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Volatile Components of Chickpea (*Cicer arietinum* L.) Seed

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Headspace material was collected from floured *Cicer arietinum* seed and analyzed by capillary GC–mass spectrometry. Structural assignment was achieved for 132 components of the chickpea volatiles by mass spectra, by cochromatography, and through their Kovats indices. All the substances, with two exceptions, present in chickpea headspace in an amount above 0.5% of the total volatiles, could be identified. Besides aliphatic hydrocarbons, the dominant chemical classes in the chickpea volatiles were terpenoids (35%) and alcohols (18%). With the sampling method applied for this analysis, a total of 2.4 μg of volatiles was collected from 1.5 L of headspace volume. The main individual component was α-pinene (≈0.3 μg).

Chickpea (*Cicer arietinum* L., Fabaceae) is a legume of economic importance, which is mainly grown in the hot climates of India, Pakistan, Iran, Ethiopia, Mexico, and the Mediterranean area. Its most important insect pest is *Heliothis armigera* Hübner (Lepidoptera: Noctuidae), a polyphagous night-active moth identified in India on 181 host plant species (Manjunath et al., 1985). On chickpea, the larvae fed on leaves, flowers, buds, pods, and seeds of different maturation stages. Preliminary studies have demonstrated an attraction of *H. armigera* larvae by chickpea seed volatiles (Saxena and Rembold, 1984). The present study was undertaken to characterize such headspace material, which is volatile at 43 °C. Its volatiles profile could also be of interest for food chemists if used as chemical fingerprint for identification of chickpea seed samples. Such an analytical characterization is still missing, according to corresponding literature (van Straten and Maarse, 1983).

EXPERIMENTAL SECTION

Materials. For this stock-taking study, one batch of commercial standard chickpea seed of kabuli type (Scandimport, Maisach, FRG) was used. The material was dried at 40 °C overnight and, if required, ground in 25-g portions in an IKA-M-20 Universal mill (Janke & Kunkel, Staufen, FRG) under intensive water cooling. Headspace was collected immediately afterward.

Isolation of Volatiles Using Tenax Traps. The seed flour was placed in a 110-mL graduated flask fitted with three ground glass stoppers and maintained at 43 °C in a water bath. Two traps filled with Tenax TA (150 mg, 80–100 mesh, package of 56-mm length fixed with silanized glass wool in the middle of a glass tube with 20-cm length and 4-mm i.d.) were directly connected through ground-glass connections with the flask. The third inlet was for sample introduction and was closed with a ground-in stopper. After 10 min, purified nitrogen was flown (100 mL/min) via a Teflon tube connection through one of the Tenax tubes into the flask. Headspace was collected in the second Tenax tube for 15 min. The commercially available authentic chemical samples used for identification purposes were trapped in a similar way as the headspace material.

Capillary Gas–Liquid Chromatography–Mass Spectral (GC–MS) Analysis. The method of thermal desorption of the Tenax trap and transfer of the volatiles via an intermediate trap onto the capillary column has been described already (Nitz et al., 1984; Wächter et al., 1986). For the present study, a desorption temperature of 150 °C for the Tenax traps, helium flux of 20 mL/min, and time period of 10 min were applied. The chickpea volatiles are completely desorbed under these conditions. A Finnigan 1020 quadrupole automated GC–MS system, directly coupled to a Sigma III gas chromatograph (Perkin-Elmer) equipped with a modified PTV injector from Dani as described elsewhere (Nitz et al., 1984), was used. Separation was performed with a J&W fused silica capillary column (30 m × 0.25 mm (i.d.)) coated with SE54 (film thickness 0.25 μm). Carrier gas was helium (29 cm/s), and the oven, after having been kept at 0 °C for 12 min, was programmed to 250 °C at a rate of 2 °C/min. The mass spectra were measured by electron impact at 70 eV.

RESULTS AND DISCUSSION

With the technique described, a solvent-free sample collection is achieved. Control experiments using the empty manifold under our standard sampling conditions showed that only insignificant impurities were present. Practically no serious breakthrough effect of the volatiles was discernible in the second trap if two Tenax traps were used in line for sample collection. Only some part of the

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Table I. Volatile Compounds from floured *C. arietinum* Seed Identified after GC–MS Analysis

no.[a]	peak no.[b]	chem name	Kovats index[c]	reliability of ident[d]	rel %[e]	no.[a]	peak no.[b]	chem name	Kovats index[c]	reliability of ident[d]	rel %[e]
					Aliphatic Alcohols						
1	1	ethanol	503	a	3.7	11	44	heptan-2-ol	904	a	0.1
2	7	propan-1-ol	574	a	0.7	12		2-methylpropan-2-ol	540	b	
3	19	butan-1-ol	671	a	0.5	13	14	2-methylpropan-1-ol	636	a	0.8
4	31	pentan-1-ol	775	a	2.3	14	27	2-methylbutan-1-ol	743	a	0.4
5	40	hexan-1-ol	878	a	5.3	15	26	3-methylbutan-1-ol	741	a	0.4
6	56	heptan-1-ol	972	a	0.2	16		(*E*)-2-buten-1-ol	654	d	
7	4	propan-2-ol	524	a	2.5	17		(*E*)-2-hepten-1-ol	970	c	
8	11	butan-2-ol	612	a	1.1	18	20	1-penten-3-ol	687	a	<0.1
9	24	pentan-2-ol	705	a	0.1	19		1-octen-3-ol	980	b	
10		hexan-2-ol	804	b							
					Aliphatic Aldehydes						
20	34	hexanal	798	a	0.3	26	15	(*E*)-2-butenal	645	a	<0.1
21	72	nonanal	1101	a	0.2	27	29	(*E*)-2-pentenal	751	a	<0.1
22	77	decanal	1203	a	<0.1	28	36	(*E*)-2-hexenal	849	a	<0.1
23	6	2-methylpropanal	554	a	0.3	29	51	(*E*)-2-heptenal	952	a	0.7
24	18	2-methylbutanal	658	a	0.3	30		2-methyl-2-propenal	566	d	
25	16	3-methylbutanal	649	a	0.2						
					Aliphatic Ketones						
31	3	acetone	503	a	1.4	35	42	heptan-2-one	890	a	<0.1
32	9	butan-2-one	602	a	1.1	36	28	2-methylpentan-3-one	748	a	<0.1
33	21	pentan-2-one	687	a	0.1	37		1-octen-3-one	975	b	
34	32	hexan-2-one	788	a	<0.1	38	66	3-octen-2-one	1035	a	<0.1
					Aliphatic Esters						
39	5	methyl acetate	531	a	<0.1	41		butyl acetate	817	b	
40	12	ethyl acetate	618	a	0.1	42	45	γ-butyrolactone	908	a	<0.1
					Terpenoids						
43		α-thujene	923	b		50	62	α-terpinene	1009	a	0.1
44	48	α-pinene	928	a	12.6	51	63	*p*-cymene	1019	a	2.9
45	49	camphene	938	a	0.3	52		β-phellandrene	1021	b	
46	57	β-pinene	968	a	3.4	53	64	limonene	1023	a	3.6
47	59	myrcene	989	a	1.3	54	68	γ-terpinene	1055	a	3.4
48		α-phellandrene	997	b		55	70	terpinolene	1082	a	0.8
49	61	Δ^3-carene	1004	a	6.9						
					n-Alkanes						
56	2	*n*-pentane	500	a	1.4	62	71	*n*-undecane	1100	a	1.4
57	8	*n*-hexane	600	a	0.6	63	75	*n*-dodecane	1200	a	0.8
58	22	*n*-heptane	700	a	1.1	64	79	*n*-tridecane	1300	a	0.4
59	33	*n*-octane	800	a	1.6	65	80	*n*-tetradecane	1400	a	0.3
60	43	*n*-nonane	900	a	0.3	66	81	*n*-pentadecane	1500	a	0.1
61	60	*n*-decane	1000	a	0.8	67	82	*n*-hexadecane	1600	a	<0.1
					Methyl-Branched Alkanes						
68		2-methylhexane	659	b		88		5-methylnonane	960	b	
69		2-methylheptane	763	b		89		5-methyldecane	1057	b	
70		2-methyloctane	864	b		90		5-methylundecane	1156	b	
71	54	2-methylnonane	964	a	0.1	91		5-methyldodecane	1255	b	
72	69	2-methyldecane	1064	a	0.2	92		6-methylundecane	1154	b	
73	74	2-methylundecane	1164	a	0.2	93		6-methyldodecane	1253	b	
74		2-methyldodecane	1264	b		94		2,2,4-trimethylpentane	680	b	
75		3-methylhexane	667	b		95		2,3-dimethylhexane	755	b	
76		3-methylheptane	770	b		96		2,4-dimethylhexane	729	b	
77		3-methyloctane	870	b		97		2,5-dimethylhexane	727	b	
78	55	3-methylnonane	970	a	0.2	98		2,4-dimethylheptane	821	b	
79		3-methyldecane	1070	b		99		?-dimethyloctane	933	d	
80		3-methylundecane	1171	b		100		?-dimethyldecane	1117	d	
81		3-methyldodecane	1271	b		101		?-dimethyldecane	1127	d	
82		4-methylheptane	764	b		102		?-dimethylundecane	1214	d	
83		4-methyloctane	863	b		103		?-dimethylundecane	1218	d	
84	53	4-methylnonane	962	a	0.2	104		?-dimethylundecane	1222	d	
85		4-methyldecane	1060	b		105		?-dimethylundecane	1228	d	
86		4-methylundecane	1160	b		106		?-dimethylundecane	1273	d	
87		4-methyldodecane	1259	b							
					Cycloalkanes						
107		ethylcyclopentane	725	b		112	73	pentylcyclohexane	1128	a	<0.1
108	25	methylcyclohexane	713	a	<0.1	113		hexylcyclohexane	1234	c	
109	35	ethylcyclohexane	824	a	<0.1	114	67	decahydronaphthalene	1042	a	0.3
110	47	propylcyclohexane	923	a	<0.1	115		2-methyldecahydronaphthalene	1115	c	
111	65	butylcyclohexane	1025	a	<0.1						
					Alkenes						
116		1-hexene	586	b		120		2-octene	813	d	
117		?-hexene	607	d		121		4-octene	806	d	
118		?-hexene	618	d		122		?-octadiene	824	d	
119		1-octene	790	b							

Table I (Continued)

no.[a]	peak no.[b]	chem name	Kovats index[c]	reliability of ident[d]	rel %[e]	no.[a]	peak no.[b]	chem name	Kovats index[c]	reliability of ident[d]	rel %[e]
					Aromatic Compounds						
123	17	benzene	650	a	0.6	133		1-methyl-4-ethylbenzene	955	b	
124	30	toluene	758	a	7.7	134		1-methyl-2-ethylbenzene	971	b	
125	37	ethylbenzene	853	a	0.7	135		1,3,5-trimethylbenzene	961	b	
126	38	*p*-xylene	861	a	1.4	136		1,2,4-trimethylbenzene	984	b	
127	39	*m*-xylene	862	a		137		1,2,3-trimethylbenzene	1012	b	
128		styrene	882	b		138		3,5-dimethylphenol	931	d	
129	41	*o*-xylene	884	a	0.5	139	52	benzaldehyde	949	a	0.6
130	46	cumol (isopropylbenzene)	917	a	<0.1	140	58	benzonitrile	973	a	<0.1
131	50	propylbenzene	946	a	0.1	141	76	estragole	1192	a	0.6
132		1-methyl-3-ethylbenzene	954	b		142	78	anethol	1279	a	0.9
					Furans						
143	10	2-methylfuran	604	a	0.3	146		2-butylfuran	889	b	
144	23	2-ethylfuran	701	a	0.3	147		2-pentylfuran	989	b	
145		2-propylfuran	787	b		148		2-hexylfuran	1079	b	
					Others						
149		dimethyl disulfide	734	b		152		trichloromethane	616	b	
150	13	tetrahydrofuran	621	a	1.8	153		1,2-dichloroethane	641	c	
151		dichloromethane	531	c		154		trichloroethene	693	c	

[a] Current number of compounds. [b] Peak number in Figure 1. [c] Kovats indices calculated for the SE54 capillary column of the GC–MS system (for details see Materials and Methods). [d] The reliability of the identification or structural proposal is indicated by the following symbols: a = mass spectrum and retention time consistent with those of an authentic sample; b = mass spectrum and Kovats index in agreement with the corresponding values found in literature; c = mass spectrum consistent with spectra found in literature; d = tentative identification by mass spectrum (e.g., position of methyl branching unknown). [e] Relative percentage of total peak area.

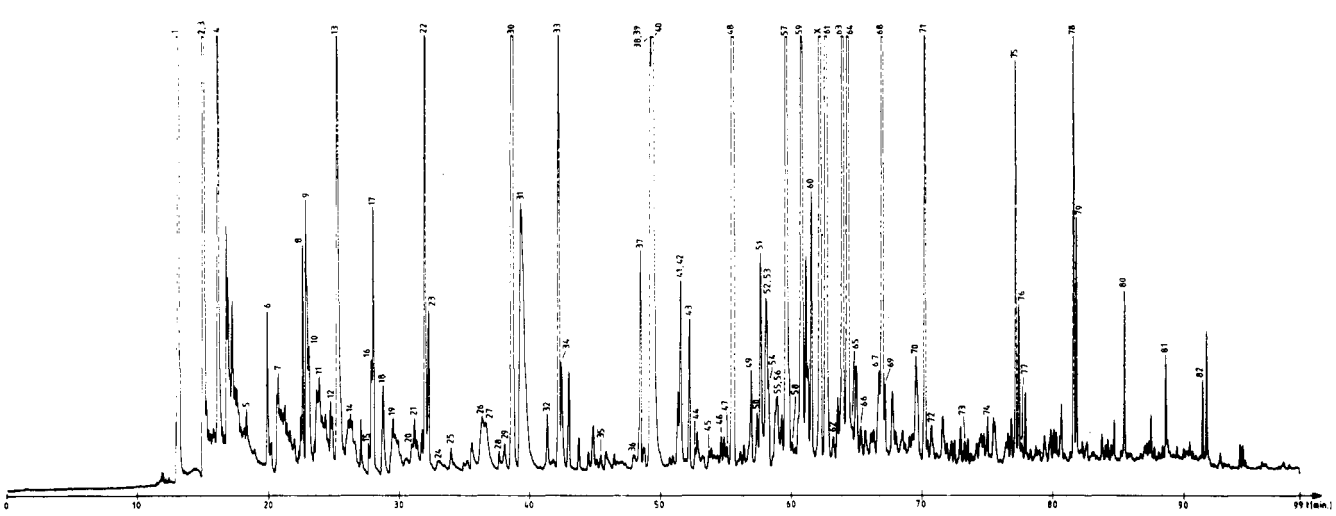

Figure 1. Gas chromatogram of *C. arietinum* volatiles. Total chickpea headspace volatiles of floured seed were adsorbed on Tenax, thermally desorbed, and separated by capillary gas chromatography. The numbers mark such compounds identified by cochromatography of the original substances. For more details, see Table I and Materials and Methods.

extremely volatile compounds had passed under these conditions into the second trap.

A typical gas chromatogram of chickpea volatiles is shown in Figure 1. About 200 individual peaks were detected, and for 154 of them structural proposals are given based on mass spectral data. By cochromatography with authentic reference substances, 82 compounds could be identified. They represent 84% of the total peak area in Figure 1. For 50 of the 154 proposed structures, Kovats indices (Kovats, 1958) were calculated after van den Dool and Kratz (1963) and compared with available literature data. From the substances with amounts above 0.5% of the total volatiles only two could not be identified.

Table I collates all 154 volatile compounds including some information on the means used for their identification (mass spectra, Kovats indices, coinjection of reference substances). Some of the volatile aldehydes and alcohols with C_6 carbon chains, which were present in the chickpea volatiles, may be derived from oxidation of higher unsaturated fatty acids. The source of the many aliphatic hydrocarbons is not clear. They cannot be artifacts, however, as they are absent in control analyses.

Sixteen prominent components of the chickpea volatiles were tested singly with first-instar *H. armigera* larvae in an olfactometer assay. Significantly attractive were the compounds pentanol, Δ^3-carene, myrcene, and α-pinene (Rembold et al., 1989). Whether these four components constitute the whole chickpea kairomone or whether additional, even less volatile compounds add to the chemical information that lures the larva to its host plant is being studied in our laboratory.

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Registry No. **1**, 64-17-5; **2**, 71-23-8; **3**, 71-36-3; **4**, 71-41-0; **5**, 111-27-3; **6**, 111-70-6; **7**, 67-63-0; **8**, 78-92-2; **9**, 6032-29-7; **10**, 626-93-7; **11**, 543-49-7; **12**, 75-65-0; **13**, 78-83-1; **14**, 137-32-6; **15**, 123-51-3; **16**, 504-61-0; **17**, 33467-76-4; **18**, 616-25-1; **19**, 3391-86-4; **20**, 66-25-1; **21**, 124-19-6; **22**, 112-31-2; **23**, 78-84-2; **24**, 96-17-3; **25**, 590-86-3; **26**, 123-73-9; **27**, 1576-87-0; **28**, 6728-26-3; **29**, 18829-55-5; **30**, 78-85-3; **31**, 67-64-1; **32**, 78-93-3; **33**, 107-87-9; **34**, 591-78-6; **35**, 110-43-0; **36**, 565-69-5; **37**, 4312-99-6; **38**, 1669-44-9; **39**, 79-20-9; **40**, 141-78-6; **41**, 123-86-4; **42**, 96-48-0; **43**, 2867-05-2; **44**, 80-56-8; **45**, 79-92-5; **46**, 127-91-3; **47**, 123-35-3; **48**, 99-83-2; **49**, 13466-78-9;

50, 99-86-5; 51, 99-87-6; 52, 555-10-2; 53, 138-86-3; 54, 99-85-4; 55, 586-62-9; 56, 109-66-0; 57, 110-54-3; 58, 142-82-5; 59, 111-65-9; 60, 111-84-2; 61, 124-18-5; 62, 1120-21-4; 63, 112-40-3; 64, 629-50-5; 65, 629-59-4; 66, 629-62-9; 67, 544-76-3; 68, 591-76-4; 69, 592-27-8; 70, 3221-61-2; 71, 871-83-0; 72, 6975-98-0; 73, 7045-71-8; 74, 1560-97-0; 75, 589-34-4; 76, 589-81-1; 77, 2216-33-3; 78, 5911-04-6; 79, 13151-34-3; 80, 1002-43-3; 81, 17312-57-1; 82, 589-53-7; 83, 2216-34-4; 84, 17301-94-9; 85, 2847-72-5; 86, 2980-69-0; 87, 6117-97-1; 88, 15869-85-9; 89, 13151-35-4; 90, 1632-70-8; 91, 17453-93-9; 92, 17302-33-9; 93, 6044-71-9; 94, 540-84-1; 95, 584-94-1; 96, 589-43-5; 97, 592-13-2; 98, 2213-23-2; 99, 4032-94-4; 100, 2801-84-5; 102, 17312-80-0; 107, 1640-89-7; 108, 108-87-2; 109, 1678-91-7; 110, 1678-92-8; 111, 1678-93-9; 112, 4292-92-6; 113, 4292-75-5; 114, 91-17-8; 115, 2958-76-1; 116, 592-41-6; 117, 25264-93-1; 119, 111-66-0; 120, 111-67-1; 121, 592-99-4; 122, 63597-41-1; 123, 71-43-2; 124, 108-88-3; 125, 100-41-4; 126, 106-42-3; 127, 108-38-3; 128, 100-42-5; 129, 95-47-6; 130, 98-82-8; 131, 103-65-1; 132, 620-14-4; 133, 622-96-8; 134, 611-14-3; 135, 108-67-8; 136, 95-63-6; 137, 526-73-8; 138, 108-68-9; 139, 100-52-7; 140, 100-47-0; 141, 140-67-0; 142, 104-46-1; 143, 534-22-5; 144, 3208-16-0; 145, 4229-91-8; 146, 4466-24-4; 147, 3777-69-3; 148, 3777-70-6; 149, 624-92-0; 150, 109-99-9; 151, 75-09-2; 152, 67-66-3; 153, 107-06-2; 154, 79-01-6.

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Stanol and Sterol Esters of Ferulic and *p*-Coumaric Acids in Wheat, Corn, Rye, and Triticale

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Sitostanyl and campestanyl ferulates, and lesser amounts of sitosteryl and campesteryl ferulates, were found in corn, wheat, rye, and triticale grains. Corn also contained minor amounts of sitostanyl and campestanyl *p*-coumarates. Identification of individual esters isolated by thin-layer and high-performance liquid chromatography (HPLC) was confirmed mainly by ultraviolet and ^1H nuclear magnetic resonance spectra of the esters and by gas chromatography–mass spectroscopy of products from transesterification using potassium carbonate in methanol. A reversed-phase (C_{18}, methanol–water) HPLC system equipped with a photodiode array detector was used to determine the esters in extracts cleaned by a base–acid procedure. Analyses of dissected tissues from corn and wheat indicated that the esters were associated mostly with inner pericarp. The ferulates alone did not stimulate *Aspergillus amstelodami* spore germination and in the presence of nutrient did not inhibit its spore germination or mycelial growth.

The stanols (saturated sterols) corresponding to cholesterol, campesterol, and sitosterol are found only rarely in tracheophytes (Nes, 1977). Among the cereal grains, stanols have been reported only in corn (Knights, 1967; Kemp and Mercer, 1968), wheat (Knights, 1967), rye (Knights, 1967), triticale (Dominguez et al., 1972), and oats (Knights and Laurie, 1967). Campestanol and sitostanol were found in nonsaponifiable extracts of the whole grains and wheat flour (MacMurray and Morrison, 1970). Concerning dissected grain fractions, relatively little information is available on sterol content and essentially none on stanol content (Barnes, 1983).

The presence of ferulic (4-hydroxy-3-methoxycinnamic), *p*-coumaric (4-hydroxycinnamic), and other hydroxycinnamic acids (mainly in the trans form) in cereal grains is well documented (Collins, 1986; Sosulski et al., 1982). Ferulic acid, the most abundant, is associated with autofluorescence of aleurone cell walls (Fulcher, 1982) and is an indicator of nonendosperm tissues in wheat milling fractions (Pussayanawin et al., 1988). Ferulic and *p*-coumaric acids are known to be esterified to cell wall polysaccharides (Hartley and Jones, 1977). Ferulic acid bound to carbohydrate in wheat bran cell walls is released with a cellulase (Smith and Hartley, 1983). Rice bran oil contains the ferulates cycloartenyl (Ohta and Shimizu, 1957), 24-methylenecycloartenyl (Ohta, 1960), an unidentified C_{28} steryl (Kato, 1961), sitosteryl (Tanaka et al., 1964) and methyl (Tanaka et al., 1971). By using a normal-phase HPLC system, Tanaka et al. (1977) found that total ferulate content in 13 rice bran oils ranged from 1.47 to 1.97%. Dihydro-β-sitosteryl ferulate was found in corn oil (Tamura et al., 1958; Nilsson et al., 1968), and dihydro-γ-sitosteryl ferulate was found in wheat oil (Tamura

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