SPME-GC-MS analysis of volatile compounds in Czech white wines from five grape varieties

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29 samples of white wines, produced from five varieties of Vitis vinifera *L. grown in South Moravia, were analysed by Solid Phase Micro-Extraction (SPME) in the vapour phase by using a CAR-PDMS fibre. With the direct extraction of wine samples by SPME, statistical evaluation resulted in clear-cut discrimination of two groups of varieties, 'Riesling' and 'Gewürztraminer' on the one hand, and 'Pinot gris', 'Sauvignon blanc' and 'Grüner Veltliner' on the other. The dominant terpenes found in the analysed wines were linalool and terpineol. The variety 'Riesling' had concentrations of 185.2 µg/l and 127.8 µg/l, respectively, and the variety 'Gewürztraminer' contained lower concentrations (linalool 103.2 ± 29.8 µg/l, terpineol 65.3 ± 29.1 µg/l, citronellol 42.4 ± 11.5 µg/l, geraniol 179.4 ± 67.4 µg/l, nerol < 26.19 µg/l (= LOD - limit of detection).*
Keywords: grape varieties, SPME-GC-MS-analysis, aromatic composition, terpenes, esters

SPME-GC-MS-Analyse flüchtiger Substanzen in tschechischen Weißweinen fünf verschiedener Rebsorten. *Neunundzwanzig Weißweine fünf verschiedener Rebsorten aus dem Anbaugebiet Südmähren wurden mittels Solid Phase Micro-Extraction (SPME) in der Dampfphase unter Verwendung von CAR-PDMS-Fasern analysiert. Nach der direkten Extraktion der Proben mittels SPME zeigte die statistische Auswertung eine deutliche Differenzierung zweier Sortengruppen, 'Riesling' und 'Gewürztraminer' einerseits, und 'Pinot gris', 'Sauvignon blanc' und 'Grüner Veltliner' andererseits. Die vorherrschenden Terpene in den analysierten Weinen waren Linalool und Terpineol. Die Sorte 'Riesling' wies Konzentrationen von 185,2 µg/l bzw. 127,8 µg/l auf, die Sorte 'Gewürztraminer' enthielt niedrigere Konzentrationen (Linalool 103,2 ± 29,8 µg/l, Terpineol 65,3 ± 29,1 µg/l, Citronellol 42,4 ± 11,5 µg/l, Geraniol 179,4 ± 67,4 µg/l, Nerol < 26,19 µg/l (= NWG - Nachweisgrenze).*
Keywords: Rebsorten, SPME-GC-MS-Analyse, Aromazusammensetzung, Terpene, Ester

Analyse de substances volatiles par SPME-GC-MS dans des vins blancs tchèques de cinq cépages différents. *Vingt-neuf vins blancs de cinq cépages différents de la région viticole Moravie du Sud (Südmähren) ont été analysés par microextraction sur phase solide (Solid Phase Micro-Extraction, SPME) dans la phase vapeur en utilisant des fibres CAR-PDMS. Après l'extraction directe des échantillons par SPME, le dépouillement statistique a montré une différenciation claire entre deux groupes de cépages, à savoir 'Riesling' et 'Gewürztraminer' d'un côté et 'Pinot gris', 'Sauvignon blanc' et 'Grüner Veltliner' de l'autre côté. Les terpènes prédominants dans les vins analysés étaient le linalool et le terpinéol. Le cépage 'Riesling' présentait des concentrations de 185,2 µg/l et/ou 127,8 µg/l, le cépage 'Gewürztraminer' contenait des concentrations moins élevées (linalool 103,2 ± 29,8 µg/l, terpinéol 65,3 ± 29,1 µg/l, citronellol 42,4 ± 11,5 µg/l, géraniol 179,4 ± 67,4 µg/l, nérol < 26,19 µg/l (= limite de détection).*
Mots clé : cépages, analyse par SPME-GC-MS, composition aromatique, terpènes, ester

Wine is a very complex beverage and many volatiles contribute to its final aroma. Varietal wines are usually classified by monitoring the content of volatile aroma using techniques of GC and various statistical methods. The aroma of wines consists of a mixture of substances and up to date some hundreds of different compounds

have been described in literature (RAPP, 1988). This makes the instrumental analysis of wine aroma a difficult task. Depending on the nature of analytes to be determined, the instrumental analysis of wines involves a number of techniques comprising both spectroscopic and separation methods.

GC analysis of less volatile compounds was carried out mainly by liquid-liquid extraction. The quantification of minor compounds needs a prior concentration step such as liquid microextraction with Freon 113 (FERREIRA et al., 1993) or extraction with dichloromethane. The analysis is usually performed by GC with Flame Ionization Detection (FID) or mass-selective detection (ORTEGA et al., 2001). Compared to other extraction techniques (solid-phase extraction, Purge-and-Trap, supercritical fluid extraction, etc.) SPME is an appropriate technique for the quantitative analysis of the major analytes (ethyl acetate, methanol, 1-butanol, 2-butanol, 1-propanol, isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol) in wine (ROCHA et al., 2001; MARÍN et al., 2002; RODRÍGUEZ-BENCOMO et al., 2003; AZNAR and ARROYO, 2007). This technique has found several applications in headspace analysis in wine, both for the evaluation of single compounds and for characterizing complex aromas. The analytical properties of the SPME fibres vary depending on the extraction conditions and the composition of the sample matrix (TAT et al., 2005; ROCHA et al., 2006; CÁMARA et al., 2007). SPME is equally quick and efficient as Purge-and-Trap (P&T) methods for headspace analysis under dynamic or static conditions (POVOLO and CONTARINI, 2003).

The objective of this work was to analyse selected volatile compounds, especially terpenes, from samples of wine made in South Moravia and to determine if each variety has a unique profile by which it could subsequently be recognised.

Material and methods

Preparation of samples

Stabilised and bottled wines made from *Vitis vinifera* varieties (vintages 2003 to 2006), were collected and stored at +3 °C (Table 1). The SPME fibres and the SPME holder for manual sampling used in this study were purchased from Supelco (Bellefonte, PA, USA) and the PMDS coated fibre was chosen for measuring and validation studies. Volatiles from the wine samples were extracted by HS-SPME. Optimal conditions

Table 1: List of selected wines

	Variety	Quality	Production
1	Grüner Veltliner	Spätlese	Reisten
2	Sauvignon blanc	Spätlese	Mikrosvin
3	Riesling	Spätlese	Nove vinarstvi
4	Pinot gris	Spätlese	Nove vinarstvi
5	Sauvignon blanc	Auslese	Sonberk
6	Riesling	Spätlese	Jiri Hort
7	Grüner Veltliner	Spätlese	Jiri Hort
8	Pinot gris	Spätlese	Reisten
9	Sauvignon blanc	Spätlese	Jiri Hort
10	Grüner Veltliner	Spätlese	Mikrosvin
11	Pinot gris	Spätlese	Sonberk
12	Grüner Veltliner	Qualitätswein	Rodina Spalkova
13	Sauvignon blanc	Spätlese	Mikrosvin
14	Sauvignon blanc	Spätlese	Vinarstvi Kovacs
15	Grüner Veltliner	Spätlese	Mikrosvin
16	Grüner Veltliner	Spätlese	Vinarstvi Kovacs
17	Grüner Veltliner	Spätlese	Nove vinarstvi
18	Riesling	Auslese	Vinarstvi Kovacs
19	Sauvignon blanc	Spätlese	Reisten
20	Riesling	Kabinett	Reisten
21	Sauvignon blanc	Kabinett	Jiri Hort
22	Traminer	Auslese	Sonberk
23	Gewürztraminer	Auslese	Reisten
24	Gewürztraminer	Auslese	Vinarstvi Kovacs
25	Pinot gris	Spätlese	Vino Martincak
26	Pinot gris	Auslese	Reisten
27	Gewürztraminer	Spätlese	Vino Martincak
28	Riesling	Spätlese	Sonberk
29	Riesling	Spätlese	Reisten

for extraction were obtained using the following procedure: 1.2 ml of wine were transferred to a 2 ml vial (headspace volume: 0.8 ml), 1 µl of the internal standard 2-octanol (CAS 123-96-6, purity $\geq$ 97 %, Sigma-Aldrich) was added. The vial was closed and headspace extraction was performed for 20 min at 20 °C with 100 µm CAR^{TM}/PDMS fibre, while the sample was continuously stirred. The compounds were desorbed by inserting the fibre into the gas chromatograph injector for 3 min at 300 °C.

Retention times for all analysed compounds are listed in Table 2. The stock solutions were prepared by weighing terpene compounds separately and then dissolving them in 96 % (v/v) ethanol to obtain 30 µg in 50 ml of solvent. Various standard mixtures were then made by taking measured amounts of these solutions and adding them to a stock solution of 12 % (v/v) ethanol, to achieve a range of concentrations from 1.00 to 15.00 µg/ml. Linalool (CAS 78-70-6, purity $\geq$ 95 %, Fluka), terpineol (CAS 7785-53-7, purity $\geq$ 97 %, Fluka), DL-citronellol (CAS 106-22-9, purity $\geq$ 95 %, Sigma-Aldrich), nerol (CAS 106-25-2,

Table 2: Selected volatiles with retention times (R_t)

R_t (min.)	Compounds
3.06	Ethylbutyrate
3.94	2-Methylbutyl acetate
5.07	3-Methylbutanol-1
6.81	Ethyl lactate
7.86	Ethyl caprylate
8.23	Furfural
8.57	Decanal
8.88	Benzaldehyde
9.16	(+)-Linalool
9.67	Methylcaprinate
10.16	Ethylcaprate
10.69	Diethylsuccinate
10.86	Terpineol
11.60	DL-Citronellol
11.97	Nerol
12.13	2-Phenethyl acetate
12.34	Ethyllaurate
12.38	Caproic acid
12.45	Geraniol
13.23	2-Phenylethanol
13.57	1,4-Butanediol
15.19	Ethylmyristate
15.37	Diethylmalate
15.48	Octanoid acid
18.79	Methylpalmitate
19.40	Ethylpalmitate
19.79	Decanoic acid
21.09	Phenylethyloctanoate

purity ⩾ 97 %, Sigma-Aldrich), geraniol (CAS 106-24-1, purity 98 %, Sigma-Aldrich).

GC-MS conditions
The samples were analysed by GC-MS using a Finnigan Trace GC-ultra with a mass selective detector Finnigan Trace DSQ, using a DB-WAX column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Splitless injections were performed at 300 °C injector temperature for 3 min. The temperature of the column oven was increased in three steps: 55 °C (3 min) to 150 °C (5 min) to 200 °C (5 min) at a rate of heating

of 10 °C/min. The gas flow of the carrier gas helium was programmed from 1.0 ml/min (for 1 min) rising to 2.5 ml/min (for 3 min) at a rate of 0.2 ml/min and then rising to 3.5 ml/min (for 5 min) at a rate of 0.1 ml/min. The detection was performed by a Finnigan Trace DSQ with a full scan between 20.00 to 300.00 (EI+).

Method of validation
Synthetic solutions containing known amounts of terpene compounds were extracted and analysed by the described procedure. The concentration range of compounds studied is given in Table 3. The relative area was plotted against the relative concentration. The linear model was adjusted by the least-squares method. The limits of detection (LOD) were estimated as the concentration of the analyte that produced a signal-to-noise ratio of 3. The linear range experiments provide the necessary information to calculate the limits of detection, by extrapolating from the lowest concentration point. The limit of quantification (LOQ) can also be estimated as the concentration of analytes producing a signal 10 times that of the noise. The synthetic wine, which contained known amounts of these compounds, was extracted and analysed by the described procedure. The results were used to construct calibration graphs. Calibration graphs with the standards in aqueous solution were used for the terpenes.
Quantification was performed by GC-MS. Duplicate calibration graphs, at five concentration levels, were constructed by least-square linear regression using the results for a standard solution, using the same procedure as for the samples. The calibration graphs were linear with r^2-values between 0.9996 (DL-citronellol) and 1.000 (terpineol). The repeatability study, calculated from six analyses of a standard solution in 12 % ethanol (v/v) varied between 1.25 % (for DL-citronellol) and 12.1 % (for nerol).

Table 3: Validation parameters for analysis of terpenes by HP-SPME/MS using 100 μm PDMS fibre; 12 % (v/v) ethanol solution

Peak No	Compound	R_t	Range of concentration (ml/l)	a	b	r^2	LOD (μg/l)	LOQ (μg/l)	RSD (%)
6	(+)-Linalool	9.16	9 - 2633	23853	4947	0.9999	2.29	6.68	3.40
8	Terpineol	10.86	7 - 2240	-16532	2742	1.0000	1.94	5.83	3.65
9	DL-Citronellol	11.60	4 - 1097	-54539	4550	0.9996	0.95	2.86	1.25
10	Nerol	11.97	17 - 50028	-88004	2813	0.9999	7.83	26.19	12.10
13	Geraniol	12.45	16 - 4648	-70121	2174	0.9999	4.03	12.10	6.23

a = intercepts; b = slopes; r^2 = regression coefficients; LOD = limits of detection; LOQ = limits of quantification

Statistical analysis

The data for statistical analysis were based on the 15 analysed compounds (2-methylbuthyl acetate, 3-methylbutanol, ethyl lactate, furfural, ethyl caprylate, diethyl succinate, 2-phenetyl acetate, caproic acid, 2-phenyl ethanol, decanoic acid, (+)-linalool, terpineol, DL-citronellol, nerol, geraniol) obtained from the 29 wines used in this study. Prior to computing the discriminate functions by canonical correlation analysis, it was necessary to check for redundance. To establish the relationship between chemical composition and the grape variety, linear discriminant analysis was carried out using the Statgraphic program, version 7.1. These techniques were applied to the five grape varieties first, then for all the determined compounds taken together, and then also for the terpenes taken separately.

Results and discussion

Amount of selected volatiles in the wines

A chromatogram of volatile compounds detected by SPME analysis is shown in Figure 1. Table 4 summarises the non-terpene compounds identified in each of the five varieties. Likewise, the presence of other pre-fermentative and fermentative aromatic compounds, such as hexanols and hexenols, other alcohols, acetates, ethyl esters, fatty acids, and some volatile phenols, is also important, as many of them surpass their respective perception thresholds and therefore also contribute to the bouquet of these wines. The concentration of the aromatic compounds of fermentative origin ranged for 3-methylbutanol from 102 to 326 mg/l, for 2-methylbutyl acetate from 1.3 to 5.l mg/l and for ethyl lactate from 17.8 to 29.8 mg/l in wines of all five varieties ('Riesling', 'Sauvignon blanc', 'Grüner Veltliner', 'Ge-

würztraminer' and 'Pinot gris'). Other fermentative aroma compounds showed small differences but not big enough to be used as distinguishing parameters. CALLEJA and FALQUE (2005) reported that in red wines of Galicia only nine volatile compounds showed significant differences due to geographical origin (trans-3-hexenol, 1-butanol, isobutanol, ethyl acetate, hexyl acetate, butyric acid, δ-butyrolactone, methionol, and N-(2-hydroxy-ethyl)-acetamide).

Concentrations of terpene compounds

Terpenes determine the varietal aroma of wines and can be effectively used in the task of identifying the origin and variety of a given wine sample (RAPP, 1988). The average values for the free terpenes found in the studied wine samples are shown in Table 5. Differences in composition were observed between the varieties studied. The dominant terpenes found in the analysed wines were linalool and terpineol, with an average value of 185.2 µg/l and 127.8 µg/l, respecitvely, in the variety 'Riesling'. For comparison in the wines of the variety 'Gewürztraminer' the average content of linalool was 103.2 $\pm$ 29.8 µg/l and that of terpineol 65.3 $\pm$ 29.1 µg/l. The highest values of the monoterpen geraniol were found in wines of the variety 'Gewürztraminer' (Table 5).

Esti and Tamborra (2006) reported, that in red wines the amounts of terpineol (89.3 $\pm$ 12.6 µg/l) and geraniol (22.7 $\pm$ 3.9 µg/l) obtained by enzymatic hydrolysis of glycosidated precursors, is lower than in white wines. These compounds contribute to the pleasant flowery, citrus and tropical fruit odours of wines (COELHO et al., 2007). Using discriminant analysis based on ten main compounds (2-methylbutyl acetate, 3-methylbutanol, ethyl lactate, ethyl caprylate, furfural, diethyl succinate, 2-phenyl acetate, caproic acid, 2-phenyl

Table 4: Concentration of selected volatile compounds found in different wines (mg/l)

|	Riesling	Pinot gris	Sauvignon blanc	Gewürztraminer	Grüner Veltliner
	Means $\pm$ SE	Means $\pm$ SE	Means $\pm$ SE	Means $\pm$ SE	Means $\pm$ SE
3-Methylbutanol-1	214 $\pm$ 35	246 $\pm$ 30	257 $\pm$ 15	228 $\pm$ 45	251 $\pm$ 13
2-Methylbutyl acetate	1.37 $\pm$ 0.99	5.18 $\pm$ 3.12	3.54 $\pm$ 1.95	2.26 $\pm$ 1.03	3.55 $\pm$ 1.91
Ethyl lactate	17.8 $\pm$ 6.9	18.4 $\pm$ 7.6	19.4 $\pm$ 7.0	19.6 $\pm$ 6.6	29.8 $\pm$ 5.6
Ethyl caprilate	0.43 $\pm$ 0.15	0.52 $\pm$ 0.21	0.52 $\pm$ 0.12	0.70 $\pm$ 0.23	0.90 $\pm$ 0.18
Furfural	0.10 $\pm$ 0.05	0.08 $\pm$ 0.04	0.11 $\pm$ 0.06	0.05 $\pm$ 0.04	0.24 $\pm$ 0.08
Diethylsuccinate	2.75 $\pm$ 1.3	4.00 $\pm$ 1.9	0.82 $\pm$ 0.2	1.64 $\pm$ 1.1	3.79 $\pm$ 1.8
2-Phenyl acetate	0.06 $\pm$ 0.03	0.08 $\pm$ 0.04	0.05 $\pm$ 0.02	0.15 $\pm$ 0.08	0.13 $\pm$ 0.03
2-Phenylethanol	10.7 $\pm$ 2.3	19.2 $\pm$ 8.9	5.8 $\pm$ 1.2	15.5 $\pm$ 5.4	12.2 $\pm$ 4.2
Caproic acid	9.2 $\pm$ 2.9	6.9 $\pm$ 2.2	6.1 $\pm$ 0.7	9.8 $\pm$ 2.7	6.8 $\pm$ 2.5
Octanoic acid	10.3 $\pm$ 3.0	8.3 $\pm$ 4.3	4.8 $\pm$ 0.9	11.6 $\pm$ 4.4	9.2 $\pm$ 4.0
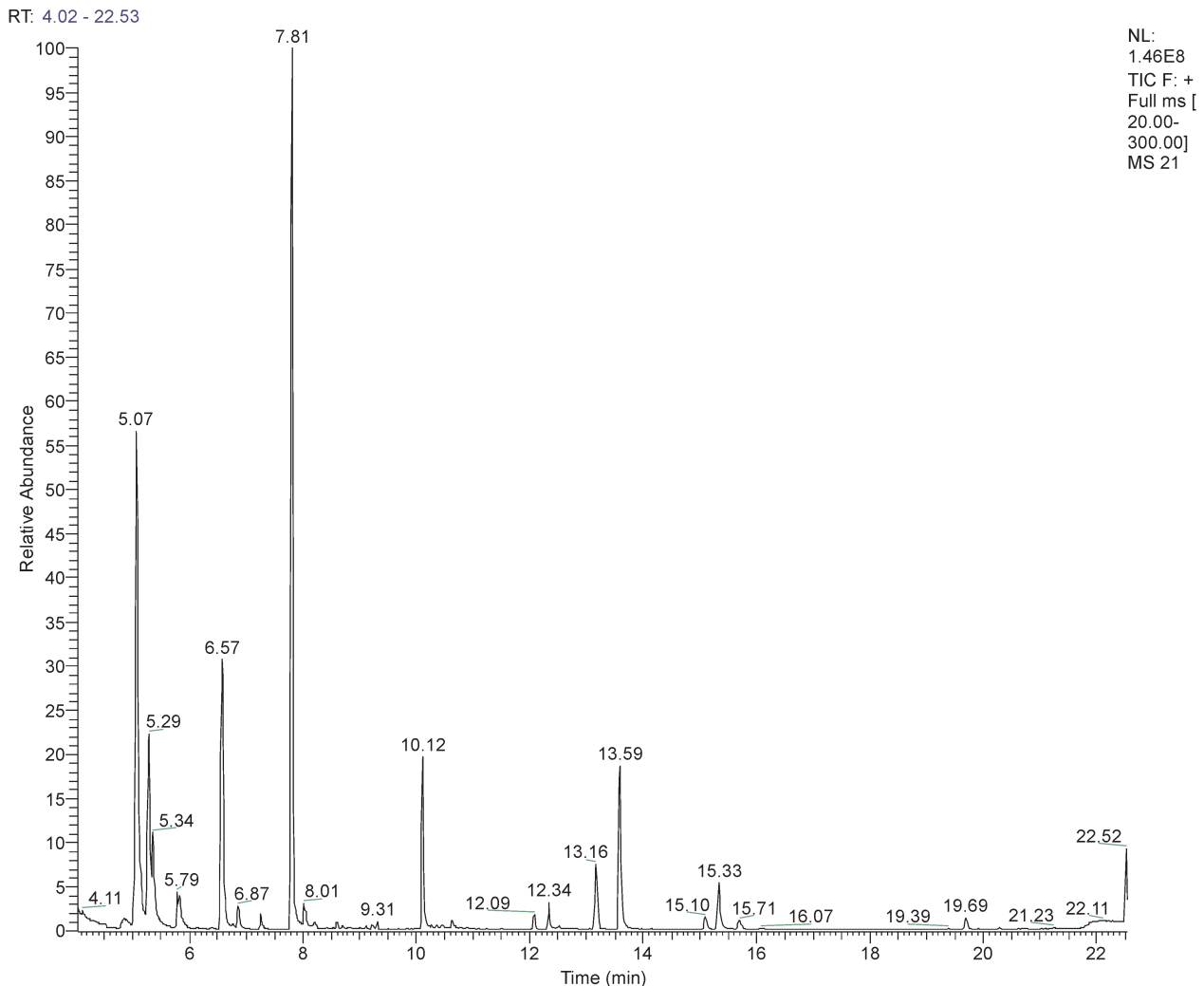
Fig. 1: Chromatogram obtained from SPME extraction. Peaks are identified by reference to retention times in Table 2. The observed minor discrepancies are due to sampling errors.

ethanol, octanoid acid) only, it was not possible to differentiate the varieties, but only a small degree of discrimination was demonstrated for 'Gewürztraminer' (Fig. 2). The results shown in Figure 3 indicate partial discrimination with fairly low variance for individual varieties (only a limited number of wines were analy-

Table 5: Concentration of monoterpenes found in different wines (µg/l)

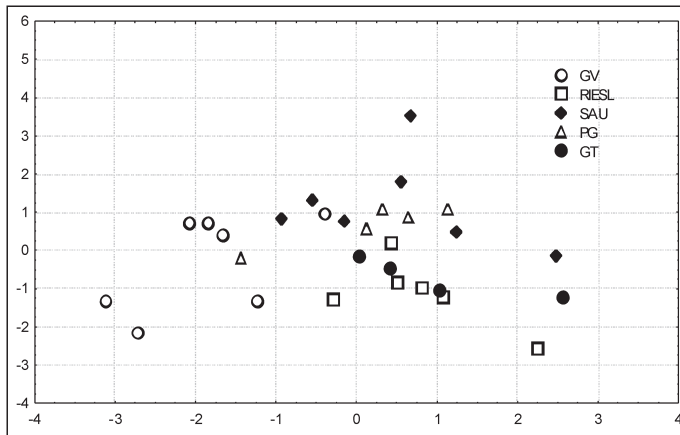|	Riesling	Pinot gris	Sauvignon blanc	Gewürztraminer	Grüner Veltliner
	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE
Linalool	185.2 ± 40.5	11.5 ± 6.1	20.2 ± 8.3	103.2 ± 29.8	15.4 ± 5.5
Terpineol	127.8 ± 23.0	16.0 ± 2.9	15.6 ± 3.3	65.3 ± 29.1	6.4 ± 2.6
Citronellol	8.4 ± 1.0	8.0 ± 2.6	6.8 ± 1.5	42.4 ± 11.5	6.7 ± 1.2
Geraniol	46.1 ± 7.1	9.8 ± 6.9	13.3 ± 3.9	179.4 ± 67.4	14.9 ± 2.7
Nerol	< 26.19	< 26.19	< 26.19	< 26.19	> 72.55

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Fig. 2: Discriminant analysis by origin for all samples of white wines; two first canonical discriminant functions for 10 main compounds
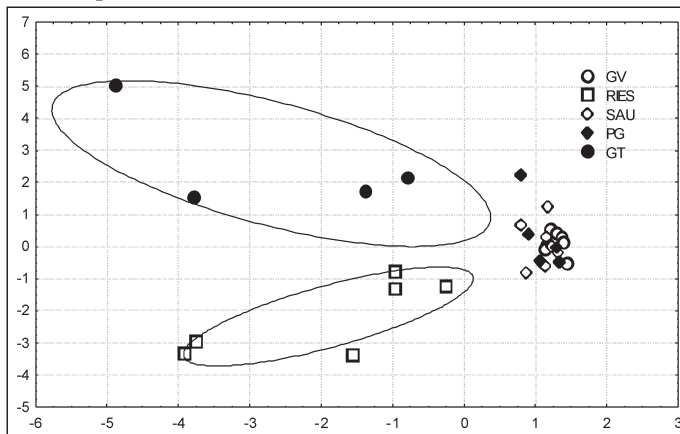

Fig. 3: Discriminant analysis by origin for white wines; two first canonical discriminant functions plotted for five terpene compounds only
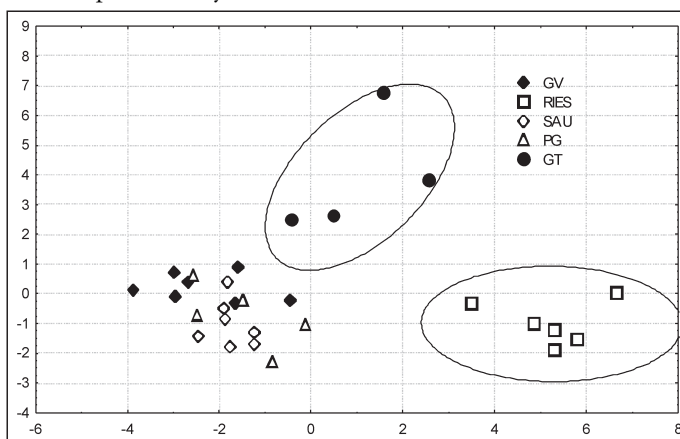

Fig. 4: Discriminant analysis by origin for white wines; two first canonical discriminant functions plotted for 10 main compounds and 5 terpenes together

sed) and a distinct separation between 'Riesling' and 'Gewürztraminer' and the other three varieties. Using discriminant analysis of monoterpenes (Table 3) it was possible to distinguish between 'Gewürztraminer' and 'Riesling', but other varieties were not separated (Fig. 3). Discriminant analysis between wines of 'Riesling' and 'Gewürztraminer' from all regions, using ten non-terpene compounds together with five monoterpenes (Fig. 4), has almost the same discriminant power as using terpenes alone.

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