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Policosanol in Tomato (*Solanum lycopersicum* L.) Seed Oil: the Effect of Cultivar

Angelo Maria Giuffrè* and Marco Capocasale

Università degli Studi "Mediterranea" di Reggio Calabria (Italy). Dipartimento AGRARIA.

Abstract: Soxhlet-petroleum ether extraction was used to obtain oil from tomato seeds. Three tomato cultivars from South Italy (Principe Borghese, Rebelion F1 and San Marzano) were studied. Policosanol is a mixture of long chain linear fatty alcohols (*n*-alkanols), its content and composition was found to be highly significantly influenced by cultivar. Seven fatty alcohols were detected: docosanol (C22-*ol*), tricosanol (C23-*ol*), tetracosanol (C24-*ol*), pentacosanol (C25-*ol*), hexacosanol (C26-*ol*), heptacosanol (C27-*ol*) and octacosanol (C28-*ol*). The highest policosanol content was found in Principe Borghese 71.88 mg/Kg. Octacosanol was the linear alcohol present in highest quantity, i.e. 38-42% of the total linear alcohols detected in tomato seed oils (TSO). Chemometrics was applied to study the differences among cultivars. The sum of even long chained fatty alcohols was always more than 95% of the total policosanol content. One-way ANOVA and principal component analysis well differentiated the three cultivars.

Key words: *n*-alkanols, linear fatty alcohols, minor components, unsaponifiable, vegetable edible oil

1 INTRODUCTION

Vegetable oil is mainly composed of glycerides (>98%) and of the so called unsaponifiable fraction (1-2%). The unsaponifiable fraction contains many components which characterize each vegetable oil. Policosanol is one of the important classes contained in the usaponifiable fraction and it is a term describing a mixture of long chain fatty alcohols (LCFAs) with a linear chain from 20 to 34 carbon atoms[1].

VLCFA precursors for cuticular wax production are synthesized in the epidermal cells by the multienzyme fatty acid elongase complex found on the endoplasmic reticulum[2].

LCFALs were proved to have beneficial effects on human health, such as cholesterol lowering in patients with type II hypercholesterolemia[3] and to contrast the inflammatory damage in different pathologies, including atherosclerosis[4].

The LCFA fraction, isolated from pomace olive oil, can reduce the release of different inflammatory mediators (eicosanoids, cytokines and nitric oxide) by interfering in different stages of their metabolic pathways, such as inducible nitric oxide synthetase expression or PLA2 activity[4]. The importance of LCFALs is also due to their attitude to the possibility of their use in recognizing adulterated vegetable oils[5].

The even to odd long chain fatty alcohols predominance in the policosanol mixture is important for the positive effect on human and plant health. Relevant human health benefits were found from even-long chain fatty alcohols: docosanol showed an anti-herpes virus activity[6]; hexacosanol encapsulated in wall matrix of solid lipospheres provided the means to enhance the encapsulation efficiency of allopurinol[7]; octacosanol showed an antiangiogenic action in preclinical set up[8] and an effect in decreasing of neutral sterol and bile acid concentration in feces[9]. In studies conducted on plants, triacontanol was proved to have an effective growth regulator in the micropropagation of *Melissa officinalis* L.[10] and in the *Erythrina variegata* protection from cadmium toxicity[11]. Even-long chain fatty alcohols are the major lipid compounds of the vegetable (fruit and leaves) surface as components of waxes; the latter were studied in the surface of sunflower seed[12], olive fruit[13], sesame leaves[14], raspberry[15]. Also the C12-C30 homologues *n*-alkanols found in the lipid molecular loess-paleosol from northwest China showed an even-over-odd carbon number predominance[16].

The tomato is one of the most widely diffused plants in the world and studies are usually conducted on the components of its pulp: antioxidant activity of dried tomatoes[17]; carotenoids in dried tomato peel[18]; carotenoids at different ripening stages[19]; the effect of culture methods on vitamin

*Correspondence to: Angelo Maria Giuffrè, Università degli Studi "Mediterranea" di Reggio Calabria (Italy). Dipartimento AGRARIA.
E-mail: amgiuffre@unirc.it.
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C, firmness, titratable acidity and soluble solids[20]; aroma composition[21]; antioxidant activity, ascorbic acid and lycopene[22]. Million of tons of tomatoes are produced each year in the world and consequently seeds and TSO in large quantity could be produced, for this reason the knowledge of this oil must to be increased and in a previous paper the physico-chemical properties of TSO for biodiesel production were studied[23]. Also the influence of factors, such as cultivar, affecting the TSO composition are very important. The cultivar effect was proved to influence olive oil composition and in particular: sterols[24, 25], fatty alcohols[26–28], wax esters[29, 30] triglycerides[31, 32].

The majority of scientific studies regarding policosanol in vegetable oils deal with olive oil and no information we have found about these minor components in TSO. The aim of this paper was to study the policosanol composition of an unusual vegetable oil (i.e. TSO). At the same time, the influence of the cultivar factor on policosanol was studied. This is the first study on policosanol in TSO produced in Calabria (South Italy).

2 EXPERIMENTAL

2.1 Vegetable material

Three tomato cultivars (Principe Borghese, Rebelion F1 and San Marzano) were selected in 2014 and cultivated in greenhouses at Roccella Ionica (Reggio Calabria, Southern Itay). Plants were spaced 70 cm apart within the row and 80 cm between the rows. A random and manual fruit picking was conducted in mid-July. After separation from the pulp and the skins, seeds were placed in a dark room to dry and no heating was applied to preserve oil from oxidation.

2.2 Chemicals

Standard samples of policosanol (purity ≥ 97%) were from Sigma-Aldrich, Steinheim-Germany. Glass plates coated with silica gel, without fluorescence indicator, thickness 0.25 mm (commercially available ready for use) for policosanol analysis were from Merck (Germany). All other reagent were purchased from Panreac (Barcelona, Spain).

2.3 Tomato seed oil extraction

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 using a Soxhlet apparatus with petroleum ether (boiling point = 40-60℃) as a solvent under the following conditions: firstly in dark conditions and at room temperature overnight (12 hours in a static extraction) and secondly 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℃. Finally the obtained oil was filtered throughout a paper filter and stored in a 50 mL amber glass bottle until analysis, i.e. in 7 days after oil extraction.

2.4 Unsaponifiable matter, policosanol separation

Policosanol was determined as described in the XIX of the CONSLEG 2003 for olive oil analysis[33]. An aliquot of 5 g of TSO was added with eicosanol as internal standard for LCFALs; 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 policosanol band was scraped and extracted by hot chloroform. After solvent evaporation and silylation, the policosanol fraction was ready for GC analysis.

A Fisons GC 8000 gas chromatograph equipped with a split-splitless injector and a F.I.D. (Flame Ionization Detector) was used. Policosanol 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℃), detector temperature (290℃), capillary column SE 54 (30 m length × 0.32 mm ID, 0.5 μm film thickness, Mega, Milan - Italy), injection volume of 1 μL. The temperature program used for the policosanol analysis was as follows: initial temperature at 180℃, held for 2 min, and ramped at 2℃/min to 260℃ held for 15 min and ramped at 6℃/min to 270℃.

2.5 Statistical analysis

Statistical significance was assayed using a one-way analysis of variance (ANOVA); the Tukey test was used to determine the differences between cultivars at $p < 0.05$. The cultivar effect was taken into consideration. SPSS version 15.0 (SPSS Inc., Chicago, IL, U.S.A.) was used to determine the significant differences and for cluster analysis. Principal component analysis (PCA) was performed on all samples using the software XLSTAT version 2009.1.01. All other statistical analyses were conducted by Excel for Windows software (2003 version).

3 RESULTS AND DISCUSSION

3.1 Oil, unsaponifiable matter and policosanol content

The oil percentage was calculated on dry weight of seeds; it ranged from 23.44% of Principe Borghese to

19.83% of Rebelion F1 (Table 1), in higher amount with respect to the 17-18% of TSO obtained by a mechanical screw pressing system[23].

The unsaponifiable matter was 1.25% in Principe Borghese, 1.49% in Rebelion F1 and 1.29% in San Marzano, with similar findings of other Authors: 1.4% in crude TSO[34], 1.75% in cold pressed TSO[35].

Seven fatty alcohols were detected: docosanol, tricosanol, tetracosanol, pentacosanol, hexacosanol, heptacosanol and octacosanol. Data are presented in Table 2.

Docosanol was the linear alcohol with the shortest carbon chain length. It ranged from 21.03% (15.12 mg/Kg) in Principe Borghese, to 25.18% (16.85 mg/Kg) in San Marzano. The absolute docosanol content of TSO was similar to the content found in olive oil of three cultivars (Leccino, Pendolino and Sinopolese) from olives picked on November and in higher quantity compared to the oil extracted from other cultivars[27]. In TSO, docosanol was found in higher quantity (five-fold more) compared to crude wheat germ clear oil (2.8 mg/Kg), and three-fold less than in the solid deposit of the same oil (47 mg/Kg)[1]. A study on docosanol demonstrated that inhibition of fusion between the plasma membrane and the herpes simplex virus envelope, and the subsequent lack of replicative events, may be the predominant mechanism for the anti-herpes simplex virus activity of *n*-docosanol[36].

Tricosanol considered as relative content in TSO was less than 1 mg/Kg in all cultivars, ranging from 0.69% in San Marzano to 1.02% in Principe Borghese. Tricosanol of TSO was lower than in passion fruit seed oil (2.12%, 0.18 mg/Kg)[37], and lower than in olive oil[26–28].

Tetracosanol in TSO was 9.09% (6.08 mg/Kg) in San Marzano and 10.59% (7.61 mg/Kg) in Principe Borghese. Findings of other vegetable oils are 27.7 - 52.2 mg/Kg in Spanish virgin olive oil[38]; 16.81 - 46.99 mg/Kg in Tunisian virgin olive oil[39]; 23.49% (2.05 mg/Kg) in passion fruit seed oil[37]. Of all the LCFALs, tetracosanol was present in the largest quantity in sunflower seed oil (34.2 -39.5%)[40].

Pentacosanol of TSO prevailed in Principe Borghese (1.83%, 1.32 mg/Kg) and it was found in the lowest content in San Marzano (1.05%, 0.70 mg/Kg).

Hexacosanol was present in the second largest quantity after octacosanol in Principe Borghese (25.15%, 18.08 mg/Kg) and in Rebelion F1 (24.23%, 15.67 mg/Kg). Findings of other athor showed that hexacosanol was the second represented fatty alcohol also in sunflower seed oil (22.8 - 27.3%)[40]. A study on the activity of hexacosanol demonstrated its effect on diabetic nephropathy. Treatment with

Table 1 Oil content (% on Dry Weight), unsaponifiable matter (% on oil content), Policosanol content (mg/Kg of oil), data are mean values ± Standard Deviation. Means in the same row with different lowercase letters differ significantly. Significance level: *** $p < 0.001$.

	Principe Borghese	Rebelion F1	San Marzano	Sign.
Oil content (% on DW)	23.44a ± 1.11	19.83c ± 1.38	22.71b ± 1.30	* * *
Unsaponifiable matter (%)	1.25c ± 0.02	1.49a ± 0.02	1.29b ± 0.08	* * *
Total Policosanol (mg/Kg)	71.88a ± 0.64	64.69c ± 0.10	66.92b ± 0.10	* * *

Table 2 Policosanol composition (%) of tomato seed oil (mean values ± Standard Deviation). Means in the same row with different lowercase letters differ significantly. Significance level: *** $p < 0.001$.

	Principe Borghese	Rebelion F1	San Marzano	Sign.
Docosanol	21.03c ± 0.06	23.23b ± 0.04	25.18a ± 0.11	* * *
Tricosanol	1.02a ± 0.04	0.99a ± 0.03	0.69b ± 0.04	* * *
Tetracosanol	10.59a ± 0.04	10.22b ± 0.08	9.09c ± 0.13	* * *
Pentacosanol	1.83a ± 0.03	1.77a ± 0.05	1.05b ± 0.07	* * *
Hexacosanol	25.15a ± 0.11	24.23b ± 0.19	21.24c ± 0.07	* * *
Heptacosanol	1.16a ± 0.03	0.97b ± 0.04	0.84c ± 0.04	* * *
Octacosanol	39.22b ± 0.06	38.60c ± 0.10	41.91a ± 0.13	* * *
Σ even LCFALs	95.99c ± 0.04	96.27b ± 0.04	97.42a ± 0.05	* * *
Σ odd LCFALs	4.01a ± 0.04	3.73b ± 0.04	2.58c ± 0.05	* * *
Σ Even / Σ Odd	23.92c ± 0.24	25.84b ± 0.27	37.72a ± 0.70	* * *

n-hexacosanol (2 and 8 mg/Kg) did not alter rats' 8-week diabetic status, including body weight, serum glucose levels, serum insulin levels, and urine output, but it did significantly improve serum creatinine levels and urinary albumin levels, and it inhibited the increase in kidney weight[41].

Heptacosanol was found in the highest quantity in Principe Borghese (1.16%, 0.83 mg/Kg), in a lower quantity compared to olive oil where a tendency was found to decrease during fruit ripening[27].

Of all the LCFALs, octacosanol was always present in the highest quantity in TSO, from 39.22% (28.19 mg/Kg) in Principe Borghese to 41.91% (28.04 mg/Kg) in San Marzano. Octacosanol content in virgin olive oil of Chemlali variety decreased during olive ripening from 100-120 mg/Kg to 20-30 mg/Kg from September to February[42]. Octacosanol is one of the most studied linear fatty alcohols, especially for its cytoprotective effects. This affords an opportunity for octacosanol to be taken as an alternative to aspirin in patients who have a history of or suffer from gastric irritation[43].

The sum of even-LCFALs was more than 95% of the total content (62.28 mg/Kg in Rebelion F1 and 69.00 mg/Kg in Principe Borghese), in a similar amount to Coratina and Leccino olive oil at the end of fruit ripening[27].

The sum of odd-LCFALs was always less than 5% (1.73 mg/Kg in San Marzano and 2.88 mg/Kg in Principe Borghese), similar to Nocellara Messinese olive oil at the end of olive ripening[27].

The even/odd LCFAL ratio was higher in San Marzano 37.72 and lower in Principe Borghese (23.92).

The total policosanol content of the TSO was 71.88 mg/Kg in Principe Borghese, 66.92 mg/Kg in San Marzano and 64.69 mg/Kg in Rebelion F1. Lazzez *et al.*[42] have found a similar content in olive oil from the Chemlali variety of olives cultivated at Sfax (Tunisie) from December to January. Similar or lower content was found in extra virgin olive oil from Morocco extracted on December (46.0 - 65.1 mg/Kg)[44].

3.2 ANOVA analysis

Principe Borghese showed the significantly highest values for tetracosanol, pentacosanol, hexacosanol, heptacosanol and odd-LCFALs espressed as a percentage (Table 2), and the significantly highest policosanol content expressed as an absolute content (Table 1).

Rebelion F1 showed the significantly highest value for tricosanol and pentacosanol expressed as a percentage, the significantly lowest content of octacosanol and total policosanol (Table 2) and the significantly highest unsaponifiable matter content (Table 1).

San Marzano showed the significantly highest docosanol, octacosanol, sum of even-LCFALs and even/odd ratio.

3.3 Correlation matrix

The correlations among the percentage content of policosanol of TSO are reported in Table 3. The most significant correlations were found between tricosanol and pentacosanol and between tetracosanol and hexacosanol (R = 1.000). All other correlations were higher than R = 0.691 found between heptacosanol and octacosanol.

3.4 Principal Component Analysis

Policosanol parameters were subjected to the PCA analysis (Fig. 1 a-b). Only two Eigen values were obtained for each studied parameter. All the values accounted for 100% of the cumulative variance. Their values (and the percentage of total variance) were 9.53 (95.29%) and 0.47 (4.71%). 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, which explains a high significant separation among cultivars. San Marzano was in the left bottom corner of the diagram, Principe Borghese was in the right top corner and Rebelion F1 in the right bottom corner.

The vector direction suggests that even-LCFALs were present at the expense of odd-LCFALs and docosanol was present at the expense of heptacosanol. Tetracosanol and hexacosanol were highly correlated.

4 CONCLUSION

Policosanol content of TSO from the three tomato cultivars after Soxhlet-petroleum ether extraction is similar to the policosanol content of some virgin olive oils and similar to other vegetable edible oils. Cultivar highly significantly influenced policosanol content and each LCFAL. Principe Borghese showed the highest policosanol content. Of all the LCFALs, octacosanol was the present in the greatest quantity, followed by docosanol and tetracosanol. Even-LCFALs accounted for more than 95% of the total policosanol content.

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Table 3 Correlation matrix of policosanol composition (%) of tomato seed oil.

Variables	Docosanol	Tricosanol	Tetracosanol	Pentacosanol	Hexacosanol	Heptacosanol	Octacosanol	Σ even LCFALs	Σ odd LCFALs	Σ Even/ Σ Odd
Docosanol	**1**									
Tricosanol	−0.889	**1**								
Tetracosanol	−0.949	0.988	**1**							
Pentacosanol	−0.883	1.000	0.986	**1**						
Hexacosanol	−0.946	0.990	1.000	0.988	**1**					
Heptacosanol	−0.997	0.853	0.924	0.846	0.919	**1**				
Octacosanol	0.742	−0.967	−0.915	−0.970	−0.919	−0.691	**1**			
Σ even LCFALs	0.931	−0.995	−0.999	−0.993	−0.999	−0.902	0.935	**1**		
Σ odd LCFALs	−0.931	0.995	0.999	0.993	0.999	0.902	−0.935	−1.000	**1**	
Even/Odd	0.909	−0.999	−0.994	−0.998	−0.995	−0.876	0.954	0.998	−0.998	**1**

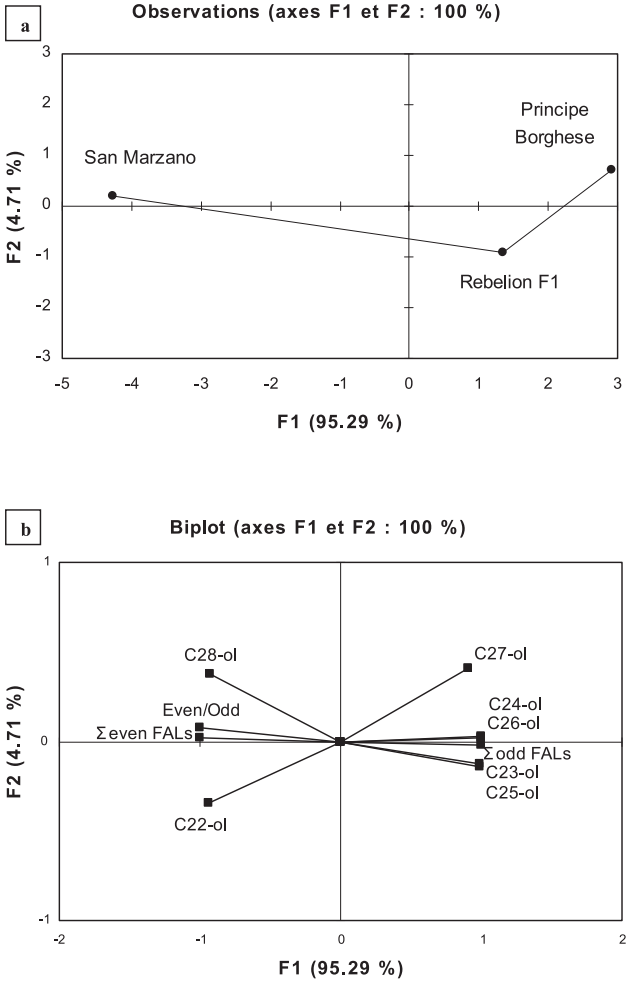

Fig. 1 (a-b) Principal component diagram of the policosanol content.
Components 1 and 2 account for 100% of the total variance.

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