HortScience 25(5):556-559. 1990.

Aromatic Volatile Composition of Celery and Celeriac Cultivars

Fredy Van Wassenhove, Patrick Dirinck
Department of Organic Chemistry, Faculty of Agricultural Sciences, State University of Ghent, Coupure 653, B-9000 Ghent, Belgium

Georges Vulsteke
Provincial Research and Information Center for Agriculture and Horticulture, Iperweg 87, B-8810 Roeselare–Rumbeke, Belgium

Niceas Schamp
Department of Organic Chemistry, Faculty of Agricultural Sciences, State University of Ghent, Coupre 653, B-9000 Ghent, Belgium

Additional index words. Apiurn graveolens, phthalides, terpenes, two-dimensional gas chromatography

Abstract. **A two-dimensional capillary gas chromatographic method was developed to separate and quantify aromatic volatiles of celery in one analysis. The isolation, identification, and quantification of the volatile compounds of four cultivars of blanching celery *(Apium graveolens* L. var. dulce) and six cultivars of celeriac *(Apium graveolens* L. var. rapaceum) are described. The qualitative composition of Likens-Nickerson extracts of both cultivars is similar. The concentration of terpenes and phthalides, the key volatile components, found in various cultivars of both celery and celeriac varied over a wide range.**

The quality judgment of celery is based principally on visual or physical characteristics. The main quality criteria for celeriac are color of the root, external and internal hollowness, flesh firmness, sensitivity to black-cooking, purple spotting, presence of cracks in the peel, sensitivity to scab, and rustspot. For blanching celery most attention is given to color, visibility of the heart leaves, formation of side shoots, and susceptibility to rotten heart leaves and leafspot. Cultivars for commercial production are selected primarily for their cultural characteristics (e.g., yield, resistance to diseases) and appearance and secondarily for their flavor characteristics. The consumer and the industry are showing a growing interest in the flavor of celery. The aim of this research was to develop an improved method for analyzing aromatic volatiles of celery and celeriac that can be used to classify cultivars for their aromatic quality.

The celery plant and especially the seed have been studied in the past for their medicinal properties. For instance, celery is recommended for the treatment of gout, rheumatism, pneumonia (Thellung, 1925), and tumors (Hartwell, 1971), while the roots are mainly used as diureticum (Limpinuntana and Chaiarj, 1977). For this reason many researchers have shown interest in knowing what compounds are present in celery.

Research on sensory quality of celery has been performed by only a few authors. From the studies of Gold and Wilson (1963), it appeared that two categories of compounds, terpenes and phthalides, are important for the typical aroma of celery. Terpenes are important aroma compounds in several plants, and the principal ones in celery are described in Fig. 1. Some of the phthalide compounds have the typical odor of celery (Gijbels et al., 1985) and are present in smaller amounts than the terpenes, but their contribution to the total celery aroma is dominant. Figure 2 describes a few of the phthalides present in celery.

Four cultivars of blanching celery and six of celeriac were grown on light sandy loam fields at Roeselare-Rumbeke (Belgium) during the 1986 and 1987 seasons. We grew 'Blancato', 'Avon Pearl', 'Golden Spartan', and 'Loret' blanching celery and 'Monarch', 'Cobra', 'Snehvide', 'Tropa', 'Correcta', and 'Mentor' celeriac. The plants were all treated in the same way.

Celery oil was extracted by simultaneous steam distillation-extraction using a Likens-Nickerson apparatus (Schreier and Idstein, 1985). A 400-g sample of finely chopped celery was mixed with 2500 ml distilled water and introduced into the Likens-Nickerson apparatus. The extraction liquid was dichloromethane, to which a series of internal standards (Fig. 4) were added. Previous research has shown that a minimum extraction time of 10 hr was necessary to achieve a

Table 1. Concentration of the volatiles (µg·kg fresh material) in several cultivars of self-blanching celery (1986).

Compound	Cultivar			
	Blancato	Avon Pearl	Golden Sparten	Loret
3-methylbutanal	520	229	334	611
2-methylbutanal	224	97	180	322
2-methylhexane	129	78	129	130
pyridine	1,703	438	1,222	1,499
hexanal	141	180	149	543
furfural	431	135	562	664
3-methyl-4-ethylhexane	479	599	459	895
α-thujene	113	63	58	56
α-pinene	1,205	436	852	719
camphene	123	18	92	57
sabinene	434	161	258	233
β-pinene	3,073	5,121	1,791	3,102
myrcene	1,125	739	744	944
p.cymene	252	439	628	499
limonene	35,917	18,708	31,261	36,687
ocimene-x	2,099	2,242	7,471	5,718
ocimene-y	65	81	124	218
γ-terpinene	16,352	6,216	9,750	9,130
n.pentylcyclohexadiene	160	176	429	171
terpinene-4-ol	59	18	31	48
β-caryophyllene	1,085	394	444	612
α-humulene	103	66	73	120
β-selinene	868	370	225	274
α-selinene	86	48	38	29
butylhexahydrophthalide	108	35	30	107
Z-butylidenephthalide	240	19	105	209
cnidilide	41	*	16	30
Z-ligustilide	410	89	681	247
butylphthalide	901	310	893	834
trans-neocnidilide	2,063	630	743	765
cis-neocnidilide	173	88	280	389
senkyunolide	3,266	1,073	3,104	4,818
E-liguistilide	73	*	28	46
ϵ-terpenes =	62,959	35,120	53,840	58,446
ϵ-phthalides =	7,275	2,244	5,880	7,445

*Not detectable.

Received for publication 22 Nov. 1988. We thank the "Instituut ter bevordering van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw" for financial support. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 2. Concentration of the volatiles (µg·kg^{-1} fresh material) in several cultivars of self-blanching celery (1987).

Compound	Cultivar			
	Blancato	Avon Pearl	Golden Spartan	Loret
3-methylbutanal	423	163	354	509
2-methylbutanal	111	62	164	301
2-methylhexane	90	78	123	132
pyridine	1,832	454	1,161	1,603
hexanal	157	123	137	615
furfural	496	194	555	736
3-methyl-4-ethylhexane	509	658	421	1,019
α-thujene	114	49	57	59
α-pinene	1,368	399	1,085	806
camphene	131	17	101	57
sabinene	443	157	241	258
β-pinene	2,274	4,544	1,880	3,000
myrcene	1,091	653	765	1,049
p.cymene	333	384	846	470
limonene	37,668	18,193	28,200	35,066
ocimene-x	2,241	2,166	6,945	5,470
ocimene-y	73	87	127	227
γ-terpinene	15,578	5,819	8,561	7,570
n.pentylcyclohexadiene	161	167	437	153
terpinene-4-ol	58	23	31	41
β-caryophyllene	1,208	385	472	551
α-humulene	104	57	79	104
β-selinene	937	386	225	263
α-selinene	82	34	46	29
butylhexahydrophthalide	119	19	33	87
Z-butylidenephthalide	253	21	106	207
cnidilide	41	*	16	32
Z-ligustilide	437	62	623	234
butylphthalide	871	356	958	821
trans-neocnidilide	2,130	650	683	612
cis-neocnidilide	195	74	300	447
senkyunolide	3,727	1,086	3,032	4,583
E-ligustilide	76	*	20	47
ε-terpenes =	63,703	33,353	49,661	55,020
ε-phthalides =	7,849	2,268	5,771	7,070

*Not detected.

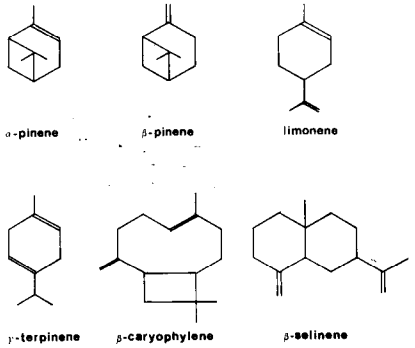

Fig. 1. The principal terpenes in celery.

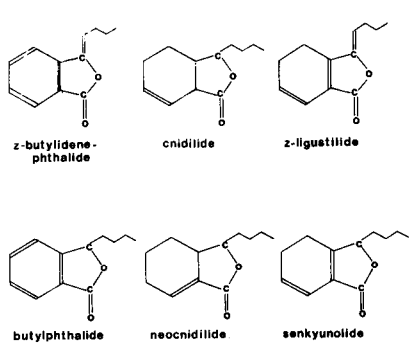

Fig. 2. A few phthalides present in celery.

quantitative aroma isolation (Van Wassenhove et al., 1988).

High-resolution multi-dimensional gas chromatography was performed with the aid of the Multiple Switching Intelligent Controller (MUSIC) (Chrompack Ltd., Middelburg, the Netherlands) built into a Varian 3700 gas chromatography equipped with two flame ionization detectors (FID) and coupled to a Nelson Analytical 3000 Chromatography Data System. The celery extracts were analyzed on a wide-bore fused silica pre-column of 30 m × 0.53 mm id. coated with OV1 (0.8-µm film thickness) (Chrompack NV). The column was programmed from 20 to 220C (2C/min) with a helium carrier flow of 4 ml·min^{-1}. After 73 rein, the unresolved phthalide peaks were trapped into a CO_2-cooled fused silica capillary during a 10-min span and the contents of the trap were reinfected and analyzed on a narrow-bore fused silica analytical column of 25 m × 0.25 mm id. coated with Cyanopropylsilicone (0.21 - µm film thickness) (CP-SIL-88, Chrompack Ltd.). The analytical column was programmed from 160 to 220C (2C/min) with a helium flow of 1 ml·min^{-1}. The injection port of the MUSIC system was at 220C and detection at 240C.

Quantitative data of the various volatiles were obtained using the Nelson Analytical 2600 Chromatography software on an IBM PC/AT computer. A user program was written for direct calculation of the amount of volatiles expressed as µg·kg^{-1} of fresh celery or celeriac. These quantitative data were corrected for evaporation of the lower boiling volatiles during extraction by calculating internal standards with the same retention indices as the unknowns through interpolation between added hydrocarbons.

For identification of the individual phthalides, the unseparated group of phthalides was isolated with a Varian 1420 gas chromatography equipped with a microkatharometer. The isolation was done on a packed glass column, 2 m x 2 mm id. filled with 10% Carbowax 20M on Chromosorb W (60-80 mesh). The column was programmed from 130 to 220C (6C/min) with a hydrogen gas flow rate of 20 ml·min^{-1}. The injection port was at 220C and the detector at 240C. The phthalide fraction to be isolated was trapped into an ice-cooled glass tube.

After isolation, the phthalides were analyzed with a Hewlett-Packard 5890 gas chromatography equipped with a fused silica column of 25 m × 0.25 mm id. coated with cyanopropylsilicone (film thickness 0.21 µm), identical to the analytical column used by multi-dimensional capillary gas chromatography, and coupled to a Hewlett-Packard 5970A series mass selective detector. The column was programmed from 100 to 200C (2C/min) with a helium carrier flow of 1 ml·min^{-1}. The injection port was at 220C and the detector at 240C.

For identification of the terpenes the same GC-MS unit was used, but the separation was done on a wide-bore fused silica column of 30 m × 0.53 mm id. coated with OV1 (0.8-µm film thickness) that was identical to

Table 3. Concentration of the volatiles (μg·kg⁻¹ fresh material) in several cultivars of celeriac (1986).

Compound	Cultivar				
	Monarch	Tropa	Cobra	Correcta	Snehvide
3-methylbutanal	1,486	3,380	1,588	1,626	4,038
2-methylbutanal	700	1,474	772	857	1,745
2-methylhexane	571	1,056	347	450	551
pyridine	7,623	12,288	7,619	8,907	9,144
hexanal	224	10,770	1,071	304	256
furfural	2,132	2,437	3,888	4,712	4,543
3-methyl-4-ethylhexane	9,105	12,335	9,087	8,140	7,232
α-thujene	94	356	226	68	78
α-pinene	467	241	240	392	305
camphene	21	35	23	24	30
sabinene	555	867	347	512	475
β-pinene	13,951	7,677	7,103	8,393	8,116
myrcene	4,298	6,641	2,054	2,777	2,722
p.cymene	1,900	2,086	1,057	1,736	1,719
limonene	29,365	27,296	13,775	21,035	21,040
ocimene-x	4,883	3,411	2,568	4,769	4,394
ocimene-y	180	318	35	69	69
γ-trerpinene	5,031	1,341	2,295	7,496	7,971
n.pentylcyclohexadiene	394	917	276	362	421
terpinene-4-ol	18	24	28	22	24
β-caryophyllene	385	516	265	466	536
β-humulene	16	24	20	14	21
β-selinene	91	396	430	1,745	1,602
α-selinene	21	41	30	33	41
butylhexahydrophthalide	200	37	20	49	42
Z-butylidenephthalide	122	637	56	70	67
cnidilide	221	294	70	78	74
Z-ligustilide	463	330	222	177	175
butylphthalide	1,028	1,616	556	432	404
trans-neocnidilide	1,446	3,942	370	633	612
cis-neocnidilide	1,164	4,118	379	529	544
senkyunolide	1,230	431	470	355	370
E-ligustilide	22	18	16	28	32
ϵ-terpenes	61,276	51,270	30,496	49,551	46,643
ϵ-phthalides	4,786	11,423	2,159	2,351	2,320

Table 4. Concentration of the volatiles (μg.·kg⁻¹ fresh material) in several cultivars of celeriac (1987).

Compound	Cultivar				
	Monarch	Tropa	Cobra	Snehvide	Mentor
3-methylbutanal	1,159	3,367	1,599	4,580	3,110
2-methylbutanal	442	1,663	808	1,780	1,538
2-methylhexane	662	1,145	354	616	1,852
pyridine	6,893	13,131	8,949	10,823	12,346
hexanal	186	12,349	1,198	209	13,818
furfural	2,361	2,606	4,205	5,034	2,826
3-methyl-4-ethylhexane	8,459	12,864	9,920	7,865	8,958
α-thujene	90	390	267	79	220
α-pinene	434	250	248	323	223
camphene	29	20	27	26	26
sabinene	465	869	447	477	666
β-pinene	14,808	7,453	7,471	7,988	6,161
myrcene	4,216	6,431	2,542	2,725	3,493
p.cymene	1,808	2,201	1,116	1,674	1,646
limonene	29,636	24,540	14,901	20,960	12,804
ocimene-x	4,501	3,255	2,685	4,586	1,049
ocimene-y	189	260	41	71	398
γ-terpinene	5,376	1,188	2,327	7,819	962
n.pentylcyclohexadiene	350	819	325	345	458
terpinene-4-ol	23	21	27	19	22
β-caryophyllene	393	572	307	473	260
α-humulene	20	12	15	14	23
β-selinene	104	386	486	1,784	195
α-selinene	20	41	31	34	25
butylhexahydrophthalide	161	48	20	39	116
Z-butylidenephthalide	95	731	69	60	159
cnidilide	190	333	47	64	189
Z-ligustilide	375	315	240	156	318
butylphthalide	844	1,795	542	383	772
trans-neocnidilide	1,280	3,911	401	568	589
cis-neocnidilide	883	4,023	433	473	766
senkyunolide	1,183	446	448	339	360
E-ligustilide	25	12	18	20	15
ϵ-terpenes	62,112	47,889	32,938	49,052	28,173
ϵ-phthalides	5,036	11,614	2,175	2,102	3,284

the pre-column used by multi-dimensional capillary gas chromatography. The column was programmed from 20 to 220C (2C/min) with a helium carrier flow of 4 ml·min⁻¹.

A typical gas chromatogram of a Likens-Nickerson extract from self-blanching celery analyzed on the pre-column is presented in Fig. 3. The phthalides, which are the key components of this investigation, are not separated on this column. After cold trapping of the unresolved phthalide group and reinfection in the analytical column (heart cut), a clear separation of the target compounds without disturbance of the other peaks was obtained (Fig. 4).

Analysis of Likens-Nickerson extracts of four self-blanching cultivars by GC-MS indicated the presence of 33 compounds that were identical in all four. These compounds, quantified with the aid of internal standards, are given in Tables 1 and 2. The concentration of the compounds expressed as μg·kg⁻¹ fresh celery are the averages of the results of four separate Likens-Nickerson extractions. Because the phthalides, and to a lesser degree the terpenes, are the aroma determining components in celery, and because the research on the importance of the individual phthalides in the total aroma is still ongoing, the sum of the concentrations of terpenes and phthalides is taken as a measure of the aroma concentration. 'Blancato' had the highest concentration, 'Avon Pearl' the lowest, and those of 'Golden Spartan' and 'Loret' were in between.

Concentrations of individual terpenes and phthalides inceleriac cultivars are given in the Tab1es 3 and 4. 'Tropa' had the highest content in phthalides, 'Monarch' contained about half as much, while 'Cobra', 'Snehvide', 'Corrects', and 'Mentor' contained about one-fourth as many phthalides. The terpene concentrations of 'Monarch', 'Tropa', 'Corrects', and 'Snehvide' were higher than in 'Cobra' and 'Mentor'.

In summary, the two-dimensional capillary gas chromatography method described here: 1) is a convenient procedure for determination of terpenes and phthalides in celery and celeriac; 2) detects important differences in concentration of terpenes and phthalides among cultivars of self-blanching celery and celeriac; and 3) makes impossible to classify cultivars of self-blanching celery and celeriac according to volatile component.

Literature Cited

Gijbels, M. J. M., F.C. Fischer, J.J.C. Scheffer, and A. Baerheim Svendson. 1985. Phthalides in roots of *Apium graveolens*, *A. graveolens* var. rapaceum, *Bifora testiculata* and *Petroselinum crispum* var. tuberosum. Fitoterapia 56:17-23.

Gold, H.J. and C.W. Wilson. 1963. The volatile flavor substances of celery. J. Food Sci. 28, 484-488.

Hartwell, J.L. 1970. Plants used against cancer. Lloydia 33(3):288-392 (Survey.)

Limpinuntana, C. and P. Chaiarj. 1977. Hypotensive effect of the extract of *Apium graveolens* L. Varasam Paesachasarthara 4(1): 10-13.

Schreier, P. and H. Idstein. 1985. Advances in the Instrumental Analysis of Food Flavours. Z.

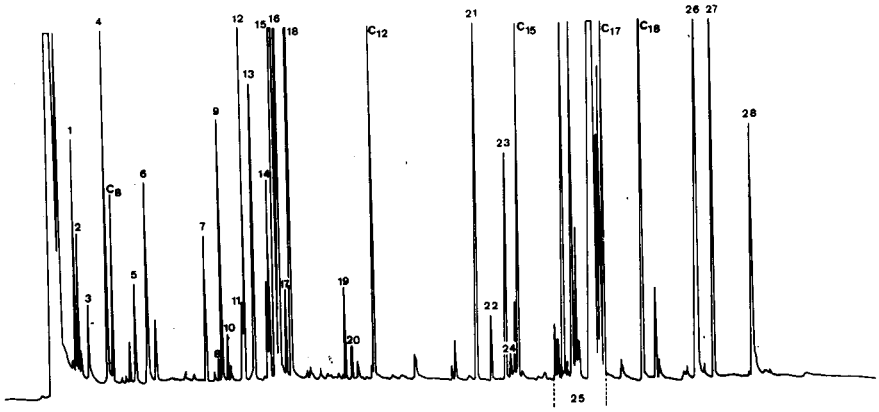

Fig. 3. GC-MS analysis of celery essential oil isolated by Likens-Nickerson extraction of self-blanching 'Golden Spartan' celery. Normal alkanes, used as internal standard, are indicated on the chromatogram by their carbon numbers. 1 = 3-methylbutanal, 2 = 2-methylbutanal, 3 = 2-methylhexane, 4 = pyridine, 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-terpinene-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.

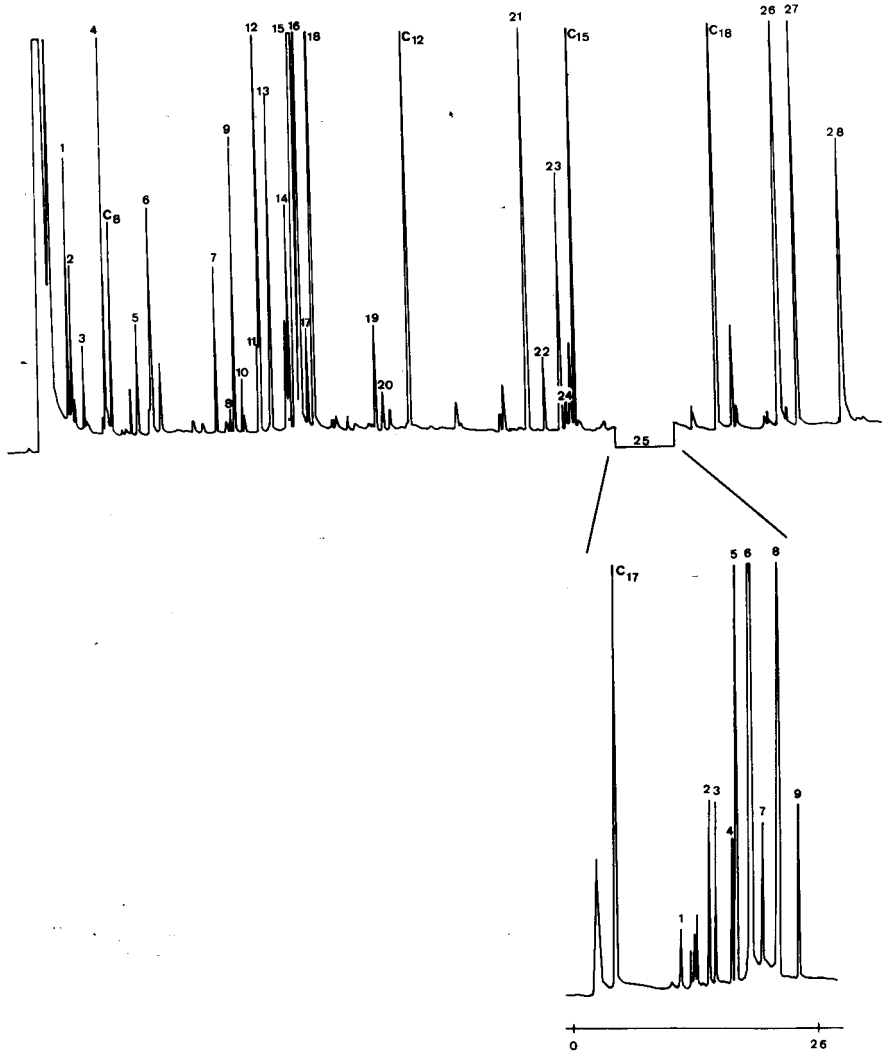

Fig. 4. Chromatogram of "phthalide cut" of celery oil obtained by Likens–Nickerson extraction of self-blanching celery ('Golden Spartan'). 1 = butylhexahydrophthalide, 2 = Z-butylidenephthalide, 3 = cnidilide, 4 = Z-ligustilide, 5 = butylphthalide, 6 = trans-neocnidilide, 7 = *cis* -neocnidilide, 8 = senkyunolide.

Lebensm. Unters. Forsch 180:1-14.
Thellurg, A. 1925. Illustrierte Flora von Mitteleuropa, Band V, Teil 2. J.F. Lehmanns Verlag, München. [Reprint published by Verlag Paul Parey, Berlin, 1975.]
Van Wassenhove, F., P. Dirinck, and N. Schamp. 1988. Analysis of the key components of celery by two dimensional capillary gas chromatography. Bioflavour '87. Walter de Gruyter & Co., Berlin, p. 137-144.