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Sterol composition of tomato (*Solanum lycopersicum* L.) seed oil: the effect of cultivar

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Abstract

The effect of cultivar on sterol composition of tomato (*Solanum lycopersicum* L.) seed oil from three industrial cultivars (Principe Borghese, Rebelion F1 and San Marzano) grown in Calabria Region (Southern Italy) was investigated. Sterol composition is presented both as percentage on the total sterol content (%) and as an absolute value (mg/kg). Rebelion F1 produced the worst seed oil in terms of cholesterol content (15.76%, 275.90 mg/kg). Principe Borghese produced the oil with the highest *β*-sitosterol (57.47%, 1088.33 mg/kg) and the highest apparent *β*-sitosterol (67.59%, 1279.62 mg/kg). One-way ANOVA and PCA analyses demonstrated a high and significant difference among the seed oils of the studied tomato cultivars.

Introduction

Sterols are one of the most important classes of components contained in the unsaponifiable fraction of a vegetable oil. They are classified in accordance to the position of a double bond in the B-ring. Δ^5 sterols have a double bond in position 5,6 (cholesterol, *β*-sitosterol, campesterol, stigmasterol, avenasterol and brassicasterol). Δ^7 sterols have a double bond in position 7,8 (sitosterol and avenasterol). Their importance is double. On the one side, sterol components are useful to characterize a vegetable oil, on the other side they can validate its biological properties. Edible oils are desterolized in order to render them "undetectable" when admixed to other oils (Grob *et al*., 1994a; Grob *et al*., 1994b). In general, sterols derive from squalene and consist of a tetracyclic cyclopenta[α]-phenanthrene structure with a hydroxyl group at C-3 and a flexible side chain with 8–10 carbons at C-17. Sterols can be classified according to their origin as animal sterols or plant sterols (García-Llatas *et al*., 2011). Plant sterols are natural plant components that work as cholesterol-lowering agents. Plant sterols could be added to a wide variety of food products and combined with other beneficial substances. Factors that may affect the efficacy of plant sterols are the nature of the food matrix and frequency and time of intake. Nevertheless, the addition of plant sterols to the diet is suggested by health experts as a safe and effective means to reduce the risk of coronary heart disease (AbuMweis and Jones, 2008). The most common plant sterols or phytosterols are: *β*-sitosterol, Δ^5-campesterol, Δ^5-stigmasterol and Δ^5-brassicasterol. Δ^5-Cholesterol is mainly contained in animal fats in a much higher quantity compared to vegetable oils. Tomato seeds contain 17-18% oil (w/w of dry seed weight) when the oil is mechanically extracted (Giuffrè *et al.*, 2015); 20-23% oil (w/w of dry seed weight) after solvent (petroleum ether) extraction (Giuffrè and Capocasale, 2015) 10-12% oil (w/w of wet seed weight) when the oil is mechanically extracted (Giuffrè *et al*., in press);. The tomato cultivars actually known can be divided in two main groups: the first is the salad tomato type and the second is the industrial type for sauce tomato and other products. All parts of the salad tomato are generally eaten and no waste is produced. By contrast tomato sauce processing produces a quantity of waste. In particular, only the peel is obtained as a waste in the case of peeled tomato production when the whole fruit is stored after blanching and peeling. Peels and seeds are derived as a waste in the case of the industrial tomato sauce processing. The variability in the amount of wastes is strongly affected by both the time of year and the origin (cultivar and agronomic treatments) of the waste production. In 2012, Italy produced 4,792,568 tons of sauce tomato: the Region of Apulia was the first Italian producer (1,739,850 tons) and

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the Region of Calabria was the sixth Italian producer (134,231 tons). TSO could be used as an edible oil and/or for an industrial purposes. Malecka (2002) studied the effect of the unsaponifiable components of tomato seed oil, oat grain oil and wheat germ oil on the oxidation of refined rapeseed oil and compared their anti-oxidative effectiveness to that of common synthetic antioxidant butylhydroxyanizole (BHA). He found that the 0.3% addition of the unsaponifiable matter isolated from tomato seeds oil showed a higher anti-oxidative activity than 0.02% of BHA or 0.02% unsaponifiable matter from oat grain or wheat germ oil, considered separately. One of the advantages of the use of TSO is that it is not necessary to cultivate a specific area for its production but seeds discarded after tomato sauce production can be used. The aim of this paper was to study the effect of cultivar on the composition of sterols of the tomato seed oil from three industrial cultivars. To our knowledge this is the first paper regarding the characteristics of the tomato seed oil produced in Calabria (Southern Italy). In the existing literature about this argument there is a scarce differentiation between cultivars.

Materials and Methods

Vegetable material

The experiment was conducted in the harvest year 2014. Tomato plants of three industrial cultivars (Principe Borghese, Rebelion F1 and San Marzano) were cultivated in three different greenhouses, one for each cultivar, at Roccella Ionica, on the South Calabria, (Southern Italy). Plant spacing was 70 cm within the row and 80 cm between the rows. Irrigation was applied every other day in an amount equal to pan evaporation and transpiration. Tomatoes were manually and randomly collected in mid-July. Seeds were separated from berries and then air-dried in dark conditions and without heating until constant weight.

Chemicals

Standard samples of α-cholestanol (95% purity), cholesterol (99% purity), stigmasterol (95% purity), *β*-sitosterol (95% purity), sitostanol (97% purity), were from Sigma-Aldrich, Steinheim-Germany. Glass plates coated with silica gel, without fluorescence indicator, thickness 0.25 mm (commercially available ready for use) were from Merck (Germany). All other reagent were from Panreac (Barcelona, Spain).

Tomato seed oil extraction

The tomato seeds were dried in the dark at room temperature until constant weight (i.e. for a one month period). Seeds were coarsely ground by an electrical home grinder. The ground seeds were submitted to oil extraction by a Soxhlet apparatus with petroleum ether (boiling point = 40-60 ^{o}C) as a solvent. Firstly, ground seeds were left in dark conditions and at room temperature overnight (12 hours in a static extraction with petroleum ether), secondly Soxhlet was activated at the minimum boiling point temperature of the solvent, for two hours to ensure full oil recovery. At this point the solvent was completely evaporated in a Rotavapor under vacuum at 25 ^{o}C. Finally the obtained oil was filtered through a paper filter and stored in a 50 mL amber glass bottle until analysis, i.e. 7 days after oil extraction.

Unsaponifiable matter and sterols

Sterols were determined as described in Annexes V and XIX of the CONSLEG 2003 for olive oil analysis (CONSLEG, 2003). An aliquot of 5 g of TSO was added with α-cholestanol as internal standard for sterols. The mixture was submitted to saponification. Subsequently the unsaponifiable fraction was separated with a liquid/liquid extraction and weighed. An aliquot of 300 μL (5% unsaponifiable matter in chloroform) was streaked on a glass chromatographic plate. The plate was placed in a chromatographic chamber with hexane/ethyl ether 65:35 as an eluent. After migration, the plate was removed and the solvent was evaporated at room temperature. A 2,7-dichlorofluorescein ethanolic solution was sprayed on the plate to identify bands. The sterolic band was scraped and sterols were extracted by hot chloroform. After solvent evaporation and silylation, the sterol fraction was ready for GC analysis. Silylation was conducted with a mixture of hexamethyldisilazane + trimethylclorosilane + pyridine (3:1:9) from Supelco (Bellefonte, PA, USA). A Fisons GC 8000 gas chromatograph equipped with a split-splitless injector and a F.I.D. (Flame Ionization Detector) was used. Sterol analysis: carrier gas (helium) 10 psi of pressure, auxiliary gas (hydrogen at 15 psi and air at 22 psi), split/splitless injector (operating in the split mode) temperature (280 ^{o}C), detector temperature (290 ^{o}C), oven temperature (270 ^{o}C), a capillary column SE 54 (30 m length x 0.32 mm ID, 0.25 μm film thickness, Mega, Milan - Italy) and an injection volume of 1 μL. The identification was made by comparison of detected peaks with authentic standards and with comparison with the literature data.

Statistical analysis

Analyses were conducted in triplicate. Statistical

Table 1. Sterol composition of tomato seed oil. Each sterol is calculated as a percentage on total sterol content (mean values ± Standard Deviation). Means in the same row with different lowercase letters differ significantly. Significance level: n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Apparent β-sitosterol is the sum of: clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol

	Principe Borghese	Rebelion F1	San Marzano	Sign.
Cholesterol	13.15±0.09 b	15.76±0.07 a	11.80±0.07 c	* * *
Brassicasterol	1.19±0.03 a	1.22±0.03 a	0.07±0.01 b	* * *
24-Methylene-cholesterol	0.41±0.03 a	0.40±0.02 a	0.45±0.02 a	n.s.
Campesterol	5.36±0.06 a	5.01±0.03 b	5.01±0.05 b	* * *
Campestanol	0.58±0.03 a	0.58±0.03 a	0.57±0.02 a	n.s.
Stigmasterol	10.64±0.07 c	13.09±0.05 b	13.87±0.06 a	* * *
Δ^7-Campesterol	0.57±0.03 a	0.40±0.03 b	0.22±0.03 c	* * *
Clerosterol	1.36±0.04 a	1.01±0.03 c	1.19±0.03 b	* * *
β-Sitosterol	57.47±0.12 a	53.58±0.10 c	57.03±0.08 b	* * *
Sitostanol	1.86±0.04 b	2.10±0.03 a	1.72±0.03 c	* * *
Δ^5 – Avenasterol	6.83±0.06 a	6.35±0.04 c	6.57±0.04 b	* * *
$\Delta^{5,24}$ – Stigmastadienol	0.06±0.01 b	0.14±0.02 a	0.11±0.02 a	* *
Δ^7 - Stigmastenol	0.44±0.02 b	0.30±0.03 c	0.69±0.02 a	* * *
Δ^7 - Avenasterol	0.08±0.01 b	0.05±0.01 c	0.69±0.02 a	* * *
Campesterol/Stigmasterol	0.50±0.01 a	0.38±0.00 b	0.36±0.00 c	* * *
Apparent *β*-sitosterol	67.59±0.26 a	63.19±0.21 c	66.63±0.19 b	* * *
Unsaponifiable (wt %)	1.25±0.02 c	1.49±0.02 a	1.29±0.08 b	* * *

significance was assayed using a one-way analysis of variance (ANOVA); the Tukey test was used for data processing (ANOVA, post-hoc test) between cultivars at $p < 0.05$. SPSS version 15.0 (SPSS Inc., Chicago, IL, U.S.A.) was used to determine the significant differences. Principal component analysis (PCA) was carried out using the software XLSTAT version 2009.1.01. All other statistical analyses were conducted by Excel for Windows software (2007 version).

Results and Discussion

Oil and unsaponifiable content

The oil content on dry seeds was 19.83% in Rebelion F1, 22.71% in San Marzano and 23.44% in Principe Borghese (Giuffrè and Capocasale, 2015). The unsaponifiable matter of the vegetable oils is a quantitatively small but important fraction in which the so called minor components are present. It gives important information about the quality of the oil. In the TSOs an unsaponifiable content ranging from 1.25% of Principe Borghese to 1.49% of Rebelion F1 was found (Tab.1), slightly more than in unroasted white sesame seed oil (1.2%) or than in unroasted brown sesame seed oil (1.1%) (Mohamed and Awatif, 1998).

Sterol content

Sterol composition is reported in Tables 1 and 2. Gas chromatogram of the TSO sterolic fraction consisted of 14 compounds. One of the chemical quality parameters for olive oil as a food is the apparent *β*-sitosterol which is the sum of $\Delta^{5,23}$-stigmastadienol, clerosterol, *β*-sitosterol, sitostanol, Δ^5-avenasterol and $\Delta^{5,24}$-stigmastadienol (CONSLEG, 2003). The highest quantity of *β*-sitosterol, the primary sterol, was found in Principe Borghese (57.47%, 1088 mg/kg) and the lowest was found in Rebelion F1 (53.58%, 938 mg/kg), in a slightly higher amount compared to the findings of Lazos *et al.* (1998) who quantified 52% of this phytosterol in Greek crude TSO. The *β*-sitosterol content of TSO was similar to sesame seed oil (57.7-61.9%), or to sunflower mid-oleic acid type (56-58%), (Codex Stan 210-1999), or to sunflower high oleic type (49.8-57.2%), (Anastasi *et al*., 2010). Apparent *β*-sitosterol in TSO is 63-67% of the total sterols, i.e. 1100-1300 mg/kg. Cholesterol, a zoo-sterol, was the sterol present with the second highest quantity in the studied TSOs. Cholesterol is an indispensable component of biological membranes and a precursor to numerous signalling molecules including steroid hormones. Its provision and disposal in all organs of the mammalian body – except for the brain – relies on dietary uptake by the intestine, de novo synthesis in every organ, and lipoprotein-mediated transport via the blood circulation (Pfrieger and Ungerer, 2011). With its large, rigid core, the cholesterol molecule affects membrane organization and physical properties by modulating the coexistence of lipid-disordered and lipid-ordered phases, which is a critical determinant of membrane bilayer permeability and fluidity. Within this context, it is essential to maintain a physiological range of free cholesterol/phospholipid ratio in cellular membranes (Musso *et al*., 2013). Cholesterol can have indirect effects, by changing the membrane properties, or direct effects, by interacting in ways that affect protein structure. The most important membrane parameters affected by cholesterol, which are of key importance for

Table 2. Sterol composition (mg/kg) of tomato seed oil (mean values ± Standard Deviation). Means in the same row with different lowercase letters differ significantly. Significance level: n.s. = not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; *** $p < 0.001$. Apparent β-sitosterol is the sum of: clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol

	Principe Borghese	Rebelion F1	San Marzano	Sign.
Cholesterol	248.89±3.15 b	275.90±0.25 a	201.71±1.72 c	* * *
Brassicasterol	22.58±0.45 a	21.36±0.54 b	1.22±0.18 c	* * *
24-Methylene-cholesterol	7.69±0.43 a	7.06±0.34 a	7.74±0.24 a	n.s.
Campesterol	101.55±1.72 a	87.66±0.25 b	85.66±0.54 b	* * *
Campestanol	10.91±0.42 a	10.20±0.48 ab	9.72±0.37 b	*
Stigmasterol	201.52±0.38 c	229.19±1.67 b	237.10±1.73 a	* * *
Δ^7-Campesterol	10.86±0.49 a	7.01±0.55 b	3.82±0.43 c	* * *
Clerosterol	25.82±0.80 a	17.63±0.60 c	20.42±0.49 b	* * *
β-Sitosterol	1088.03±3.91 a	938.24±1.89 c	975.27±1.50 b	* * *
Sitostanol	35.27±0.86 a	36.84±0.58 a	29.38±0.35 b	* * *
Δ^5 – Avenasterol	129.28±1.84 a	111.18±1.05 b	112.37±1.01 b	* * *
$\Delta^{5,24}$ – Stigmastadienol	1.22±0.24 b	2.54±0.37 a	1.99±0.26 a	* *
Δ^7 - Stigmastenol	8.28±0.25 b	5.33±0.46 c	11.85±0.23 a	* * *
Δ^7 - Avenasterol	1.43±0.09 b	0.86±0.17 c	11.73±0.22 a	* * *
Total sterol content	1893±10.41 a	1751±6.56 b	1710±5.00 c	* * *
Apparent β-sitosterol	1279.62±7.65 a	1106.43±4.49 c	1139.42±3.61 b	* * *

membrane proteins, are membrane thickness, which affects hydrophobic matching, the lateral pressure profile associated with membrane elasticity, and membrane fluidity/viscosity, which is related to the lifetime of functional complexes formed by proteins and lipids (Róg and Vattulainen, 2014). The detected cholesterol was: 13.15% (249 mg/kg) in Principe Borghese, 15.76% (276 mg/kg) in Rebelion F1, 11.80% (202 mg/kg) in San Marzano. Other Authors studied the cholesterol content in tomato seed oil and found it in these quantities: 15% in Greek tomato seed oil (Lazos *et al*., 1998); 7.5% in Italian TSO (Zuorro, 2012); 17% in ultrasonic extracted TSO (Kazamani, 2014). Cholesterol in TSOs was present in a higher amount compared to other vegetable oils: 0.2% in corn seed oil, 0.3% in cotton seed oil, 2.4% in palm oil, 0.2% in peanut seed oil, 0.1 in safflower seed oil, 0.4 in soybean oil (Reina *et al*., 1999); 0.1-2.6% (5.0-153.4 mg/kg) in different varieties of Hibiscus seed oil (Holser *e al*., 2004); 0.34% (11.32 mg/kg) in passion fruit seed oil (Giuffrè, 2007); 0.17-0.29% (6.89-53.74 mg/kg) in grape seed oil in different stage of berry development (Rubio *et al.*, 2009); 0.10-0.33% in olive oil (Giuffrè, 2012; Giuffrè *et al*., 2012; Giuffrè and Louadj, 2013);. According to Lazos *et al.* (1998), stigmasterol was found in a similar amount to cholesterol, ranging from 10.64% to 13.87%, also stigmasterol is present in a higher quantity if compared with other edible vegetable oils (1.8% in blackberry seed oil; 0.3% in blueberry seed oil; 1.3% in cranberry seed oil, 1.2% in red raspberry seed oil; 2.3% in strawberry seed oil and 2.4% in kiwi seed oil (Van Hoed, 2009). The campesterol/ stigmaterol ratio was between 0.36 in San Marzano and 0.50 in Principe Borghese, similar to passion fruit seed oil (Giuffrè, 2007) and different to other common edible vegetable oils, where campesterol is in a higher quantity compared to stigmasterol (Van Hoed, 2009). The fourth and the fifth sterols were Δ^5-avenasterol (6.35-6.83%) and campesterol (5.01-5.36%). Each other sterol accounted for less than 2%.

One way ANOVA analysis

Differences were statistically considered for each cultivar and data were evaluated. Principe Borghese showed the significantly highest values ($p < 0.001$) for brassicasterol, campesterol, Δ^7-campesterol, clerosterol, β-sitosterol, Δ^5 – avenasterol, apparent β-sitosterol for both relative and absolute content (Tables 1, 2). The absolute content of 24-methylene-cholesterol and campestanol (%) showed no significant differences among the three cultivars.

Correlation matrix

The correlations among the percentage content of sterols of TSO are reported in Table 3. The most significant correlations were found between brassicasterol and campestanol, sitostanol and cholesterol (R = 1.000), Δ^7-avenasterol and campestanol (R = -0.999), Δ^5-avenasterol and clerosterol (R = 0.998). Sitostanol and β-sitosterol are in opposition.

Principal component analysis

All the sterol parameters studied in this paper were subjected to PCA analysis. Two Eigen values were obtained, which were higher than 1.00. The values for sterol composition were 8.28 (51.72%)

Table 3. Correlation matrix of sterol parameters expressed as percentage on the total sterol content. Apparent β-sitosterol is the sum of: clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol

Variables	Cholesterol	Brassicasterol	24-Methylene-cholesterol	Campesterol	Campestanol	Stigmasterol	Δ^7 - Campesterol	Clerosterol	β-Sitosterol	Sitostanol	Δ^5 - Avenasterol	$\Delta^{5,24}$ Stigmastadienol	Δ^7 - Stigmasterol	Δ^7 - Avenasterol	Campesterol/ Stigmasterol	Total β-sitosterol
Cholesterol	1															
Brassicasterol	0.776	1														
24-Methylene-cholesterol	-0.870	-0.986	1													
Campesterol	-0.181	0.480	-0.327	1												
Campestanol	0.761	1.000	-0.982	0.500	1											
Stigmasterol	-0.052	-0.670	0.537	-0.973	-0.687	1										
Δ^7-Campesterol	0.351	0.863	-0.767	0.858	0.874	-0.953	1									
Clerosterol	-0.661	-0.039	0.205	0.858	-0.016	-0.715	0.471	1								
β-Sitosterol	-0.902	-0.429	0.573	0.587	-0.408	-0.383	0.087	0.920	1							
Sitostanol	1.000	0.795	-0.885	-0.150	0.781	-0.083	0.380	-0.637	-0.889	1						
Δ^5 – Avenasterol	-0.611	0.025	0.142	0.889	0.048	-0.759	0.527	0.998	0.892	-0.586	1					
$\Delta^{5,24}$ – Stigmastadienol	0.533	-0.120	-0.047	-0.929	-0.143	0.817	-0.606	-0.987	-0.845	0.506	-0.995	1				
Δ^7 - Stigmastenol	-0.942	-0.943	0.985	-0.161	-0.935	0.385	-0.645	0.370	0.705	-0.952	0.309	-0.217	1			
Δ^7 - Avenasterol	-0.788	-1.000	0.989	-0.464	-0.999	0.656	-0.853	0.058	0.445	-0.806	-0.007	0.102	0.949	1		
Campesterol/Stigmasterol	-0.049	0.592	-0.449	0.991	0.610	-0.995	0.918	0.782	0.475	-0.018	0.821	-0.871	-0.290	-0.577	1	
Apparent *β*-sitosterol	-0.852	-0.331	0.484	0.669	-0.309	-0.479	0.191	0.956	0.994	-0.835	0.935	-0.897	0.626	0.349	0.565	1

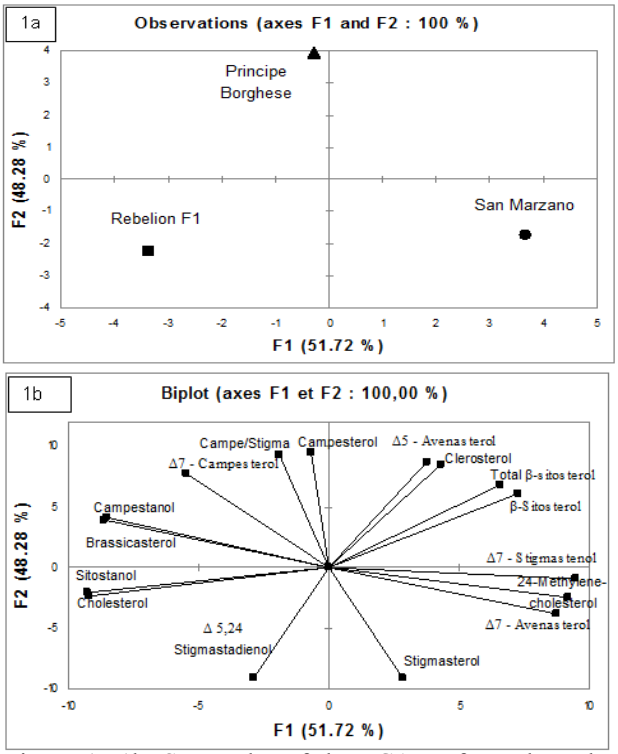

Figure 1a-1b. Score plot of the PCA performed on the sterol composition (%) of the three tomato cultivars. PCA of the studied sterol components: relationships between variables considered as percentage of the total sterol. Apparent *β*-sitosterol is the sum of: clerosterol, *β*-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol

and 7.73 (48.28%) (Fig. 1a - 1b). Visualization of the discrimination among cultivars on the plane of the first two functions led to a fairly good separation among the different groups. The three cultivars Principe Borghese, Rebelion F1 and San Marzano are represented in three different sides of the plane for all the studied parameters, which explains a high significant separation among cultivars. San Marzano was placed in the right bottom corner whereas Principe Borghese and Rebelion F1 were placed in the left side of the diagram, in the top and in the bottom corner respectively (Fig.1a -1b). The similarity in vector length and direction indicates that *β*-Sitosterol and apparent *β*-sitosterol, as well as cholesterol and sitostanol, and also campestanol and brassicasterol were highly correlated. *β*-Sitosterol was present at the expense of sitostanol, campesterol was present at the expense of stigmasterol (Fig. 1a - 1b). The orthogonal direction of vectors demonstrated the independence of *β*-sitosterol and apparent *β*-sitosterol from cholesterol (Fig. 1a - 1b).

Conclusions

Chemometrics applied to the results presented in this study proved that cultivar highly significantly influenced the sterol content and composition of the tomato seed oil. Principe Borghese showed the significantly highest sterol content. Rebelion F1 showed the significantly highest cholesterol content for both percentage and absolute values. Tomato seed oil of the three studied cultivars was

characterized by a total sterol content of 1700-1900 mg/kg (similar to a virgin olive oil), a high cholesterol concentration (11.80-15.76%) dissimilar to common edible vegetable oils, a low campesterol/stigmasterol ratio (0.36-0.50) (similar to a rapeseed oil) and a *β*-sitosterol content of 54-58% (similar to a sunflower seed oil, a corn seed oil, a safflower seed oil or a soybean oil).

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References

AbuMweis, S. S. and Jones, P. H. J. 2008. Cholesterol-Lowering Effect of Plant Sterols. Current Atherosclerosis Report 10(6): 467-472.

Anastasi, U., Santonoceto, C., Giuffrè, A. M., Sortino, O., Gresta, F. and Abbate V. 2010. Yield performance and grain lipid composition of standard and oleic sunflower as affected by water supply. Field Crop Research 119(1): 145-153.

Codex standard for named vegetable oils. Codex. CODEX-STAN 210-1999 (Revision 2001, 2003, 2009 - Amendment 2005, 2011, 2013).

CONSLEG (2003). Consolidated text 1991R2568 - 01/11/2003, on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, Annex IV.

García-Llatas, G. and Rodríguez-Estrada, M.T. 2011. Current and new insights on phytosterol oxides in plant sterol-enriched food. Chemistry and Physics of Lipids 164(6): 607–624.

Giuffrè, A.M. 2007. Chemical composition of purple passion fruit (*Passiflora edulis* Sims var. edulis) seed oil. Rivista Italiana delle Sostanze Grasse 84(2): 87-93.

Giuffrè, A.M. 2012. Steroli, Eritrodiolo e Uvaolo in olio di oliva da cultivar coltivate in Calabria. Industrie Alimentari 51(6): 20-26.

Giuffrè, A. M., Louadj, L., Poiana, M. and Macario, A. 2012. Composition en sterols des huiles extraites d'olives de cultivars de la province de Reggio Calabria (Sud d'Italie). Rivista Italiana delle Sostanze Grasse 89(3): 177-183.

Giuffrè, A. M. and Louadj, L. 2013. Influence of crop season and cultivar on sterol composition of monovarietal olive oils in Reggio Calabria (Italy). Czech Journal of Food Science 31(3): 256-263.

Giuffrè, A. M., Sicari, V., Capocasale, M., Zappia, C., Pellicanò, M. T. and Poiana, M. 2015. Physico-chemical properties of tomato seed oil (*Solanum lycopersicum* L.) for biodiesel production. Acta Horticulturae 1081: 237-244.

Giuffrè, A. M. and Capocasale, M. 2015. Policosanol in tomato (*Solanum lycopersicum* L.) seed oil. The effect of cultivar. Journal of Oleo Science 64(6): 625-631.

Giuffrè, A.M., Capocasale, M., Zappia, C., Sicari, V., Pellicanò, T.M., Poiana, M., Panzera G. Tomato seed oil for biodiesel production. European Journal of Lipid Science and Technology (in press). DOI: 10.1002/ejlt.201500002

Grob, K., Giuffrè, A. M., Biedermann, M. and Bronz M. 1994a. The detection of adulteration with desterolized oils. Fat Science and Technology 96(9): 341-345.

Grob, K., Giuffrè, A. M., Lezzi, U. and Mincione, B. 1994b. Recognition of adulterated oils by direct analysis of the minor components. Fat Science and Technology 96(8): 286-290.

Holser RH, Bost G, Van Boven M. 2004. Phytosterol composition of hybrid Hibiscus seed oils. Journal of Agriculture and Food Chemistry 52(9): 2546-2548.

Kamazani, N. A., Tovakolipour, H., Hasani, M. and Amiri, M. 2014. Evaluation and analysis of the ultrasound-assisted extracted tomato seed oil. Journal of Food Bioscience and Technology 4(4): 57-66.

Lazos, E. S., Tsaknis, J. and Lalas, S. 1998. Characteristics and composition of tomato seed oil. Grasas y Aceites 49(5-6): 440-445.

Malecka, M. 2002. Antioxidant properties of the unsaponifiable matter isolated from tomato seeds, oat grains and wheat germ oil. Food Chemistry 79(3): 327-330.

Mohamed, H. M. A. and Awatif, II. 1998. The use of sesame oil unsaponifiable matter as a natural antioxidant. Food Chemistry 62(3): 269-276.

Musso, G., Gambino, R. and Cassader, M. 2013. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. Progress in Lipid Research 52(1): 175–191.

Pfrieger, F. W. and Ungerer, N. 2011. Cholesterol metabolism in neurons and astrocytes. Progress in Lipid Research 50(4): 357–371.

Reina, R. J., White, K. D. and Firestone, D. 1999. Sterol and triterpene diol contents of vegetable oils by high-resolution capillary gas chromatography. Journal of AOAC International 82(4): 929-935.

Róg, T. and Vattulainen I. 2014. Cholesterol, sphingolipids, and glycolipids: What do we know about their role in raft-like membranes? Chemistry and Physics of Lipids 184(1): 82–104.

Rubio, M., Alvarez-Ortí, M., Alvarruiz, A., Fernández, E. and Pardo J. E. 2009. Characterization of oil obtained from grape seeds collected during berry development. Journal of Agricultural and Food Chemistry 57(7): 2812-2815.

Van Hoed, V., De Clercq, N., Echim, C., Andjelkovic, M., Leber, E., Dewettinck, K. and Verrhé, R. 2009. Berry

seed: a source of speciality oils with high content of bioactives and nutritional value. Journal of Food Lipids 16(1): 33-49.

Zuorro, A., Lavecchia, R., Medici, F. and Piga, L. 2013. Enzyme-assisted production of tomato seed oil enriched with lycopene from tomato pomace. Food Bioprocess Technology. 6(6): 3499-3509.