JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Some *Allium* Hybrids by Aroma Precursors, Aroma Profiles, and Alliinase Activity

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Various *Allium* hybrids, obtained by the crossbreeding of *Allium cepa* (onion) as the mother plant and six taxonomically distant wild species obtained by embryo rescue, were investigated with special respect to their individual profiles of cysteine sulfoxides as well as enzymically and nonenzymically formed aroma substances. Alliinase (EC 4.4.1.4) catalyzes the conversion of odorless (+)-*S*-alk(en)yl-L-cysteine sulfoxides into volatile thiosulfinates. These thiosulfinates were converted to a variety of sulfides by steam distillation. SPME-gas chromatography (GC) and high-performance liquid chromatography (HPLC) used for the analysis of aroma components and their precursors permitted a high sample throughput, so that numerous gene bank accessions and *Allium* breeding materials were analyzed within a comparatively short time. Cysteine sulfoxides as well as alliinase activity were found in all investigated samples at different levels, but (+)-*S*-methyl-L-cysteine sulfoxide (methiin) was the most abundant sulfoxide present. (+)-*S*-(*trans*-1-Propenyl)-L-cysteine sulfoxide (isoalliin) is typical for onion and was found in all investigated hybrids. The pattern of the other cysteine sulfoxides depended strongly on the parent plants used. The profile of aroma components corresponded with the related pattern of aroma precursors (cysteine sulfoxides). Successful hybridization was proven by randomly amplified polymorphic DNA analysis. Together with these established marker techniques, HPLC and SPME-GC analysis provide support to breeding projects designed to improve the health and aroma properties of *Allium* hybrids.

KEYWORDS: ***Allium*** **hybrids; cysteine sulfoxides; alliinase; sulfides; aroma profiles**

INTRODUCTION

Since ancient times, garlic (*Allium sativum*), onion (*Allium cepa*), and related species of the leek family (*Alliaceae*) have been used as foods, spices, and herbal remedies in many parts of the world. Volatile sulfur-containing flavor compounds are responsible for the characteristic smell and taste of members of the *Alliaceae*. These compounds are alk(en)yl-thiosulfinates formed by the action of alliinase (EC 4.4.1.4) on odorless and nonvolatile cysteine-derived substances (*S*-alk(en)yl-L-cysteine sulfoxides; **Figure 1**), when these reactants are released by plant material disruption (*1*−*3*). Intact garlic bulbs contain mainly (+)-*S*-(2-propenyl)-L-cysteine sulfoxide (alliin) and to lesser extent (+)-*S*-methyl-L-cysteine sulfoxide (methiin), whereas (+)-*S*-(1-propenyl)-L-cysteine sulfoxide (isoalliin) and (+)-*S*-propyl-L-cysteine sulfoxide (propiin) typify onion and leek, respectively (*1*, *4*). If aroma components of *Allium* species are produced by steam distillation, the initially formed thiosulfinates are rapidly converted into their corresponding alk(en)yl(poly)sulfides (**Figure 2**).

Aroma components of *Allium* species are pharmacologically active substances that exhibit antibiotic and lipid-lowering effects, inhibition of thrombocyte aggregation, and antioxidant and antitumor activities. Additionally, epidemiological studies have shown that regular consumption of onion and garlic may prevent stomach cancer (*2*, *5*−*7*). Sulfur-containing substances with one or more allyl residues (e.g., allicin, diallylsulfide, and diallyltrisulfide) have tumor preventative effects (*8*, *9*).

Furthermore, *Allium* oils produced from onion, garlic, or leek by steam distillation are widely used for flavoring food. Breeding experiments involving wild *Allium* species and onion have the potential to increase yields of the volatile sulfur

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10.1021/jf011331d CCC: $22.00
Published on Web 04/03/2002

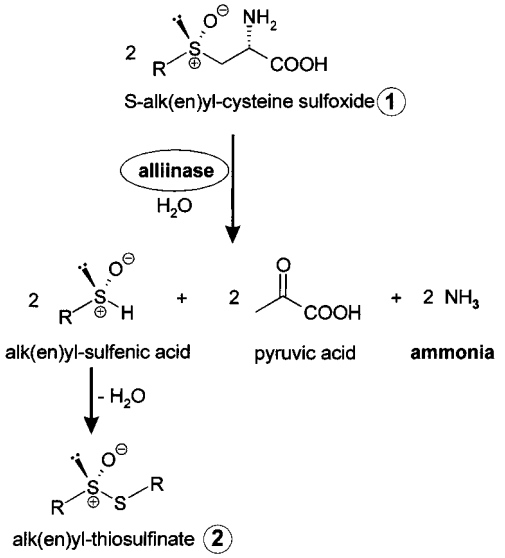

Figure 1. Alliinase-catalyzed reaction of cysteine sulfoxides (**1**) into thiosulfinates (**2**), pyruvic acid, and ammonia. R = methyl, ethyl, propyl, and butyl. Alliin, R = 2-propenyl; isoalliin, R = 1-propenyl. Conversion of alliin by the enzyme results in the production of the thiosulfinate allicin, which is characteristic for garlic.

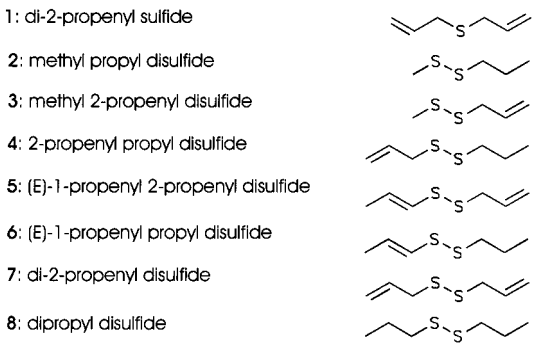

Figure 2. Chemical structures of typical sulfides formed by steam distillation of *Allium* plant material.

substances. Such experiments could also produce new aroma profiles possessing novel and more potent pharmacological or flavoring effects. The investigation of aroma precursors, aroma profiles, and alliinase activity of the hybrids *A. cepa* × *Allium globosum*, *A. cepa* × *Allium obliquum*, *A. cepa* × *Allium senescens*, *A. cepa* × *Allium chevsuricum*, and *A. cepa* × *Allium altyncolicum* in comparison to the parent plants is the subject of the studies presented here.

MATERIALS AND METHODS

Reagents. All chemicals unless otherwise specified were purchased from Merck (Darmstadt, Germany) or Fluka (Neu-Ulm, Germany) and were purified by standard procedures if necessary (distillation of solvents). Standards of molecular masses for gel electrophoresis (14.4−97.4 kDa) were obtained from Promega (Madison, WI), and polyacrylamide and Bradford reagent were purchased from Sigma (Deisenhofen, Germany). Authentic reference samples for gas chromatography (GC) analysis were obtained from Oxford Chemicals (Harlepool, U.K.) and Dragoco Gerberding & Co. AG (Holzminden, Germany).

Plant Material. The following *Allium* species, cultivated at the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, were used for this study: *A. altyncolicum* Friesen, *A. cepa* L., *A. chevsuricum* Tscholok., *A. globosum* M. Bieb. ex Ried., *A. obliquum* L., *Allium saxatile* M. Bieb., and *A. senescens* L. Hybrids were generated by hand pollination and in vitro cultures (embryo rescue) (*10*). The successful hybridization was proven by RAPDs (randomly amplified polymorphic DNAs). All plant material (bulbs) was harvested in the dormant phase (late summer and autumn).

Sample Preparation. For high-performance liquid chromatography (HPLC) analyses, 0.2−0.8 g (depending on the amount of cysteine sulfoxides in the plant material) of an exactly weighed sample was heated for 10 min in 20 mL of MeOH under reflux in order to inhibit enzymatic reactions, crushed in a mortar, and returned to the MeOH for further extraction with the addition of 20 mL of H_2O (*11*). The resultant extract was filtered, and the residue was washed with 3 × 3 mL of MeOH. The combined filtrates were evaporated to dryness under reduced pressure and stored at −20 °C before further use. The residue was then redissolved in phthaldialdehyde (OPA) derivatization reagent to give a final volume of 5 mL. The OPA reagent was prepared according to the method of Ziegler and Sticher (*12*). Volumes of 15 μL each were analyzed after 30 min of incubation in the dark. Alternatively, the residue was dissolved in 2 mL of H_2O, and the solution was divided into two equal parts. One part was digested with 200 units of alliinase for 30 min followed by derivatization with OPA reagent as described above. The second half was immediately derivatized with the OPA reagent. Authentic reference material was used as standard and derivatized in the same manner (*11*).

For GC analysis, approximately 1−2 g of the freshly minced sample, combined with 5 mL of distilled water, was transferred into a 20 mL headspace vial. The sample suspension was thermostated at 32 °C for 30 min until most of the enzymatic reactions were finished. Headspace sampling was done by piercing the septa with the SPME syringe and exposing the poly(dimethylsiloxane)-coated fiber (film thickness of 100 μm) to the sample for 10 min. The fiber was then retracted, immediately inserted into the injection port of the gas chromatograph, and thermally desorbed at 230 °C. The identification of the detected GC signals was confirmed by cochromatography and mass spectra of authentic standards.

HPLC Analysis. Quantitative analyses were performed on a Shimadzu LC-4A chromatograph equipped with a Chromatopac C-R3A integrator and a Spherimarge 80-ODS2 RP column 5 μm particle size, 250 mm × 4 mm with integrated guard column (Knauer, Berlin, Germany), operating at a constant flow rate of 1.0 mL/min. Detection was carried out using a UV detector set at 335 nm.

Best results for the analysis of cysteine sulfoxides were obtained using a modified procedure described by Ziegler and Sticher (*12*). A solvent gradient starting at 19% acetonitrile in phosphate buffer (pH 6.5, 0.05 M), 24% acetonitrile between 10 and 20 min, up to 27% at 50 min and 30% after 55 min was most suitable for the analysis of cysteine sulfoxides. Peaks were identified by cochromatography with standards as well as by comparison with samples after alliinase digestion, giving selective degradation of cysteine sulfoxides. Isoalliin was isolated from an authentic sample of *A. cepa* (*4*). Standards of cysteine sulfoxides were synthesized following the procedures formerly described (*13*−*17*). The quantitative HPLC determination was calibrated over the range of 0.01−0.4 mg/mL cysteine sulfoxides (injection volume 15 μL). Standards were derivatized in the same manner as described above.

GC Analysis. A Hewlett-Packard chromatograph model HP 5890 Series II equipped with an FID and a HP INNOWAX fused silica capillary column with a 0.5 μm bonded PEG phase, 60 m × 0.25 mm i.d., was used to analyze the volatiles desorbed from the SPME fiber. The operating conditions were as follows: injector temperature, 250 °C; detector temperature, 280 °C; H_2 flow rate, 1 mL/min.; linear oven temperature program, 35−220 °C raised at 10 °C/min; split ratio, 1:10. Mass spectrometric analyses were carried out in a Hewlett-Packard gas chromatograph (HP 5890 Series II, same column as for FID detection) directly coupled to a Hewlett-Packard MSD, model HP 5972. Operational parameters were as follows: carrier gas (He) velocity, 1 mL/min; ionization voltage, 70 eV; source temperature, 195 °C; scan range, *m*/*z* 27−300; MS libraries, Wiley 138 and NBS 75 K. Differentiation between (*E*) and (*Z*) isomers was carried out by reference substances, retention time, and mass spectra.

Alliinase Analysis. Extraction, isolation, and characterization of alliinase are described in detail in references *11* and *18*. Briefly, 1.5−3

g (fresh weight) of cleaned and peeled samples were crushed in an ice-cold mortar and extracted for 5 min at 4 °C with 10 mL of phosphate buffer (60 mM, pH 7.0). The extract was centrifuged for 30 min at 11 500*g*, and the supernatant was filtered and dialyzed overnight at 8 °C. Protein concentrations of alliinase preparations were determined following the methods described by Lowry et al. (*19*) using bovine serum albumin as standard. Enzyme activity was calculated from the amount of enzymically formed pyruvate as published by Schwimmer and Mazelis (*20*; **Figure 1**). The standard reaction mixture contained 18 U lactate dehydrogenase, 0.4 μmol NADH, and 4 μmol L-(+)-alliin. Volume was adjusted with phosphate buffer (60 mM, pH 7.0) to give 1.00 mL. The decrease of NADH was followed at 340 nm. Standard experiments were performed at 25.0 °C.

Substrate specificity of alliinase was determined analogously. Activity toward racemic alliin was used to set 100%. Homologous substances of alliin were used as racemates (with the exception of isoalliin), and enzyme activity was related to racemic alliin. For determination of enzyme activity at different pH values, phosphate buffer (60 mM, pH 7.0) was replaced by a citric acid/phosphate buffer (0.2 M) in the range of pH 3−7, phosphate buffer (0.2 M) in the range of pH 6−8, and borate buffer (0.2 M) in the range of pH 7.5−11. Temperature optimum of the alliinase was investigated in a range from 20 to 56 °C in steps of 4 °C. K_M and V_{max} values were deduced from a Hanes plot. l-(+)-Alliin in concentrations from 0.04 to 4 mM was used as substrate. Protein samples (20−30 μg of protein) were also subjected to dissociating sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS−PAGE) as described by Lämmli (*21*). A polyacrylamide concentration of 10% was used throughout all experiments. Proteins were visualized by staining with Coomassie blue, and masses were determined by calibration proteins.

RAPD Analysis. DNA was isolated from young leaves according to Rogers and Bendich (*22*). The RAPD assay was performed in a volume of 25 μL with 1 U of TAQ polymerase (Perkin-Elmer, Norwalk, CT), 20 ng of DNA, and 5 pmol of primers in polymerase chain reaction (PCR) buffer II (Perkin-Elmer). The primers were selected from sets OPD, OPE, and OPF (Operon Technologies, Alameda, CA) according to their efficiency to produce polymorphic fragments. Forty PCR cycles (95 °C for 20 s, 42 °C for 20 s, and 72 °C for 2 min) were run, and the products were separated on 1.5% agarose gels. For comparison of the plants, all bands occurring in the regenerated plantlets were numbered and related to the parent plants.

RESULTS AND DISCUSSION

RAPD Analysis of Hybrids. For parentage analysis, the PCR samples were arranged in a design that allows direct comparison of the parental lanes with those of the hybrid plants. All presumptive hybrids of interest for this study so far possess at least one discriminative band, which they have in common with only one of the parents and which is not present in the other one. **Figure 3** depicts an example for a cross between *A. cepa* and *A. obliquum* covering six different hybrid plants together with their parents. For all investigated hybrids, several discriminative bands were found, which they share with the corresponding paternal plants. These results indicate that all hybrids contain chromosomal material from paternal plants and that hybridizations were performed successfully.

Cysteine Sulfoxides. Determination of cysteine sulfoxides was carried out by means of HPLC analysis. With the exception of isoalliin, which was isolated from authentic *A. cepa*, reference material was synthesized at the beginning of the study. Structures were confirmed by means of nuclear magnetic resonance (NMR), mass spectrometry (MS), and IR analysis (*11*). In agreement with previous investigations (*2*), only the L-(+)-diastereomers of methiin, alliin, isoalliin, and propiin were found as naturally occurring cysteine sulfoxides (**Figure 4**).

Methiin was most abundant and was detected in all investigated samples. In particular, *A. chevsuricum* (48%), *A. globosum* (69%), *A. saxatile* (82%), and the hybrids *A. cepa* × *A. altyncolicum* (42%), *A. cepa* × *A. globosum* (67%), and *A. cepa* × *A. senescens* (45%) exhibited relatively high levels. However, methiin is less valuable as a precursor for aroma components because substances resulting from its alliinase reaction and steam distillation are common to most *Allium* species, rather than restricted to species such as garlic, leek, or onion. In contrast, the cysteine sulfoxide alliin is the characteristic precursor for garlic, leading to S-rich compounds with distinct aroma and pharmacological activity. High amounts were found in *A. globosum* and *A. obliquum* and also in both corresponding hybrids *A. cepa* × *A. globosum* (12%) and *A cepa* × *A. obliquum* (49%), respectively.

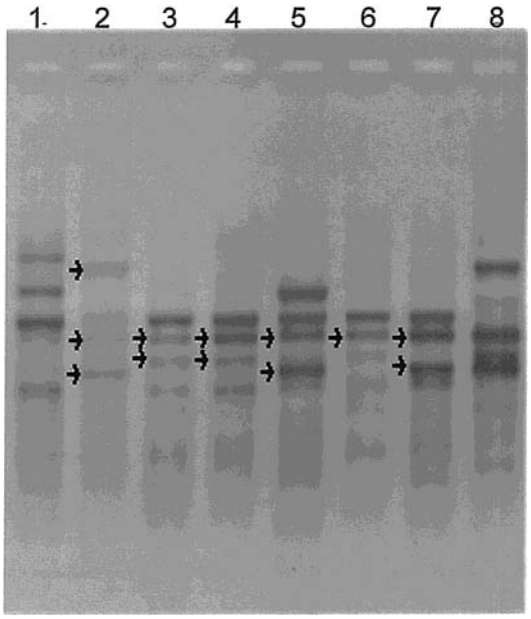

Figure 3. Amplified RAPD fragments of different hybrids of *A. cepa* × *A. obliquum* (**2**–**7**), the maternal plant *A. cepa* (**1**), and the paternal plant *A. obliquum* (**8**). Important hybrid bands are marked by arrows. Six different hybrids were proven. Arrows of these mark the discriminative bands, which hybrids have in common with the father plant.

Isoalliin is typical of onion (*A. cepa*) and is the main cysteine sulfoxide of this species. Remarkable relative amounts were also found in the wild species *A. altyncolicum* (74%), *A. chevsuricum* (49%), and *A. senescens* (43%). These three wild species are related to *A. cepa* and belong also to the same subgenus *Rhizirideum*. Isoalliin was previously described for wild species belonging to this subgenus (*11*).

For the breeding of hybrids, *A. cepa* was used as the maternal plant and the wild species were used as paternal parents. Consequently, isoalliin should be expected in all hybrids and was found in all investigated *Allium* hybrids. Those species exhibiting low levels of isoalliin in the paternal plants showed especially significant increases of isoalliin after hybridization with onion. This effect could be demonstrated for *A. globosum* (6%; *A. cepa* × *A. globosum*, 20%), *A. obliquum* (4%; *A cepa* × *A. obliquum*, 16%), and *A. saxatile* (10%; *A. cepa* × *A. saxatile*, 55%). Among these hybrids, *A cepa* × *A. globosum* and *A. cepa* × *A. obliquum* are of great interest, because levels of alliin are also relatively high in these plants (12 and 49%, respectively). The occurrence of alliin in these two hybrids is predictable from the father plants, which are also rich in alliin. Consistent with this, the aroma of these two hybrids is more reminiscent of garlic than of onion. Interestingly, the same two hybrids (*A. cepa* × *A. globosum* and *A. cepa* × *A. obliquum*) exhibited the highest levels of total cysteine sulfoxides of all

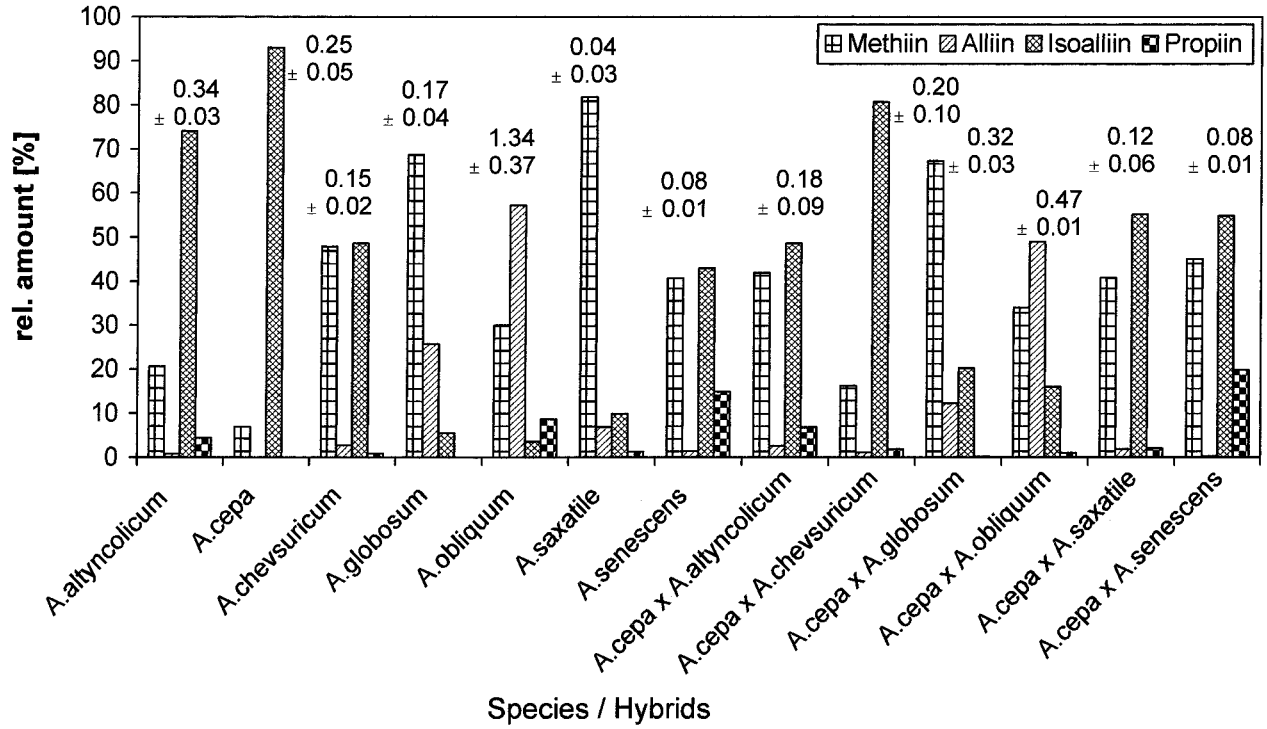

Figure 4. Relative amounts of cysteine sulfoxides of different *Alliaceae* (total cysteine sulfoxides = 100%). Absolute amounts, related to the fresh weight of bulbs, are given at the top of bars ($n = 4$).

Table 1. Characteristics of Crude Alliinase Preparations Obtained from *Allium* Hybrids and *A. altyncolicum*[a]

species	pH activity range	pH at half V_{max}	pH optimum	temp optimum (°C)	K_M (mM)	V_{max} (μmol/min/mg)
A. altyncolicum	4.5–8.5	6.0, 8.0	7.0	36	2.45	4.02
A. cepa × *A. altyncolicum*	6.0–8.5	6.5, 8.0	7.5	34	3.08	0.06
A. cepa × *A. chevsuricum*	4.0–9.0	5.5, 7.5	7.0	40	2.33	12.14
A. cepa × *A. globosum*	4.0–9.0	5.5, 8.0	7.5	40	1.04	9.28
A. cepa × *A. obliquum*	4.0–9.0	5.0, 8.0	7.5	38	1.92	16.90
A. cepa × *A. saxatile*	4.0–9.0	5.5, 7.5	6.5	36	0.88	5.76
A. cepa × *A.senescens*	4.0–9.0	5.5, 8.0	7.0	36	1.44	4.20

[a] L-(+)-Alliin was used for the determination of kinetic parameters.

hybrids investigated (0.32 and 0.47%, respectively, relative to the fresh weight of the bulbs).

Only low levels of propiin were detected in the wild species investigated. Propiin is the characteristic compound for leek (*A. porrum*; *2*). Remarkable amounts were also found in *A. senescens* (15%) and in the corresponding hybrid (*A. cepa* × *A. senescens*), which showed comparable amounts (20%). Total amounts of all cysteine sulfoxides for the wild species were between 0.08 (*A. senescens*) and 1.34% (*A. obliquum*); total amounts for hybrids were between 0.08 (*A. cepa* × *A. senescens*) and 0.47% (*A. cepa* × *A. obliquum*). Evidently, the amount of total cysteine sulfoxides of investigated hybrids is determined by the paternal plants used for hybridization.

Alliinase Activity. As shown in **Figure 1**, alliinase action is necessary to convert odorless cysteine sulfoxides into characteristic smelling thiosulfinates. An aqueous extract of hybrids was therefore prepared, and alliinase activity was investigated. Results of protein analysis are listed in **Table 1**. All enzyme extracts exhibited alliinase activity in a pH range between 5 and 8. The temperature optimum for alliinases was found between 34 (*A. cepa* × *A. altyncolicum*) and 40 °C (*A. cepa* × *A. chevsuricum*, *A. cepa* × *A. globosum*). K_M values were in the range between 0.88 (*A. cepa* × *A. saxatile*; high specificity for alliin) and 3.08 mM (*A. altyncolicum*; low specificity for cysteine sulfoxides).

The highest alliinase activity V_{max} of protein extracts was found for *A. cepa* × *A. obliquum* (16.9 μmol/min/mg). Samples of *A. cepa* × *A. chevsuricum* and *A. cepa* × *A. globosum* also showed a relatively high alliinase activity (12.1 and 9.3 μmol/min/mg, respectively). All other samples exhibited a V_{max} lower than 9 μmol/min/mg. The protein extract of *A. cepa* × *A. altyncolicum* was found to be nearly free of alliinase activity (0.06 μmol/min/mg). Therefore, alliinase activity of the parent species was determined and found to be 4.0 μmol/min/mg, a value that is also rather low. Alliinase from *A. cepa* was investigated in detail previously and its activity was found to be sufficient for the conversion of cysteine sulfoxides into the corresponding thiosulfinates (*20*, *23*−*26*). It must be assumed that the low alliinase activity *of A. altyncolicum* is negatively influenced by the hybridization with *A. cepa*, resulting in a nearly inactive enzyme in the hybrid. In contrast, levels of cysteine sulfoxides of both parents and the hybrid are sufficient for the production of volatile sulfur compounds by steam distillation.

In addition to *A. altyncolicum*, the activity of the paternal plants *A. obliquum* and *A. saxatile* was investigated previously (*11*). The V_{max} of the latter species was found to be 13 and 18 μmol/min/mg, respectively. For *A. cepa* × *A. obliquum*, V_{max} was 16.9 μmol/min/mg, indicating that hybridization of this species had little influence on alliinase activity. However, alliinase activity of *A. cepa* × *A. altyncolicum* and *A. cepa* × *A. saxatile* was much lower than that of the parent plants (0.06 vs 4.0 μmol/min/mg and 5.8 vs 18 μmol/min/mg).

In previous studies with purified alliinase, mainly obtained from cultured varieties, molecular masses of subunits were reported to be 48−57 kDa (*25*−*34*). At least two of these subunits are necessary to form an active enzyme. In addition, isoforms of the alliinase of *A. cepa* were described recently

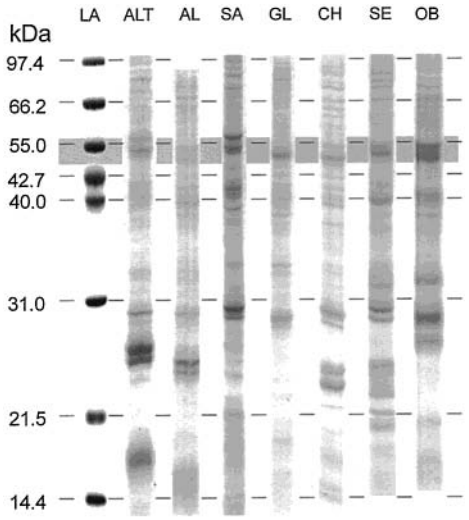

Figure 5. SDS–PAGE of protein fractions obtained from various *Allium* samples. Subunits of alliinase are expected to have a molecular mass of 48–57 kDa (gray bar). ALT, *A. altyncolicum*; AL, *A. cepa* × *A. altyncolicum*; SA, *A. cepa* × *A. saxatile*; GL, *A. cepa* × *A. globosum*; CH, *A. cepa* × *A. chevsuricum*; SE, *A. cepa* × *A. senescens*; OB, *A. cepa* × *A. obliquum*.

(*25*−*26*). Results from SDS−PAGE are shown in **Figure 5**. As expected, the electrophoretograms of all investigated species exhibited protein bands in the range of positions described for alliinase subunits in earlier studies (see above). In this range, *A. cepa* × *A. altyncolicum* gave protein bands of very low intensity, which correlate well with the low alliinase activity described above. However, exact identity of these bands must be proved by further studies. All investigated protein extracts exhibited a characteristic protein pattern in the range between 21 and 31 KDa that might be useful for the identification of species (*11*).

Relative enzyme activities toward different cysteine sulfoxides are depicted in **Figure 6**. The activity toward racemic L-(±)-alliin was used to set 100% because synthetic methiin, ethiin, propiin, and butiin could not be obtained as pure L-(+)-isomers and were therefore used as their racemates. In addition, enzyme activities for pure L-(+)-alliin, L-(+)-isoalliin, and for the unnatural L-(−)-isomer of alliin were determined. With the exception of *A. cepa* × *A. globosum* and *A. cepa* × *A. saxatile*, all samples showed highest activity toward isoalliin. Also, L-(+)-alliin was converted by all investigated protein extracts at high rates, whereas the corresponding L-(−)-isomer and the other investigated cysteine sulfoxides were enzymically converted at much lower rates. The alliinase activity of the protein extract obtained from *A. cepa* × *A. altyncolicum* was most stereoselective, whereas alliinase activity of *A. cepa* × *A. saxatile* was the most unspecific. The sample obtained from *A. cepa* × *A. obliquum* gave the highest relative activity toward isoalliin.

Alliinase of *A. obliquum* and *A. saxatile* were most active toward L-(+)-alliin (*11*). The enzymes of the hybrids *A. cepa* × *A. obliquum* and *A. cepa* × *A. saxatile* digested L-(+)-alliin at similar rates. L-(−)-Alliin, methiin, ethiin, propiin, and butiin were also accepted by alliinases obtained from both paternal species and hybrids but converted at much lower rates. Also, for *A. altyncolicum* and *A. cepa* × *A. altyncolicum*, it was shown that substrate specificity of both species is very similar (**Figure 6**). This leads to the assumption that the substrate specificity of the alliinase encoded by the alliinase gene(s) of the paternal plants is little affected by hybridization, but alliinase activity

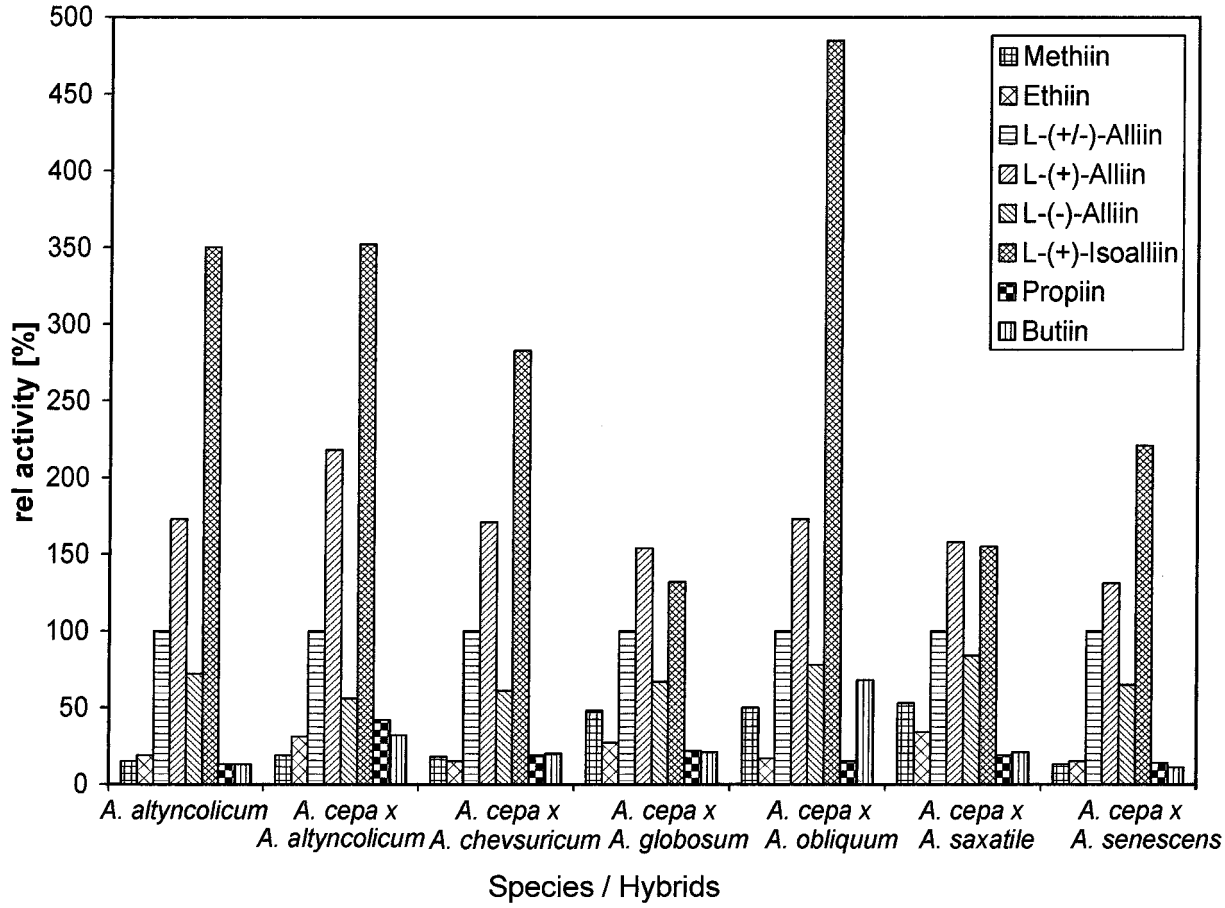

Figure 6. Relative alliinase activity of protein extracts of *Allium* hybrids. Activity toward L-(±)-alliin was set to 100%.

Table 2. Average Relative Contents and Standard Deviations of Volatile Sulfur Compounds Obtained by GC Analysis ($n = 8$)[a]

species	content (%) of sulfur compds							
	1	2	**3**	**4**	**5**	**6**	**7**	**8**
A. cepa × *A. altyncolicum*	0.8 (0.43)	9.0 (2.3)	0.1 (0.05)	0.9 (0.17)	0.1 (0.06)	20.6 (1.9)	0	43 (3.0)
A. cepa × *A. chevsuricum*	1.3 (0.38)	0.9 (0.38)	0	1.2 (0.35)	0.3 (0.13)	22.2 (2.1)	0	38.9 (5.7)
A. cepa × *A. globosum*	2.2 (0.29)	10.5 (2.1)	17.5 (5.7)	13.9 (1.3)	8.2 (3.4)	6.6 (2.7)	14.1 (1.6)	3.4 (1.2)
A. cepa × *A. obliquum*	19.8 (3.3)	0.1 (0.01)	6.0 (0.63)	1.4 (0.12)	3.0 (0.38)	0.2 (0.05)	68.5 (3.1)	0
A. cepa × *A. saxatile*	0.7 (0.14)	14.7 (3.3)	0.3 (0.08)	1.5 (0.13)	0.2 (0.13)	11.7 (5.6)	0.1 (0.08)	38.2 (3.5)
A. cepa × *A. senescens*	0.5 (0.13)	1.7 (0.41)	0	2.9 (0.43)	0.2 (0.09)	9.4 (4.9)	0	68.1 (4.5)
A. altyncolicum	0.3 (0.13)	2.2 (0.45)	0	1.0 (0.07)	0.1 (0.01)	14.4 (1.9)	0	66.5 (2.6)
A. chevsuricum	0.9 (0.41)	10.9 (3.4)	0.2 (0.11)	1.8 (0.23)	0.1 (0.05)	15.7 (3.4)	0	46.2 (5.3)
A. globosum	4.4 (1.2)	0.4 (0.11)	25.2 (5.1)	1.1 (0.16)	3.0 (0.61)	0.1 (0.04)	62 (3.6)	0
A. obliquum	4.3 (1.4)	0.1 (0.01)	5.7 (1.6)	1.0 (0.17)	4.9 (3.1)	0.1 (0.01)	81.3 (4.2)	0
A. saxatile	1.6 (0.41)	4.3 (0.87)	44.5 (5.7)	4.2 (0.9)	3.4 (3.0)	0.1 (0.05)	26 (3.9)	0.2 (0.06)
A. senescens	0.6 (0.41)	9.4 (1.3)	0	0.8 (0.17)	0.05 (0.05)	14.1 (4.9)	0.2 (0.04)	59.6 (5.4)
A. cepa	0.91 (0.19)	1.2 (0.08)	0	0.5 (0.08)	0	7.7 (0.7)	0	46.3 (1.80)

[a] Detected substances are as follows: **1**, di-2-propenyl sulfide; **2**, methyl propyl disulfide; **3**, methyl 2-propenyl disulfide; **4**, 2-propenyl propyl disulfide; **5**, (*E*)-1-propenyl 2-propenyl disulfide; **6**, (*E*)-1-propenyl propyl disulfide; **7**, di-2-propenyl disulfide; **8**, dipropyl disulfide.

might be decreased (*A. cepa* × *A. altyncolicum*, *A. cepa* × *A. saxatile*). The reason for this behavior is unknown and needs further investigation.

Aroma Profiles. In comparison to the usually performed aroma isolation techniques such as steam distillation, a surprisingly high reproducibility, sensitivity, and selectivity of all relevant S-containing aroma compounds were achieved by solvent extraction or simultaneous distillation/extraction (SDE) (*35*−*36*). Although several competing consecutive reactions prevent the establishment of a stable equilibrium, the analysis of the adsorbed sulfur components leads to reproducible results. Even at 10 min, the adsorption of the analyte molecules on the SPME fiber is almost complete; from that time onward, an optimal detector response is reached. In contrast to related investigations performed with *Allium* samples processed by the SDE method, the SPME-GC results show few discrimination effects in the response of the individual substances. Only components with a higher amount of sulfur in the molecule such as dialk(en)yl trisulfides showed lesser tendencies to adsorb on the fiber. As shown in **Table 2**, the genetic influences of the individual parent genomes are very different. Generally, a very good correlation exists between the analyzed cysteine sulfoxides, aroma precursors, and each resulting aroma profile. Depending on the *Allium* species involved, the hybrids correspond more or less to a "garlic type", characterized by the main components di-2-propenyl disulfide and methyl 2-propenyl disulfide, or an "onion type", containing predominantly dipropyl disulfide and (*E*)-1-propenyl propyl disulfide in the aroma fraction. The aroma profile of *A. cepa* × *A. globosum* is characterized mainly by (*Z*)-1-methyl propenyl disulfide, di-2-propenyl disulfide, and 2-propenyl propyl disulfide, which are all aroma substances derived from alliin. A similar situation is to be recognizable in *A. cepa* × *A. obliquum*, which contains mostly di-2-propenyl sulfide and di-2-propenyl disulfide. The gene expression in both of these hybrids, leading to a relatively high alliin content and the related, very specific "garlic-type" aroma, is dominated by the paternal wild type (*37*). Hybridization of *A. cepa* with the wild species *A. senescens*, *A. altyncolicum*, and *A. chevsuricum* results in *Allium* types, which show high similarity to the individual aroma profiles of both parent plants. In all cases, the aroma fraction is dominated by dipropyl disulfide, (*E*)-1-propenyl propyl disulfide, and propanthiol. This finding also corresponds with the individual cysteine sulfoxide profiles of the hybrids, presented in **Figure 4**.

It was demonstrated in summary that varied aroma profiles could be generated by breeding experiments using wild *Allium* species as paternal plants and *A. cepa* as maternal. These experiments resulted in isoalliin-rich hybrids and were of special interest when alliin-rich species were used as paternal plants (e.g., *A. globosum* and *A. obliquum*). Hybridization of these species with *A. cepa* results in plants showing high levels of both alliin and isoalliin. As expected, the aroma of these hybrids was also influenced by both parent plants, depending on the ratio of individual cysteine sulfoxides. It was also demonstrated that wild species with high levels of cysteine sulfoxides are highly beneficial for breeding experiments (*A. globosum* and *A. obliquum*). Hybrids of these species appear to have potential uses in the production of new *Allium* oils, spices, and probably also as medicinal plants. In conclusion, *A. cepa* × *A. globosum* and *A. cepa* × *A. obliquum* are the most interesting hybrids determined by this set of experiments and may lead to the production of vegetable plants with an increased health benefit. The alliinase activity of both hybrids was also found to be sufficient to guarantee full conversion of cysteine sulfoxides into volatile aroma components.

ACKNOWLEDGMENT

We thank Mrs. B. Zeiger for the careful performance of the GC and GC-MS measurements. Thanks for technical assistance are due to students, who participated at the phytochemical course of the Institute for Pharmaceutical Biology in winter 1999/2000.

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Received for review October 5, 2001. Revised manuscript received February 8, 2002. Accepted February 11, 2002. We are grateful to Drs. K.-W. Glombitza and E. Leistner for helpful discussions and financial support. This research was also supported by the German Fonds der Chemischen Industrie and the DPhG-Stiftung as part of the Stifterverband für die Deutsche Wissenschaft.

JF011331D