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Changes in Composition of Sunflower Oil Extracted from Achenes of *Sclerotium bataticola* Infected Plants

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Changes in physicochemical components and fatty acid composition of sunflower oil due to *Sclerotium bataticola* infection are reported. In both varieties, cvs. Gloriasol and Romsun HS 301, achenes from infected plants showed a percent reduction in crude fiber content, oil, ash content, and weight of 1000 achenes. However, there were increases in protein content and nitrogen-free extract. Fatty acid composition of oil extracted from achenes of infected plants showed appreciable differences compared to healthy plants. Differences were also observed in cation concentration.

The sunflower (*Helianthus annuus* L.) is one of the most widely grown spring crops in Italy. In 1986, the area under cultivation exceeded 100 000 ha, and oil seed production reached about 1.9 million tons. However, the variety, cultural practices, and environmental conditions, as well as diseases, may adversely affect yield and reduce seed quality. Both fatty acid composition and physical and chemical properties are important for evaluating oil quality. Since genetic factors control oil quality independently of the environment (Putt et al., 1969), it differs considerably from one sunflower variety to another. In addition, diseases such as downy mildew (*Plasmopara helianthi* Novot.) and verticillium wilt (*Verticillium albo-atrum* Reinke and Berth) may modify fatty acid composition and so affect the quality of the oil (Zimmer and Zimmerman, 1972).

The casual agent *Sclerotium bataticola* Taub. [*Macrophomina phaseolina* (Tassi) Goid.], which has a wide host range and grows best at high temperatures, is responsible for charcoal rot, one of the major sunflower diseases in all Italian growing areas (Zazzerini et al., 1985). Attacks by this fungus reduce both seed yield and the oil content of the seeds (Zazzerini et al., 1987).

The present study was undertaken to investigate the effect of *S. bataticola* on the physicochemical components and the fatty acid composition of sunflower achenes.

MATERIALS AND METHODS

Ten healthy and ten *S. bataticola* infected plants were randomly collected from each of the cvs. Gloriasol and Romsum HS 301 at seed development (stage 5.3) (Siddiqui et al., 1975). Both varieties were susceptible to the fungus, but attacks were heavier on Romsun HS 301 (69%) than on Gloriasol (33%). Achenes from both healthy and infected plants were stored for physical and chemical analysis.

The weight of 1000 achenes was determined for each of the six samples. After the achenes had been frozen in liquid nitrogen and ground to meal in an Osterizer mill, the following analyses were carried out.

Moisture of the meal was calculated and used as the basis for estimating dry matter components. A Kjeltec system apparatus (Tecator) was employed for measuring protein. The methods adopted are those recommended in Official Methods of Cereal Analysis (Italian Ministery of Agriculture and Forestry, 1987). After ash content had been calculated, 10 mL of a 50% HCl solution was added to the ash and slowly evaporated to dryness in a water bath. The treatment was repeated, the resulting solution filtered, and the volume adjusted to 100 mL with distilled water. A Pye Unicam SP 9 atomic absorption spectrophotometer was adopted for determining cation concentration (ppm).

Whole seed oil content was measured both with an NMR analyzer and by extraction with diethyl ether using a Soxhtec system (Tecator). The methods used for estimating fatty acid composition are those described in the Gazzetta Ufficiale (1981). Briefly, methyl esters of the fatty acids were prepared by methanolysis and the fatty acid composition of the oil extracted from each sample was analyzed. A 250-μL portion of a 2 N KOH solution in methanol and 5 mL of *n*-hexane were added to 500 mg of oil. The solution was mechanically shaken for 2 min, and phases were separated for GLC analysis. Fatty acid composition of the methyl esters was determined by capillary gas chromatography on a Carlo Erba 4160 gas chromatograph equipped with a split/splitless injector and a flame ionization detector. The column was a fused silica capillary column (30 m × 0.32 mm (i.d.)) coated with a 0.25-μm film SP 2340 (Supelco). Oven temperature and injector and detector temperatures were maintained at 180 and 210 °C, respectively. The flow of helium carrier gas was 1.5 mL/min and the split ratio 1:80.

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Table I. Physicochemical Components of Achenes Harvested from Healthy Sunflower Plants and Plants Affected with *S. bataticola*

parameter	Gloriasol			Romsun HS 301		
	healthy plants	infected plants	% inc (+) or dec (−) over healthy	healthy plants	infected plants	% inc (+) or dec (−) over healthy
wt of 1000 achenes, g	55.40 ± 0.71	48.70 ± 2.24[a,b]	−12.09	74.1 ± 1.05	65.0 ± 3.10[b]	−12.28
crude fiber content, %	16.05 ± 0.49	13.65 ± 2.24[b]	−14.95	23.15 ± 1.50	20.17 ± 1.48[b]	−12.87
oil, %	59.95 ± 1.21	57.90 ± 2.43[c]	−3.42	44.90 ± 3.96	41.35 ± 1.15[c]	−7.91
protein, %	15.85 ± 0.60	17.55 ± 1.23[b]	10.72	17.00 ± 0.56	17.90 ± 1.12[c]	5.29
ash, %	3.80 ± 0.54	3.15 ± 0.70[b]	−17.1	3.52 ± 0.15	3.13 ± 0.15[b]	−11.08
N-free extr, %	4.35 ± 1.23	7.75 ± 2.67[b]	78.16	11.43 ± 2.77	17.45 ± 1.99[b]	52.67
peroxide value, mequiv of O_2/kg	8.21 ± 1.00	8.76 ± 1.08[c]	6.7	8.10 ± 2.55	9.03 ± 1.53[c]	11.48

[a] Means ± standard deviation of six replicates. [b] Significant at $P \leq 0.05$. [c] Difference not significant.

Table II. Fatty Acid Composition (Percent of Total Fatty Acid) in Sunflower Oil Extracted from Healthy and *S. bataticola* Infected Plants

fatty acid	Gloriasol			Romsun HS 301		
	healthy plants	infected plants	% inc (+) or dec (−) over healthy	healthy plants	infected plants	% inc (+) or dec (−) over healthy
myristic	0.08 ± 0.03	0.04 ± 0.08[a,b]	−50.00	0.1 ± 0.004	0.06 ± 0.01[b]	−40.00
palmitic	5.90 ± 0.24	6.05 ± 0.39[c]	2.54	5.57 ± 0.15	5.95 ± 0.10[b]	6.82
palmitoleic	0.10 ± 0.004	0.05 ± 0.05[b]	−50.00	0.055 ± 0.01	0.09 ± 0.02[b]	63.64
margaric	0.10 ± 0.004	0.04 ± 0.05[b]	−50.00	0.01 ± 0.005	0.03 ± 0.01[b]	200.00
stearic	4.55 ± 0.36	4.45 ± 0.62[c]	−2.20	4.40 ± 0.04	4.70 ± 0.07[b]	6.82
oleic	30.57 ± 1.38	26.90 ± 0.55[b]	−12.00	30.15 ± 2.24	34.18 ± 0.35[b]	13.37
linoleic	57.85 ± 0.24	61.00 ± 1.03[b]	5.44	59.05 ± 1.17	53.90 ± 0.20[b]	−8.72
arachidic	0.27 ± 0.12	0.24 ± 0.30[c]	−11.11	0.15 ± 0.08	0.25 ± 0.14[b]	66.67
linolenic	0.05 ± 0.02	0.055 ± 0.05[c]	−10.00	0.055 ± 0.02	0.09 ± 0.02[b]	63.64
eicosenic	0.06 ± 0.01	0.12 ± 0.05[b]	100.00	0.055 ± 0.02	0.09 ± 0.01[b]	63.64
NI[d]	0.55 ± 0.19	0.44 ± 0.39[c]	−20.00	0.36 ± 0.12	0.55 ± 0.15[b]	57.14

[a] Means ± standard deviation of six replicates. [b] Significant at $P \leq 0.05$. [c] Difference not significant. [d] NI = not identified.

Table III. Cation Concentration (ppm) of Achenes Harvested from Healthy and *S. bataticola* Infected Plants

cation	Gloriasol		Romsun HS 301	
	healthy plants	infected plants	healthy plants	infected plants
sodium	1171.1 ± 130.50	1757.1 ± 301.65[a,c]	1203.2 ± 8.85	1018.3 ± 596.8[c]
potassium	7202.9 ± 170.53	6852.5 ± 135.14[c]	6518.2 ± 307.80	6356.6 ± 80.24[c]
magnesium	3236.5 ± 315.85	3090.1 ± 91.85[c]	2925.8 ± 52.70	2873.2 ± 91.40[c]
calcium	887.6 ± 0.34	900.8 ± 16.90[c]	634.6 ± 25.70	723.9 ± 51.30[c]
zinc	98.2 ± 36.60	61.2 ± 2.55[c]	57.3 ± 2.35	64.0 ± 5.90[c]
copper	21.6 ± 0.35	20.8 ± 0.35[c]	23.3 ± 0.90	23.2 ± 0.35[c]
manganese	15.2 ± 1.25	13.6 ± 0.60[c]	14.7 ± 0.35	16.5 ± 2.05[c]
iron	240.6 ± 2.75	163.7 ± 9.40[b]	289.4 ± 1.90	416.7 ± 8.10[b]
lead	1.1 ± 0.07	1.6 ± 0.36[c]	0.7 ± 0.05	1.1 ± 0.65[c]
nickel	3.8 ± 0.65	3.6 ± 0.45[c]	2.3 ± 0.40	16.7 ± 1.00[b]
cobalt	0.8 ± 0.15	0.8 ± 0.15[c]	0.6 ± 0.15	0.9 ± 0.60[c]
chromium	0.9 ± 0.30	0.7 ± 0.35[c]	0.6 ± 0.06	12.0 ± 0.75[b]
cadmium	2.5 ± 2.20	0.4 ± 0.10[c]	0.3 ± 0.05	0.4 ± 0.05[c]

[a] Means ± standard deviation of three replicates. [b] Significant at $P \leq 0.05$. [c] Difference not significant.

The percentage composition of fatty acids was estimated by measuring the area under the peaks obtained with a Spectra Physics 4290 integrator.

Peroxide value (mequiv of O_2/kg of fat) was calculated according to the method of Takagi et al. (1977).

The Student's *t*-test was employed for analysis of the variations the disease induced in the different factors.

RESULTS

Achenes harvested from infected plants were smaller than achenes from healthy plants. Achenes (1000) from infected cv. Gloriasol weighed 12.09% and those from infected cv. Romsun HS 301 12.28% less than the achenes of the respective healthy plants. Crude fiber content and ash content were also significantly lower in the infected plants. Protein content and nitrogen-free extract were both higher in infected cv. Gloriasol, whereas only nitrogen-free extract was significantly increased in infected cv. Romsun HS 301. There were no marked differences in the oil content and peroxide value of healthy and infected plants of either varieties. However, a 3.42% (Gloriasol) and a 7.91% (Romsun HS 301) reduction in oil content was documented in plants affected by *S. bataticola* (Table I).

Table II shows that there were considerable differences in the fatty acid contents of oils extracted from infected and healthy plants of cv. Romsun HS 301. In cv. Gloriasol, only myristic, palmitoleic, margaric, oleic, linoleic, and eicosenic acids differed significantly. The percentages of myristic and oleic acid were lower in achenes from infected plants of both varieties, while there were no appreciable differences in palmitic and stearic acids. Palmitoleic, margaric, arachidic, and linoleic acid percentages were reduced in achenes from infected cv. Romsun HS 301 plants, while linoleic acid content was higher in achenes from infected plants of cv. Gloriasol. The oleic/linoleic

ratio of oil extracted from achenes of infected cv. Gloriasol plants (0.44) was lower than that of infected cv. Romsun HS 301 (0.63).

Except for iron, which was lower in infected plants, there were no differences in the cation concentration of achenes harvested from healthy and infected cv. Gloriasol plants. In contrast, iron, nickel, and chromium were all higher in achenes from infected plants of cv. Romsun HS 301 (Table III).

DISCUSSION

S. bataticola is a soil-borne pathogen that causes root and stem rot in sunflowers. Infected plants ripen prematurely, there is gray discoloration on the stems, the vascular system is completely invaded by the parasite, and black sclerotia fill the pith. Because the plants are subjected to water stress due to altered transport of nutrients and translocation of toxins, this fungal infection affects both the quantity and quality of the yield (Chan and Sackston, 1973; Zimmer and Hoes, 1978). It may be that the pathogen interrupts transpiration, so causing the plants to wilt and produce smaller seeds, in which case the seed composition could be that of an immature seed whose development, was arrested prematurely.

Despite the fact that all alterations documented in the present study were revealed in achenes harvested from *S. bataticola* infected plants, the seeds themselves were not directly invaded by the fungus. Achene weight and crude fiber, oil, and ash contents were reduced with respect to healthy plants, and appreciable differences in fatty acid composition were also recorded.

Physical and chemical alterations were greater, but the oleic/linoleic ratio was higher in Romsun HS 301 than in Gloriasol. When the degree of unsaturation in an oil is high, that is when the oleic/linoleic ratio is low, the autoxidative process take place more easily. The decomposition products derived from autoxidation of fat are thought to be dangerous to health and, certainly, an oil with a high content of autoxidative products requires additional refining, especially during the bleaching step. Greater amounts of bleaching earth are needed in the treatment of peroxidized oils and contact with the bleaching earth transforms peroxides, as well as epoxides to carbonyl compounds.

Although oxidation products have occasionally been detected in stored seeds (Conte et al., 1979), the level of peroxides is usually low in fresh seeds, as it was in this study.

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Registry No. Na, 7440-23-5; K, 7440-09-7; Mg, 7439-95-4; Ca, 7440-70-2; Zn, 7440-66-6; Cu, 7440-50-8; Mn, 7439-96-5; Fe, 7439-89-6; Pb, 7439-92-1; Ni, 7440-02-0; Co, 7440-48-4; Cr, 7440-47-3; Cd, 7440-43-9; myristic acid, 544-63-8; palmitic acid, 57-10-3; palmitoleic acid, 373-49-9; margaric acid, 506-12-7; stearic acid, 57-11-4; oleic acid, 112-80-1; linoleic acid, 60-33-3; arachidic acid, 506-30-9; linolenic acid, 463-40-1; eicosenoic acid, 28933-89-3.

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