Distribution of Volatile Sulfur Compounds in an Interspecific Hybrid between Onion (*Allium cepa* L.) and Leek (*Allium porrum* L.)

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Volatile sulfur compounds of an interspecific hybrid between *Allium cepa* and *Allium porrum* were analyzed by gas chromatography (GC) and GC/mass spectrometry prior to isolation of the individual oils by a simultaneous distillation/extraxtion (SDE) method. Furthermore, the aroma profiles of various onion and leek cultivars were investigated. Major volatile components detected in onion were 2-methyl-2-pentenal, (*E*)-methyl 1-propenyl disulfide, methyl propyl trisulfide, and propanethiol, whereas dipropyl trisulfide, dipropyl disulfide, and (*E*)-propenyl propyl disulfide predominated in leek oils. According to the higher amount of leek chromosomes in the cell nucleus, the percentages of the measured sulfur volatiles in the hybrid material correspond more to the leek than to the onion flavor profile. Discrimination analysis was successfully applied to classify the three predefined *Allium* groups. It has been found that at minimum five of the most important aroma volatiles were necessary to receive sufficient differentiated clusters without any overlapping areas. This graphical display demonstrates very clearly the influence of the genetic background of the parent varieties on the sulfur volatile composition in the *Allium* hybrid.

Keywords: *Interspecific allium hybrid; onion; leek; GC analysis; volatile aroma compounds; discrimination analysis*

INTRODUCTION

Allium vegetables, such as onion, garlic, leek, and chives, are traditionally known for their fresh flavors but also for their antibacterial and fungicidal properties. The characteristic flavor of *Allium* species is due to the volatile oil, which consists mainly of sulfur compounds. When the cells of the *Allium* plants are ruptured, a reaction between the enzymes (lyase, alliinase) and the individual flavor precursors takes place, resulting in the formation of an individual flavor profile (Tressl et al., 1975; Whitaker, 1976; Boelens et al., 1980; Kuo et al., 1990; Lancaster and Boland, 1990; Stephani and Baltes, 1992) as presented generally in Figure 1. It has been found that these sulfur volatiles play also a defensive role against animal pests and microorganisms (Block, 1985; Carson, 1987).

On the basis of the results from case-control studies performed in different parts of the world, *Allium* vegetable consumption has been found to be associated with a reduction of stomach cancer risk in humans (You et al., 1989; Hansson et al., 1993; Graham et al., 1990). It has been proved that some of the organosulfur compounds present in raw and cooked *Allium* vegetable act as antimutagens in in-vitro models and even as anticarcinogens in in-vivo test systems (Dorant et al., 1993).

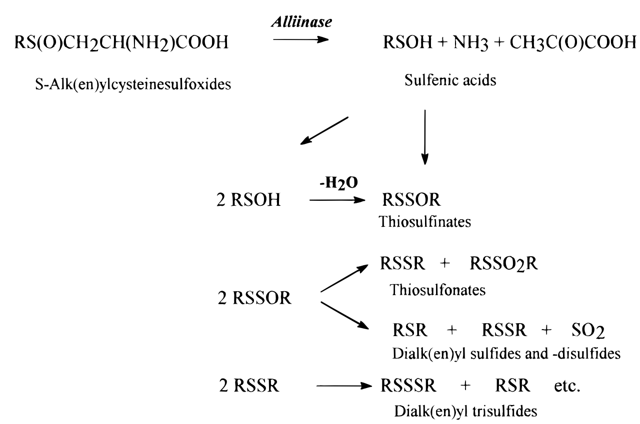

Figure 1. General scheme for the formation of alk(en)yl sulfur components in *Allium* plants.

In the past the composition and formation of volatiles in onion, garlic, leek, and also in ornamental *Allium* species have been extensively studied (Brodnitz et al., 1969; Freeman, 1975; Yu et al., 1989). It has been found that the characteristic aroma impressions recognized in the individual *Allium* species or varieties are predominantly attributed to a different pattern of nonvolatile alk(en)ylcysteinsulfoxides in the plant. Depending on the species the alk(en)yl groups are mainly a combination of propyl, 1-propenyl, allyl, and methyl substituents. So far as *Allium* species have been analyzed, they can be divided in the following three groups (Freeman and Whenham, 1975): high proportions of propyl/propenyl cysteinsulfoxide (e.g., onions), high proportions of allyl cysteinsulfoxide (e.g., garlic), and high methyl cysteinsulfoxide portions (e.g., ornamental *Allium* spe-

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cies). According to these results it must be assumed that the formation of the flavor precursors is genetically controlled. Up to now no single *Allium* species has been described in the literature to produce different sulfur amino acids as the major flavor precursors.

Recently, the aroma composition of an interspecific hybrid between *Allium fistolosum* and *Allium tuberosum*, formed by embryo culture, has been investigated (Kobayashi et al., 1997). According to the published results there is only little difference between the compositions of *A. tuberosum* and the hybrid; however, much more of the allyl-substituted sulfur components occur in the hybrid plant, which explains its stronger garlic-like aroma.

The aim of the study reported here is to characterize an interspecific hybrid of *Allium cepa* and *Allium porrum* with special respect to its very distinctive flavor profile, influenced by the genetic background of the parent plants.

MATERIALS AND METHODS

Cultivation and Sample Preparation of *Allium* Plants. Onion varieties (Hystar, Summit, Stuttgarter Riesen, Vitesso, Romeo, Bristol, Macho, Boston, Trefford) and leek cultivars (Upton, Erik, Lanzelot, Parton, Porbella, Gloria, Nepal, Profina) were cultivated in the experimental garden of the Landesversuchsanstalt Gartenbau at Ditfurt (Germany). The leek variety Pollux was grown under comparable growing conditions in the experimental garden of the Federal Centre for Breeding Research on Cultivated Plants in Quedlinburg (Germany). The interspecific hybrid 99/1 was obtained from the cross of onion Summit with leek Pollux (Peterka et al., 1997).

Normal farming practice was employed. Onion bulbs and leek plants were harvested from July to August. Approximately five to eight randomly taken ripe onions formed one sample. In the case of leek cultivars and hybrid material, the edible parts of approximately four to five plants, freshly homogenized prior to analysis, were taken for one investigation. Two identical replica samples of each variety were analyzed under the same conditions.

The washed *Allium* samples were cut into pieces and macerated carefully. A sample amount of ~120−160 g was divided in two equivalent portions, each of them transferred into a 250 mL round-bottom flask and blended with 100 mL of water. The volatile constituents were extracted for 3 h at boiling temperature in a Likens−Nickerson water distillation/solvent extraction (SDE) apparatus. *n*-Pentane (15 mL, purity = 99.0%) was used as extracting solvent. One milliliter of a 2.5×10^{-3} M internal standard solution (6-methyl-5-hepten-2-one in *n*-pentane) was added to the received aroma extract.

The following authentic reference samples were obtained from commercial suppliers (Oxford Chemicals, Harlepool, U.K., and Dragoco Gerberding & Co. AG, Holzminden, Germany): allyl propyl disulfide, allyl propyl trisulfide, 2,5-dimethylthiophene, dimethyl trisulfide, dipropyl disulfide, dipropyl trisulfide, methyl propyl disulfide, and methyl propyl trisulfide.

Gas Chromatographic and Mass Spectrometric (GC/MS) Analysis. A Hewlett-Packard chromatograph model HP 5890 Series II equipped with an FID and a fused silica capillary column [HP INNOWAX with a 0.5 μm bonded PEG phase, 60 m × 0.25 mm (i.d.)] was used to analyze the isolated volatiles. The operating conditions were as follows: injector temperature, 250 °C; detector temperature, 280 °C; H_2 flow rate, 1 mL/min; oven temperature, 80−220 °C raised at 4 °C/min; injection volume, 1 μL; split ratio, 1:40. Linear retention indices were calculated against C_7−C_{20} *n*-paraffins as references according to the method of van den Dool and Kratz (1963).

Mass spectral analyses were carried out in a Hewlett-Packard gas chromatograph (HP 5890 Series II) directly coupled to a Hewlett-Packard MSD, model HP 5972. Operational parameters were as follows: carrier gas, He, 1 mL/min; ionization voltage, 70 eV; source temperature, 195 °C; scan region, 27−300 *m*/*z*.

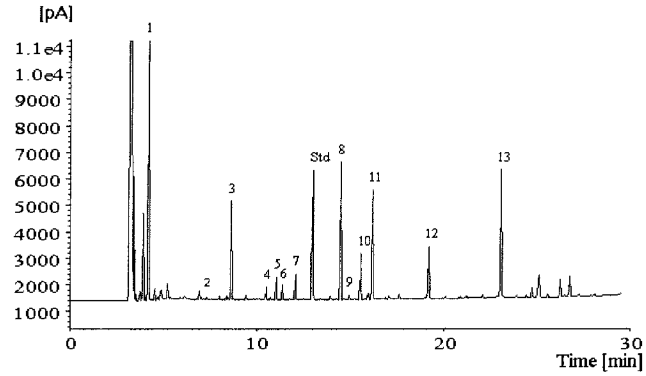

Figure 2. Gas chromatogram of the oil obtained from an interspecific *Allium* hybrid (*A. cepa* × *A. porrum*) by SDE procedure. Peak identification is according to Table 1. Internal standard (Std): 6-methyl-5-hepten-2-one.

The GC separation conditions applied were the same as described above.

Statistics. Discrimation analysis of the collected analytical data was carried out using the statistical software program SYSTAT 7.0 of SPSS Inc., Chicago, IL.

RESULTS AND DISCUSSION

The oils received from the *Allium* hybrid and from different onion and leek varieties have been analyzed to identify the most important aroma components as well as to describe the hybrid species on the basis of the characteristic profile of volatile sulfur substances. The individual oil constituents are identified by comparing their mass spectra and retention indices with those of reference substances.

Representing the high resolution of the separated components, Figure 2 shows a typical gas chromatogram of an *Allium* hybrid oil received by the SDE procedure. It has to be mentioned here that the sample preparation process was performed very accurately and reproducibly due to the fact that the rates of formation and half-lives of the volatile di-, tri-, and tetrasulfides vary widely (Kallio et al., 1994). The concentrations of the individual sulfur volatiles identified in the studied *Allium* oils are presented in Table 1. As can be seen, the leek and onion varieties, cultivated under the same growing conditions, do not show any strong differences in their flavor profiles within each species.

The composition of the investigated leek oils corresponds very well with the data already indicated in earlier studies (Schreyen et al., 1976; Stephani and Baltes, 1992). Main components in the leek oil are dipropyl trisulfide, dipropyl disulfide, propanethiol, and (*E*)-1-propenyl propyl disulfide. In a similar way the composition of onion oils received from different varieties shows no significant differences concerning the flavor profile, so the variability of the volatile sulfur components is also here comparatively small within the different cultivars.

Only the variety Stuttgarter Riesen indicates relatively high contents of 2-methyl-2-pentenal, whereas the variety Hystar contains clearly lower contents of this flavor material. As main constituents in the onion oil, 2-methyl-2-pentenal, (*E*)-methyl 1-propenyl disulfide, methyl propyl trisulfide, and propanethiol are detected.

Concerning the GC profile the aroma extract of the *Allium* hybrid presents more similarity with leek than

Table 1. Composition[a] of Volatile Compounds Detected in Different Onion and Leek Varieties as well as an Interspecific Hybrid of *A. cepa* × *A. porrum*

	1,[b] 1-propanethiol, RI[c] = 855	2, dimethyl disulfide, RI = 1110	3, 2-methyl-2-pentenal, RI = 1190	4, methyl propyl disulfide, RI = 1263	5, dimethylthiophene, RI = 1285	6, (*Z*)-methyl 1-propenyl disulfide, RI = 1298	7, (*E*)-methyl 1-propenyl disulfide, RI = 1322	8, dipropyl disulfide, RI = 1413	9, dimethyl trisulfide, RI = 1430	10, (*Z*)-1-propenyl propyl disulfide, RI = 1450	11, (*E*)-1-propenyl propyl disulfide, RI = 1473	12, methyl propyl trisulfide, RI = 1576	13, dipropyl trisulfide, RI = 1713
onion cv.													
Hystar	174	63	424	42	114	179	245	36	245	58	93	263	62
Stuttgarter Riesen	243	121	642	60	173	298	419	26	375	93	107	378	72
Vitesso	168	31	510	27	136	133	178	35	124	59	84	193	62
Romeo	161	52	420	29	122	196	262	5	217	54	66	209	40
Bristol	159	33	520	24	135	156	206	21	158	54	75	189	46
Macho	104	16	401	10	118	107	135	0	82	31	37	76	8
Boston	217	16	589	18	167	89	118	29	47	56	83	117	50
Trefford	258	35	490	36	136	127	172	42	112	74	104	214	86
Summit	246	25	509	31	131	214	153	46	99	72	107	210	96
hybrid													
Summit × Pollux	502	9	326	52	93	80	118	348	32	187	383	301	555
leek cv.													
Pollux	861	24	336	90	148	90	154	545	24	377	682	428	1248
Upton	932	76	378	256	106	131	230	968	112	289	558	851	1230
Erik	1099	52	412	237	114	160	297	954	89	443	957	993	2044
Lanzelot	1083	0	410	45	126	30	49	823	0	365	722	182	1460
Parton	851	30	301	170	84	130	223	918	72	360	870	813	1672
Porbella	663	80	392	269	75	229	453	1016	252	330	884	1303	1463
Gloria	602	36	135	266	41	90	234	1298	102	261	1056	902	1609
Nepal	1028	57	358	241	97	178	339	991	107	360	845	900	1389
Profina	885	14	178	161	47	60	143	1283	27	308	1027	519	1843

[a] Amounts calculated as micrograms per 100 g of fresh material by an internal standard method, average of two samples. [b] Peak number in Figure 2. [c] Retention index.

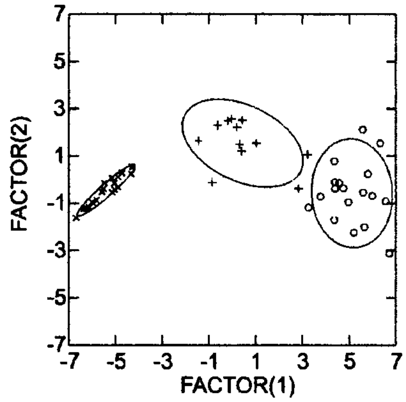

Figure 3. DA scores of leek (○) and onion (×) varieties as well as samples of the interspecific *Allium* hybrid (+) based on the following volatile aroma components: dipropyl disulfide, (*Z*)-1-propenyl propyl disulfide, (*E*)-1-propenyl propyl disulfide, dipropyl trisulfide, propanethiol, and 2-methyl-2-pentenal.

with onion varieties. Major sulfur components of the oil are dipropyl trisulfide, propanethiol, (*E*)-1-propenyl propyl disulfide, and dipropyl disulfide. In accordance with the composition of leek the percentage of 2-methyl-2-pentenal is comparatively low. This flavor substance, occurring in onion in relatively large amounts, is formed from the amino acid 1-propenyl-L-cysteine sulfoxide by the enzymatic reaction with alliinase (Freeman and Whenham, 1975). Thus, it can be assumed that the different genetic backgrounds of leek and onion are responsible for the different biochemical production yields of the individual alk(en)yl cysteine sulfoxides in the investigated *Allium* plants.

Discrimination analysis (DA) involving all 13 detected volatile compounds analyzed in this study gives some visual evidence of the ability to differ between onion and leek cultivars and the hybrid material as clearly separated factor groups. A differentiation of these three groups can be also achieved when only six of the most important aroma components are involved in the statistical calculations (Figures 3 and 4). The graphical display of the resulting scores using factors 1 and 2 shows that all *Allium* hybrid samples build up a single cluster without any overlapping areas with the clusters of the parents varieties.

It should be noted here that all points in the DA plot correspond to physically different samples, and therefore these samples cover a wide range in terms of analytical reproducibility, variety, time of harvesting, and cultivation influences. However, these factors seem to introduce a variability into the data set, which is not as important as the variability from the different *Allium* species and the hybrid material, respectively. Regarding the percentages of the individual sulfur volatiles, it can be seen very easily that the hybrid material corresponds more to the leek than to the onion profile. As already mentioned above, this is attributed to the fact that the genetic information coming from the leek supplies a larger contribution for the biogenesis of the analyzed volatile flavor materials or precursors than the onion genome does. The hybrid plants are allotriploid, which means they possess $3x = 24$ chromosomes in the cell nucleus. From the allotetraploid leek here come 16 chromosomes, whereas the diploid onion plant contributes to the hybrid only 8 chromosomes.

The results presented in this study clearly demonstrate the potential for DA to facilitate the differentiation between similar *Allium* species or genotypes on the basis of the most characteristic volatile sulfur components. It can be assumed that interspecific *Allium* hybrids are a useful approach to increase the genetic variability of *Allium* flavor characteristics for further improvement in breeding.

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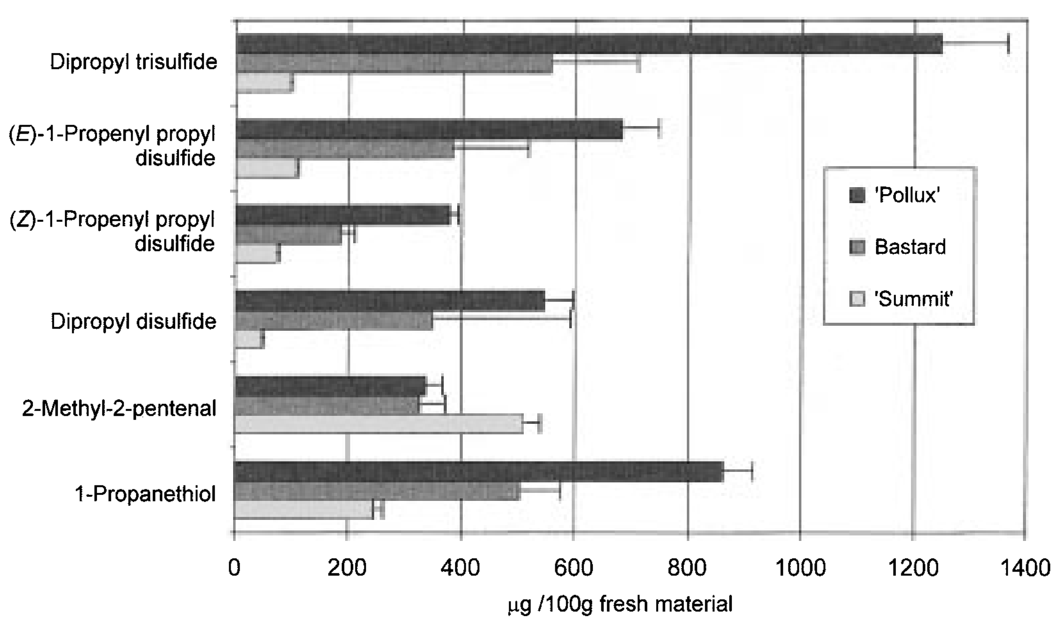

Figure 4. Contents [micrograms per 100 g of fresh material] with confidence limits of most important aroma compounds in the *Allium* hybrid and the relating parent varieties Summit and Pollux.

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Registry No. Supplied by the Author: 1-propanethiol, 75-33-2; dimethyl disulfide, 624-92-0; 2-methyl-2-pentenal, 623-36-9; methyl propyl disulfide, 2179-60-4; 2,5-dimethylthiophene, 638-02-8; (*Z*)-methyl 1-propenyl disulfide, 23838-19-9; (*E*)-methyl 1-propenyl disulfide, 23838-19-9; dipropyl disulfide, 629-19-6; dimethyl disulfide, 624-92-0; dimethyl trisulfide, 3658-80-8; (*Z*)-1-propenyl propyl disulfide, 23838-20-2; (*E*)-1-propenyl propyl disulfide, 23838-21-3; methyl propyl trisulfide, 17619-36-2; dipropyl trisulfide, 6028-61-1; 6-methyl-5-hepten-2-one, 110-93-0.

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