Δ^7-stigmastenol	ND	7.43	0.24	5.33	0.68
Δ^7-avenasterol	0.82	2.87	0.86	3.06	0.76
Others	2.15	-	2.41	2.53	8.36

Sample A presented a desmethylsterol composition close to literature values for sesame oil (Table 3 and 4), except for the high content of Δ^5-avenasterol. However, the alpha-tocopherol content in sample A (2.4 ± 0.1 mg/100g) was above the limit for sesame seed oil while gamma-tocopherol contents (42 ± 1mg/100g) were below the reference values (Table 1).

The Alpha-tocopherol content of samples B and E was above and gamma-tocopherol below reference values (Table 1). The desmethylsterol composition of sample B showed that Δ^7-stigmastenol percentage was above, and Δ^5-avenasterol was below the expected values for sesame seed oil (Tables 3 and 4). The results suggest that sunflower oil was probably added to sample B.

It is well known that sesame oil contains natural antioxidants, as sesamin and sesamolin (lignans). These are characteristic components of sesame seed oil (Hemaltha and Ghafoorunissa, 2004) and are eluted and identified during HPLC analyses of tocopherols by the fluorescent detector (Antoniassi and Souza, 2001; Coors and Montag, 1985; Kamal-Eldin, 1994a). In all samples, except in sample E, the chromatographic profile of tocopherols revealed the presence of peaks 2 and 5 in Figures 2A and 2B, probably the lignans mentioned above.

The isolation, analysis and identification of sesamolin and sesamin from sesame seed oil along with desmethylsterol fraction had been carried out in previous investigations (Antoniassi *et al.*, 2002a, b). The desmethylsterol fraction isolated by TLC analysis (70:30 petroleum ether/diethyl ether) was analysed by GC-MS. The analysis was performed on an Agilent 6890 chromatograph coupled with an Agilent 5973N mass spectrometer. Mass spectrum was obtained by electron impact ionization at 70 eV. Sesamolin and sesamin were detected in considerable amounts in the desmethylsterol fraction of sesame seed (*Sesamun indicum*), under these conditions. Figure 1 shows a desmethylsterol chromatogram of authentic sesame seed oil analyzed at the same time. The natural antioxidants co-eluted before the sterols are indicated in the chromatogram of Figure 1. They were not observed in sample E. The absence of these compounds can indicate that the sample was not sesame oil or it was submitted to a process that eliminated those compounds. Sample E presented an alpha-tocopherol content above (7.4 ± 0.1mg/100g) and gamma-tocopherol (25 ± 1mg/100g) below the ranges for sesame seed oil (Table 1). Fatty acid composition was inside the range referred to in the literature for corn oil and tocopherol contents were close to the values for this oil. The campesterol percentage stood above for sesame seed oil. The desmethylsterol profile of sample E could be easily found in other vegetable oils, like corn oil (Table 5). The low contents of Δ^7-stigmastenol and Δ^7-avenasterol indicate that adulteration was probably not elaborated with sunflower oil. The results suggest that corn oil was the adulterant of sample E.

The composition of fatty acids, tocopherols and desmethylsterols of sample D were over the limit

Table 4
Composition of desmethylsterols of sesame seed oil (*Sesamum indicum*) (%).

	Campesterol	Stigmasterol	β-Sitosterol	Δ^5-avenasterol	Δ^7-stigmastenol	Δ^7-avenasterol	Others
Codex Stan 210 (1999)	10.1-20.0	3.4-12.0	57.7-61.9	6.2-7.8	0.5-7.6	1.2-5.6	0.7-9.2
Firestone (1999)	10.1-20.0	3.4-6,4	57.7-61.9	6.2-7.8	1.8-7.6	1.2-5.6	0.7-9.2
Bocca *et al.* (1988)	17.6-20.2	6.2-8.1	53.0-60.4	8.0-14.2	0.2-0.4	0.4-0.8	-
Kamal-Eldin *et al.* (1992)	15.4-20.3	5.4-8	60.3-66.9	6.4-10.6	0.8-2.7	0.3-1.4	-
Kamal-Eldin and Appelqvist (1994)	12.5-16.9	6.0-8.7	57.5-62.0	8.1-11.5	0.4-3.1	0.3-1.3	-

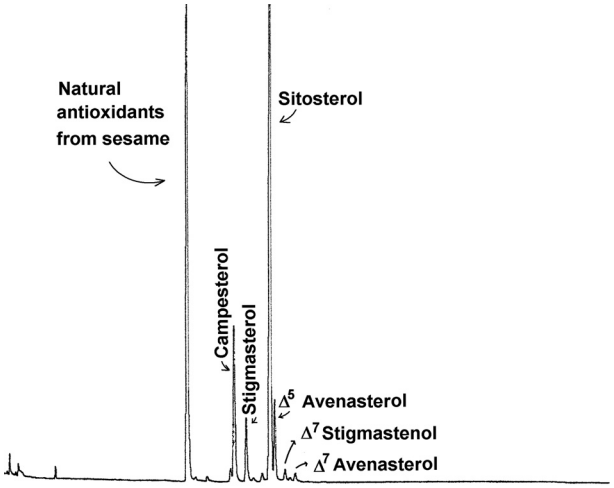

Figure 1
GC-FID desmethylsterols chromatogram of an authentic sesame seed oil

values referred to in the literature for sesame seed oil (Tables 1-4). The percentage of palmitic, linolenic, miristic and short-chain fatty acids (below $C_{14:0}$) were above the reference values for sesame seed oil (Table 2), while the percentage of oleic acid was below the expected**.** The quantity of linolenic acid found in sample D (3.5%), could indicate the presence of canola or soybean oil. Regarding the composition of tocopherols, the contents of alpha and delta tocopherols found were above the values referred to for sesame oil (Table 1). Soybean oil presents quantities of those tocopherols similar to the values found in sample D. The content of campesterol was compatible with reference values for sesame seed oil and stigmasterol was above expected values (Tables 3 and 4). Soybean oil presents similar quantities of campesterol and stigmasterol which are higher than 15%. Similar proportions of campesterol and stigmasterol were observed in sample D, close to 15% (Table 3). The results indicate that the most probable alterations adulterants of sample D would be the addition of soybean and lauric oils.

Figure 2A shows a chromatogram from pure sesame seed oil tocopherols; Figure 2B: sample D, probably adulterated with soybean oil; and Figure 2C: pure soybean oil.

Sample C showed fatty acid, desmethylsterols and tocopherol profiles close to the values from the literature for authentic sesame seed oil.

Briefly, the results showed that through fatty acid composition the adulteration of sesame oil was not conclusive, with the exception of sample D (inconclusive for D or only conclusive for D?)**.** Considering the composition of tocopherol, the other three samples presented alteration in the profile for sesame oil (Samples A, B and E). The adulterations

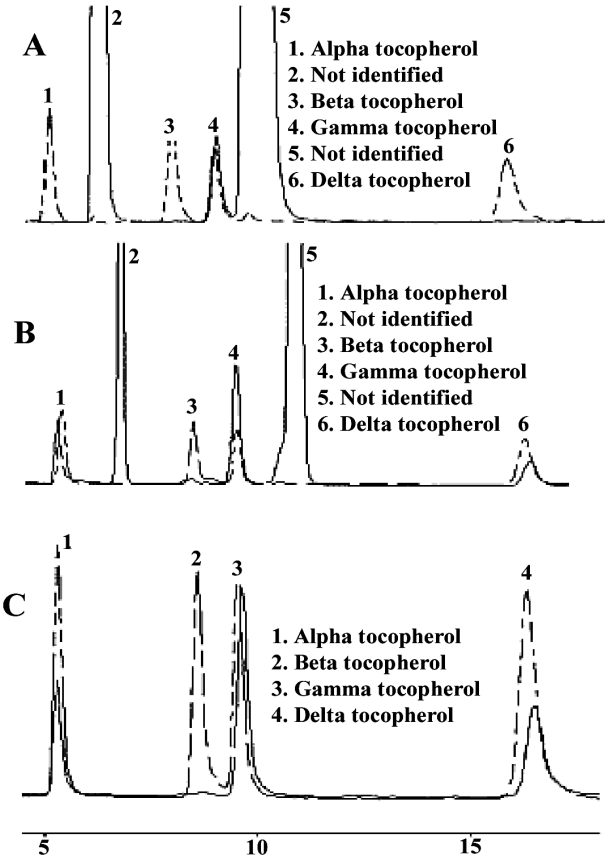

Figure 2
HPLC with fluorescence detection chromatograms of tocopherols in vegetable oils. A) Authentic sesame seed oil. B) Authentic soybean oil C) Sesame seed oil probably adulterated with soybean oil (sample D).
- - - - Tocopherol standards
------- Vegetable oil samples

Table 5
Composition of desmethylsterols in vegetable oils (%)*

Desmethylsterol	Soybean oil *(Glycine max)*	Sunflower oil *(Helianthus annuus)*	Corn oil *(Zea mays)*
Campesterol	15.8 - 24.2	6.5-13.0	16.0 - 24.1
Stigmasterol	14.9 - 19.1	6.0-13.0	4.3 - 8.0
β-Sitosterol	47.0 - 60.0	50.0 -70.0	54.8 - 66.6
Δ^5-avenasterol	1.5 - 3.7	ND - 6.9	1.5 - 8.2
Δ^7-stigmastenol	1.4 - 5.2	6.5 - 24	0.2 - 4.2
Δ^7-avenasterol	1.0 - 4.6	3.0 - 7.5	0.3 - 2.7
Others	ND - 1,8	ND - 5.3	ND- 2.4

*Codex Stan 210, 1999. ND – Non detectable, defined as ≤ 0.05%

or alterations were confirmed through desmethylsterol analysis. The percentage of some desmethylsterols was over the limits found in the literature consulted. The observed alterations suggest the addition of other vegetable oils of low commercial value in Brazil, like soybean oil (sample D) and corn oils (sample E).

Due to the wide range that has been found in fatty acid and desmethylsterol profiles of sesame seed oil, the application of these parameters to uncover frauds or alterations is useful only when great differences are observed.

The evaluation of the tocopherol profile of vegetable oils could supply important information about identity and alterations of the vegetable oils studied.

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