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Characteristics and composition of tomato seed oil

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RESUMEN

Características y composición del aceite de semilla de tomate

Se separaron semillas de tomate de la pulpa seca y posteriormente se trituraron y extrajeron con éter de petróleo en caliente. El aceite extraído se desgomó, neutralizó y decoloró, y luego se determinaron las características físicas y químicas de los aceites crudos y purificados. La purificación produjo una disminución en la acidez, color, insaponificables, $E^{1\%}_{1cm}$ 232 y estabilidad oxidativa y un aumento en el punto de humo y $E^{1\%}_{1cm}$ 270. Las propiedades físico-químicas no se afectaron por la purificación. Se encontró que el aceite de semilla de tomate contenía altos niveles de linoleico (54%), seguido por oleico (22%), mientras que los ácidos grasos saturados mayoritarios fueron el palmítico (14%) y el esteárico (6%). La purificación produjo también un aumento en el C 18:2 trans, mientras que el perfil de ácidos grasos del aceite permaneció inalterado. Se detectaron α- y δ- tocoferoles a niveles de 202 y 1059 mg/kg; la purificación redujo el contenido en tocoferol a 161 y 898 mg/kg, respectivamente. El β-sitosterol fue el componente predominante de la fracción esterólica del aceite de semilla de tomate. Se detectó un alto porcentaje de colesterol (16%). Otros esteroles encontrados en porcentajes más elevados del 1.5% fueron el campesterol, estigmasterol y Δ^5-avenasterol. Además, se encontraron como componentes minoritarios 24-metilenecolesterol, brasicasterol, Δ^7-campesterol, clerosterol, $\Delta^{7,24}$-estigmastadienol, Δ^7-estigmastanol, Δ^7-avenasterol y eritrodiol. El perfil de esteroles no se afectó por la purificación.

PALABRAS-CLAVE: Aceite de semilla de tomate — Acido graso — Estabilidad — Esterol — Purificación — Tocoferol.

SUMMARY

Characteristics and composition of tomato seed oil

Tomato seeds were separated from dried pomace, and seeds were ground and extracted with hot petroleum ether. The extracted oil was degummed, neutralised and bleached, and then the physical and chemical characteristics of crude and purified oils were determined. Purification led to a decrease in acidity, colour, unsaponifiables, $E^{1\%}_{1cm}$ 232 and oxidative stability, and to an increase in smoke point and $E^{1\%}_{1cm}$ 270. The fundamental physicochemical properties of the oil were not affected by purification. Tomato seed oil was found to contain high levels of linoleic (54%), followed by oleic (22%), while the dominant saturated acids were palmitic (14%) and stearic (6%). Purification led to an increase in C18:2*trans*, while the fatty acid profile of the oil remained unchanged. α- and δ-tocopherols were detected at levels of 202 and 1059 mg/kg; purification reduced tocopherol content to 161 and 898 mg/kg, respectively. β-sitosterol was found as the most predominant component of the sterolic fraction from tomato seed oil. A large percentage (16%) of cholesterol was detected. Other sterols found in percentages higher than 1.5% were campesterol, stigmasterol and Δ^5-avenasterol. In addition, trace to minor amounts of 24-methylenecholesterol, brassicasterol, Δ^7-campesterol, clerosterol, $\Delta^{7,24}$-stigmastadienol, Δ^7-stigmastanol, Δ^7-avenasterol and erythrodiol were found. Sterol profile was not affected by purification.

KEY-WORDS: Fatty acid — Purification — Stability — Sterol — Tocopherol — Tomato seed oil.

1. INTRODUCTION

Tomato pomace, a waste product from tomato processing plants, consists of skins, pulp and seeds. The major component of tomato pomace is the seeds, which are considered to be a good source of an edible oil (1, 2, 3, 4, 5). Tomato is one of the most widely cultivated vegetable crops in Greece. According to data received from the Ministry of Agriculture of Greece, the amounts of tomatoes produced for industrial processing were for the year 1994 1,105,984 metric tons, for 1995 1,020,740 tons and for 1996 1,268,930 tons, while additional amounts of 565,712, 513,217 and 600,040 tons, respectively for the three years, have been produced for raw consumption (salad and cooking). Thus, the amount of seeds resulting annually is quite enough to produce 1,300 to 2,000 tons of edible oil. The oil has a high unsaturated acid content, with over 50% linoleic acid (3, 4) and could be used in cooking. Besides, tomato seed cake was found to contain 23-39% protein with good functional and nutritional properties (6, 7, 8). In addition, the seeds have been reported to posses antioxidant properties (9, 10).

Though data on tomato seed oil composition are already available in literature, more detailed information is needed, as in many cases they remain controversial. The objective of the present work was to contribute to extent the knowledge of chemical composition of tomato seed oil as well as to determine the effect of purification on it. Such data will be useful for studying the behavior of the oil during heating and processing.

2. MATERIALS AND METHODS

Sample preparation and oil purification

Samples of tomato pomace were obtained from tomato processing plants of Kopais region, Greece. Quantities of 20 kg of pomace were collected twice per week and for a total period of one month, from two different plants. After receiving, the samples were dried in an air-dryer (Apex Construction Ltd., England) at 60°C, ground in a blender and the seeds were separated using a sieve system of 2.0 and 1.6 mm. The separated seeds of all samples were combined and then ground to a powder by a Brabender mill to pass through a 0.70 mm sieve. Batches of ground seeds were extracted for 15 hr with petroleum ether (b.p. 40-60°C) in 2L Soxhlet extractors. The solvent was evaporated under reduced pressure, and the oils from different batches were combined and kept in sealed bottles under refrigeration (0-4°C) for further processing and analysis.

Tomato seed oil was degummed using the following procedure developed in our laboratory. Fifty grams of oil were put in 100 mL capacity pyrex test tubes and then were immersed in a water-bath adjusted at 90°C. Afterwards, a 3% (w/w) water at 80°C and 0.3% (w/w) phosphoric acid were added under continuous stirring. The mixture was stirred for 10 min, cooled, centrifuged for 5 min at 3000 rpm, and decanted to obtain degummed oil.

Batches of 500g of oil were put into 1L beakers, heated at 80°C, and then 4.7 mL of 4.125 M NaOH were added under continuous stirring. Stirring was continued for 40 min to coagulate the soap, and then left to stand for soap separation. Most of the neutralized oil was obtained by decanting, and the remaining soap-stock was centrifuged at 3000 rpm for further oil separation. Afterwards, the oil was transferred into a separatory funnel and was washed with 15% (w/w) distilled water (80°C) by shaking vigorously the separatory funnel, and allowed to stand for 15 min. The lower aqueous layer was separated and discarded.

The purified oil was heated at 105°C for moisture removal, and afterwards, was bleached by adding 3% Tonsil Optimum earth technical powder, plus 2% activated carbon. The mixture was stirred for 15 min and then filtered under vacuum using a Whatman No 1 filter paper. The color of the oil as measured by a Lovibond tintometer was changed from the initial 22 red and 10 yellow units to 10 red and 2 yellow units.

Analytical procedures

Free fatty acids (FFA), peroxide value (PV), and ultra-violet absorption at 232 and 270 nm were determined using the IUPAC methods (11). Iodine value (IV) was determined using the Wijs method as described in Pearson's (12). Refractive index of the oil was measured at 40°C with an Abbe refractometer with temperature adjustment; color was measured with a Lovibond tintometer; viscosity was measured using a Brookfield LVT rotational viscometer equipped with a No 2 spindle rotated at 60 rpm at 21°C; and smoke point with a method adapted from that described by BS 648:Section 1.8 (13). Oil stability was evaluated by the Rancimat method using the Rancimat 679 until the oil batch reached the temperature of 120°C and with an air flow rate set at 20 L/hr. Extra virgin olive oil and sunflower oil (ELAIS SA, Athens, Greece) without added antioxidants were examined concurrently for comparison reasons.

Fatty acids were characterized by gas-liquid chromatography (GLC). The fatty acid methyl esters (FAME) were prepared according to the following method optimized in our laboratory and based on the well known methanolic NaOH - BF_3-MeOH method (14,15). About 25 mg of oil was accurately weighed into a screw cap tube and 1.5 mL 0.5M methanolic sodium hydroxide was added, mixed and heated at 100°C for 7 minutes. After cooling 2 mL of boron trifluoride was added and heated at 100°C for 5 minutes. The tube was cooled to 30-40°C and 1 mL iso-octane was added, capped and shaken using a whirli mix for 30 seconds. 5 mL of saturated sodium chloride solution was immediately added and the tube was shaken again. The tube contents were allowed to separate and the top (iso-octane containing FAME) layer was removed and the lower layer was re-extracted with an additional 1 mL iso-octane. The two iso-octane extracts were combined, dried over anhydrous sodium sulfate if necessary, and concentrated to approximately 1 mL using a nitrogen stream. Internal standard solution for FAME determination was prepared by dissolving 100 mg (±0.1 mg) pentadecanoic acid (C15:0) in 50 mL iso-octane (16). One mL and/or 500 μL of the internal standard solution were added into oil and treated as previously described.

The analysis of FAME were performed with a Carlo Erba 5600 gas-liquid chromatograph equipped with a 50 m, 0.22 mm ID, 0.25 μm thickness, BPx70 capillary column. Operating conditions were as follows: Helium flow rate 25.6 mL/min; injector and detector (FID with hydrogen flow 40 mL/min and air flow 450 mL/min) temperatures were 235°C. The temperature programme was 160 - 210°C at a rate of 1.5°C/min, initial time at 160°C was 0.0 min and final time at 210°C was 5.0 min. Methyl esters were identified and quantified by comparing the retention time and peak area of the unknowns with those of FAME standards (British Greyhound, Chromatography and Allied Chemicals).

Tocopherols were determined by high performance liquid chromatography (HPLC) using a modification

of Carpenter's method (17). One gram of oil was accurately weighed into a 3 dram sample vial wrapped in foil paper to prevent oxidation. The oil was dissolved in 5 mL n-hexane, HPLC grade, before injection. A 20 μL sample was injected into a Waters 600E HPLC fitted with a μ-Polarsil, 125Å, 10 μm, 3.9x300 mm HPLC column with the following characteristics: irregularly-shaped silica. Detection was made with a Waters 486 Tunable Absorbance Detector set at 295 nm. Iso-propanol:n-hexane: absolute ethanol (2:97.5:0.5) at 1 mL/min was used as mobile phase. Waters Baseline 815 programme running on a computer was used as integrator for the determination of the standard calibration curves and for the calculation of the amounts of tocopherols in the oil samples. Tocopherols obtained from Merck (DL-α-tocopherol) and Sigma ((+)-δ-tocopherol), were used as standards.

Unsaponifiable matter and sterols were determined using the standard method of the Official Journal of the European Communities (18). Unsaponifiables were prepared by saponification with ethanolic KOH 2M, washing and ether extraction using 500 μL/5 g solvent of a 0.2% α-cholestanol solution as an internal standard. Sterolic fraction was separated by TLC using silica gel plates, developed with a 65:35 (v/v) hexane:ethyl ether mixture, spraying with 2,7-dichlorofluorescein, scraping and extracting with chloroform and ethyl ether. Sterols were converted to trimethylsilyl (TMS) ethers using a silylation reagent consisting of a 9:3:1 (v/v/v) mixture of pyridine:hexamethyldisilazane: trimethylchlorosilane in the ratio of 50 μL for every milligram of sterols. The measurement was done on a Carlo Erba 5600 Gas Chromatograph using a 25m x 0.35mm x 0.25 μm WCOT Fused Silica 7452 CP-Sil-8CB capillary column. The pressure of the carrier gas (H_2) was 110 kPa. Injector and FID temperatures were 280°C and 300°C respectively. The temperature programme was isothermal 260°C for 40 min at least. Sterols obtained from Laroden AB (Sweden), were used as standards.

3. RESULTS AND DISCUSSION

The oil content of tomato seeds was found to be 21.8%, and fell in the range previously reported for seeds received from tomato processing plants (2, 4, 5, 19). The seed oil was a red-yellowish liquid at ambient temperature and had a mild tomato fruit-like odor.

The physical and chemical characteristics of crude and purified tomato seed oil are shown in Table I. It is apparent that purification did not alter the fundamental physicochemical characteristics of the oil. As expected, the basic changes happened are referred to a decrease in acidity, color and unsaponifiables, as a result of degumming, neutralisation and bleaching. An increase in smoke point was observed due to free fatty acid reduction, as they are much more volatile than glycerides, and hence, smoke point depends on the free fatty acid content (20). The obtained values for density and refractive index are in line with those reported in literature (2, 4). Viscosity values were similar to those for olive and cottonseed oils and much higher than those for corn, peanut, soybean, and safflower oils (20,21). The peroxide value of tomato seed oil was lower than 20 meq/kg and fell in the range adopted as satisfactory. There was no change in peroxide value during purification and bleaching. This may be due to tocopherols and hence, purified tomato seed oil should expected to show an overall lower stability compared to crude oil. A 4.85% reduction in induction time, as measured by Rancimat, was observed (Table I), which could be attributed to oil purification. Tomato seed oil has values for specific extinction in UV at 232 and 270 nm similar to other vegetable oils such as olive and sunflower oils (22). Generally, R-value is higher if secondary oxidative changes are lower, i.e., the value for $E^{1\%}_{1cm}$ 270, though the same peroxide values show different values for specific extinction. Laboratory purification led to a decrease in $E^{1\%}_{1cm}$ 232 and to an increase in $E^{1\%}_{1cm}$ 270, resulting in a decrease in R-value (Table I). Similar findings have been reported by Vogel (23) for pumpkin seed oil, who furthermore reported that bleaching and deodorisation exert the main action, while degumming and neutralization had no actual effect on specific extinction.

The saponification value was in the reported range (2,4). The oil had a high iodine value, thus reflecting a high degree of unsaturation.

Table II shows the fatty acid composition of tomato seed oil. Total unsaturated fatty acids were 80%, while the major fatty acid was linoleic (C18:2) in a concentration of 54%, followed by oleic (C18:1), 22%. Generally, the level of linoleic acid falls in the range of 50-60%, while Swern (20) reports concentrations of oleic acid higher (46%) than those of linoleic (35%). Palmitic acid (C16:0) was found to be the dominant saturated fatty acid and was in line with values reported (1, 4, 5, 20, 24, 25). Higher palmitic acid concentrations, in the range of 20.28-24.81%, have been reported by El-Tamimi *et al.,* (3). The same authors have also reported lower concentrations for stearic acid (C18:0), 2.36-2.96%, and relatively high concentrations of arachidic acid (C20:0), 1.93-2.20%. The concentrations of C20:0 found in the present study were in agreement with those reported by Tsatsaronis and Boskou (26), and Lazos and Kalathenos (4). Small to trace amounts of C14:0, C16:1, C17:0, C18:2*trans*, C20:0, C20:1, C22:0 and C24:0 were also found in tomato seed oil. Tsatsaronis and Boskou (26) have reported that odd- and even-chain length saturated acids from C12:0-C28:0, except C21:0, were present in the oil. Based on results obtained the fatty acid composition of tomato seed oil showed that it falls in the linoleic-oleic acid oils category. Therefore the oil could be useful for edible purposes and for some industrial applications like hydrogenation, shortening production and others.

Table I
Physical and chemical characteristics of tomato seed oil

Characteristic	Value [1]	
	Crude	Purified
Density, 25°C	0.9160 ± 0.00024	0.9156 ± 0.00019
Refractive index, 40°C	1.4603 ± 0.00022	1.4610 ± 0.00016
Viscosity (21°C), mPa.s	75 ± 0.4	74 ± 0.3
Smoke point, °C	176 ± 63.0	208 ± 5.4
Colour		
red units	22 ± 0.5	10 ± 0.1
yellow units	10 ± 0.1	2 ± 0.0
Saponification number	184 ± 6.9	186 ± 2.5
Iodine value	105 ± 0.7	104 ± 0.9
Unsaponifiable matter, %	1.4 ± 0.02	0.9 ± 0.01
Acidity, % as oleic	1.01 ± 0.0008	0.05 ± 0.0001
Peroxide value, meq/kg	9.3 ± 0.055	9.1 ± 0.054
Induction time		
tomato	5.15 ± 0.012	4.9 ± 0.016
olive	7.22 ± 0.027	
sunflower	3.40 ± 0.014	
$E^{1\%}_{1cm}$ 232	2.40 ± 0.0001	2.23 ± 0.0001
$E^{1\%}_{1cm}$ 270	0.63 ± 0.00004	0.72 ± 0.00003
R = $E^{1\%}_{1cm}$ 232 / $E^{1\%}_{1cm}$ 270	3.81 ± 0.0002	3.11 ± 0.00009

[1] Average of three samples with three to five replications, ± standard deviation.

Table II
Fatty acid composition of tomato seed oil [1]

Fatty acid	%	
	Crude	Purified
C14:0	0.2 ± 0.0001	0.1 ± 0.0000
C16:0	14.0 ± 0.12	13.6 ± 0.10
C16:1*cis* ω9	0.4 ± 0.0004	0.6 ± 0.0001
C16:1*cis* ω7	0.1 ± 0.0000	ND[2]
C17:0	0.3 ± 0.003	0.1 ± 0.0002
C18:0	6.0 ± 0.01	6.0 ± 0.05
C18:1ω9	22.0 ± 0.21	22.0 ± 0.12
C18:1ω7	0.6 ± 0.001	0.1 ± 0.0001
C18:2	53.6 ± 0.28	54.0 ± 0.65
C18:2*trans*	0.1 ± 0.0000	0.8 ± 0.0002
C18:3	2.0 ± 0.003	2.1 ± 0.002
C20:0	0.3 ± 0.0001	0.2 ± 0.0001
C20:1	0.1 ± 0.0000	0.2 ± 0.0000
C22:0	Trace[3]	0.1 ± 0.0000
C24:0	0.1 ± 0.0000	0.1 ± 0.0000

[1] Average of three samples, ± standard deviation; [2] ND = not detected; [3] trace < 0.1%

Furthermore, it should be pointed out that the fatty acid pattern has not changed during purification and bleaching of the oil. However, the concentration of C18:2*trans* was increased from 0.1% in the crude oil to 0.8% in purified oil (Table II). This is due to high temperature clay bleaching, which causes *cis-trans* isomerization (20).

A high tocopherol content was found in tomato seed oil, consisting of α-and δ-tocopherol, at concentrations of 202 and 1059 mg/kg, respectively. Dabrowska (1) and Nadirov *et al.* (27) reported total tocopherol contents from 418 to 767 mg/kg, which can influence the stability of the oil during storage. Purification and bleaching led to a reduction in tocopherol contents to values of 161 and 898 mg/kg, for α- and δ-tocopherol respectively, corresponding to a 80 and 85% retention, respectively. It should be noted that the variation in the amount of tocopherols lost during refining depends on the severity of the process with respect to temperature and time, and to crude oil condition. McLaughlin and Weihrauch (28) reported that deodorization causes the greatest loss of tocopherols, while little or no tocopherol is destroyed during hydrogenation. More recently, Jung *et al.* (29) showed that the most substantial loss during refining of soybean oil occurred at the deodorization stage, while degumming, neutralization and bleaching led to an average retention of 88%. This value is very close to the value found in this work.

Tomato seed oil showed lower induction time than olive oil and higher than that of sunflower oil (Table I). Although tomato seed oil shows a similar fatty acid profile with sunflower oil (20), the latter showed lower induction time, which among others could be attributed to lower tocopherol content in sunflower, 636 mg/kg, of which α-tocopherol accounts for 96%, followed by β-tocopherol, 2.5%, and γ-tocopherol, 1.5% (30). Nevertheless, the higher oxidative stability of tomato seed oil over sunflower oil should be attributed to other constituents of non glyceride fraction of the oil, which posses antioxidant properties.

The results of qualitative and quantitative sterol determinations in the sterolic fraction of tomato seed oil unsaponifiables are summarised in Table III. Total sterol content in crude oil varied between 325 and 533 mg/100g with an average value of 455±89 mg/100g, while in purified oil between 225 and 368 mg/100g with an average of 315±60 mg/100g. The sterol fraction of the tomato seed oil consisted mainly of campesterol, stigmasterol, β-sitosterol and Δ^5-avenasterol, among of which β-sitosterol was the most predominant, accompanied with trace or minute amounts of 24-methylenecholesterol, brassicasterol, Δ^7-campesterol, clerosterol, $\Delta^{7,24}$-stigmastadienol, Δ^7-stigmastanol, Δ^7-avenasterol and erythrodiol. In addition, cholesterol at a level of 16% was found, which was the second quantitatively important sterol of tomato seed oil after β-sitosterol. Yamamoto and

Mackinney (31) mentioned only the existence of stigmasterol, β-sitosterol and possibly campesterol in tomato fruit and seeds. The results obtained in this study were in good agreement with those previously reported (4, 32, 33, 34), although higher concentrations for cholesterol approaching 27% have been reported. An unusually high concentration (up to 41%) of cholesterol has been reported by Kiosseoglou and Boskou (35). Ismail *et al.* (36) have reported a total cholesterol concentration of 30 mg/100 g for the edible portion of tomatoes. The high cholesterol content in tomato seed oil gives rise to problems in analytical interpretation when controlling the purity of vegetable as well as animal fats. Moreover, the results of the present study confirmed the wide distribution of 24-methylenecholesterol in vegetable oils, which initially was believed to be widely distributed in marine organisms (37, 38).

Table III
Composition of sterol fraction of tomato seed oil

Sterol	RRT[a]	%	
		Crude	Purified
Cholesterol	0.64	15 ± 0.2	16 ± 0.2
Brassicasterol	0.71	1.5 ± 0.04	1.1 ± 0.02
24-Methylenecholesterol	0.80	1.2 ± 0.02	0.9 ± 0.004
Campesterol	0.83	6.7 ± 0.14	6.1 ± 0.06
Stigmasterol	0.89	14.4 ± 0.24	14.6 ± 0.17
Δ^7-Campesterol	0.92	0.3 ± 0.0003	Trace
Clerosterol	0.96	Trace[3]	ND[2]
β-Sitosterol	1.00	52 ± 2.22	53 ± 1.17
Δ^5-Avenasterol	1.04	6.7 ± 0.022	7 ± 0.029
$\Delta^{7,24}$-Stigmastadienol	1.09	0.5 ± 0.0005	0.3 ± 0.0003
Δ^7- Stigmastenol	1.11	0.4 ± 0.0003	0.2 ± 0.0001
Δ^7-Avenasterol	1.17	0.1 ± 0.0001	Trace
Erythrodiol	1.47	0.1 ± 0.0000	ND

[a] Relative retention time for β-sitosterol (retention time 20.7 min) taken as 1.00.
[1] Average of three samples, ± standard deviation; [2] ND = not detected; [3] trace < 0.1%.

The sterol fraction from tomato seed oil consists mainly of Δ^5-sterols with small proportions of Δ^7-sterols as in most vegetable oils (38). Oil purification and bleaching did not change the sterol pattern of the oil due to the fact that they are relatively inert. It is known that alkali refining of oils removes a portion of the sterols (20). As a result sterols present in trace or minor amounts in crude oil were not detected in purified oil (Table III).

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