Composition of Organic and Conventionally Produced Sunflower Seed Oil

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ABSTRACT: The aim of the present study was to highlight the main differences between seed oils produced from conventionally cultivated crops and organically cultivated ones and processed using mild extraction procedures. The composition and the nutritional and health aspects of both types of sunflower seed oils were compared and were analytically tested to determine the macroscopic differences in proximate composition, the main differences in the minor components, the main quality parameters, the *in vitro* antioxidant activity, and the presence of *trans*-ethylene stereoisomers in FA. No significant trends were found in the oil samples for TAG and FA composition, but remarkable differences were found in the composition of minor components and in the main chemical and analytical quality properties. The organically grown samples had a higher total antioxidant activity compared with the conventional samples. *Trans* FA were found only in the conventional oils.

Paper no. J10754 in *JAOCS 81,* 1119–1123 (December 2004).

KEY WORDS: Antioxidant activity, mechanical extraction, organic oil, seed oil refining, β-sitosterol, sunflower seed oil, tocopherols.

Organic foods are derived from raw materials produced by organic agriculture, which prohibits the use of any chemicals. Such products are regulated by laws that mandate the framework and establish the production, labeling, and control system standards. The control system is based on European regulations (CEE 2092/91) enforced by certified institutions in each country (in Italy D.M. 220/94). Fears caused by scares such as methanol-adulterated wine, massive pesticide use, the outbreak of bovine spongiform encephalopathy (BSE), and the presence of dioxin in chicken meat have prompted consumers to increase the use of organic products as a way to protect their health and, at the same time, safeguard the environment (1,2).

Although organic production is expanding worldwide, consumption is still at the level of a niche market. The reasons for this apparently weak demand for organic products are: insufficient availability and standardization, higher prices (higher labor cost and lower production), and scarce availability of qualitative and quantitative information about processes and control systems (3,4). Moreover, some processes in conventional agri-food industries have become well consolidated over the years, and there is a strong resistance to change. One example is the seed oil industry.

The aim of the present study is to highlight the main differences between seed oils produced from conventionally cultivated crops and organically cultivated ones, as processed using mild extraction procedures. The composition and nutritional and health aspects of different sunflower oils are compared. Organic and conventional oils available on the market were analytically tested to determine: (i) the macroscopic differences in proximate composition; (ii) the main qualitative/quantitative differences in the minor components (tocopherols, polyphenols, and β-sitosterol); (iii) the main quality parameters (free acidity, peroxide number, induction time by Rancimat); (iv) the *in vitro* antioxidant activity; and (v) the presence of trans-ethylene stereoisomers in FA. The correlations between composition, functional and health aspects of the tested oils are discussed with respect to their origin.

EXPERIMENTAL PROCEDURES

Materials. Sunflower seed oil samples mechanically extracted from organically grown sunflowers (A–D), and sunflower seed oil samples obtained from conventionally grown crops and extracted using the conventional solvent method (E, F) were purchased on the local market. Three bottles or cans (750 mL or 1 L) of each brand were blended together for homogeneous sampling and kept at +4°C until analyzed.

Chemicals. Myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosanoic acid, eicosenoic acid, eptadecanoic acid, α-tocopherol, γ-tocopherol, δ-tocopherol, and β-sitosterol were purchased from Sigma Aldrich (Steinheim, Germany). Acetone HPLC grade 99.8%, acetonitrile HPLC grade 99.9%, methyl alcohol 99.9%, *n*-hexane HPLC grade 99.9%, H_2O, and isopropyl alcohol HPLC grade were purchased from Carlo Erba (Milano, Italy). Chloroform 99.8%, potassium hydroxide 87%, and anhydrous sodium sulfate (Na_2SO_3) were purchased from J.T.Baker (Deventer, Holland). Single-use syringe filters with pore size 0.45 μm were purchased from Sartorius AG (Göttingen, Germany). Helium was purchased from Linde (Roma, Italy).

Equipment. The HPLC system consisted of two 2010 pumps and a gradient 2020 programmer (Varian, Torino, Italy); an ELSD 500 Evaporative Light Scattering Detector (Alltech Associates Inc., Deerfield, IL); a model 7025 Rheo-

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dyne injector with a 10 µL loop (Rheodyne, Cotati, CA); a Hypersil ODS C_{18} column (4.6 × 250 mm) equipped with a Hamilton C_{18} precolumn, in inverse phase; a 25 cm × 4.6 mm silica packed stationary column (silica Adsorbosil 5 µm; Alltech, Italy); and a precolumn (silica Adsorbosil 5 µm, Alltech, Italy)

There were two GC systems: (i) a 30 m (i.d. 0.25 µm) Supelco 2330 column (Bellefonte, PA) and a FID with a split/splitless injection system at a 1:10 ratio; and (ii) a Chrompack 9001 instrument with a split/splitless injection system and flow injection analysis; the column was a CP-Sil 88 (60 m × 0.25 mm i.d., film thickness 0.2 µm, Chrompack). The acquisition and integration of the chromatograms were carried out by means of the Chrompack MOSAIC software.

A rotovapor manufactured Labo-rota SEM-320 (Resona Technics, Germany) was used.

METHODS

The following analyses were carried out at least in duplicate: (i) *free acidity*, expressed in % (w/w) of oleic acid, using the titration method described in the *EEC Reg. 2568/91*. (ii) *peroxide number*, expressed as meq O_2/kg of oil, using the titration method described in *EEC Reg. 2568/91*. (iii) *total polyphenols*, expressed as mg/L of dihydroxytyrosol in oil, determined using the Folin-Ciocalteau colorimetric method (5); (iv) *stability to oxidation*, expressed as induction time (h), determined by the Rancimat method (6); (v) *TAG,* determined by using the HPLC/ELSD method (7) and reported as percentage (w/w). (vi) *FA*, determined using a GC and GC system A, *EEC Reg. 2568/91*; (vii) trans-*FA*, determined using a high-resolution GC method and GC system B (8); (viii) *antioxidant activity*, determined using the carotenoid crocin method (9–11). This method is based on bleaching of crocin as a result of its oxidation through a free radical source, 2,2′-azobis (2,4-dimethylvaleronitrile) (AMVN). The reaction can be monitored by recording the corresponding decrease in absorbance at 443 nm. When an antioxidant, such as the unsaponifiable fraction of the samples, is added, it reacts with the free radicals and, consequently, the degree of bleaching of crocin is reduced (V_a). The equation of the competitive reaction is:

$$V_o/V_a = \frac{K_a}{K_c} \times \frac{[\text{antioxidant } (A)]}{[\text{crocin } (C)]} \quad [1]$$

where K_a and K_c are the respective absolute second-order constants.

The K_a/K_c ratio can be calculated and described by linear regression, and the relative slope of the straight line expresses the ratio between the constants of the velocity of the interaction of the antioxidant with the free radicals. Therefore, the K_a/K_c value indicates the relative activity of the molecules interacting with radicals (ROO•). Moreover, the kinetic test is able to show pro-oxidant activity (negative slope of the straight line in the kinetic equation), due to the reaction of the newly formed radicals that react even faster with the crocin. The reactions were conducted at 40°C. The crocin index of bleaching was recorded for 10 min after adding the AMVN to the toluene solution. The toluene solution contained 40 mM of AMVN, 0.24 mM of crocin, and the unsaponifiable fraction at five different antioxidant/crocin (A/C) concentrations (A/C = 0.0 A/C= 0.4; A/C = 0.8; A/C =1.2; A/C = 1.6; A/C = 2). The final volume of each solution was 1 mL. Each kinetic analysis was compared with the kinetics of crocin containing only AMVN (V_o) and was used for the calculations of V_o/V_a in Equation 1.

Additionally, the following analyses were performed at least in duplicate: (i) *total unsaponifiable fraction,* determined using the 28.092 ether extraction method, AOAC (12); (ii) *α-tocopherol and β-sitosterol,* determined in the unsaponifiable fraction using the GC method, *EEC reg. 2568/91* (14); and (iii) *tocopherol content in oil*, determined using the HPLC method of Pocklington and Dieffenbacher (13).

Data were calculated using an electronic spreadsheet for SD. The unpaired *t*-test (SigmaStat; SPSS Science, Chicago, IL) was used at $P < 0.05$ to determine the statistically significant difference between analytical methods.

RESULTS AND DISCUSSION

The TAG composition of the sunflower seed oil samples (% of the total TAG) is reported in Table 1. For all the samples, the LLL, OLL, OOL, and PPL (where L = linoleate, O = oleate, and P = palmitate) values were comparable to those

TABLE 1
TAG in Different Sunflower Seed Oils[a] (%, values ± SD)

Sample[b,c]	LLL	OLL	PLL	OOL	POL	PPL	OOO	POO
A	23.2 ± 0.0^{a}	30.9 ± 0.4^{ca}	8.2 ± 0.1^{ac}	16.0 ± 0.3^{a}	3.8 ± 0.4^{a}	6.2 ± 0.2^{a}	7.2 ± 0.0^{a}	2.1 ± 0.3^{ab}
B	32.2 ± 0.8^{b}	30.9 ± 0.3^{a}	10.5 ± 0.2^{b}	9.3 ± 0.2^{b}	8.0 ± 0.4^{b}	0.3 ± 0.0^{b}	1.3 ± 0.0^{b}	2.4 ± 0.2^{a}
C	19.8 ± 0.4^{c}	31.9 ± 0.5^{ad}	8.0 ± 0.1^{a}	17.8 ± 0.3^{c}	7.6 ± 0.8^{bd}	0.4 ± 0.2^{b}	5.5 ± 0.3^{c}	2.9 ± 1.5^{ab}
D	22.0 ± 0.2^{d}	33.1 ± 0.3^{bde}	8.9 ± 0.2^{c}	19.1 ± 2.5^{ac}	7.7 ± 0.1^{be}	0.4 ± 0.1^{b}	4.0 ± 0.1^{d}	2.4 ± 0.6^{ab}
E	23.9 ± 2.6^{acde}	30.6 ± 1.0^{ae}	7.7 ± 1.3^{ac}	14.4 ± 1.5^{ac}	5.4 ± 0.7^{ade}	0.9 ± 0.8^{b}	6.8 ± 0.0^{e}	2.1 ± 0.3^{ab}
F	24.7 ± 0.4^{e}	32.5 ± 0.5^{bcd}	8.9 ± 0.3^{ac}	16.2 ± 0.3^{a}	6.3 ± 0.3^{cd}	0.3 ± 0.1^{b}	5.5 ± 0.2^{c}	1.4 ± 0.0^{b}
Literature[d]	14–36	29–9	10–12	7–19	≅ 4	≅ 0.5	≅ 0.6	≅ 0.4

[a]Means with different superscript letters in the same column differ ($P < 0.05$).
[b]$n = 2$; $n = 3$ for sample B.
[c]A, B, C, D = mechanically extracted organic sunflower oils; E, F = conventional sunflower oils.
[d]Capella *et al.* (14).

TABLE 2
FA Content (%) in Different Sunflower Seed Oils[a]

Sample[b]	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
A	0[a]	7.50 ± 0.88[c]	0[a]	4.04 ± 0.92[a]	29.51 ± 2.33[ab]	58.95 ± 2.64[a]	0.4	0.3	0
B	0[a]	12.30 ± 2.48[b]	0[a]	7.22 ± 0.20[bc]	20.51 ± 5.35[a]	59.97 ± 3.07[a]	0.1	0.1	0
C	0[a]	7.11 ± 3.00[bc]	0[a]	5.11 ± 4.18[ac]	30.72 ± 0.73[b]	57.05 ± 6.46[a]	0.1	0.2	0.1
D	0[a]	7.60 ± 1.82[bc]	0.90 ± 0.13[b]	4.41 ± 1.14[a]	23.64 ± 5.40[ab]	63.45 ± 2.44[a]	0.1	0.1	0
E	0[a]	9.26 ± 3.85[bc]	0[a]	6.97 ± 4.09[ac]	43.07 ± 3.60[ab]	42.33 ± 6.65[a]	0.1	0.3	0.1
F	0[a]	6.92 ± 4.01[bc]	0[a]	5.31 ± 1.18[ac]	28.30 ± 1.73[ab]	59.46 ± 3.46[a]	0.1	0.2	0.1
Literature[c]	0–0.1	5–8	0–0.5	2.5–70	13–40	40–74	0–0.3	0–1	0–0.5

[a]Means with different superscript letters in the same column differ ($P < 0.05$).
[b]$n = 3$; $n = 1$ for C18:3, C20:0, C20:1. A, B, C, D = mechanically extracted organic sunflower oils; E, F = conventional sunflower oils.
[c]Capella *et al.* (14).

reported in the literature. The PPL concentration in sample A was higher and statistically different from the other samples. The OOO and POO concentrations were higher in all the samples with respect to the literature. The statistically significant differences among the samples do not highlight any particular trends for the conventional or organic samples.

The differences found between the literature values and the samples analyzed in this work do not appear to be due to the extraction technologies and refining, but rather to the different origin of the sunflower seed (seed variety, cultivation, environment, etc.).

The percentages of FA in the sunflower seed oil samples are reported in Table 2. In particular, the content of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2n-6), linolenic acid (C18:3n-3), arachidic acid (C20:0), and *cis*-9 eicosenoic acid (C20:1) were determined. Only traces of the other FA were found. The data were within the ranges found in the literature. When statistically significant differences were found, no particular trends were found among the samples; in these cases, the differences between the conventional samples were not statistically significant. The statistically significant differences do not appear to be due to the extraction technologies, but may depend on the different cultivars, as already stated for TAG. The data presented in Tables 1 and 2 show that the six oil samples had no particular trends, and this is important for the discussion of the other analytical and qualitative parameters. The organic sample B is an exception, due to its high LLL content, which indicates a greater predisposition to oxidative rancidity. In any case, this difference is not due to the technology used, but rather to the sunflower cultivars with different oleic/linoleic acid ratios.

The concentrations of total unsaponifiable fraction, α-tocopherol, β-sitosterol, and total polyphenols in the oils are reported in Table 3. α-Tocopherol and β-sitosterol were determined in the unsaponifiable fraction. Total polyphenols were directly determined in the oil. For all the samples, the total unsaponifiable fraction concentration was higher than that reported in the literature. This may be due to the high wax content.

With the exception of organic sample C, the concentration of α-tocopherol was higher in the conventional oils, even if the values in the organic oils varied greatly (1 to 30 μg/mg). As far as the unsaponifiable fraction is concerned, the total polyphenol content varied greatly between the organic samples (4.8 to 16.4 μg/g), but there were no significant differences between the organic oils and the conventional ones.

There were significant differences for β-sitosterol in all the samples. It is important to assess the relative amounts of the phenols, because they are mainly responsible for the antioxidant activity in the unsaponifiable fraction and therefore act as a chemical barrier to oxidative stress by to protecting the FA, especially PUFA.

Free acidity, peroxide number, and oxidation stability in the oils are reported in Table 4. The samples obtained from organic seeds had a higher free acidity than what is reported in the literature. Indeed, the conventional oils had very low free acidity values (0.06%). Among the organic oils, sample A had the highest free acidity (1.63%) and sample B the lowest, (0.72%); the latter value is close to the legal limit for seed oils in which the free acidity is reduced by refining. The free acidity was higher for the organic samples due to the lack of refining, while it was much lower for the conventional oils (less than 0.5%).

In organic oils, however, the peroxide number was significantly higher than in the conventional samples; the values ranged from 6 to 11 meq O_2. Nevertheless, these values fall lower within the permitted limit for virgin olive oils (20 meq O_2). This indicates good preservation with respect to oxidative damage.

TABLE 3
Total Unsaponifiable Fraction, α-Tocopherol, β-Sitosterol, and Total Polyphenols in Sunflower Seed Oils[a]

Sample[b]	Unsaponifiable fraction (mg/g)	α-Tocopherol (μg/mg)	β-Sitosterol (μg/mg)	Total polyphenols (μg/g)
A	40.5 ± 0.3[a]	10 ± 0.01[a]	3 ± 0.002[a]	16.4 ± 1.7[a]
B	79.0 ± 0.2[b]	1 ± 0.001[b]	20 ± 0.02[b]	4.8 ± 0.8[b]
C	95.0 ± 0.4[c]	30 ± 0.01[c]	32 ± 0.01[c]	7.7 ± 1.8[bc]
D	44.9 ± 0.4[d]	27 ± 0.01[d]	90 ± 0.02[d]	9.9 ± 0.4[c]
E	49.6 ± 0.3[e]	30 ± 0.01[c]	41 ± 0.02[e]	12.1 ± 8.4[abc]
F	51.1 ± 0.2[f]	90 ± 0.02[e]	70 ± 0.03[f]	10.1 ± 2.1[c]
Literature[c]	5–20	N.A.	N.A.	Traces

[a]Means with different superscript letters in the same column differ ($P < 0.05$).
[b]$n = 2$; A, B, C, D = mechanically extracted organic sunflower oils; E, F = conventional sunflower oils; N.A. = not available.
[c]Capella *et al.* (14).

TABLE 4
Chemical-Physical Characteristics of Sunflower Seed Oils[a]

Sample[b]	Free acidity (% oleic acid)	Peroxide number (meq O_2/kg)	Rancimat (i.t. in h:min)
A	1.63 ± 0.04[a]	8.81 ± 1.95[a]	7:58 ± 0:18[ae]
B	0.72 ± 0.01[b]	10.62 ± 3.27[a]	6:45 ± 0:05[b]
C	0.83 ± 0.01[c]	6.4 ± 1.83[a]	8:38 ± 0:07[a]
D	1.04 ± 0.03[d]	7.39 ± 1.83[a]	7:23 ± 0:18[e]
E	0.06 ± 0.02[e]	0.8 ± 0.74[b]	11:41 ± 0:10[c]
F	0.06 ± 0.03[e]	2.78 ± 0.59[c]	9:35 ± 0:08[d]
Literature[c]	0–0.5%	0–10	—

[a]Means with different superscript letters in the same column differ ($P < 0.05$). i.t. = induction time.
[b]$n = 2$; A, B, C, D = mechanically extracted organic sunflower oils; E, F = conventional sunflower oils.
[c]Capella *et al.* (14).

The shelf life, reported as induction time determined by the Rancimat method, was longer for conventional oils. This is clearly correlated to the lower free acidity and the lower peroxide number (predisposition to oxidation) found in those oils. Organic sample B, having a low concentration of total polyphenols and a-tocopherol (see Table 3), is more subject to oxidative damage; this is confirmed by the low induction time (6 h 45 min).

The results reported in Table 5 show that all the samples had a positive K_a/K_c value and therefore high antioxidant activity. Total antioxidant activity takes into account the amount of the unsaponifiable fraction and the antioxidant activity for each oil. Except for sample C, the organic samples had a higher total antioxidant activity. Considering that α-tocopherol and total polyphenols contribute to the antioxidant activity, whereas β-sitosterol has a pro-oxidant effect (negative K_a/K_c), it's due to the difference of these three factors in the unsaponifiable fraction (10,15–17). Even though sample A had a low α-tocopherol content, it had high antioxidant activity because of the high amount of total polyphenols as well as the low β-sitosterol content. The total antioxidant activity of sample B, which was lower than for sample A, was high compared to the conventional samples (E and F). This may have been due to the low concentration of α-tocopherol and total polyphenols, and the amount of β-sitosterol was probably not enough to affect the antioxidant activity. Sample C had the lowest total antioxidant activity, despite its high α-tocopherol content. The α-tocopherol concentration and low total polyphenol content were probably not sufficient to counteract the negative effect of β-sitosterol. Samples E and F had high α-tocopherol and total polyphenol values, but they also had high β-sitosterol values; these led to low values for the total antioxidant activity of the unsaponifiable fraction. The behavior of the unsaponifiable fraction with respect to the antioxidant activity does not correspond to the shelf-life of the oils (see Table 4). This anomaly may be explained by the effect induced by the FFA (predisposition to oxidation). The synergistic effect that occurs between α-tocopherol and total polyphenols should be considered, because the antioxidant activity value is positive, even if their absolute concentrations would not justify this. Previous studies conducted by Finotti *et al.* (10,15) support these results.

The tocopherol composition directly determined on the six oil samples is reported in Table 6. The α-tocopherol values correspond to the literature data, and no particular trend was found among conventional and organic oils. β- and γ-Tocopherol values were determined only on samples A and B. Sample B had a lower concentration of β-tocopherol, and sample A had a higher concentration of γ-tocopherol than those reported in the literature. The presence of these tocopherols may contribute to the resistance of the oil to oxidation (18).

Samples A–F were analyzed for *trans*-C18:1 and *trans*-C18:2. No *trans* C18:1 was found in samples A–F; *trans* C18:2 was found in sample E at 0.4%, and in sample F at 0.5%. The presence of these *trans* forms is important from a nutritional and health point of view; the correlation between the consumption of these trans forms and the development of coronary pathologies has been widely studied (19). These *trans* FA are incorporated into the cell membranes in different ways with respect to *cis* isomers, block the use of phospholipids, and increase the concentration of lipids and plasma lipoproteins, in particular LDL. A high consumption of *trans* FA increases the cholesterol level in blood notably. Positive correlations have been found between *trans* FA consumption and the prevalence of allergies, atopic asthma and eczema in the children and between the consumption of *trans* FA and the risk of cardiovascular diseases, thrombosis, and cancer (19,20).

TABLE 5
Unsaponifiable Fraction and Antioxidant Activity in Sunflower Seed Oils

Sample	Relative antioxidant activity (K_a/K_c)	Total antioxidant activity[b] (K_a/K_c × mg of unsaponifiable fraction)
A	2.70	109.35
B	0.76	60.04
C	0.43	40.85
D	2.40	107.76
E	0.84	41.66
F	0.88	44.97

[a]A, B, C, D = mechanically extracted organic sunflower oils; E, F = conventional sunflower oils.
[b]K_a/K_c ratio of rate constants for antioxidant and crocin.

TABLE 6
Tocopherols Determined in the Sunflower Seed Oils[a]

Sample[b]	α-Tocopherol (μg/g)	β-Tocopherol (μg/g)	γ-Tocopherol (μg/g)
A	650 ± 35.75[ac]	364 ± 11.46[a]	506 ± 30.18[a]
B	562 ± 5.26[ac]	111 ± 1.31[b]	239 ± 36.09[b]
C	518 ± 16.36[a]	ND	ND
D	702 ± 43.86[bc]	ND	ND
E	685 ± 24.64[d]	ND	ND
F	614 ± 0.54[b]	ND	ND
Literature[c]	400–1179	132–720	0–493

[a]Means with different superscript letters in the same column differ ($P < 0.05$). ND, not detected.
[b]$n = 2$. A, B, C, D = mechanically extracted organic sunflower oils; E, F = conventional sunflower oils.
[c]Capella *et al.* (14).

The total absence of *trans* FA in the organic samples confirms the validity of "mild technologies" (mechanical extraction) to obtain high nutritional value products.

No significant trends in TAG and FA composition were found among the conventional and organic oil samples. The differences found in the composition of the minor components between the two types of oils considered appeared random. Free acidity, peroxide number, and induction time showed statistically significant differences between organic and conventional oils. The high free acidity and peroxide numbers of the organic oils indicate that they were obtained from old seeds, even if they had been stored in proper conditions. The organic oils had the lowest α-tocopherol content determined in the unsaponifiable fraction. The induction time for the organic oils was shorter than for conventionally processed oils because of the high presence of β- and γ-tocopherol. The *in vitro* test for antioxidant activity showed that all the samples had a positive activity. This was probably due to the synergistic effect between α-tocopherol and total polyphenols.

The organic oils had worse quality parameters than conventional ones. This does not represent a direct risk to consumer health, but it does indicate that greater attention has to be given to processing and storage of the raw material and end products. In contrast, conventional oils had worse antioxidant activity and showed the presence of *trans* FA.

ACKNOWLEDGMENTS

The authors gratefully thank Professor Fabio Favati, University of Basilicata (Italy), for assistance in carrying out the tocopherol analysis.

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[Received November 20, 2003; accepted November 23, 2004]