



Comparative study on the interrelation between flavor related parameters of different onion (*Allium cepa* L.) cultivars and their applicability to forecasting onion oil yield

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Abstract

Different flavor related parameters along the biochemical and chemical way of aroma genesis of onions (*Allium cepa* L.), i.e. bulb sulfur, γ -glutamyl-1-propenyl-L-cysteine sulfoxide (γ -GPeCSO), alk(en)yl-L-cysteine sulfoxides (ACSOs), enzymatically developed pyruvate (EPY), amount of distilled volatiles (onion oil) and headspace volatiles were compared and correlated with respect to their suitability for the evaluation of onion flavor potential and for prediction of onion oil yield. Identical material (lyophilisate and juice from same bulbs) from four onion cultivars with two of them grown at different sulfur fertilization levels (0 and 100 kg S /ha) was analyzed. For the cultivars in this study (cvs. Copra, Sturon, Golden Bear, Matador), all parameters were generally in good correlation with EPY, underlining their significance with regard to raw material assessment. Among the cultivar lines under fieldgrowing conditions the ratios of the ACSOs methyl-L-cysteine sulfoxide (MCSO) and *E*-1-propenyl-L-cysteine sulfoxide (PeCSO) varied only within a limited range (8:92-14:86 % μ mol MCSO:PeCSO, resp.). A shift in proportions towards methylated derivatives after steam distillation was not attributable to the variability in ACSO ratios, but was assumed to be caused by the thermal lability of 1-propenyl radicals during distillation and analysis. A rapid semiquantitative gaschromatographic solidphase microextraction (SPME-GC/MS) procedure of heated onion juice was evaluated and proved to be superior to fresh juice sampling in terms of quantitative correlation to onion oil ($r=0.98$ vs. $r=0.71$) and reproducibility (CV 2.7% vs 4.7%). Neither for different cultivars nor for the two applied sulfur rates a significant qualitative change in the relative peak areas of the headspace could be observed.

Key words: Onion, flavor, alk(en)yl-L-cysteine sulfoxides, SPME, onion oil.

Introduction

The extracted distillate from *Allium cepa* L., commonly referred to as "onion oil", is extensively used in the food industry as seasoning and flavoring agent for savory products. Because of its low yields (<0.01%), onion oil belongs to the high price products. It is therefore of importance to secure a high aroma potential of raw materials used for processing by employing predictive methods for quality control. Numerous pungency markers have been described and suggested in literature^{1,2}. However, correlations between different pungency parameters appeared not always to be consistent and reliable³⁻⁵. As a standard pungency evaluation procedure the determination of enzymatically produced pyruvate (EPY) is widely used among breeders in screening programs⁶. This test is convenient to perform, however it provides only an estimate of gross flavor intensity, since it is based on a by-product of the aroma reaction. Using EPY as a predictive parameter for raw material control and cultivar selection, it is essential to secure a quantitative correlation with onion oil yield and to gain information about the qualitative variability of flavor related constituents among different cultivars of *A. cepa* and their impact on the quality of onion oil. The biochemical precursors to virtually all onion flavor are three alk(en)yl-L-cysteine sulfoxides (ACSOs), comprising methyl- (MCSO), propyl- (PCSO), and *E*-1-propenyl-L-cysteine sulfoxide (PeCSO). PeCSO, the predominant aroma precursor (>80%), is biosynthetically released from its glutaminated storage form γ -glutamyl-1-propenyl-L-cysteine sulfoxide (γ -GPeCSO) by the action of γ -glutamyl-transpeptidase [EC 2.3.2.1.]⁷⁻⁹. γ -GPeCSO is accumulated in amounts comparable to PeCSO and therefore represents a source of potential flavor¹⁰⁻¹². Onion aroma is produced enzymatically by the action of alliinase [EC 4.4.1.4.] upon cell

disrupture and subsequent hydrolysis of the ACSOs. The reaction products derived from this 'aroma reaction' are highly unstable sulfenic acids, pyruvate and ammonia^{1,13}. Sulfenic acids immediately condense to form thiosulfonates¹⁴. In the case of PeCSO the sulfenic acid instantly isomerises in an enzymatically mediated way to the lachrymatory principle *E/Z*-thiopropional *S*-oxide¹⁵. Fresh onion aroma is believed to solely consist of those primary products^{16,17}. In onion oil, which underwent a thermal treatment, di- and polysulfides prevail beside dimethylthiophenes, propanethiol and 2-methyl-2-pentenal¹⁸⁻²¹. Some heterocyclic *S*-compounds and substances resulting from Maillard reactions are found in minor quantities.

The objective of this study was to coherently compare different parameters associated with onion flavor intensity in respect to the standard marker pyruvate. Because earlier works^{3-6,10,11,17-21} on onion flavor were mainly focused on either biochemical precursors or aroma volatiles and distillates, a comprehensive study of correlation between these parameters and with the EPY marker has not been conducted yet. Therefore, emphasis was put on monitoring the whole way from the biochemical precursors to the successive stages of aroma development by applying appropriate methods on identical sample material. The intention was the coverage of the complete onion processing line from the bulbs to the onion oil. The chosen flavor related parameters were bulb-sulfur, γ -GPeCSO, ACSOs, EPY, headspace (HS) volatiles and steam distillate (onion oil).

Reviewed literature reports onion oil profiles extremely differing in composition and complexity^{18-20,24-26}. The role of the ACSOs in this respect is unclear. Randle et al.^{22,4} and Coolong and Randle²³ reported wide variations of ACSO ratios in onions at different N and S fertility levels and claimed an important influence of the differing proportions on the perceived flavor of fresh onions. In

contrast, when examining different onion cultivars Schulz et al.²⁴ found no significant change in the chemical profile of their distillates. In the view of these data, variability of the ACSO proportions between cultivars under fieldgrowing conditions and its possible influence on the resulting aroma profiles requires a closer examination.

A further aim was to evaluate the suitability of a HS solidphase microextraction sampling procedure, so far merely used in qualitative considerations of the HS of freshly cut onions^{44,46-48}, as a rapid method for the forecast of onion oil yield. Cultivars, representing a range of pungency intensities, were grown for this investigation. For onion pungency intensification, sulfur was applied to the growing plant. Hence, qualitative and quantitative effects of additional sulfur were taken into consideration.

Material and Methods

Plant material: Onions (*A. cepa*) were fieldgrown at the Experimental Station for Horticulture, Hohenheim University, Stuttgart, Germany. The cultivars Copra, Golden Bear, Sturon, and Matador (shallot) were grown from seeds. 50 kg/ha NPK was given as starter fertilizer. In response to soil analysis, fertilization with N to a final content of 130 kg N /ha at the beginning of bulbing was conducted. In case of sulfur fertilization experiments 100 kg S /ha ($MgSO_4$) was applied. Onions were harvested at maturity indicated by 80% foliar collapse among the respective population, cured in a shed and stored for 6 weeks at 15°C / 75% R. H. until analyzed.

Preparation for analysis: All analyses were conducted from tissue of the same bulbs. Onions were cooled to 4°C 12 h prior to preparation. After removing dry outer scales, 20 bulbs of each variety were longitudinally cut in two pieces, assuring a precise halving of the basal plate. One set was used for juicing with a centrifugal juicer in a minicoldlab at 4°C. Enzymatic reactions were sufficiently suppressed in the fresh juice until given into a closed vessel, as no lachrymatory effect was observed. For pyruvate determinations and distillation, juice was incubated at 35°C for 40 min. From wedges of this set background pyruvate was determined. The second set of bulbs was subjected to lyophilization. Homogeneity was secured by pulverizing and mixing the dried material under liquid nitrogen in a mortar.

Bulb sulfur analysis: Bulb sulfur was determined from lyophilized and pulverized samples on a CE Elantech Flash Elemental Analyzer (NA 2500, Thermo Electron Inc., Waltham, MA, USA).

Analysis of ACSOs and γ -GPeCSO: The analysis of ACSOs and γ -GPeCSO was conducted using an HPLC method based on that described by Randle et al.⁴. However, several modifications were made: From the pulverized sample, a quantity equivalent to 20 g fresh weight was extracted twice using the fivefold amount of methanol (80% v/v) at each extraction step. All steps of extraction were carried out at -30°C. The pulverized samples were soaked in 100 ml methanol for 24 h, filtered and the remainder extracted for additional 24 h. To 10 ml of the pooled and filtered extracts (0.1 g fresh weight ml⁻¹) 500 μ l internal standard solution were added containing 2 mg ml⁻¹ ethyl-L-cysteine sulfoxide and 0.4 mg ml⁻¹ γ -glutamyl glutamic acid (Acros Organics, Geel, Belgium). Extracts were dried under vacuum (20 mbar) in a rotary evaporator at 35°C and rehydrated with 2 ml dd H₂O. Ion chromatography and

phenylisothiocyanate-derivatization steps were carried out according to the original method⁴, obtaining an ACSO and a γ -glutamyl-peptide (γ -GP) fraction. HPLC analysis was run on LaChrom[®] modules equipped with a cold rack (5°C) using a Brownlee Spheri-5 RP 18 column (5 μ m, 220 x 4.6 mm) and a RP-18 guard column (Perkin Elmer, Shelton, CT, USA). 20 μ l were injected and the eluting substances were detected at 254 nm. Data acquisition and analysis were conducted on HPLC-software D-7000 HSM 3.1 (Merck-Hitachi, Darmstadt, Germany). A buffer system consisting of 0.14 M ammonium formate (eluent A, pH 6.32) was employed. In combination with eluent B (ACN:dd H₂O, 60:40 v/v) the following two gradient elution programs (% eluent A:B, v/v) were used: ACSO fraction: 15. min (87:13), 36. min (45:55), 39. min (0:100), 55. min (0:100), 56. min (87:13), 65. min (87:13), flow rate 0.8 ml min⁻¹. γ -GP fraction: 20. min (85:15), 21. min (45:55), 35. min (0:100), 36. min (85:15), 46. (85:15), flow rate 1 ml min⁻¹. Thioethers of the ACSO-reference compounds were prepared according to Theodoropoulos²⁷ with subsequent oxidation as described by Stoll and Seebeck²⁸. 2-PeCSO (Alliin) instead of 1-PeCSO was synthesized and used for calibration. γ -GPeCSO was isolated using the procedure of Shaw et al.⁹. Purity of all reference compounds exceeded 98.5%. ECSO was taken as internal standard compound for ACSO calibration and γ -glutamyl glutamic acid for the peptide fraction. Authenticity and purity of standards and analytes were confirmed by means of HPLC and LC/MS as described by Resemann et al.²⁹.

Pyruvate determination: Enzymatically produced pyruvate (EPY) was determined in a spectrophotometric assay based on a lactate dehydrogenase (LDH)-mediated reduction of pyruvate and a corresponding oxidation of NADH according to the official ASU-method L 01.0019. (DIN 10193)³⁰. Solutions were diluted to the required concentration range. Correction for physiological levels of pyruvate (background pyruvate) were conducted through inactivating alliinase by microwave heating (600 W, 1 s g⁻¹ fresh weight) prior to maceration.

Simultaneous distillation and extraction (SDE): After incubating for 40 min in a closed bottle at RT, 200.0 g of juice were transferred into a 2 l round-bottom flask. 800.0 g of phosphate buffer solution (0.5 M, pH 5.60) were added and the flask was connected to a Likens-Nickerson type distillation apparatus. Volatiles were extracted for 3 h with 30 ml dichloromethane (GC grade, > 99.9%). 1 ml internal standard solution containing 2 mg diisopropyl disulfide (DiPDS) and 2 mg 6-methyl-5-hepten-2-one was added to the aroma extract. After drying over anhydrous Na₂SO₄, the extract was concentrated on a Snyder-column with subsequent N₂ purging to a final volume of 1 ml. Onion oil was quantified as mg DiPDS by gas chromatography (GC). Detection and peak assignment was performed by mass spectrometry (MS). The response factors were assumed to be 1.

GC/MS-analysis: Instrumentation consisted of a Varian GC 3400 (1077 split/splitless injector), equipped with a BPX-5 column 30 m, 0.25 mm ID, 0.5 μ m (SGE, Darmstadt, Germany), coupled to a Varian Saturn II mass spectrometer. Operating conditions were as follows: Carrier gas He (>99.999 % vol), flow rate 1 ml min⁻¹, 1 μ l injection volume, injector temperature 220°C, transferline 220°C, run 45°C/3.33 min, 45-200°C/58.33 min, 220°C/3.34 min. Ion trap 180°C, ionization voltage 70 eV, scanned mass range 33-249 m/z, emission current 10 μ A.

Table 1. Contents of bulb sulfur, γ -GPeCSO^a and EPY^b of different onion cultivars and correlations between the respective parameters.

Cultivar	Bulb S [g kg ⁻¹ dw]	γ -GPeCSO [μ mol g ⁻¹ fw]	EPY [μ mol g ⁻¹ fw]	EPY/S
Copra	4.55 ± 0.03	4.38 ± 0.081	4.99 ± 0.152	1.097
Golden Bear	4.37 ± 0.04	3.81 ± 0.085	5.50 ± 0.085	1.259
Matador	5.92 ± 0.08	5.67 ± 0.092	10.34 ± 0.090	1.746
Matador +S ^c	6.66 ± 0.04	6.18 ± 0.129	11.47 ± 0.003	1.722
Sturon	4.36 ± 0.11	4.06 ± 0.309	4.95 ± 0.047	1.135
Sturon +S ^c	5.37 ± 0.09	4.31 ± 0.276	6.19 ± 0.104	1.152
γ -GPeCSO	r = 0.941			
EPY	r = 0.947	r = 0.968		
ACSO ^d	r = 0.901	r = 0.931	r = 0.984	

Values are given as means (n=3) ± SD; all correlations were significant at p < 0.05 (Pearson product moment correlation)

^a γ -GPeCSO: γ -glutamyl-L-propenyl-L-cysteine sulfoxide

^bEPY: enzymatically produced pyruvate

^c+S: sulfur fertilization at a rate of 100 kg S/ha

^d values given in Table 2

Headspace solidphase microextraction (HS-SPME): Fresh juice samples: 1.5 ml cold onion juice were pipetted immediately after juicing into a 15 ml vial containing 3.5 ml internal standard solution (5.9 mg l⁻¹ n-heptanol) and a magnetic stirring bar. Sample weights were recorded and the vials immediately capped and incubated at 35°C in a waterbath (±0.2°C) for 40 min. The HS part of the vial was completely immersed. A 100 μ m polydimethylsiloxane (PDMS) coated SPME-fiber (Supelco, Taufkirchen, Germany) was used, conditioned at 200°C in a helium flushed parallel injection port between sampling. For sampling the fiber was cooled for 2 min and exposed to the HS for 10.00 min (35°C), desorption was conducted with closed splitter for 2.00 min.

Heated juice samples: 5 ml cold juice were pipetted into a Pyrex test tube, immediately capped, incubated at 35°C for 30 min, exposed to 110°C in a test tube heater (SHT, Stuart Scientific, Redhill, UK) for 5 min and cooled in ice water. After centrifugation 1.5 ml of the supernatant was used as described above. The vial was conditioned at 35°C for 5 min prior to sampling. SPME-sampling was conducted as mentioned above.

GC/MS-analysis: Same instrumentation as described for SDE-GC/MS was used, except the temperature was programmed from 60°C/1min, 60-200°C/25.45min, 200-220°C/1min and 220°C/2.55 min. A linear response to a series of concentrations was ascertained and differences in the weighted samples were corrected.

Statistical analysis: All statistical procedures were conducted using SigmaStat[®] Software (SPSS Inc, Chicago, IL, USA).

Results and Discussion

Bulb-sulfur, biosynthetic precursors and pyruvate: Table 1

shows the contents of bulb sulfur, the biosynthetic intermediate γ -GPeCSO and EPY. Total bulb S displayed a trend following that of pyruvate and ACSO values (Table 1 and 2, resp.). However, as indicated by differing EPY/bulb S ratios a phenotypic variability in S-utilization for flavor precursors may be assumed. With a significantly higher EPY/bulb S ratio cv. Matador appears more efficient in incorporating S into flavor substances, thus corroborating the findings of Randle³¹ and Randle et al.²², who demonstrated that allocation of S into the flavor pathway was cultivar dependent. However, in contrast to Randle et al.²² a significant decrease of S-utilization for biogenesis of aroma precursors with increased S nutrition could not be confirmed in this study. The determined contents of γ -GPeCSO are in agreement with data of other authors^{4,11,32,33}. Although onion varieties were found to differ greatly in their initial γ -GPeCSO:ACSO ratio¹¹, in this investigation the order of γ -GPeCSO contents was in agreement with the respective ACSO contents except for cv. Golden Bear, where an inversed ratio of γ -GPeCSO to EPY and ACSOs was observed (Table 1 and 2, resp.). 'Golden Bear' is an early sprouting cultivar with low storability. With breaking of dormancy the enhanced transformation of γ -GPeCSO to PeCSO is triggered by the activation of γ -glutamyl-transpeptidase^{8,10}. As a function of storage duration, a reciprocal change of the γ -GPeCSO and PeCSO contents has been observed by Kopsell et al.¹¹. An advanced developmental stage of cv. Golden Bear may thus be responsible for this effect.

In ACSO analysis, propyl-L-cysteine sulfoxide was detected in trace amounts only (<0.2% of total ACSOs), rendering this precursor negligible in quantitative considerations. Amounts of total

Table 2. Contents of alk(en)yl-L-cysteine sulfoxides (ACSO) in different onion cultivars.

Cultivar	ACSO ^a [μ mol g ⁻¹ fw]	MCSO ^b [μ mol g ⁻¹ fw]	PeCSO ^c [μ mol g ⁻¹ fw]	MCSO : PeCSO [% μ mol]
Copra	6.86 ± 0.17	0.55 ± 0.01	6.31 ± 0.19	8.0 : 92.0
Golden Bear	8.48 ± 0.34	1.02 ± 0.04	7.46 ± 0.33	12.0 : 88.0
Matador	13.64 ± 0.10	1.46 ± 0.10	12.19 ± 0.18	10.7 : 89.3
Matador +S ^d	14.82 ± 0.35	1.93 ± 0.05	12.90 ± 0.32	13.0 : 87.0
Sturon	5.88 ± 0.41	0.76 ± 0.21	5.13 ± 0.62	12.8 : 87.2
Sturon +S ^d	7.71 ± 0.19	1.04 ± 0.06	6.67 ± 0.16	13.5 : 86.5

Values are given as means (n=3) ± SD

^a ACSO = MCSO + PeCSO

^b MCSO: Methyl-L-cysteine sulfoxide

^c PeCSO: E-1-propenyl-L-cysteine sulfoxide

^d+S: sulfur fertilization at a rate of 100 kg S/ha

Table 3. Comparison of total peak areas obtained by SDE and SPME methods and their correlation to enzymatically produced pyruvate (EPY).

Cultivar	SDE (tpa) [mg DiPDS]	SPME-F ^a (tpa) [μg heptanol g ⁻¹ juice]	CV [%]	SPME-H ^a (tpa) [μg heptanol g ⁻¹ juice]	CV [%]
Copra	16.06 ± 0.12	215.9 ± 29.0	13.43	262.6 ± 19.9	7.58
Golden Bear	16.38 ± 0.43	206.9 ± 0.1	0.05	285.0 ± 3.4	1.19
Matador	33.30 ± 0.82	233.2 ± 8.9	3.82	597.2 ± 11.6	1.94
Matador +S ^c	37.76 ± 1.61	274.3 ± 5.6	2.04	670.2 ± 6.0	0.90
Sturon	18.31 ± 0.67	217.5 ± 11.5	5.29	278.3 ± 8.3	2.98
Sturon +S ^c	22.90 ± 0.72	247.9 ± 9.8	3.94	348.8 ± 4.9	1.40
averaged CV [%]			4.7		2.7
EPY ^b	r = 0.985	r = 0.591		r = 0.997	
SDE		r = 0.706		r = 0.984	

^a SPME volatiles are arbitrarily expressed as [μg n-heptanol g⁻¹ juice], total peak areas (tpa) from HS of fresh onion juice (F) and heated onion juice (H)

^b Linear correlation (p<0.05)

^c +S: sulfur fertilization at a rate of 100 kg S/ha

ACSOs (Table 2) were highly correlated with EPY values ($r = 0.984$, $p < 0.001$, Table 1). Randle et al.⁴ and Kopsell et al.¹¹ found only poor correlations between EPY and total ACSOs, but significant correlations between EPY and PeCSO. However, using the original method and instrumentation of Randle et al.⁴ we found MCSO very difficult to resolve clearly. Therefore, modifications of chromatographic conditions were necessary to achieve baseline resolution of the MCSO peak. On a molar basis, pyruvate determination exhibited lower values as expected from stoichiometric considerations. This effect of unmeasurable 'cryptopyruvate' has been observed and discussed by Lancaster et al.³⁴ and Shen and Parkin³⁵. ACSOs ratios were found to be in a narrow range between 8:92 to 14:86 [% μmols] MCSO:PeCSO (Table 2), which is in good agreement with the data reported by Yoo and Pike³⁶, Thomas and Parkin³⁷, Keusgen et al.³⁸ and Bacon et al.³⁹. Although different onion cultivar lines have been selected intentionally for this investigation, ACSO ratios varied only within a limited range. High rates of applied sulfur (100 vs 0 kg S/ha) were reflected in a higher S uptake. S was obviously incorporated into the flavor pathway as indicated by the increased γ -GPcCSO and ACSO contents of the sulfur fertilized varieties (Table 1 and 2). However, increased sulfur nutrition did not result in a significant change in ACSO ratios. Whereas Randle et al.⁴ reported an increase in the proportion of PeCSO at higher sulfate levels, the present results corroborated earlier observations from separately designed field trials on S fertilization and screening data of previous years (Resemann, J., unpublished).

Onion oil by SDE-GC/MS: As a measure of obtainable distillate, total peak areas (tpa) of total ion chromatograms were expressed as mg of the internal standard diisopropyl disulfide (DiPDS). Surprisingly, distilled onion oil yield displayed a close correlation with the EPY values (Table 3). For a qualitative comparison of the respective cultivars, 21 major peaks (substances marked in Table 4 by an asterisk) were selected. The sums of the areas of these 21 peaks were in good correlation with the total peak area ($R^2 = 0.987$), nonetheless contributing only about 50% to the latter. A large proportion has to be assigned to unidentified substances and elemental sulfur. Neither among the screened cultivars nor at the different sulfur fertilization levels the single percent areas of the respective 21 compounds were statistically distinguishable (Kruskall-Wallis ANOVA on ranks, chromatograms and data not shown). Although limited equipment allowed only determinations

in duplicate, a tendency towards higher proportions (45% to 60%) of the four summed up dimethyl homologous DMDS, DMTS, DMTeS, DMPeS (abbr. see Table 4) with increasing pungency was observable. As ACSO ratios proved rather uniform (Table 2), these changes may be ascribed to the instability of the 1-propenyl groups leading with higher concentrations to more unknown reaction products, thus overrating the more stable methyl-containing sulfides. Mass spectra of a considerable number of remaining unknown peaks were characterized by fragments with m/z 45, 59 and 99 which are likely to originate from a 1-propenyl residue⁴⁰. Dimethyl trisulfide (40%) was found to be the most abundant compound. Whereas other workers^{19,20,26,42,43} found dipropyl disulfide (DPDS) to be a major component of onion oil, DPDS was a minor constituent in this study (<1% of total peak area), which is in agreement with other authors^{18,24,25,41}. From these inconsistencies it can be assumed, that the DPDS content and consequently the quality of onion oil is strongly influenced by external, partly unknown factors in preparation, rather than on variabilities in the ACSO proportions.

Headspace volatiles by HS-SPME-GC/MS: Due to thermal degradations, cyclization and Maillard reactions during sample preparation and analysis, SDE-chromatograms are extremely complex (>180 peaks detected). Because of the reactivity and volatility of *Allium* aroma substances, adherence to a stringent procedure is a prerequisite to acquire reproducible data^{44,45}. Providing the possibility of good control over sampling conditions in terms of time and temperature, a SPME method, offering rapid and solvent free sample preparation, was evaluated for semiquantitative analysis^{44,46,47}. In respect to homogeneity of sample material, onion juice was used for sampling. In Figure 1a a typical chromatogram from the headspace of a fresh juice sample is presented. Bis-1-propenyl disulfide isomers (# 12,13, Table 4), *E/Z* 1-propenyl methyl trisulfides (# 17,18), *E/Z* 1-propenyl propyl trisulfide (# 22,23) and the unknown peaks # 24 and # 25,26 (*E/Z* isomers) were the predominating peaks. Since it reproducibly appeared in all GC-runs as a major peak and no record of such spectrum was found in the literature reviewed, the spectrum of the unidentified peak # 24 is shown in Figure 2. The series of fragments with m/z 32n_(n=1-5) and their relation to the M+2 isotopes may point to a S₅ or S₄O₂-derivative. In contrast to the HS of fresh juice (Fig. 1a), the unknown peaks # 24-26, comprising up to 25% of the total peak area, were completely absent in SPME-chromatograms from onion oil headspace (Fig. 1c). Since thiosulfonates cannot be

Table 4. Table of major substances from the headspace of fresh (F) and heated (H) onion juice, transferred by solidphase microextraction (cv. Golden Bear).

Peak#	compound (abbreviation)	Mw	ID	SPME-F[% area]	SPME-H[% area]
1	* dimethyl disulfide (DMDS)	94	R, MS	0.1 ± 0.02	3.4 ± 0.23
2	* 2-methyl 2-pentenal (2Me2Pe)	98	R, MS	1.1 ± 0.20	0.9 ± 0.02
3	* 2,4-dimethyl thiophene	112	MS	0.5 ± 0.09	1.3 ± 0.08
4	* 3,4-dimethyl thiophene	112	R, MS	0.6 ± 0.08	2.7 ± 0.17
5	* methyl <i>E/Z</i> -1-propenyl disulfide (MPeDS) I	120	MS	1.2 ± 0.57	3.1 ± 0.15
6	* methyl propyl disulfide	122	R, MS	0.1 ± 0.15	2.9 ± 0.23
7	* methyl <i>E/Z</i> -1-propenyl disulfide (MPeDS) II	120	MS	3.5 ± 0.41	1.5 ± 0.35
8	* dimethyl trisulfide (DMTS)	126	R, MS	1.2 ± 0.29	18.8 ± 1.01
9	* dipropyl disulfide (DPDS)	150	R, MS	4.4 ± 0.69	1.4 ± 0.14
10	* <i>E/Z</i> -1-propenyl propyl disulfide (PePDS) I	148	MS	1.0 ± 0.01	2.1 ± 0.08
11	* <i>E/Z</i> -1-propenyl propyl disulfide (PePDS) II	148	MS	5.6 ± 0.60	3.8 ± 0.18
12	bis <i>E/Z</i> -1-propenyl disulfide (PePeDS) I	146	MS	7.4 ± 0.24	2.5 ± 0.19
13	bis <i>E/Z</i> -1-propenyl disulfide (PePeDS) II	146	MS	9.1 ± 0.30	0.3 ± 0.07
14	unidentified isomer I #14/19 [113 (100), 79 (30), 45 (28), 97 (27), 77 (24), 111 (21), 146 (20), 112 (12), 85 (10)]			2.4 ± 0.33	0.1 ± 0.02
15	methyl allyl trisulfide (MATS)	152	R, MS	0.2 ± 0.03	1.6 ± 0.11
16	* methyl propyl trisulfide (MPTS)	154	R, MS	0.9 ± 0.24	21.5 ± 1.33
17	* methyl <i>E/Z</i> -1-propenyl trisulfide (MPeTS)	152	MS	7.6 ± 1.59	4.3 ± 0.31
18	* methyl <i>E/Z</i> -1-propenyl trisulfide (MPeTS)	152	MS	12.2 ± 1.16	6.1 ± 0.60
19	unidentified isomer II # 14/19			3.9 ± 0.43	0.1 ± 0.03
20	* dimethyl tetrasulphide (DMTS)	158	R, MS	2.1 ± 0.53	6.9 ± 0.50
21	* dipropyl trisulfide (DPTS)	182	R, MS	0.9 ± 0.18	3.3 ± 0.07
22	* <i>E/Z</i> -1-propenyl propyl trisulfide (PePTS) I	180	MS	5.8 ± 0.02	5.0 ± 0.45
23	* <i>E/Z</i> -1-propenyl propyl trisulfide (PePTS) II	180	MS	3.5 ± 0.07	5.0 ± 0.34
24	unidentified compound [spectrum Fig 2]			14.1 ± 0.91	0.1 ± 0.00
25	unidentified isomer I # 25/26 [73 (100), 147 (73), 45 (50), 41 (20), 39 (19), 64 (14), 138 (13), 146 (10)]			5.6 ± 0.54	0.6 ± 0.29
26	unidentified isomer II # 25/26			5.3 ± 0.17	0.6 ± 0.10

*Peaks included in SDE comparison, except for dimethyl pentasulfide (DMPeS)

Mw: mol weight/molecular ion

ID: Identification with mass spectra (MS), with retention times and spectra of reference substances (R)

transferred via the PDMS-SPME fiber⁴⁸, the disulfides detected are thought to be formed over the headspace or to be artefacts generated during GC analysis¹⁶. Onion oil consists of substances originated from the heated headspace. Consequently, we attempted to simulate such conditions by heating the sample. Heat treatment of the juice prior to SPME-sampling resulted in chromatograms obviously approaching towards the characteristic HS-profile of onion oil (Fig. 1b-c). Chromatograms displayed less complexity and peak assignment was easier to accomplish. Some major peaks, like the above mentioned unknown peaks (# 24-26), were characterized by their thermolability. 26 of the most abundant substances from fresh and heated juice headspace, representing >90% of the total peak areas, are listed in Table 4. In Table 3 the total amounts of volatiles, expressed as µg heptanol g⁻¹ juice from HS analyses are shown. For the heated samples a remarkably higher correlation of the total peak areas with the SDE or EPY values compared to the fresh juice was found. Presumably this is associated with the unstable unknown substances (peaks # 24-26), since extracting peak areas from groups like dimethyl homologues (DMDS, DMTS, DMTeS) and thiophenes or 2-methyl-2-pentenal resulted in a strong correlation to SDE and EPY (R²>0.98) values. Heated samples were devoid of the unknown compounds and a good correlation with the SDE and EPY values was given for the

total peak area. Moreover reproducibility was slightly increased, as the averaged coefficient of variation (CV) was lowered from 4.7% for the fresh samples to 2.7% for the heated samples (Table 3). As a result of single substance comparison (ANOVA, n=3), no significant qualitative shift in the profile of the heated HS samples could be observed among different sulfur levels and different cultivars (data not shown). Randle et al.⁴⁹, employing GC/MS instrumentation, found with increasing sulfur fertility levels a significant shift in proportions from methyl- towards 1-propenyl-radicals of certain thiosulfonates in the juice. For the methyl derivatives determined in this study, a similar trend was not detected. Analogous to the SDE-chromatograms DPDS appeared as a minor component in both heated and fresh juice samples. However, varying the conditions of maceration and preparation we observed in separate examinations a striking variability of DPDS formation using the HS-SPME method. Yagami et al.⁵⁰ and Mazza et al.⁴⁵ also found different rates of DPDS development, depending on mode and extent of tissue disintegration. Nevertheless, more precise information about factors influencing the DPDS proportion in HS and onion oil is still demanded. Small scale preparation with a SPME methodology offers a promising approach for these future investigations.

Conclusions

Generally, analysis of different markers along the stages of flavor development displayed a consistent and conclusive pattern. This investigation, however, comprised only a restricted number of cultivars. A good overall correlation of the widely used EPY value with the other flavor related parameters determined underlines its usefulness for screening programs. Furthermore, different onion cultivars grown under identical conditions exhibited only minor differences in ACSO ratios. Likewise the profile was unaffected by sulfur fertilization under conditions of fieldgrowing. Thus, qualitative differences in onion oil are mainly governed by their thermal history, rather than by different ACSO proportions. Solidphase microextraction proved to be a method of choice for a semiquantitative rapid determination of flavor potential as demanded from the aspect of raw material assessment. Exact control of heating of the juice prior to headspace sampling provides a good estimate for the yield of distillate and simplifies peak assignment. With the background of the rather stable ACSO ratios and to speed up analysis time the potential significance of selecting marker peaks (e.g. dimethyl di-, tri-, and tetrasulfides) is an option to be evaluated in more detail. Correlation coefficients in this study were surprisingly high, however SPME was only evaluated with a limited number of replications, hence a more comprehensive analytical validation should be a future task.

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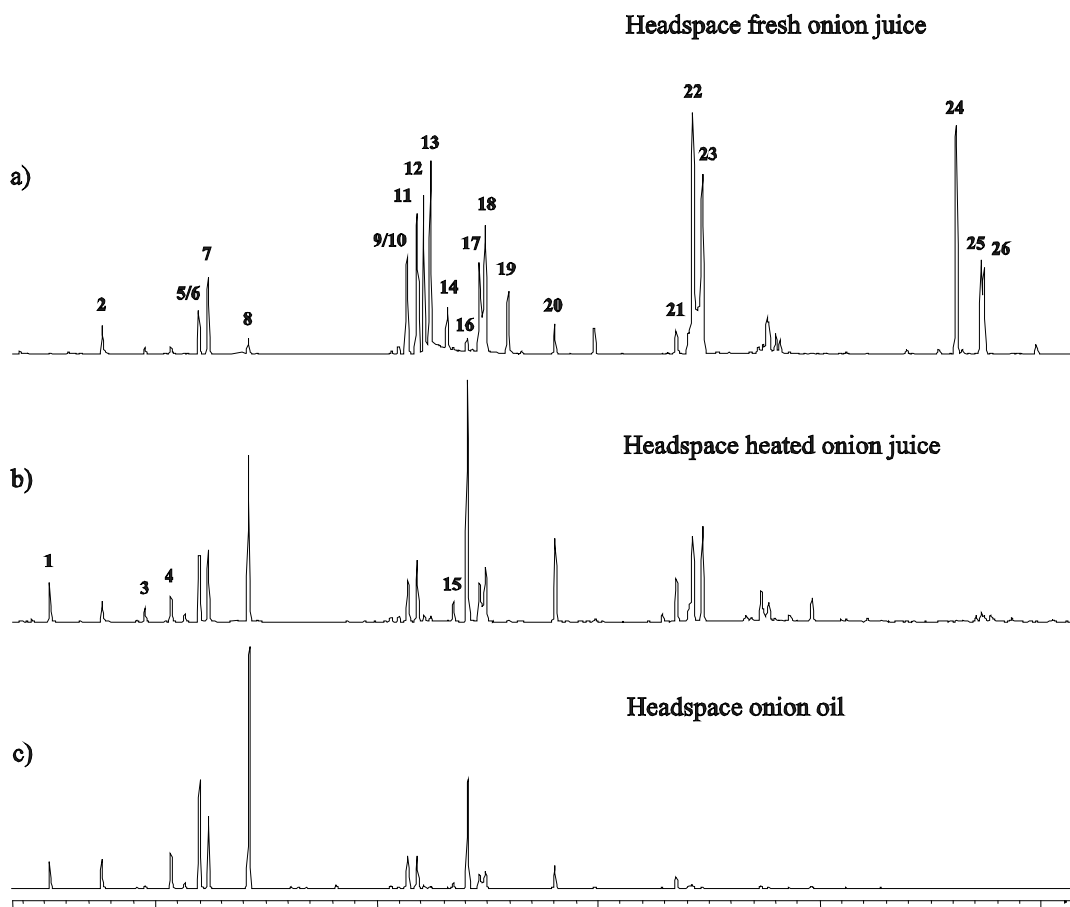


Figure 1. Headspace chromatograms from fresh onion juice (a), heated onion juice (b) and onion oil (c), transferred by solidphase microextraction.

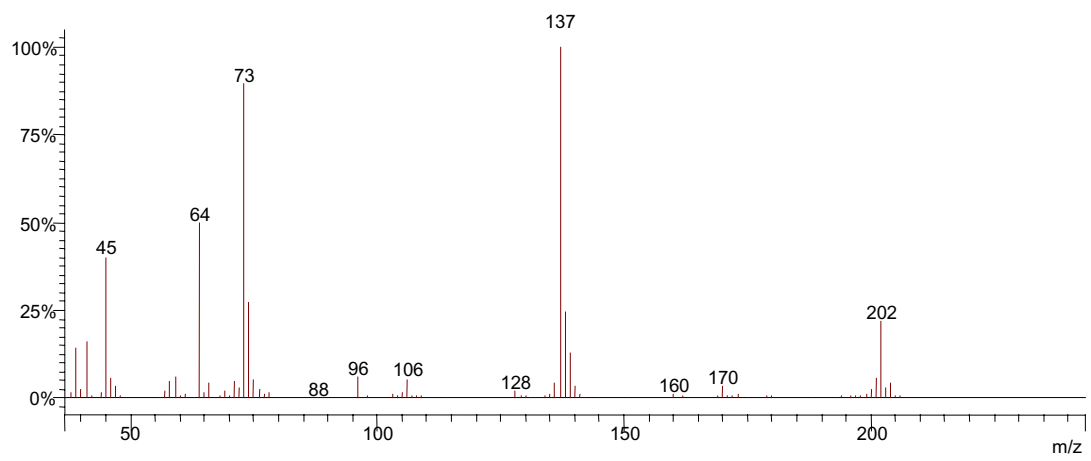


Figure 2. Mass spectrum (EI) of the unidentified compound # 24.