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Analysis of volatile fraction, fixed oil and tegumental waxes of the seeds of two different cultivars of *Helianthus annuus*

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Dedicated to the memory of Prof. Serena Catalano

Abstract

The chemical composition of volatile fraction, fixed oil and tegumental waxes of the seeds of two *Helianthus annuus* L. cultivars (Carlos and Florom 350) were examined by GC and GC–MS. Many qualitative and/or quantitative differences were observed. α-Pinene, *cis*-verbenol and β-gurjunene were in both the main volatiles but with significant quantitative differences; moreover, Florom oil was characterized by a greater variety of constituents. The fixed oil and the waxes composition showed a general qualitative homogeneity, for both cultivars, even though marked quantitative differences were observable. The data obtained could be useful for the correct identification of the cultivars.

Keywords: *Helianthus annuus* L.; Seeds; Cultivars; Florom; Carlos 350; Volatile compounds; Fixed oil; Waxes

1. Introduction

The cultivated sunflower (*Helianthus annuus* L.) is counted among the most important oil crops all over the world (Jonic, Skoric, Lecic, & Molnar, 2000; Putt, 1978).

It has excellent nutritional properties, in fact it is the major polyunsaturated oil (high content in linoleic acid) used in human nutrition. It could be used to replace saturated fats in an attempt to reduce cardio-vascular diseases linked to athero-thrombosis. Linoleic acid, which is the predominant dietary polyunsaturated fatty acid present in sunflower oil, gives consistently low plasma cholesterol and slightly reduces triglycerides (Delplanque, 2000). Moreover, it is an essential fatty acid, which cannot be synthetized by humans and is the precursor of gamma linolenic and arachidonic acids (Dorell, 1978).

Sunflower oil is used for cooking, margarine preparation and salad dressings. Kernels are eaten raw, roasted and salted by humans or made into flour (Duke & Wain, 1981). In India, sunflower seeds are used to prepare biscuits and snack food items with a high content of proteins. Decorticated press cake is used as a high proteic food for livestock, while seed hulls provide filler in livestock feeds (Praveena, Srinivas, & Nagaraj, 2000).

Recently, various experiments have been performed, using sunflower seeds, in the food-processing industry, in order to obtain provisions of higher quality from a nutritional point of view, having controlled levels of oleic and linoleic acids.

For example, sunflower seeds have been used to feed pigs (Gundel et al., 2000) cows (Lightfiel, Baer, Schingoethe, Kasperson, & Brouk, 1993; Stegeman, Casper, Schingoethe, & Baer, 1992), lambs (Rizzi, Simioli, Sardi, & Monetti, 1999) and hens (Jiang, Ahn, & Sim, 1991). In most cases, these vegetable fat sources proved to be superior to animal fats usually used in that they elevated the percentage of lean meat with respect to back fat; moreover, the levels of unsaturated fatty acids were increased without influencing the flavour and storage characteristics of the food (Lightfiel et al., 1993).

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From this point of view, it becomes important to investigate the fixed oil composition of various cultivars and to analyse the differences among them. In our work the fixed oil composition of two different cultivars of *H. annuus* L. (Florom 350 and Carlos) were investigated. Among the main fatty acids that constitute the fixed oil of sunflower linoleic (17.0–85.0%), oleic (6.0–78.0%), stearic (3.1–6.2%) and palmitic (4.9–6.9%) acids are reported in the literature (Ucciani, 1995).

Following the developing interest in the allelopathic properties of volatiles, we also decided to investigate these chemicals obtained from the seeds of the two cultivars to illustrate the qualitative and/or quantitative differences which might occur between them. In fact, such compounds represent natural phytotoxins which the plant can use against weeds as herbicides or microbes. The accumulation of volatiles could indicate more resistant crop varieties, partly overcoming crop loss problems due to weeds and insects (Macias, Oliva, Varela, Torres, & Molinillo, 1999). The sunflower heads are known to contain a strong smelling essential oil (0.2%) (Marechal & Rigal, 1999), but the authors do not reported its composition. Recently, Ceccarini et al. (2004) reported the composition of the volatile oils obtained separately from leaves and heads of two cultivated hybrids, Carlos and Florom 350. They showed many differences between the different organs, but the resulting oils were quite similar.

Little is known about the hydrocarbon composition of the seed teguments on the role they play. Probably they provide a protective barrier against climate changes and infectious processes; furthermore, they may influence the absorption of chemicals, including pollutants and agrochemicals, providing a measure of plant resistance to diseases.

Although the qualitative pattern of waxy coatings of different vegetal organs is relatively similar from plant to plant, considerable quantitative variations, having a potential taxonomic value, are often seen (Martins, Mesquita, & Vaz, 1999).

In this work, we have analysed the alkane composition of the seeds of Florom 350 and Carlos cultivars, showing significant quantitative differences between them.

2. Materials and methods

2.1. Plant material

H. annuus L. was cultivated in a field lot within the Centro Interdipartimentale di Ricerche Agro-Ambientali "Enrico Avanzi" of Pisa University. Soil chemical-physical properties were as follows: sand 29.3%, silt 37.6%, clay 33.1%, pH 8.5, organic matter (Lotti method) 1.66%, total nitrogen (Kjeldahl method) 1.23%, assimilable P (Olsen method) 4.75 ppm, exchangeable K (Intern. method) 175 mg/kg. On field plots, deep ploughing was performed in January 2001. Soil fertilisation was carried out before sowing by 124, 96 and 96 kg/ha, of N, P_2O_5 and K_2O, respectively. Sunflower hybrids, Florom 350 and Carlos, were sown in May 2001 by a precision drill to obtain 8 plants/m crop density. Pre-emergence herbicide Duasol (Metolaclor + Metobromuron) was sprayed at the rate of 4 l/ha.

2.2. Volatile fraction analyses

Five plants belonging to the Carlos cultivar and five plants belonging to the Florom 350 cultivar were collected during the flowering phase. A sample (100 g) of crushed seeds of each cultivar was hydrodistilled for two hours using a Clevenger-type apparatus and volatile compounds were collected in *n*-hexane (HPLC grade).

The GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature programme: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250 °C; carrier gas nitrogen (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl. The identification of the components was performed, for both the columns, by comparison of their retention times with those of pure authentic samples and by mean of their linear retention indices (LRI) relative to a series of *n*-hydrocarbons.

The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalisation, all relative response factors being taken as one.

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 ml/min; injection of 0.2 ml (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings & Shibamoto, 1980; Massada, 1976; Stenhagen, Abrahamsson, & McLafferty, 1974; Swigar & Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

2.3. Fixed oil analysis

2.3.1. Oil extraction

100 g of crushed seeds, obtained from each cultivar (Florom 350 and Carlos), were separately submitted to three successive 48 h extractions using *n*-hexane. After filtration and evaporation under reduced pressure the oil was obtained.

2.3.2. Transesterification reaction

The reaction was performed by adding, drop wise, sulphuric acid to 2–3 ml of oil in excess methanol. The reaction mixture was refluxed in a steam-bath and stirred continuosly for two hours.

The solution was further diluted using water and then extracted with Et_2O in a separatory funnel (3×5 ml) The Et_2O solution, containing the fatty acid methylesters, was evaporated under reduced pressure and a sample dissolved in CH_2Cl_2 for the GC analyses.

2.3.3. GC analyses of fatty acid methylesters

The GC analyses were accomplished with a DANI GC 1000 equipped with a SUPELCOWAX 10 semicapillary column (15 m × 0.53 mm, 0.5 μm film thickness), working with the following temperature programme: 185 °C for 13 min, ramp of 7.5 °C/min up to 200 °C for 5.0 min, ramp of 10 °C/min up to 220 °C; injector and detector temperatures 260 °C; carrier gas nitrogen (4 ml/min); detector FID; split ratio 1:10; injection of 1μl. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and comparing their linear retention indices relative to a series of *n*-hydrocarbons.

The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalisation, all relative response factors being taken as one.

2.4. Seed waxes analysis

2.4.1. Wax extraction

To obtain the tegumental waxes, 10 g of seeds, belonging to each cultivar, were separately extracted using *n*-hexane for 15 min, yielding 4.8 mg of residue for Carlos and 8.4 mg of residue for Florom.

2.4.2. GC analyses

The GC analyses were accomplished with a DANI GC 1000 equipped with a 3% SE 30 glass packed column (2.5 m × 3 mm ID, 80/100 Supelcoport) working with the following temperature programme: 160–270 °C with a ramp of 5.0 °C/min; injector and detector temperatures 300 °C; carrier gas nitrogen (40 ml/min); detector FID; injection of 2 μl. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and comparing their linear retention indices relative to a series of *n*-hydrocarbons.

The relative proportions of each constituent were percentages obtained by FID peak-area normalisation, all relative response factors being taken as one.

3. Results and discussion

The main constituents of the volatile fraction obtained from the seeds of Carlos cultivar were the monoterpene hydrocarbon, α-pinene (53.6%); followed by the monoterpene alcohol *cis*-verbenol (16.7%) and the sesquiterpene hydrocarbon β-gurjunene (7.2%) (Table 1).

In the volatile fraction obtained from the seeds of Florom cultivar, the principal constituent was again α-pinene (43.1%), while β-gurjunene (13.0%) became the second most represented compound, followed by *cis*-verbenol (7.2%), and the aldehyde α-campholenal (4.4%) (Table 1).

Table 1
Volatile fraction compositions[a] of Carlos and Florom 350 cultivars

Constituents	Carlos	Florom 350	LRI[b]
α-Pinene	53.6	43.1	940
Camphene	1.0	1.5	955
Thuja-2,4(10) diene	–	0.2	959
Sabinene	4.2	1.7	977
β-Pinene	1.6	1.8	981
p-Cymene	–	0.2	1028
Limonene	–	0.4	1032
Phenyl-acetaldehyde	–	0.2	1044
cis-Sabinene hydrate	–	0.2	1070
α-Campholenal	2.8	4.4	1127
trans-Pinocarveol	3.0	2.4	1141
cis-Verbenol	16.7	7.2	1143
trans-Pinocamphone	–	0.5	1160
Borneol	–	0.8	1169
iso-Pinocamphone	–	0.3	1173
Terpinen-4-ol	–	0.2	1179
Myrtenol	–	1.5	1195
Verbenone	4.2	2.7	1206
trans-Carveol	–	0.9	1219
cis-Carveol	–	0.2	1229
Carvone	–	0.3	1243
Isobornyl acetate	–	2.4	1286
β-Elemene	–	0.2	1392
β-Gurjunene	7.2	13.0	1433
Germacrene D	–	0.4	1481
β-Bisabolene	Tr	1.8	1506
trans γ-Cadinene	–	0.5	1514
Kaur-16-en	–	0.2	2040
Total identified	94.3	89.2	

[a] Percentages obtained by FID peak area normalisation, all the relative response factors being taken as one (HP-5 column).
[b] Linear retention indices (HP-5 column).

The percentages of the main volatile constituents of each cultivar appeared to be significatly different between Carlos and Florom 350.

Moreover, in Florom, a greater number of constituents was detected: among them monoterpene hydrocarbons (thuja-2,4(10)diene, *p*-cymene, limonene), alcohols, such as *cis*-sabinene hydrate, borneol, terpinen-4-ol, myrtenol, *cis*- and *trans*-carveol, and ketones such as *cis* and *trans*-pinocamphone, verbenone, carvone and some sesquiterpenes, such as β-elemene, germacrene D and *trans* γ-cadinene.

The main types of compounds were monoterpene hydrocarbons; alcohols, aldehydes and ketones were detected in minor amounts; sesquiterpenes were only in small amounts, 7.2% in Carlos and 16.1% in Florom.

Regarding the fixed oil, the yields were 23.7% and 25.4% for Carlos and Florom, respectively. The principal compounds of Carlos oil were palmitic (5.7%), stearic (1.9%), oleic (34.5%), and linoleic (58.0%) acids, whereas in Florom they were palmitic (4.4%), stearic (0.7%), oleic (50.1%), and linoleic acids (43.3%); furthermore, traces of linolenic, arachidic and behenic acids were present in the latter cultivar.

These results showed a general homogeneity among the two cultivars regarding their qualitative oil composition but with significant quantitative differences, as shown in Table 2; in fact, in the case of Carlos, the main fatty acid of its oil was linoleic, followed by oleic, while, in the case of Florom cultivar oleic acid was more abundant than linoleic. Moreover, palmitic acid was more abundant than stearic in both cultivars.

The tegumental waxes of both cultivars were made up of six different *n*-alkanes ranging from C-22 to C-31: *n*-docosane (C-22), *n*-heptacosane (C-27), *n*-octacosane (C-28), *n*-nonacosane (C-29), *n*-triacontane (C-30) and *n*-hentriacontane (C-31). In both cases odd number hydrocarbons were present in greater amounts than even.

Despite this qualitative homogeneity, there were also in this case significant differences in the relative quantities of hydrocarbons between the two cultivars, as shown in Table 3.

Table 2
Fatty acid methylesters in Carlos and Florom 350 fixed oil (%)

Constituents	Carlos	Florom
Palmitic acid	5.4	4.4
Stearic acid	1.9	0.7
Oleic acid	34.5	50.1
Linoleic acid	58.0	43.3
Linolenic acid	–	Tr
Arachidic acid	–	Tr
Behenic acid	–	Tr
Yield	23.7	25.4
Total identified	99.8	98.5

Table 3
n-Alkanes present in tegumental waxes of Carlos and Florom 350 seeds (%)

Constituents	Carlos	Florom	Mean
C-22 *n*-Docosane	3.0	1.8	2.4
C-27 *n*-Heptacosane	10.7	11.7	11.2
C-28 *n*-Octacosane	1.2	0.8	1.0
C-29 *n*-Nonacosane	45.1	51.0	48.0
C-30 *n*-Triacontane	2.8	0.7	1.7
C31 *n*-Hentriacontane	37.1	33.0	35.0
Mean	16.6	16.5	
Total identified	99.9	99.0	

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