Composition of the Unsaponifiable Oil Fraction Obtained from a Number of Cultivars of Safflower

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ABSTRACT

Composition of the unsaponifiable matter obtained from oil extracted from six safflower cultivars (UC-1, Tara, S-541, S-202, S-317 and S-918) was determined. The composition of the fractions of sterols was related to fatty acid composition, and significant correlations were found between campesterol, Δ^7-stigmastenol, oleic and linoleic acids. The composition of the fractions of terpenic alcohols and methyl sterols is also reported.

INTRODUCTION

The increase in the cultivation of safflower in Italy has been predicted by various researchers (1-3), who have also considered the possibility of using this crop to improve rather arid areas such as those found in the central and southern regions of the country.

The problems relating to the fatty acid composition of safflower are well known, as are the advances in genetic improvement aimed at reducing the linoleic acid content and, at the same time, increasing the oleic acid content (4-9).

A number of varieties of safflower with a high oleic acid or a high linoleic acid content are now available; the oil extracted from the high oleic acid varieties has a more balanced fatty acid composition (approaching that of olive oil or maize oil), good stability and resistance to oxidation (10) and can therefore be used as an edible oil – either as a salad oil or cooking oil.

The oil obtained from the high linoleic acid varieties, on the other hand, can be readily used for the manufacture of paints and varnishes (11,12).

Although, as indicated above, there is considerable literature available with respect to the fatty acid composition, few studies have been carried out on the composition of the unsaponifiable fraction.

In addition, the few studies which have been carried out on the sterol fraction (13-15) date back a number of years and concern only a few cultivars of safflower.

However, recent work carried out specifically on the composition of all the principal fractions of the unsaponifiable matter and relating to different cultivars does not appear to be available.

In this study, we report the composition of the unsaponifiable fraction obtained from six cultivars of safflower. The six cultivars used are those on which attention has been focused by researchers in recent years.

EXPERIMENTAL

The safflower achenes were supplied by the Institute of Agronomy of Sassari University (Italy) and are part of a finalized research project of the Ministry of Agriculture and Forestry.

The oils used for analysis were extracted in the laboratory using *n*-hexane as a solvent in a Soxhlet apparatus.

All the unsaponifiable matter was prepared by the Official Analysis Methods for Oils and Fats (16). The unsaponifiable matter was then treated with a solution of diazomethane to convert any free fatty acids present into the corresponding methyl esters.

Separation by thin layer chromatography (TLC) and preparation of samples for gas liquid chromatographic (GLC) analysis (silanization) were carried out as described in a previous study (17).

The gas chromatographic analysis was carried out using a Carlo Erba HRGC model 4160 instrument connected to a Spectra Physics model 4100 printer-plotter.

The sterols bands (as TMS) were analyzed with an SE 52 glass capillary column, 10 m long and an id of 0.25 mm.

TABLE I

Trivial and Systematic Names of the Compounds of Unsaponifiable Matter of Safflower Oil

Trivial name	Systematic name
Sterols	
Cholesterol	Cholest-5-en-3β-ol
Campesterol	[24R]-24-Methyl-cholest-5-3β-ol
Stigmasterol	[24S]-24-Ethyl-cholest-5,22-dien-3β-ol
Δ^7-Campesterol	[24R]-24-Methyl-cholest-7-en-3β-ol
β-Sitosterol	[24R]-24-Ethyl-cholest-5-en-3β-ol
Δ^5-Avenasterol	[24Z]-24(28)-Ethylidene-cholest-5-en-3β-ol
Δ^7-Stigmastenol	[24R]-24-Ethyl-5α-cholest-7-ene-3β-ol
Δ^7-Avenasterol	[24Z]-24(28)-Ethylidene-5α-cholest-7-en-3β-ol
Triterpene alcohols	
β-Amirin	5α-Olean-12-en-3β-ol
Cycloartenol	9β,19-Cyclo-5α-lanost-24-en-3β-ol
24-Methylene-cycloartanol	24-Methylene-9β,19-cyclo-5α-lanostan-3β-ol
Cyclolaudenol	[24S]-24-Methyl-9β,19-cyclo-5α-lanost-25-en-3β-ol
Methyl sterols	
Cycloeucalenol	4α,14α-Dimethyl-9β,19-cyclo-24-methylene-5α-cholestan-3β-ol
Obtusifoliol	4α,14α-Dimethyl-24-methylene-5α-cholest-8-en-3β-ol
Gramisterol	4α-Methyl-24-methylene-5α-cholest-7-en-3β-ol
Citrostadienol	4α-Methyl-(24Z)-24-ethylidene-5α-cholest-7-en-3β-ol
24-Ethyl-lophenol	4α-Methyl-24-ethyl-5α-cholest-7-en-3β-ol

The thickness of the stationary phase film was 0.10-0.15 μ. The column was split-system connected and the carrier gas (helium) flow was 1.5 mL/min. The splitting ratio was 1:40, the oven temperature was 250 C, and the injector and flame ionization detector (FID) temperatures were 275 C.

The terpene alcohol bands and the methyl sterol bands (all silanized) were analyzed with an OV 17 glass capillary column (treated with $BaCO_3$ on the inside surface), 15 m long and of 0.25 mm id. The carrier gas used (helium) flowed at 2.5 mL/min in the split-system connected column and the splitting ratio was 1:40.

The oven temperature was 245 C, and those of the injector and detector (FID) were 270 C.

RESULTS AND DISCUSSION

Table I shows the list of trivial and systematic names of chemical compounds used in this work.

Table II shows the list of cultivars tested, the percentage content of unsaponifiable matter, and the oleic/linoleic acid ratio which allows an immediate evaluation of the cultivar type (high oleic or high linoleic).

As can be seen, four cultivars (UC-1, S-541, S-317 and Tara) contain ca. 1% of unsaponifiable matter, while the remaining two (S-918 and S-202) contain just over half that amount (ca. 0.6%).

The unsaponifiable matter content does not therefore appear to correlate with either the high oleic acid varieties or with the high linoleic acid varieties: UC-1 and S-317 are, in fact, high oleic acid cultivars whereas the others are high linoleic acid cultivars.

The composition of the sterol fraction, on the other hand, appears more interesing and significant in this respect (Table III).

As can be noted, the percentage of β-sitosterol remains reasonably constant in the various cultivars, as also does stigmasterol; on the other hand, larger variations were noted for the percentage content of campesterol, Δ^5-avenasterol and Δ^7-stigmastenol.

However, while the variation in Δ^5-avenasterol is only slightly related to the fatty acid composition, this is not so for campesterol and Δ^7-stigmastenol, variations of which seem strongly influenced by either a high oleic or high linoleic acid content as is shown in the graph of Figure 1.

In fact, campesterol shows equivalent values (ca. 12%) in cultivars UC-1 and S-317 (i.e., in the two high oleic acid cultivars) (19), while lower values (ca. 10%) are seen in the high linoleic acid cultivars. A similar distribution has already been reported by Tiscornia and Bertini (14).

On the other hand, Δ^7-stigmastenol, a characteristic sterol of the Compositae, presents a distribution which is the reverse of that seen for campesterol.

TABLE II

Analysis of Safflower Cultivars

Cultivar	Nonsaponifiable matter (%)	$\frac{\%C_{18:2}}{\%C_{18:2}}$
UC-1	0.97	1.65
Tara	1.07	0.16
S-541	0.90	0.18
S-202	0.64	0.13
S-317	1.12	2.53
S-918	0.65	0.15

TABLE III

Composition of the fraction of sterols[a]

Cultivar	Peak no. RRT/β-Sitosterol	Cholesterol 1 0.63	Campesterol 2 0.80	Stigmasterol 3 0.86	Δ^7-Campesterol 4 0.91	β-Sitosterol 5 1.00	Δ^5-Avenasterol 6 1.03	7 1.07	Δ^7-Stigmastenol 8 1.12	Δ^7-Avenasterol 9 1.16	Other peaks 10 1.22
UC-1		0.12	12.02	4.26	2.83	50.54	5.81	2.12	14.98	5.67	0.60	1.05
Tara		–	8.45	4.77	3.57	50.09	1.59	1.80	23.88	4.87	0.75	0.23
S-541		0.88	10.67	6.32	2.53	54.24	2.22	0.62	17.56	3.03	0.15	1.78
S-202		0.69	9.30	4.52	4.36	50.56	5.63	2.61	16.63	4.52	0.58	0.60
S-317		0.22	11.97	5.33	3.09	51.49	6.42	2.13	12.72	5.58	0.48	0.55
S-918		trace	10.45	6.37	2.91	49.54	5.37	2.16	17.01	5.28	0.67	0.25

[a]Identification on the basis of comparison of the relative retention time (RRT) to β-sitosterol, with those reported in the literature (17,18).

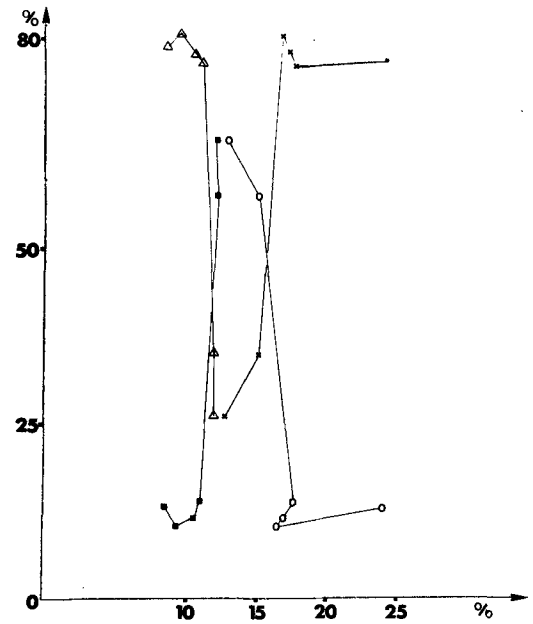

FIG. 1. The relationships between Δ^7-stigmastenol, campesterol and the main fatty acids in safflower oil. (Δ—Δ)x-axis = campesterol, y-axis = linoleic acid. (■—■)x-axis = campesterol, y-axis = oleic acid. (X—X)x-axis = Δ^7-stigmastenol, y-axis = oleic acid. (○—○)x-axis = Δ^7-stigmastenol, y-axis = linoleic acid.

In fact, as already noted by other researchers (13-15), the high oleic acid cultivars give lower Δ^7-stigmastenol percentage values with respect to high linoleic acid cultivars.

Δ^5-Avenasterol shows higher values in two high oleic acid cultivars but the percentage difference with respect to the high linoleic acid cultivars is not very great.

The distribution of the sterol fraction of the Tara cultivar (a high linoleic acid variety) is interesting: it has minimum values of Δ^5-avenasterol and campesterol but shows the highest value of Δ^7-stigmastenol (ca. 24%). The statistical evaluation of these experimental data shows a high coefficient of correlation between campesterol and fatty acids: campesterol, in fact, correlates positively with oleic and linoleic acids, and negatively with linoleic acid. The values for the coefficients of correlation between the other sterols and the fatty acid composition are, on the other hand, below the level of significance.

The composition of the terpene alcohol fraction, reported in Table IV, does not appear to be influenced by either a high oleic or a high linoleic acid content.

The percentages of β-amirin and 24-methylene-cycloartanol vary within rather narrow limits, whereas the variations in the values of cycloartenol are greater but do not appear to be linked with any other characteristic.

The presence of an appreciable quantity of a component with a retention time relative to β-sitosterol of 1.12 is confirmed: this had already been observed in the unsaponifiable matter of safflower but has not as yet been identified (17).

The composition of the methyl sterol fraction (Table V) also shows variations which do not correlate with the fatty acid composition; the largest variations were observed for dihydro-obtusifoliol and obtusifoliol which, while showing average values of ca. 24-25%, show a maximum value of 31% in Tara and a minimum of 19% in S-541.

Both cycloeucalenol and gramisterol show similar values for the high oleic acid cultivars (UC-1 and S-317), while the remaining cultivars show two sets of values: 21-23% for the Tara and S-202 cultivars, and ca. 15% for S-541 and S-918.

TABLE IV

Composition of the Fraction of Terpenic Alcohols[a]

Cultivar	Peak no. RRT/β-Sitosterol	1 0.82	2 0.86	3 0.92	4 0.95	β-Amyrin 5 1.02	6 1.12	7 1.19	Cyclortenol 8 1.24	Cyclolaudenol 9 1.26	24-Methylene-cyclortenol 10 1.32	Other peaks
UC-1		1.49	2.45	0.56	1.63	13.18	50.38	7.21	7.31	2.98	9.86	2.95
Tara		2.62	5.16	–	1.17	11.53	39.84	9.00	11.54	2.25	8.98	7.91
S-541		1.02	1.90	0.42	1.56	13.59	50.48	10.15	4.14	2.24	8.93	5.57
S-202		0.64	1.56	0.73	0.82	13.52	50.01	6.16	6.08	2.98	11.21	6.29
S-317		3.10	2.72	trace	trace	12.86	46.38	12.25	9.54	trace	9.71	3.44
S-918		2.04	2.84	0.69	3.67	16.42	52.55	6.11	4.34	1.56	7.62	2.16

[a]Identification on the basis of comparison of relative retention time (RRT) to β-sitosterol, with those reported in the literature (17,18).

TABLE V

Composition of the Fraction of Methyl Sterols[a]

Cultivar	Peak no. RRT/β-Sitosterol	1	2	3	4	5 Dihydro-obtusifoliol + obtusifoliol 0.97	6 31-nor-Cycloartenol 1.04	7	8 Cycloeucalenol + gramisterol 1.15	9 1.26	10 1.34	11 24-Ethyl-lophenol 1.44	12 1.53	13 Citrostadienol 1.62	14 1.77	15 1.92	16 2.01	Other peaks
UC-1		1.62	0.91	4.22	1.56	26.03	17.35		17.60	1.34	5.12	1.46	2.05	6.39				14.30
Tara		0.07	1.89	6.20	1.91	31.30	9.65		23.85	1.01	2.73	3.99	1.34	10.03	0.31	0.69		5.03
S-541		0.66	0.95	7.82	6.85	19.55	7.23	7.45	15.38	4.99	2.27	3.47	2.68	9.18	4.17	1.81	1.74	
S-202		0.35	1.89	9.70	2.84	25.75	13.28		21.30	trace	1.96	8.26	trace	14.64				0.03
S-317		0.54	3.18	10.29	3.13	28.01	8.67		18.36	4.26	2.66	2.54	1.45	10.58				6.34
S-918		1.94	1.74	11.13	3.38	23.42	15.47		15.84	0.37	5.35	1.75	1.61	6.94	0.30			10.76

[a]Identification on the basis of comparison of relative retention time (RRT) to β-sitosterol, with those reported in the literature (17,18).

Overall, the composition of the methyl sterol fraction appears to agree with results obtained in a previous study (17), whereas the terpene alcohol composition appears to agree somewhat less (17).

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