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Effect of Nitrogen Fertilizers on Celery Volatiles

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The volatiles of two varieties of celery (*Apium graveolens* L. var. dulce and rapaceum) were isolated both by Likens-Nickerson extraction and by Soxhlet extraction. The extracts were analyzed and quantified by two-dimensional capillary gas chromatography. Celery grown with different levels of organic and/or anorganic fertilizers was investigated. The addition of high levels of mineral and/or organic nitrogen fertilizers significantly decreased the amounts of the character-impact flavor compounds. Yields and nitrate and dry matter contents of the two vegetables were also determined.

Celery (*Apium graveolens* L.) (Umbelliferae = Apiaceae) ranks with the more important vegetables cultivated in Belgium. From the wild celery two familiar garden varieties were developed, *Apium graveolens* L. var. dulce, the common blanching celery and *Apium graveolens* L. var. rapaceum, the root vegetable celery or celeriac. Celery is used for its unique texture and appetizing flavor. Gold and Wilson (1961) were the first to investigate the volatile flavor substances of the vegetable celery. Among the components described by these authors were several phthalides. The characteristic odor of celery is due to a series of phthalide derivatives (Wilson, 1970; Fehr, 1979; Gijbels et al., 1985). The separation of phthalides in the complex matrix of the flavor compounds of celery is very difficult. Two-dimensional capillary gas chromatography gives a solution to this problem and may contribute to the objective measurement of the quality

of the celery flavor (Van Wassenhove et al., 1988).

The application of fertilizers on arable crops may have adverse effects on crop production and crop quality such as lodging of cereals, decreases in starch content of potatoes and sugar content of sugar beets (Smilde, 1980), and increase of the nitrate concentration of vegetables (Vulsteke and Biston, 1978).

The aim of this study was to determine the effect of different rates of pig and cattle manure and/or mineral nitrogen application on the flavor components of celery. The results of 2 years of work are presented in this paper.

EXPERIMENTAL SECTION

Field Experiments. The field plots were situated in the middle of a field with blanching celery or celeriac. The experimental fields were laid down at random with four replications, except for celeriac in 1987 with three parallels.

Specimens of *A. graveolens* L. var. dulce cultivar Golden Spartan and *A. graveolens* L. var. rapaceum cultivar Monarch were used in this fertilization study.

Details of the different types of fertilizers used and the rates of application are given in Table I.

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Table I. Amounts of Different Fertilizers Applied on Celery for the Years 1986 and 1987

year	variety	organic manure, ton/ha	mineral N fertilizer, kg N/ha
1986	dulce	50	0
		0	0
		50	300
	rapaceum	0	300
		50	0
		0	0
1987	dulce	25	0
		50	0
		100	0
	rapaceum	0	0
		0	200
		50	0
		0	0
		50	300
		100	0

Table II. Yields of Marketable Blanching Celery

N fertilizer		1986		1987	
organic, ton/ha	mineral, kg/ha	yield, kg/ha	Duncan's test ^a	yield, kg/ha	Duncan's test ^a
0	0	75 750	a	81 409	a
50	0	79 861	a	92 545	a
0	300	75 528	a		
50	300	80 583	a		
25	0			82 636	a
100	0			84 500	a
0	200			84 682	a

^a Objects without a common character are significantly different at $P \leq 0.05$.

The organic fertilizer was applied in the form of pig manure (PM) on both crops except for 1986 where cattle manure (CM) was applied on var. dulce. The mineral fertilizer was applied in the form of calcium nitrate (15.5%) on blanching celery and ammonium nitrate (27.5%) on var. rapaceum.

Determination of Nitrate Content. The nitrate content was determined on the dried material by the potentiometric method after extraction with potassium aluminum sulfate (Cottenie et al., 1976).

Determination of Dry Matter Content. Analysis of the dry matter content was carried out in a drying oven with ventilation at 60 °C until constant weight.

Isolation of Celery Oil. In a previous paper from this laboratory (Van Wassenhove et al., 1988), two procedures (Likens-Nickerson (LN) and Soxhlet (S) extraction) for recovering the essential oil from celery were reported.

Comparison of these methods shows that the Soxhlet extraction was less efficient for the isolation of terpenes but that both isolation procedures can be used for quantitative determination of the phthalides. In the first method, 400 g of celery (var. dulce or rapaceum) was chopped and submitted for at least 10 h to a simultaneous distillation-extraction procedure using a

Likens-Nickerson extraction apparatus. A 3-mL portion of dichloromethane, containing four internal standards, was used as organic solvent. Due to the small amount of dichloromethane, no further enrichment of the volatiles was required.

In the second method, 50 g celery (var. dulce or rapaceum) was chopped and the volatile compounds were isolated from celery by Soxhlet extraction with diethyl ether containing four internal standards. The extraction time was at least 4 h. After the mixture was dried over Na_2SO_4 and concentrated under reduced pressure to a small volume (± 1 mL), 1 μL of the extract was sufficient for quantitative analysis of the phthalides.

Two-Dimensional Capillary Gas Chromatography. Gas-liquid chromatography was performed with the aid of the MUSIC (multiple switching intelligent controller) instrument (Chrompack NV) built into a Varian 3700 gas chromatograph equipped with two flame ionization detectors (FID). The celery extracts were analyzed on a wide-bore fused silica precolumn of 30 m \times 0.53 mm (i.d.) coated with OV1 (0.8- μm film thickness). The column was temperature-programmed from 20 to 220 °C (2 °C/min) with a helium carrier flow rate of 4 mL/min. On this apolar phase the phthalides elute in one group without sufficient resolution ($R_t = 73$ min). After 73 min the phthalide peaks are trapped into a CO_2 -cooled fused silica capillary for 10 min and the content of the trap was reinjected and analyzed on a narrow-bore fused silica analytical column of 25 m \times 0.25 mm (i.d.) coated with cyanopropylsilicone (0.21- μm film thickness) (CP-Sil-88, Chrompack NV). The analytical column was temperature-programmed from 160 to 220 °C (2 °C/min) with a helium pressure of 1.1 bar.

Gas Chromatography-Mass Spectrometry. The GC-MS apparatus consisted of a Hewlett-Packard 5890 gas chromatograph directly coupled to a Hewlett-Packard 5970A series mass-selective detector.

The separation of the compounds was performed on an identical column as used by two-dimensional capillary gas chromatography. The apolar OV1 column was temperature-programmed from 20 to 220 °C (2 °C/min) with a helium carrier flow of 4 mL/min.

The polar CP-Sil-88 column was temperature-programmed from 100 to 200 °C (2 °C/min) with a helium carrier flow of 1 mL/min. The ion source temperature of the mass spectrometer was 220 °C, the electron energy 70 eV, and the accelerating voltage 250 V. Mass spectral identification was based on our own and published spectra.

RESULTS AND DISCUSSION

A. *graveolens* L. Var. Dulce (Blanching Celery). The celery yields for the different fertilization rates are given in Table II. In 1986, the application of 50 tons of cattle manure/ha resulted in a yield not significantly higher than that achieved with mineral nitrogen fertilization of 300 kg of N/ha. In 1987 the highest yields were also recorded after application of 50 tons of pig manure/ha, but the differences in marketable yield between the different objects were not significant.

The nitrate and the dry matter contents of the different fertilization rates are given in Table III. The nitrate content of the stems and the leaves in 1986 was higher without manure application. The mineral nitrogen had

Table III. Nitrate and Dry Matter Contents of Blanching Celery

N fertilizer		nitrate content, ppm/fresh matl				dry matter content, %			
organic, ton/ha	mineral, kg/ha	1986		1987		1986		1987	
		stems ^a	leaves ^a	stems ^a	leaves ^a	stems ^a	leaves ^a	stems ^a	leaves ^a
0	0	420 a	896 a	1373 a	1182 a	3.1 a	8.6 a	3.9 a	10.0 a
25	0			1284 a	1475 ab			3.6 a	9.8 a
50	0	390 a	672 a	1593 a	2169 ab	3.3 a	9.2 a	3.6 a	9.6 a
100	0			1759 a	2020 ab			3.9 a	9.5 a
50	300	1324 ab	4761 b			3.2 a	8.9 a		
0	300	3065 b	4536 b			3.5 a	7.6 a		
0	200			1385 a	2441 b			3.3 a	9.7 a

^a Duncan's test: objects without common characters are significantly different at $P \leq 0.05$.

Table IV. Concentration of Terpenes and Phthalides (Micrograms per Kilogram of Fresh Material) in *A. graveolens* L. Var. Dulce as a Function of Fertilization in 1986

compound	50 tons CM ^a /ha + 0 kg N/ha		0 kg CM/ha + 0 kg N/ha		50 tons CM/ha + 300 kg N/ha		0 kg CM/ha + 300 kg N/ha	
	LN	S	LN	S	LN	S	LN	S
α -thujene	54		92		52		89	
α -pinene	1039		1240		949		1072	
camphene	118		101		146		109	
sabinene	290		237		284		272	
β -pinene	2536		2706		2828		2436	
myrcene	1097		1248		1094		1138	
<i>p</i> -cymene	821		1183		762		901	
limonene	33025		39137		32584		35932	
ocimene-x	6480		6572		5801		6215	
ocimene-y	353		378		383		280	
γ -terpinene	10236		10273		9576		9979	
terpinen-4-ol	30		38		31		30	
β -caryophyllene	434		475		301		425	
α -humulene	87		92		73		79	
β -selinene	178		276		137		173	
α -selinene	40		74		36		36	
butyl hexahydrophthalide	35	33	117	128	40	39	61	62
(<i>Z</i>)-butylidenephthalide	108	104	142	134	96	101	131	131
cnidilide	14	17	99	86	12	17	34	41
(<i>Z</i>)-ligustilide	649	604	576	603	631	649	573	525
butylphthalide	953	902	1488	1615	973	932	1116	1222
<i>trans</i> -neocnidilide	665	713	1084	1123	645	656	722	762
<i>cis</i> -neocnidilide	311	321	641	691	301	316	454	429
senkyunolide	3137	3179	3809	3922	2849	2818	2883	2752
(<i>E</i>)-ligustilide	26	18	44	38	22	22	25	26
Σ terpenes	56818		64122		55037		59166	
Σ phthalides	5898	5899	8000	8340	5569	5580	5999	5950

^a CM = cattle manure.**Table V. Concentration of Terpenes and Phthalides (Micrograms per Kilogram of Fresh Material) in *A. graveolens* L. Var. Dulce as a Function of Fertilization in 1987**

compound	25 tons PM ^a /ha + 0 kg N/ha		50 tons PM/ha + 0 kg N/ha		100 tons PM/ha + 0 kg N/ha		0 kg PM/ha + 0 kg N/ha		0 kg PM/ha + 200 kg N/ha	
	LN	S	LN	S	LN	S	LN	S	LN	S
α -thujene	66		76		78		75		71	
α -pinene	1127		1070		617		1158		1138	
camphene	95		106		103		99		110	
sabinene	21		263		289		226		236	
β -pinene	2161		1983		1464		2688		2177	
myrcene	925		817		773		1099		915	
<i>p</i> -cymene	849		845		579		972		949	
limonene	31990		27511		26616		35035		29717	
ocimene-x	6357		6029		4595		6255		6554	
ocimene-y	252		200		197		309		221	
γ -terpinene	9235		8630		6872		9432		9328	
terpinen-4-ol	29		29		36		30		32	
β -caryophyllene	556		474		452		496		473	
α -humulene	82		80		111		85		91	
β -selinene	317		215		202		267		171	
α -selinene	75		37		46		84		29	
butyl hexahydrophthalide	82	87	36	48	22	28	127	135	68	65
(<i>Z</i>)-butylidenephthalide	111	133	103	104	79	72	162	160	106	107
cnidilide	63	79	12	21	9	9	109	119	31	28
(<i>Z</i>)-ligustilide	639	664	626	608	273	289	646	687	580	599
butylphthalide	1354	1332	954	952	629	633	1592	1601	1266	1316
<i>trans</i> -neocnidilide	863	900	702	699	830	849	1017	1054	776	780
<i>cis</i> -neocnidilide	597	586	304	320	254	264	677	703	473	474
senkyunolide	3297	3353	3087	2999	3140	3170	3672	3737	2947	3033
(<i>E</i>)-ligustilide	31	31	22	25	13	15	43	45	24	26
Σ terpenes	54137		48365		43030		58310		52212	
Σ phthalides	7037	7165	5846	5776	5249	5329	8045	8241	6271	6428

^a PM = pig manure.

a very significant effect on the nitrate content of stems and leaves in 1986 but only on the leaves during 1987. The influence of organic fertilization was less important. For both years no significant differences of dry matter content were observed.

A typical gas chromatogram of a Likens-Nickerson extract from the stems of *A. graveolens* L. var. dulce analyzed on the precolumn is presented in Figure 1. The phthalides, which are the key components of this investigation, are not separated on this column. After cold

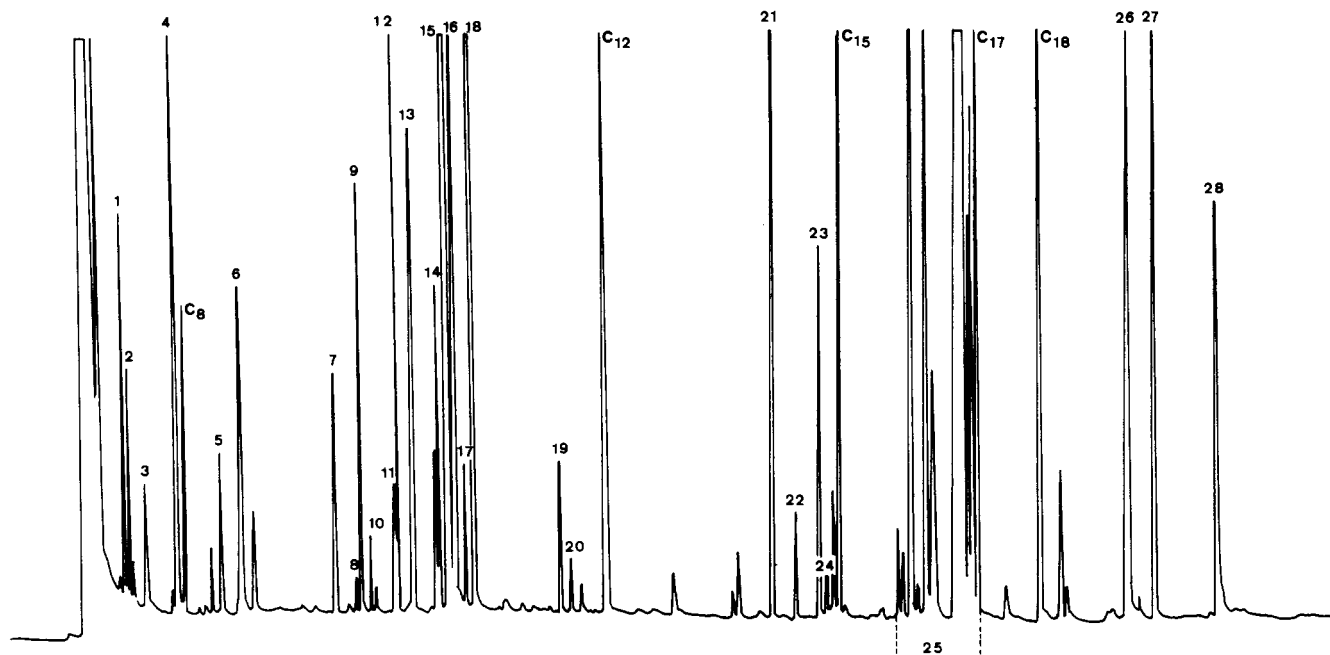


Figure 1. GC-MS analysis of celery essential oil (var. dulce) isolated by Likens-Nickerson extraction. Normal alkanes used as internal standard are indicated on the chromatogram by their carbon numbers. Key: 1 = 3-methylbutanal, 2 = 2-methylbutanal, 3 = 2-methylhexane, 4 = pyridine, C_8 = 2,5-dimethylhexane (internal standard), 5 = hexanal, 6 = furfural, 7 = 3-methyl-4-ethylhexane, 8 = α -thujene, 9 = α -pinene, 10 = camphene, 11 = sabinene, 12 = β -pinene, 13 = myrcene, 14 = *p*-cymene, 15 = limonene, 16 = oximene-x, 17 = ocimene-y, 18 = γ -terpinene, 19 = *n*-pentylcyclohexadiene, 20 = 1-terpinen-4-ol, C_{12} = dodecane (internal standard), 21 = β -caryophyllene, 22 = α -humulene, 23 = β -selinene, 24 = α -selinene, C_{15} = pentadecane (internal standard), 25 = phthalide region, C_{17} = heptadecane (internal standard), C_{18} = octadecane (internal standard), 26 = palmitic acid, 27 = unknown, 28 = linoleic acid.

Table VI. Yield of Celeriac

N fertilizn		1986		1987	
organic, ton/ha	mineral, kg/ha	yield, kg/ha	Duncan's test ^a	yield, kg/ha	Duncan's test ^a
0	0	45 060	a	43 324	a
50	0	51 331	a	39 669	a
0	300	44 657	a		
50	300	48 750	a	46 491	a
100	0			47 953	a

^a Objects without a common character are significantly different at $P \leq 0.05$.

trapping of the badly resolved phthalide region and reinjection into the analytical column (heart cut), it is possible to have a clear separation of the target components without disturbance of the other peaks (Figure 2).

Quantitative results, obtained by two-dimensional capillary gas chromatography of the Likens-Nickerson and Soxhlet extracts of *A. graveolens* L. var. dulce as a function of the fertilization levels, are shown in Tables IV and V. These data, expressed as micrograms per kilogram of fresh material, are the average values of four Likens-Nickerson extracts. Because the phthalides, and to a lesser degree the terpenes, are the aroma-determin-

ing components in celery and because the research on the importance of the individual phthalides in the total aroma is still going on, the mean sum of terpenes and phthalides is taken as a measure for the aroma concentration.

From these results it appears that unfertilized celery had the highest aroma content independent of the type of fertilization. In 1986 an organic fertilization of 50 tons/ha and 50 tons/ha plus 300 kg of mineral nitrogen/ha resulted in almost identical quantities of terpenes and phthalides. A mineral nitrogen fertilization of 300 kg of nitrogen/ha gave a higher content of aroma compounds than an organic fertilization of 50 tons/ha and 50 tons/ha plus 300 kg of nitrogen/ha.

In 1987 it was observed that increasing quantities of pig manure resulted in decreasing content of phthalides and terpenes. A fertilization of 25 tons of pig manure/ha gives almost the same result as a mineral fertilization of 200 kg of mineral nitrogen/ha.

A. graveolens L. Var. Rapaceum (Celeriac). The yields obtained by applying different fertilization rates are given in Table VI. No significant differences were observed between the different treatments. The mineral nitrogen fertilization influenced yields negatively in 1986 but positively in 1987. This was due probably to

Table VII. Nitrate and Dry Matter Content of Celeriac

N fertilizn		nitrate content, ppm/fresh matl			dry matter content, %		
organic, ton/ha	mineral, kg/ha	1986: roots ^a	1987		1986: roots ^a	1987	
			roots ^a	leaves ^a		roots ^a	leaves ^a
0	0	107a	135a	896a	9.6a	10.2a	15.7 a
50	0	609b	206a	1380a	8.3a	10.1a	15.6ab
0	300	994b			9.3a		
50	300	901b	1083b	3489b	8.8a	10.2a	12.8 b
100	0		167a	1199a		10.0a	14.8ab

^a Duncan's test: objects without a common character are significantly different at $P \leq 0.05$.

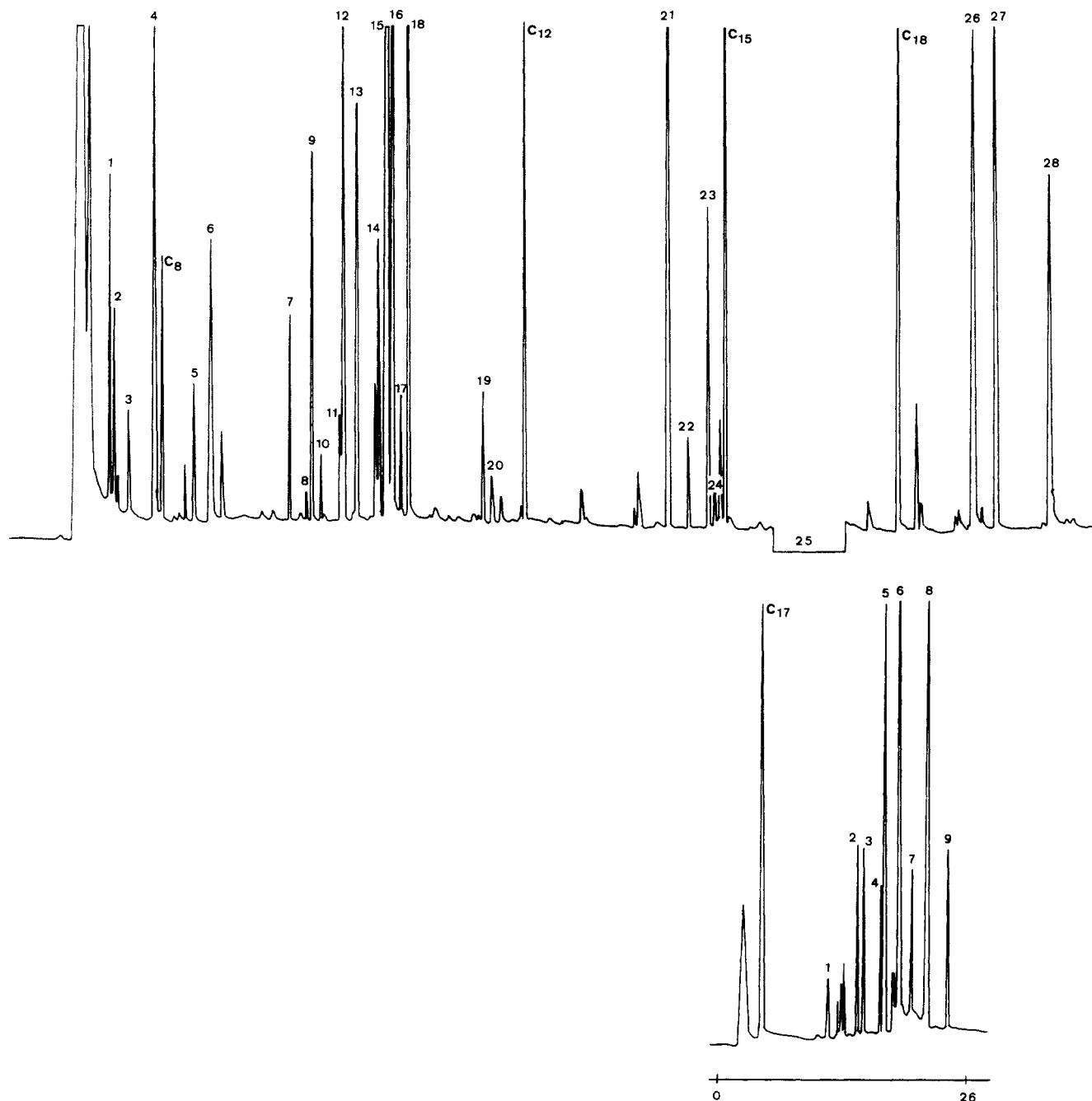


Figure 2. Phthalide cut of celery essential oil (var. dulce) obtained by Likens-Nickerson extraction. Key: 1 = butyl hexahydrophthalide, 2 = (*Z*)-butylidenephthalide, 3 = cnidilide, 4 = (*Z*)-ligustilide, 5 = butyl phthalide, 6 = *trans*-neocnidilide, 7 = *cis*-neocnidilide, 8 = senkyunolide, 9 = (*E*)-ligustilide.

the weather circumstances during the vegetation period.

The nitrate content and dry matter obtained by applying different fertilization rates are given in Table VII. The nitrate content increased with the rate of nitrogen in both forms. Pig manure caused a moderate increase of nitrates while the mineral nitrogen fertilization resulted in a significant increase of the nitrate content.

Terpenes and phthalides of the different samples of var. rapaceum were analyzed in the same way as for var. dulce. The results in Table VIII and IX show that unfertilized celery has the highest aroma content independent of the type of nitrogen fertilization. In 1986 an organic fertilization of 50 tons/ha and 50 tons/ha plus 300 kg of mineral N/ha gave almost the same quantities of terpenes and phthalides. In 1987, in comparison with unfertilized celery, a reduction in phthalide content of about 50% was obtained in celery fertilized with 50 tons of pig

manure/ha, 50 tons of pig manure/ha plus 300 kg of mineral N/ha, and 100 tons of pig manure/ha. The concentration of terpenes was reduced by 10–20% as compared to unfertilized celery.

CONCLUSION

As a general conclusion we state that two-dimensional capillary gas chromatography is a fast method allowing separation and quantification of all the volatiles in one analysis.

Unfertilized celery of both crops had a higher content of aroma compounds than fertilized celery, both manured with organic and/or mineral nitrogen fertilizers. High levels of nitrogen fertilization cause drastic reduction of the aroma-determining compounds in both varieties of celery.

Table VIII. Concentration of Terpenes and Phthalides (Micrograms per Kilogram of Fresh Material) in *A. graveolens* L. Var. *Rapaceum* as a Function of Fertilization in 1986

compound	50 tons PM ^a /ha + 0 kg N/ha		0 kg PM/ha + 0 kg N/ha		50 tons PM/ha + 300 kg N/ha		0 kg PM/ha + 300 kg N/ha	
	LN	S	LN	S	LN	S	LN	S
α -thujene	89		118		95		105	
α -pinene	557		720		316		549	
camphene	29		31		26		29	
sabinene	475		576		485		488	
β -pinene	15752		17704		14078		15156	
myrcene	6478		5060		4109		5183	
<i>p</i> -cymene	2179		2942		2893		2761	
limonene	30868		35267		30473		29875	
ocimene-x	5035		9654		7711		7025	
ocimene-y	237		280		359		274	
γ -terpinene	4120		6545		5368		5170	
terpinen-4-ol	22		24		19		22	
β -caryophyllene	335		366		297		291	
α -humulene	29		24		20		21	
β -selinene	88		119		18		80	
α -selinene	18		28		17		20	
butyl hexahydrophthalide	243	261	303	333	198	192	221	237
(<i>Z</i>)-butylidenephthalide	119	133	180	192	135	139	170	170
cnidilide	216	221	276	282	220	234	285	267
(<i>Z</i>)-ligustilide	405	403	450	450	399	410	419	432
butylphthalide	816	780	1231	1343	809	824	1047	1082
<i>trans</i> -neocnidilide	1328	1319	2174	2126	1261	1269	1644	1616
<i>cis</i> -neocnidilide	1107	1170	1909	1929	865	893	1468	1494
senkyunolide	1053	1093	1914	2063	1172	1143	1458	1489
(<i>E</i>)-ligustilide	27	31	30	39	26	24	27	24
Σ terpenes	66311		79458		66284		67049	
Σ phthalides	5314	5411	8467	7757	5085	5128	6739	6831

^a PM = pig manure.**Table IX. Concentration of Terpenes and Phthalides (Micrograms per Kilogram of Fresh Material) in *A. graveolens* L. Var. *Rapaceum* as a Function of Fertilization in 1987**

compound	50 tons PM ^a /ha + 0 kg N/ha		0 kg PM/ha + 0 kg N/ha		50 tons PM/ha + 300 kg N/ha		100 tons PM/ha + 0 kg N/ha	
	LN	S	LN	S	LN	S	LN	S
α -thujene	83		127		86		107	
α -pinene	679		681		408		533	
camphene	46		32		27		45	
sabinene	431		524		423		469	
β -pinene	14094		16205		13846		13737	
myrcene	6976		4798		4499		5928	
<i>p</i> -cymene	1876		2832		2178		2053	
limonene	28832		31404		26819		27109	
ocimene-x	4798		9241		4916		5303	
ocimene-y	241		282		252		289	
γ -terpinene	4122		6295		4308		3927	
terpinen-4-ol	24		24		17		25	
β -caryophyllene	356		349		260		330	
α -humulene	25		22		18		24	
β -selinene	76		114		93		74	
α -selinene	15		24		13		21	
butyl hexahydrophthalide	202	256	257	276	188	182	192	191
(<i>Z</i>)-butylidenephthalide	100	109	157	180	104	102	150	152
cnidilide	200	209	262	273	178	171	220	216
(<i>Z</i>)-ligustilide	387	369	444	458	394	377	367	362
butylphthalide	788	853	1235	1287	699	690	681	653
<i>trans</i> -neocnidilide	1236	1284	2029	2126	1228	1220	1253	1340
<i>cis</i> -neocnidilide	996	1015	1847	1847	1007	1051	1013	1080
senkyunolide	905	956	1910	2005	1014	1050	1037	1081
(<i>E</i>)-ligustilide	27	27	29	30	23	19	29	26
Σ terpenes	62674		72954		58163		59974	
Σ phthalides	4841	5078	8170	8482	4835	4862	4942	5101

^a PM = pig manure.

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Registry No. α -Thujene, 2867-05-2; α -pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5; β -pinene, 127-91-3; myrcene, 123-35-3; *p*-cymene, 99-87-6; limonene, 138-86-3; γ -terpinene, 99-85-4; terpinen-4-ol, 562-74-3; β -caryophyllene, 87-44-5; α -humulene, 6753-98-6; β -selinene, 17066-67-0; α -selinene, 473-13-

2; butyl hexahydrophthalide, 3553-34-2; (Z)-butylidene-phthalide, 72917-31-8; cnidilide, 3674-03-1; (Z)-ligustilide, 81944-09-4; butylphthalide, 6066-49-5; *trans*-neocnidilide, 3553-29-5; *cis*-neocnidilide, 4567-33-3; senkyunolide, 63038-10-8; (E)-ligustilide, 81944-08-3; nitrate, 14797-55-8.

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Analytical Investigation of Rio Off-Flavor in Green Coffee

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About 20% of Brazil coffee production presents the so-called Rio defect characterized by a strong off-flavor, often described as medicinal, phenolic, or iodine-like. Occasionally, this defect also occurs in coffees from other origins. An extensive investigation was carried out to identify the compound(s) responsible for the coffee samples. Volatiles were isolated from green beans by simultaneous distillation-extraction and analyzed by capillary GC, GC-sniffing, and GC-MS. 2,4,6-Trichloroanisole (TCA) was identified as the most likely key compound for the Rio off-flavor. TCA was found in all Rio samples in concentrations ranging from 1 to 100 ppb. Less than 50% of TCA present in green beans was lost during roasting. 2,4,6-Trichlorophenol (TCP), the probable precursor of TCA, was also found in most of these samples. Adding TCA to freshly brewed coffee imparted to it the same off-flavor notes as described in actual Rio coffee. The perception threshold of TCA in coffee brew was found to be 8 ppt for direct odor perception and 1-2 ppt for flavor by mouth.

Brazilian coffees are classified according to the quality of the beverage obtained after roasting and brewing. The categories range from "soft", for the best samples, to "hard" and "rio", for samples with increasing harshness (Jobin, 1982). Rio flavor is a pronounced harsh taste mainly found in coffees from the State of Rio, Minas Gerais, Zona da Mata, and the northwest of Esperito Santo. The associated olfactive note has been described alternatively as medicinal, phenolic, or iodine-like. According to our experience, however, the odor of strong Rio coffee is better depicted as a musty, cellarlike odor. This characteristic flavor, considered as an off-flavor by a majority of the consumers, develops more particularly when coffee is harvested under wet climate conditions and affects 20-25% of Brazilian coffees. Occasionally, Rio flavor has

also been found in coffees grown in other parts of the world.

Although Rio coffee has been known for generations, knowledge on the origin and the cause of this defect is still quite limited. However, it was suspected to be the consequence of some kind of fermentation. Amorim et al. (1979) related it to the action of endogenous polyphenol oxidase and peroxidase triggered by structural changes of bean cell membranes. More recently, however, microscopic and microbiological investigations undertaken in our laboratories revealed that Rio beans were heavily infested with various fungi (*Aspergilli*, *Fusaria*, *Penicillia*, *Rhizopus*, etc.) and bacteria (*Lactobacilli*, *Streptococci*), resulting in a more or less complete degradation of the cell structure (Dentan, 1988; Vanos, 1988).

We have also searched for the components responsible for the characteristic Rio off-flavor. Volatiles from healthy and Rio green coffees were isolated and analyzed by gas chromatography combined with flame ionization, mass spectrometry, and odor detection. In the

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