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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.