Wild *Amaranthus caudatus* Seed Oil, a Nutraceutical Resource from Ecuadorian Flora

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Seed oil of wild *Amaranthus caudatus* from Ecuador was analyzed for determining the tocopherol, fatty acid, and sterol contents. The data obtained were compared with the analogous chemical profile of seed oil of Italian *A. caudatus* with the objective of evaluating the nutraceutical and alimentary potential of the Ecuadorian matrix. Supercritical fluid and ultrasound-enhanced extractions were performed on both matrices. Qualitative and quantitative determinations of tocopherols were performed by HPLC, whereas GC and GC-MS were used to determine the fatty acid composition and sterols, respectively. Supercritical fluid extraction at 400 atm was the most efficient extraction method in terms of both total yield extract and tocopherol yield. Seeds of Ecuadorian of *A. caudatus* contained higher levels of tocopherols than Italian samples, whereas the fatty acid composition and sterol content were similar. From the obtained results it can be suggested that seed oil of wild Ecuadorian *A. caudatus* can prove to be an effective nutraceutical and alimentary resource and a valid alternative to the European varieties.

Keywords: *Amaranthus caudatus; tocopherols; fatty acids; sterols; squalene; supercritical fluid extraction; nutraceuticals; alimentary resource*

INTRODUCTION

Alimentary and pharmaceutical dietary research is increasingly focusing on nutraceuticals. This is reflected in the increasing interest in potentially exploitable sources of foodstuffs—such as functional foods—able to overcome any dietary insufficiencies (*1*, *2*). The sector showing the greatest development is linked to the identification of plant species and cultivars that can be used to produce dietary supplements to counteract the onset of pathologies related to fat and cholesterol consumption in the diet (*3*). Various classes of chemical compounds are deemed to be co-responsible for hypocholesterolemic activity, and many of these are present in the lipid fraction of oleaginous seeds.

Tocopherols and tocotrienols (vitamin E isomers) are well-known natural antioxidants, and their presence in oilseeds is often correlated with the relative abundance of unsaturated fatty acids. Besides their known activity as antioxidants and free radical scavengers, they have also proved to be active against hypercholesterolemic arteriosclerosis (*4*). Phytosterols—present in the unsaponifiable fraction of plant lipid matrices—are known inhibitors of cholesterol absorption as they compete for this substance at the intestinal level (*5*). Squalene is an expensive terpenoid compound derived primarily from the shark, although this component is also widespread in the unsaponifiable fraction of plant oils. Its importance as a dietary supplement is linked to its capacity to reduce cholesterol and triglyceride levels as well as to enhance the effects of some cholesterol-lowering drugs (*6*). Moreover, dietary squalene is also supposed to play a role in tumor prevention (*7*).

Amaranthus caudatus is a gluten-free pseudocereal widely found in all temperate−tropical areas of the world. Its seeds have long been used as a source of food in South America. Like other *Amaranthus* spp., *A. caudatus* has several features that make it attractive as a potential nutraceutical crop, both in the Western world and in developing countries. In fact, *A. caudatus* readily adapts to new, demanding environments, including some that are inhospitable to cereals (*8*). From the nutraceutical point of view, products derived from plants belonging to the *Amaranthus* genus are known to be antihypercholesterolemic (*9*). Indeed, products derived from flours and seeds are already being marketed as nutraceuticals (*10*).

Seeds of *A. caudatus* contain an average 16% protein, with an ideal amino acid balance. Flour made from such seeds can complement the protein intake from other cereals (*11*). Moreover, its high lysine content can make up for the lack of this amino acid from the more common cereals. All of these characteristics make *A. caudatus* an attractive, complete dietary crop. The present work uses different extraction methods to characterize the lipid and unsaponifiable fraction of *A. caudatus* seed oil—one wild species from Ecuador and another from Italy—in order to determine their potential for applica-

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tion as dietary supplements. Particular attention is focused on the quantitative characterization of their tocopherol contents because plants from different biological sources are known to differ widely (*12*, *13*). In addition, the sterol fraction and fatty acid composition have been characterized. A comparison has also been made between the classical extraction technique and a relatively new technique using supercritical fluids (SFE) to optimize isolation conditions and enhance analysis.

MATERIALS AND METHODS

Plant Material. Seeds of *A. caudatus* (Amarantaceae) from wild Ecuadorian plants (Macas, Eastern Province, Ecuador) and from plants grown in the Botanical Garden of the University of Ferrara were ground in a blade grinder (Fritsch, Idar-Oberstein, Germany) to pass a 0.2 mm mesh. During grinding provisions were made to ensure that the temperature never exceeded 30 °C. After grinding, the flour was stored in the dark at −20 °C. Wild Ecuadorian plants of *A. caudatus* were collected and identified following the taxonomic keys and botanical and ethnobotanical description given by Villacres (*14*), Martinez (*15*), and Sauer (*16*). Samples of both the Ecuadorian and Italian *A. caudatus* are held at the Herbarium Universitatis Ferrariensis of the Department of Biology, Section of Botany, University of Ferrara (codes FER 002546 2A2 and FER 002548 2A2, respectively).

Tocopherol Extraction Procedures and Analysis. The same amount of flour of each sample was subjected to SFE and organic solvent extraction, and care was taken to operate under conditions that shielded them from light and which prevented oxidation. Each extraction was performed in triplicate. All of the extracts obtained were stored in the dark at −20 °C until the moment of qualitative and quantitative HPLC analysis.

SFE. Seed flour (5 g) of both *A. caudatus* samples was subjected to supercritical CO_2 extraction using an Applied Separations Speed SFE extractor, which comprises an air-driven pump to deliver the CO_2 to the extraction cell (10 mL stainless steel vessel with 2 μm frits at either end) housed within a temperature-controlled oven. The outlet of the extraction cell was connected to a thermally controlled variable restrictor, which maintains supercritical pressure conditions in the system. Two separate extractions were performed on the flours under the following operating conditions: (extraction 1) CO_2 flow rate = 2 L/min, CO_2 density = 0.85 g/mL, extraction cell temperature = 40 °C, pressure = 200 atm, restrictor temperature = 70 °C; (extraction 2) CO_2 flow rate = 2 L/min, CO_2 density = 0.96 g/mL, extraction cell temperature = 40 °C, pressure = 400 atm, restrictor temperature = 70 °C.

For both extractions 1 min of static extraction was followed by 14 min of dynamic extraction. As the CO_2 evaporated at the restrictor outlet due to decompression, the extracted material was collected into an empty glass vial fitted with a septum and a needle vent. The SFE instrument and extraction vessel were rated to withstand pressures up to 400 atm.

Solvent Extraction. *A. caudatus* seed flour (5 g) of both samples was placed in 100 mL of methanol and subjected to ultrasound treatment for 30 min (Branson 5200, Danbury, CT) in the dark at a constant temperature of 25 °C. The sonication was performed with the objective of obtaining a better interaction between matrices and solvent. The extract obtained was then centrifuged (3000 rpm for 20 min) employing a Heraeus LaboFuge GL centrifuge to completely remove the exhausted flour. The supernatant was recovered, dried in a rotavapor, and weighed. The extract was then taken up in 100 mL of hexane and once again placed in the ultrasound bath for 30 min at a constant temperature of 25 °C to facilitate solubilization. Subsequently, the sample was centrifuged at 3000 rpm for 20 min. The supernatant recovered was then dried in a rotavapor.

HPLC Apparatus and Conditions. HPLC analyses were performed using a modular Jasco HPLC unit (Tokyo, Japan) which consisted of a PU-980 pump, an LG-1580−02 ternary gradient unit, a DG-980-503-line degasser, a UV−vis 975 detector set at an excitation wavelength of 295 nm, linked to an injection valve with a 20 μL sample loop. A Lichrosorb silica gel Si 60 (5 μm and 25 × 0.4 cm; Teknokroma, Barcelona, Spain) column was used, and the mobile phase was 0.5% 2-propanol/hexane at a flow rate of 1 mL/min. The injection volume was 80 μL. All solvents used were of chromatographic grade. Chromatograms were recorded, and α-, β-, γ-, and δ-tocopherol peaks from amaranth samples were identified by comparing their retention times with those of pure standards (Matreya Inc., Pleasant Gap, PA). The peak areas were determined by integration using dedicated Borwin software (Borwin ver. 1.22, JMBS Developments, Grenoble, France). For each extract quali-quantitative analysis was performed in triplicate.

Fatty Acid Composition. The fatty acid composition was evaluated from extracts obtained with supercritical CO_2 extraction under the above conditions and with extracts obtained by placing 5 g of *A. caudatus* seed flour in 100 mL of hexane for 1 h aided by ultrasound. Subsequently, the samples were filtered and centrifuged for 20 min at 3000 rpm. The supernatant was recovered, dried with a rotavapor, and stored at −20 °C until GC was to be performed. Each extraction was performed in triplicate.

GC of the Fatty Acid Methyl Esters (FAME). The FAME were prepared by transmethylation using sodium methoxide in the presence of methyl acetate following the method laid out by Christie (*17*). GC analysis was performed on a Fisons 9130-9000 series GC equipped with a Fisons EL980 processor, an FID detector, and a MEGA SE52 (Mega, Legnano, Italy) column (i.d. = 0.32 mm, length = 25 m, film thickness = 0.15 μm). Operating conditions were as follows: injector temperature, 300 °C; FID temperature, 350 °C; carrier (helium) flow rate, 2.5 mL/min; and split ratio, 1:40. Oven temperature was initially 150 °C and then raised to 250 °C at a rate of 5 °C/min followed by 5 min at 250 °C. 1 μL of each sample was injected. The fatty acid standards were obtained from Alltech (Deerfield, IL).

Sterol Extraction Procedures and Analysis. The same amount of amaranth flour was subjected to SFE and appropriate solvent extraction. Each extraction was performed in triplicate. The extracts obtained were stored in the dark at −20 °C until the moment of GC analysis.

SFE. Seed flour (5 g) of both samples of *A. caudatus* was subjected to supercritical CO_2 extraction using the same apparatus employed for tocopherol extractions. The extraction was performed in two steps. The first step was carried out at 200 atm, whereas the second step was performed at 400 atm. All other conditions (extraction time, temperature, and CO_2 flow rate) were as reported for the SFE of tocopherols.

Solvent Extraction. *A. caudatus* seed flour (5 g) of both samples was placed in 100 mL of methanol and subjected to ultrasound treatment in the dark at a constant temperature of 25 °C for 30 min. The extract obtained was then dried in a rotavapor and weighed.

GC Unsaponifiable Fraction. Each of the samples obtained by extraction was then treated as follows: 40 mg of oil was weighed into a 10 mL vial with a screw cap and cold saponified with 5 mL of 1 M methanolic KOH. The vial was placed under constant agitation for 24 h at a temperature of 28 °C. After this time had elapsed, the solution was extracted twice with 2 mL of hexane and 0.2 mL of ethanol using a separator funnel. The *n*-hexane fraction was then dried under a nitrogen flow, and the unsaponifiable fraction was silanized at room temperature with 2 mL of a silanizing mixture containing pyridine/hexamethyldisilazane/trimethylchlorosilane (5:2:1). After 1 h, the liquid was evaporated under a nitrogen flow in a heat bath at 80 °C and then extracted with 0.3 mL of hexane. The conical test tube was placed in ultrasound for 2 min and centrifuged, and the supernatant was withdrawn for injection into the GC.

One microliter of the solution was injected into a Fisons 9130-9000 series gas chromatograph equipped with a Mega SE52 (Mega, Legnano, Italy) column (i.d. = 0.32 mm, length

Table 1. Percentage of Total Lipid Extraction Yield[a]

yield (%) from Ecuadorian *A. caudatus* seeds				yield (%) from Italian *A. caudatus* seeds			
		sonication[b]				sonication[b]	
SFE 200[c]	SFE 400[d]	hexane	methanol	SFE 200[c]	SFE 400[d]	hexane	methanol
3.74 ± 0.49	8.25 ± 1.07	8.77 ± 1.14	8.15 ± 1.06	4.17 ± 0.54	9.92 ± 1.29	9.22 ± 1.20	6.09 ± 0.79

[a] The results are the average of three determinations ± standard deviation. [b] Solvent extraction performed with the aid of sonication. [c] SFE 200: supercritical fluid extraction performed at 200 atm. [d] SFE 400: supercritical fluid extraction performed at 400 atm.

= 25 m, film thickness = 0.15 μm) under the following conditions: 230−320 °C at 5 °C/min, carrier gas (He) inlet pressure = 40 kPa, injector temperature = 300 °C, detector temperature = 350 °C.

GC-MS Analysis. GC-MS analyses were performed on a Fisons 8060-8000 series GC equipped with a Mega SE54 (Mega) column (i.d. = 0.32 mm, length = 25 m, film thickness = 0.15 μm) and coupled with a Fisons HD800 mass spectrometer with a MassLab data system. Helium was used as carrier gas at an inlet pressure of 40 kPa. The injector temperature was 300 °C, and the samples were injected under the same conditions reported above for GC analysis. The mass spectra were recorded between 40 and 600 amu at an electron energy of 70 eV, and the ion source temperature was 300 °C. Qualitative analysis was based on a comparison of the retention times and the mass spectra with the corresponding data in the literature (*18*, *19*).

Statistical Analysis. For each of the data collected, the relative standard deviation is given. Univariate and multivariate analyses of variance were then performed with the aim to determine if the differences recorded for the chemical characterization were significant with respect to the extraction methods and the different origins of the plant matrices. All computations were made by employing the statistical software SPSS ver. 10.0.

RESULTS AND DISCUSSION

The oil samples of *A. caudatus* from Ecuador and from Italy both showed interesting chemical characteristics for potential use as nutraceuticals. SFE at 400 atm (CO_2 density = 0.96 g/mL) for 15 min provided a total yield similar to what was found with organic solvents and approximately double that obtained at 200 atm (CO_2 density = 0.85 g/mL) for the same extraction time (Table 1). No significant improvement in SFE recoveries was observed at longer operating time (30 min) or at higher CO_2 flow rate (4 L/min of expanded gas). Moreover, statistical analyses of the yield data collected showed that the differences checked among the methods employed were all significant for both matrices (Ecuadorian *A. caudatus* seeds: $F = 32.063$, dof = 2, $p < 0.01$; Italian *A. caudatus* seeds: $F = 40.413$, dof = 2, $p < 0.01$).

The tocopherol content of the Ecuadorian samples checked by HPLC analyses (Figure 1) was ∼18% higher than that of the Italian sample (Table 2), even though the latter did present a higher vitamin E isomer content in comparison with other literature data (*20*, *21*).

The most efficient extraction method for both types of seeds was SFE at 400 atm. In fact, SFE at 200 atm of Ecuadorian and Italian *A. caudatus* seeds gave, respectively, total tocopherol yields about 44.1 and 29.3% lower than the SFE at 400 atm. Comparison between the most efficient SFE at 400 atm and the ultrasound-enhanced solvent extraction gave results even more different, with a yield quantitatively lower by about half.

In terms of the individual tocopherols, in both plant matrices β-tocopherol prevailed over α-, whereas δ- and γ-tocopherols were present at lower levels. The α- and β-tocopherol contents were higher in the seeds from the Ecuadorian *A. caudatus* than in the Italian ones. Indeed, the amounts of the individual tocopherols from the Italian *A. caudatus* seeds was significantly different ($p < 0.01$). With reference to the most efficient extraction, the δ-tocopherol in Italian samples accounted for 17.5% of the total tocopherols extracted, quantitatively ∼4 times higher than in the Ecuadorian sample in which the ratio of the δ-tocopherol was ∼3.5% of the total. Likewise, the γ-tocopherol content was higher in the Italian sample, its percentage of the total tocopherol content being nearly double that found in the Ecuadorian sample. With regard to statistical considerations for each of the tocopherols, the analyses of variance showed significant differences among the extraction methods for both matrices. In fact, SFE at 400 atm proved to be significantly more effective ($p < 0.01$) for each of the tocol compounds in both Ecuadorian and Italian amaranth seeds. However, these results partially agree with the literature. In fact, some researchers report qualitative tocopherol data similar to that given here for flour of *A. caudatus* seeds (*21*), but from a quantitative point of view the concentrations detected are considerably higher for both matrices, in particular for the Ecuadorian one. Moreover, a peak between α-tocopherol and β-tocopherol was found (Figure 1). This could be due to α-tocotrienol (*20*), which has been detected in several amaranth species (*20*, *21*).

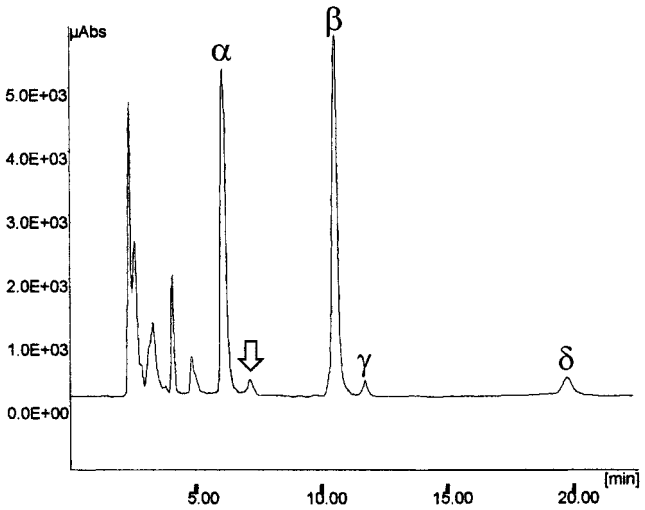

Figure 1. HPLC chromatogram of Ecuadorian *A. caudatus* seeds, supercritical fluid extracted at 400 atm. Arrows: most probably α-tocotrienol.

With regard to the alimentary potential of amaranth seeds, the Ecuadorian ones in particular, it can be pointed out that this type of result shows how the total amount of vitamin E isomers in *A. caudatus* is slightly lower than that in wheat germ oil and significantly higher than that found in other edible oils (*22*). The importance of tocopherols in the diet has been demonstrated by numerous studies ascertaining not only the average suggested daily intake (*23*) but also the levels

Table 2. α-, *β*-, *γ*-, and *δ*-Tocopherol and Total Tocopherol Content (Milligrams per Kilogram of Seeds) in Ecuadorian and Italian *A. caudatus* Seeds in Relation to the Different Extraction Methods Employed[a]

	Ecuadorian *A. caudatus* seeds			Italian *A. caudatus* seeds		
	SFE 200[b]	SFE 400[c]	sonication[d]	SFE 200[b]	SFE 400[c]	sonication[d]
α-tocopherol	27.76 ± 3.62	47.84 ± 6.25	16.88 ± 2.20	23.61 ± 3.08	32.07 ± 4.19	15.58 ± 2.03
β-tocopherol	33.85 ± 4.42	61.56 ± 8.04	34.58 ± 4.52	28.98 ± 3.78	41.54 ± 5.43	24.97 ± 3.26
γ-tocopherol	1.12 ± 0.14	2.53 ± 0.33	1.79 ± 0.23	2.98 ± 0.38	4.99 ± 0.65	3.06 ± 0.40
δ-tocopherol	2.1 ± 0.27	4.09 ± 0.53	3.47 ± 0.45	11.78 ± 1.54	16.69 ± 2.18	8.2 ± 1.07
total tocopherols	64.83 ± 8.47	116.02 ± 15.16	56.72 ± 7.41	67.35 ± 8.80	95.29 ± 12.45	51.81 ± 6.77

[a] The results are the average of three determinations ± standard deviation. [b] SFE 200: supercritical fluid extraction performed at 200 atm. [c] SFE 400: supercritical fluid extraction performed at 400 atm. [d] Solvent extraction performed with the aid of sonication.

Table 3. Fatty Acids Composition (Percent) of Ecuadorian and Italian *A. caudatus* Seeds in Relation to the Different Extraction Methods Employed[a]

	Ecuadorian *A. caudatus* seeds			Italian *A. caudatus* seeds		
	SFE 200[b]	SFE 400[c]	sonication[d]	SFE 200[b]	SFE 400[c]	sonication[d]
C16:0	17.94 ± 2.34	16.54 ± 2.16	16.51 ± 2.16	17.47 ± 2.28	12.32 ± 1.61	16.46 ± 2.15
C18:2	45.89 ± 5.99	46.91 ± 6.13	47.48 ± 6.20	43.66 ± 5.70	46.31 ± 6.05	44.94 ± 5.87
C18:1	23.85 ± 3.08	26.19 ± 3.42	25.53 ± 3.33	27.48 ± 3.59	32.88 ± 4.29	28.77 ± 3.77
C18:0	3.52 ± 0.46	4.64 ± 0.61	3.55 ± 0.46	2.71 ± 0.35	4.66 ± 0.61	3.12 ± 0.41
C20:0	0.61 ± 0.08	1.22 ± 0.16	0.74 ± 0.09	0.38 ± 0.05	1.54 ± 0.20	0.52 ± 0.07
C22:0	0.21 ± 0.03	0.6 ± 0.07	0.25 ± 0.03	0.11 ± 0.01	0.84 ± 0.11	0.2 ± 0.07
C24:0	ND	0.4 ± 0.05	ND	ND	0.78 ± 0.10	0.15 ± 0.02
squalene	7.98 ± 1.04	3.5 ± 0.46	5.94 ± 0.77	8.19 ± 1.07	0.67 ± 0.09	5.84 ± 0.76
S/U[e] ratio	0.32 ± 0.04	0.32 ± 0.04	0.29 ± 0.04	0.29 ± 0.04	0.25 ± 0.03	0.28 ± 0.04
% U[f]	75.8 ± 9.90	75.7 ± 9.89	77.6 ± 10.13	77.5 ± 10.12	79.7 ± 10.41	78.2 ± 10.22

[a] The results are the average of three determinations ± standard deviation. [b] SFE 200: supercritical fluid extraction performed at 200 atm. [c] SFE 400: supercritical fluid extraction performed at 400 atm. [d] Solvent extraction performed with the aid of sonication. [e] S/U: saponifiable/unsaponifiable ratio [f] U: unsaponifiable. [g] ND, not determined.

Table 4. Unsaponifiable Fraction (Percent) of Ecuadorian and Italian *A. caudatus* Seeds[a]

	Ecuadorian *A. caudatus* seeds			Italian *A. caudatus* seeds		
	SFE 200[b]	SFE 400[c]	sonication[d]	SFE 200[b]	SFE 400[c]	sonication[d]
squalene	86.89 ± 11.35	60.04 ± 7.84	73.31 ± 9.58	88.77 ± 11.59	30.17 ± 3.94	74.94 ± 9.79
ergost-7-en-3*β*-ol	2.77 ± 0.36	10.64 ± 1.39	6.44 ± 0.84	1.25 ± 0.16	14.84 ± 1.94	4.44 ± 0.58
chondrillasterol	6.77 ± 0.88	15.05 ± 1.97	10.01 ± 1.31	7.67 ± 1.00	26.49 ± 3.46	11.83 ± 1.54
chondrillastanol	1.99 ± 0.26	7.53 ± 0.98	6.13 ± 0.80	1.29 ± 0.17	15.99 ± 2.09	4.98 ± 0.65
24-ethylidenecholest-7-en-3*β*-ol	1.24 ± 0.16	6.12 ± 0.79	3.60 ± 0.47	0.48 ± 0.06	11.21 ± 1.46	3.16 ± 0.41

[a] The results are the average of three determinations ± standard deviation. [b] SFE 200: supercritical fluid extraction performed at 200 atm. [c] SFE 400: supercritical fluid extraction performed at 400 atm. [d] Solvent extraction performed with the aid of sonication.

Table 5. EI Mass Spectra for Amaranth Sterol-TMS Ether Derivatives[a]

sterol-TMS ether derivative	*m/z* (relative intensity)
ergost-7-en-3*β*-ol-TMS ether	M^+ 472 (27.7), 457 (11.9), 415 (4.2), 382 (9.7), 367 (26.5), 345 (5.8), 327 (5.7), 303 (8.1), 261 (6.9), 255 (100), 229 (49.5), 213 (94.6), 173 (23.6), 159 (38.2), 147 (49.3), 145 (47.4), 133 (58.3), 119 (54.9), 107 (71.4), 105 (61.2), 95 (69.5), 75 (97.5)
chondrillasterol-TMS ether	M^+ 484 (3.9), 469 (8.1), 427 (2.1), 394 (3.9), 379 (8.2), 351 (11.7), 343 (49.2), 303 (6.5), 255 (63.4), 229 (32.2), 213 (36.7), 173 (22.3), 159 (33.6), 147 (40.2), 145 (37.7), 133 (43.1), 119 (39.0), 107 (62.6), 105 (53.0), 95 (51.2), 81 (88.7), 75 (75.6)
chondrillastanol-TMS ether	M^+ 486 (23.3), 472 (12.1), 429 (3.5), 396 (6.1), 381 (20.9), 345 (6.0), 303 (8.7), 255 (100), 229 (41.9), 213 (84.3), 173 (25.4), 159 (41.6), 147 (52.2), 145 (45.8), 133 (45.7), 119 (62.4), 107 (83.5), 105 (78.6), 95 (64.0), 81 (63.5), 75 (90.1)
24-ethylidenecholest-7-en-3*β*-ol-TMS ether	M^+ 484 (0.0), 469 (8.2), 429 (2.3), 394 (3.2), 386 (14.8), 343 (100), 303 (5.4), 281 (21.4), 253 (46.3), 227 (18.7), 213 (44.1), 173 (18.4), 147 (28.7), 145 (32.2), 131 (30.1), 119 (51.8), 105 (53.2), 107 (52.5), 95 (46.3), 81 (46.4), 75 (87.5), 55 (78.2)

[a] EI: electron ionization. TMS: trimethylsilyl.

felt to be able to prevent, or cure, coronary heart disease (*24*, *25*). In the case of Ecuadorian *A. caudatus*, taking 100 g of seeds corresponds to an average daily intake of 11.6 mg, which is the average in most important European countries (*23*).

Such a high tocopherol content is most likely linked to the ability to protect unsaturated fatty acids—in particular, linoleic acid, which both samples have in abundance—against oxidation and autoperoxidation (Table 3). With a degree of unsaturation of 77.6% recorded for the extracts obtained with the sonication-enhanced hexane extraction and an average saturation/unsaturation (S/U) ratio of 0.3, Ecuadorian *A. caudatus* oil is on a dietary par with ricebran oil and just slightly lower than soybean oil, known for their dietetic properties (*26*). Besides linoleic acid (C18:2), intake of which depends on diet, the predominant fatty acids were palmitic (C16:0) and oleic (C18:1) acids, whereas the amounts of stearic (C18:0), arachidic (C20:0), behenic (C22:0), and lignoceric (C24:0) acids were lower. Statistically, the extraction performed on the Ecuadorian seeds gave significant differences ($p < 0.05$) for all of the fatty acids except for palmitic (C16:0), whereas for the Italian ones the data were all significantly different

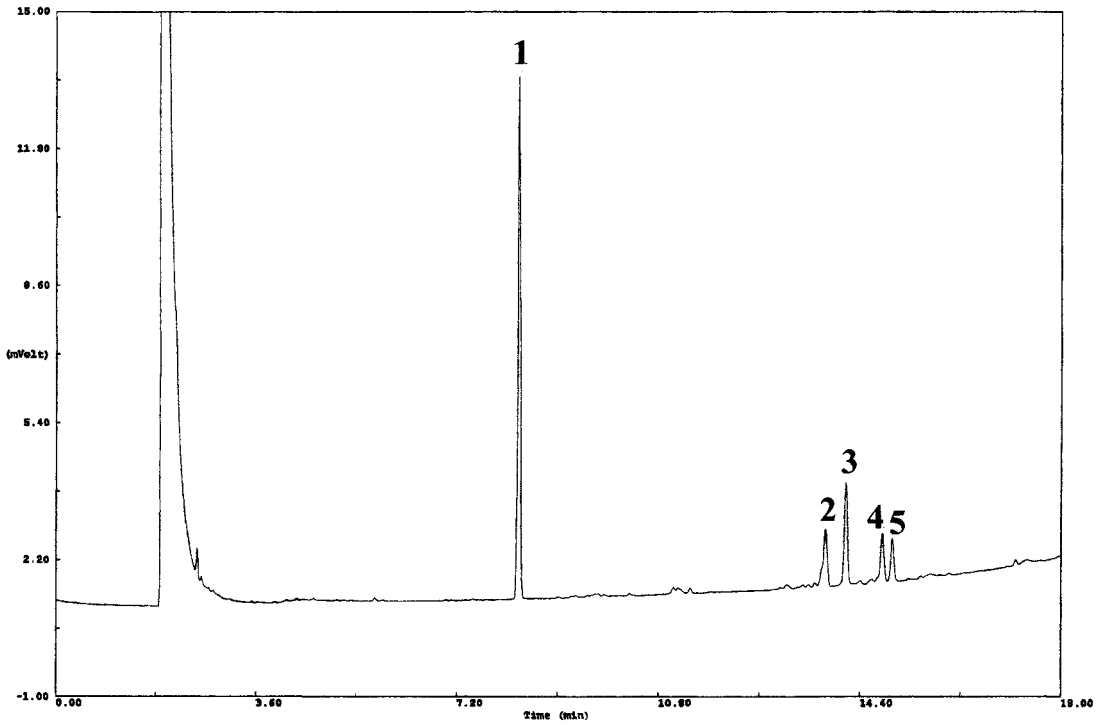

Figure 2. GC spectrum chromatogram of unsaponifiable fraction of Ecuadorian *A. caudatus* seeds: 1, squalene; 2, ergost-7-en-3β-ol; 3, chondrillasterol; 4, chondrillastanol; 5, 24-ethylidenecholest-7-en-3β-ol.

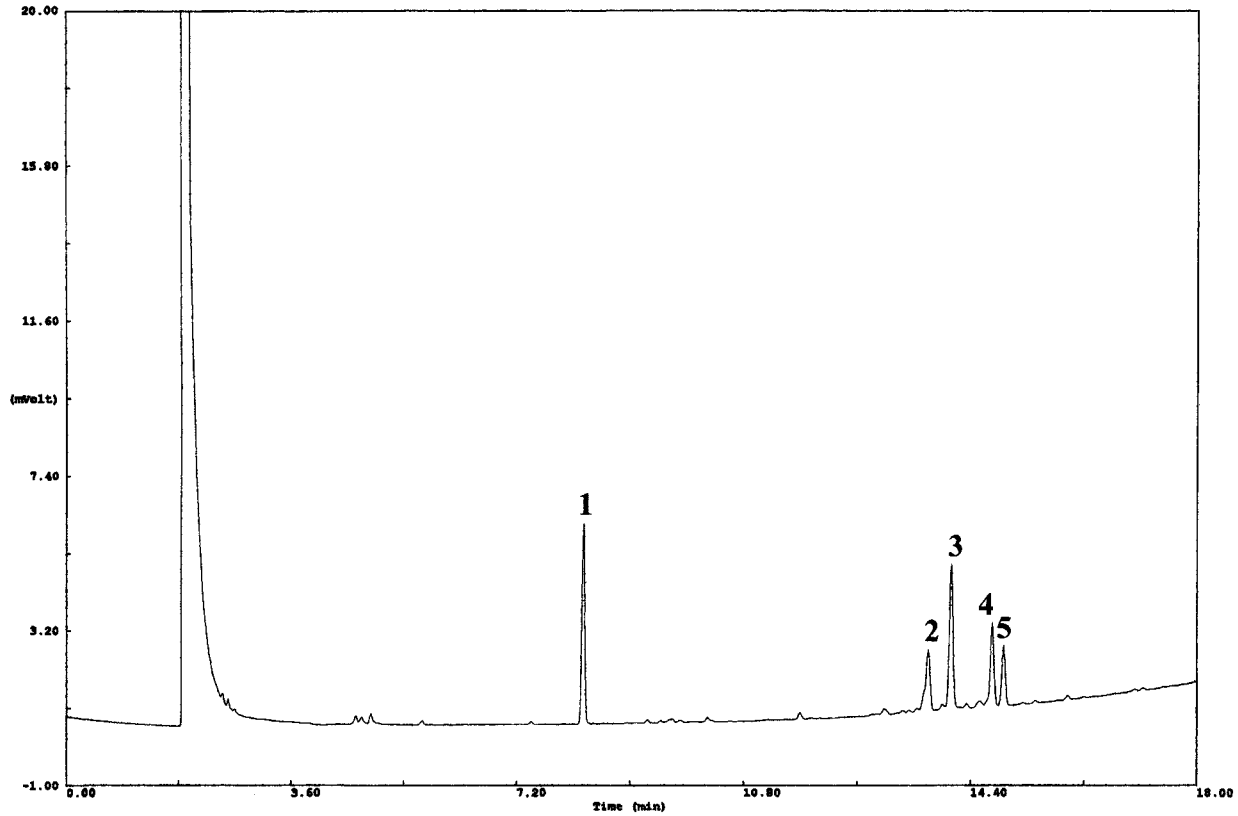

Figure 3. GC spectrum chromatogram of unsaponifiable fraction of Italian *A. caudatus* seeds: 1, squalene; 2, ergost-7-en-3β-ol; 3, chondrillasterol; 4, chondrillastanol; 5, 24-ethylidenecholest-7-en-3β-ol.

($p < 0.01$) except for linoleic (C18:2) and oleic acid (C18:1).

Within the total transmethylate lipid fraction, a single GC analysis showed the total squalene percentage. Identified by GC-MS analysis, this compound has proved to be highly abundant, accounting for 6% of the total and, whenever found in combination with Δ-7 sterols, as much as 80% of the unsaponifiable fraction (Tables 4 and 5). Such a remarkable amount could prove to be particularly interesting if we consider that dietary oils such as olive and rice oils contain lower amounts (*27*, *28*). Such availability suggests that there is potential for the use of *A. caudatus* as a renewable source of squalene, an alternative to animal sources (*29*).

The composition of the unsaponifiable fraction in the two *A. caudatus* samples did not reveal any particular qualitative differences. Besides the remarkable abundance of squalene—approximately 80% of the entire unsaponifiable fraction—the dominant sterol proved to be chondrillasterol. The different extraction conditions pointed out a significant ($p < 0.01$) higher squalene percentage at 200 atm than at 400 atm in both matrices. This difference could be mainly due to coextracted material that interferes with the squalene extraction at 400 atm. From the extraction point of view, SFE also enabled us to obtain cleaner tracings (Figures 2 and 3).

In conclusion, *A. caudatus* seed oil can be considered a rich source of dietary supplements. These characteristics proved tobe more favorable for the samples from Ecuador—due to the higher amount of tocopherols and a comparable abundance of squalene (86.89 ± 11.35 vs 88.77 ± 11.59) (Table 4)—with respect to the Italian ones. The reported data indicate that further studies should be performed on wild Ecuadorian *A. caudatus* to evaluate its use as a source of nutraceutical and alimentary alternatives to the European varieties.

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