Scientific paper

Analysis of Free and Bound Aroma Compounds in Grape Berries Using Headspace Solid-Phase Microextraction with GC-MS and a Preliminary Study of Solid-Phase Extraction with LC-MS

Helena Prosen,[a]* Lucija Janeš,[a] Matija Strlič,[a] Denis Rusjan,[b] Drago Kočar[a]

[a] *University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5 SI-1000 Ljubljana, Slovenia. Tel.: +38612419176, Fax: +38612419220, E-mail: helena.prosen@fkkt.uni-lj.si.*

[b] *University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia*

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Abstract

An extraction procedure for the aroma compounds from musts and wines has been developed, using solid-phase microextraction (DVB/CAR/PDMS fibre) from the headspace of heated samples (50 °C). Analysis was performed with gas chromatography – mass spectrometry. The method was applicable to the analysis of different aroma compounds (aliphatic, aromatic aldehydes, terpenes) in a broad concentration range (1–5000 μg L^{-1}). A stir-bar sorptive extraction procedure was also tested, but was not effective enough due to the lack of a suitable desorption device. Free aroma compounds in must samples of different grape varieties were analysed, and their release was evaluated after enzymatic or acidic hydrolysis. Different hydrolytic approaches were tested and the most successful was the enzymatic hydrolysis (with two different enzymes) and acidic hydrolysis at pH 3. Acidic hydrolysis at pH 1 resulted in substantial decomposition and re-arrangement reactions of terpenes. Non-hydrolysed terpene glycosides were extracted from the musts using solid-phase extraction and the extract was analysed with liquid chromatography – mass spectrometry (electrospray interface). Some compounds were tentatively identified as terpene glycosides.

Keywords: food aroma, grape, wine, extraction, chromatography, mass spectrometry,

1. Introduction

Wine aroma is one of the key properties determining its quality. It depends on several factors, e.g. first aroma of grapes, reactions during extraction of juice and maceration (hydrolysis, oxidation), biochemical reactions during fermentation, chemical and enzymatic reactions during the aging of wine. The most important of these factors is the grape aroma, which again depends on the grape variety and quality, soil and climate properties and also the winegrowing practice.[1]

Volatile compounds constituting the aroma of fresh grapes fall into several chemical families: terpenes, norisoprenoids, alcohols and polyols, aldehydes, organic acids, esters, methoxypyrazines, sulphur compounds.[1–7] The greatest contribution to a pleasant olfactory perception of aroma comes from the group of terpenes and norisoprenoids, while sulphur compounds and methoxypyrazines have a more unpleasant smell, but if carefully balanced add a more distinctive character to some grape varieties.[1,8–9] However, the sensory perception of grape aroma tells nothing of the actual concentrations of the involved compounds, as their olfactory thresholds can differ for a factor of 10^6 or more.[1,8–9] Low threshold values, meaning high contribution to the aroma in spite of low concentration, are typical for some monoterpene alcohols, methoxypyrazines,[1] sulphur compounds, norisoprenoids and some esters.[9]

The analytical method of choice for volatile aroma compounds is gas chromatography (GC), preferably with mass spectrometric (MS) detection,[3–4,7–17] although flame ionisation detection (FID) is also used.[5,9,11,18–21] It is sometimes combined with olfactometry[8] or aroma extract dilution analysis (AEDA)[9] in order to better evaluate the contribution of each compound to the overall aroma. Different extraction techniques are employed before the actual analysis with the aim of separating the compounds of interest from the matrix and to pre-concentrate them. While purge-and-trap,[14] liquid-liquid extraction (LLE)[8–9,14,16–17,21–22] and solid-phase extraction (SPE)[5,7,9–10,18–19] are

already well-established for this purpose, several publications have recently appeared using novel extraction techniques, e.g. simultaneous distillation-extraction (SDE),[15] solid-phase microextraction (SPME)[4,9,11–13,21] and stir bar sorptive extraction (SBSE).[3,15,21] Especially SPME and SBSE are promising as they are simple-to-use, solvent-free and efficient.

Beside the free volatiles constituting the aroma, odoriferous compounds, especially monoterpenols, terpene polyols and norisoprenoids are also present in grapes in the form of diglycosides, involving glucose, arabinose, rhamnose and apiose.[1–2,6,23] These glycosides are non-volatile and odourless, but rather constitute the aroma potential of a grape variety since they are present in much higher concentrations than the free flavour compounds.[2] Only a small part of them is hydrolysed during the various stages of wine production, e.g. due to endogenous or yeast glycosidases[1] or because of the acidic medium during wine ageing.[2] However, odoriferous compounds can be released from their glycosides by adding exogenous glycoside hydrolases to the must or wine,[2,6,23–24] resulting in an enhanced bouquet and therefore superior wine quality.

Presently, the established method for diglycoside analysis in grapes involves an extraction step, usually SPE,[10,20,22,25] followed by enzymatic[2,10,16,20,22,24,25] or acidic hydrolysis.[2,10] After the hydrolysis, free aglycones are extracted (SPE) and analysed, mainly by GC-MS.[10,25] Other methods are rarely used, e.g. enzyme analysis of liberated glucose after hydrolysis,[20] Fourier-transform infrared spectrometry,[22] droplet countercurrent chromatography or HPLC with fast atom bombardment tandem mass spectrometry.[26]

Our aim in the present work was to develop a fast and simple method for the screening of the flavour compounds in grapes. We have also tried some hydrolytic approaches for the release of bound flavour components from their glycosilated form in order to assess the aroma potential of different grape varieties. Three aromatic and three non-aromatic varieties were chosen to compare the differences in their aroma potential. The emphasis was put on locally grown varieties, for which the aroma potential has – to our knowledge – never been assessed before. We also report the preliminary results of LC-MS analysis of terpene disaccharides extracted from grape berries.

2. Experimental

2.1. Materials

Standard compounds used in this study were monoterpenes α-terpineol (99% purity), nerol (90% purity), geraniol (96% purity), linalool (97% purity), all from Fluka (Buchs, Switzerland); as well as aldehydes benzaldehyde (puris., Riedel-de Haën, Seelze, Germany), hexanal (98% purity, PolyScience, Niles, IL, USA), and (E)-2-hexen-1-al (97% purity, Fluka, Buchs, Switzerland). Solvents *n*-hexane, acetone, methanol and acetonitrile were of HPLC grade purity, obtained from Sigma-Aldrich (Steinheim, Germany). Other chemicals used were of p.a. grade quality from different producers. Pectinolytic enzymes for wine clarification with side glycosidase activity were Rohavin MX from AB Enzymes (Darmstadt, Germany) and Lallzyme BETA from Lallemand (St. Simon, France).

For solid-phase extraction, Supelclean C18 (1 g) extraction cartridges from Supelco (Bellefonte, PA, USA) were used. For stir bar sorptive extraction (SBSE) a stir bar coated with polydimethylsiloxane (PDMS) coating, dimensions 20 × 1.0 mm from Gerstel (Mülheim an der Ruhr, Germany) was used. For solid-phase microextraction, manual holder was used and fibres with different coatings: polydimethylsiloxane (PDMS), 100 μm, polyacrylate (PA), 85 μm, polydimethylsiloxane-divinylbenzene (PDMS/DVB), 65 μm, Carbowax-divinylbenzene (CW/ DVB), 65 μm, divinylbenzene-Carboxen-polydimethylsiloxane (DVB/CAR/PDMS), 50/30 μm, all from Supelco (Bellefonte, PA, USA).

For gas chromatography, helium (>99.999%) from Messer (Gumpoldskirchen, Austria) was used. The analytical capillary column was VOCOL, dimensions 60 m x 0.25 mm (i.d.), film thickness 1.5 μm, from Supelco (Bellefonte, PA, USA).

Different grape varieties were screened for their flavour compounds and their glycoside precursors: aromatic varieties "Aurora", "Beograjska rana", "Muscat blanc", and non-aromatic varieties "Perlette", "Pinot noir", "Danijela". The listed varieties were selected to be as different as possible: locally and globally known, red and white, more and less aromatic, wine and table grape varieties.

2.2. Instrumentation

Gas chromatograph was HP 5890 Series with mass spectrometric detector (MSD) 6890 from Hewlett-Packard (Palo Alto, CA, USA).

For LC-MS/MS experiments, liquid chromatograph Perkin Elmer Series 200 from Perkin Elmer (Shelton, CT, USA) and 3200 QTRAP LC/MS/MS System equipped with ESI and APCI ion sources from Applied Biosystems/MDS Sciex (Foster City, CA, USA) were used.

The analytical balance was Mettler Toledo MX5 (Mettler Toledo, Kuesnacht, Switzerland), We used a Visiprep SPE Vacuum Manifold from Supelco (Bellefonte, PA, USA).

2.3. Preparation of Standard Solutions

Stock standard solutions of compounds were prepared by dissolving the weighed solid standard in methanol to obtain the concentration of 0.8–1.2 g L^{-1}. These solutions were kept in the refrigerator and were stable for several months. They were further diluted with *n*-hexane or

methanol to obtain working solutions for injection in gas chromatograph or water for extraction optimisation.

2.4. Solid-Phase Extraction (SPE)

Extraction cartridge was conditioned with 5 mL of methanol and 5 mL of deionised water. A filtered homogenisate of grape berries (cca. 20 g) diluted to 100 mL with deionised water was passed under vacuum through the cartridge using the vacuum manifold. The cartridge was rinsed with 20 mL of deionised water. Free aroma compounds were eluted with 8 mL of *n*-hexane and the remaining water was sorbed on solid sodium sulphate. The solvent was transferred into a conical test tube and evaporated to 2 mL under the stream of nitrogen on a water bath (approx. 50 °C).

Glycoside fraction was eluted with 10 mL of methanol. The solvent was transferred into a conical test tube and evaporated to dryness under the stream of nitrogen on a water bath (approx. 50 °C). The dry residue was reconstituted in 1 mL of methanol for subsequent LC analysis.

2.5. Stir-Bar Sorptive Extraction (SBSE)

A tentative procedure for SBSE without the thermal desorption unit was as follows: a sample solution (5 mL) was extracted for 30 min on a magnetic stirrer, stir bar was transferred to 2 mL of *n*-hexane and placed in an ultrasonic bath for 10 min. The hexane extract (1 μL) was injected into the gas chromatograph.

2.6. Solid-Phase Microextraction (SPME)

Fibre with DVB/CAR/PDMS coating was used for the extraction of aroma compounds from grapes. Grape berries were homogenised (cca. 20 g diluted to 100 mL with deionised water) and 5 mL of homogenisate was measured into a 20-mL headspace vial, crimped and thermostated for 15 min at 50 °C (for analyses of terpenes and benzaldehyde). An SPME fibre was inserted in the headspace and the compounds were sampled for 35 min. The fibre was subsequently inserted into the injector port of a gas chromatograph and desorbed for 10 min. The procedure for aliphatic aldehydes was the same except that they were sampled at room temperature (25 °C) for 15 min.

2.7. GC-MS Conditions

Volatile grape aroma compounds were analysed using a gas chromatograph with mass spectrometric detector. The temperature programme was: 50 °C (2 min) –10 °C min^{-1} –210 °C (40 min). Temperature of the injector was 250 °C, temperature of the detector was 280 °C. Injection volume of *n*-hexane/methanol solutions or extracts was 1 μL (splitless). SPME fibre was left in the injector for 10 min.

In the mass spectrometer, electron impact (EI) ionisation was used and the chromatograms were recorded in the total ion current (TIC) mode. Compounds were identified on the basis of their retention times (comparison with standards) and spectra using the searchable EI-MS spectra library (NIST02). The peak area for quantitation was measured either in TIC chromatogram or in an extracted ion chromatogram in the case of coelution with other compounds.

2.8. LC-MS Conditions

Extracts of glycosides in methanol were analysed using a liquid chromatograph coupled to mass spectrometer through electrospray ion source. HPLC column was Hypersil ODS (Agilent Technologies), dimensions 250 mm x 4 mm, 5 μm particles. Mobile phase was composed of acetonitrile (phase A) and 0.5% acetic acid in deionised water (phase B). Gradient elution was applied: 0–5 min 0% A, 5–60 min from 0% to 90% A and hold for 10 min. Mobile phase flow was 0.8 mL min^{-1}, injection volume was 10 μL.

Electrospray ion source (ESI) voltage was 5500 V and the temperature was 400 °C.

2.9. Hydrolysis of Terpene Disaccharides

Two protocols for the hydrolysis of terpene disaccharides were adopted: with pectinolytic enzymes for wine clarification with side glycosidase activity; acid hydrolysis.

Enzyme hydrolysis: an enzyme preparation (50 mg) was added to the homogenisate of grape berries (20 g) diluted to 100 mL with deionised water, pH adjusted to 4.5. The flask was sealed and placed in a water bath (40 °C) for 24 h.

Acid hydrolysis: a homogenisate of grape berries (20 g) diluted to 100 mL with deionised water was prepared and the pH was adjusted to either 3 or 1 with an addition of H_2SO_4 solution. The flask was sealed and placed in a thermostated oven (100 °C) for 30 min.

3. Results and Discussion

3.1. Optimisation of SPME Conditions

Several different fibres were initially tested to establish their efficiency for extracting the selected terpenes and benzaldehyde from the headspace of homogenised grapes: polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane-divinylbenzene (PDMS/DVB), Carbowax-divinylbenzene (CW/DVB), divinylbenzene-Carboxen-polydimethylsiloxane (DVB/CAR/PDMS), as shown in Figure 1. The latter (mixed) phase was found to extract the highest amount of all analytes except geraniol (see Figure 1), for which the PDMS fibre was slightly more efficient.

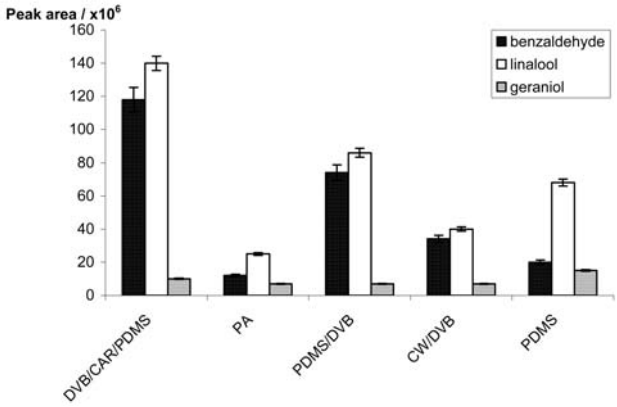

Figure 1. Comparison of different stationary phases on the SPME fibre for the extraction of grape aroma compounds.

These findings are in good agreement with the results obtained by Tat et al.,[11] who found the DVB/CAR /PDMS phase to be the most suitable for slightly less volatile and more polar compounds of wine aroma (t_R > 15 min), while for those with t_R < 15 min, the CAR/ PDMS fibre (which we did not test) was more suitable. However, the compounds of our interest are of the first type. In another study of SPME conditions,[12] CAR/PDMS fibre was also found to be the most suitable for the extraction of acids, aldehydes and esters in wine aroma, while DVB/ CAR/PDMS was not tested.

DVB/CAR/PDMS fibre was therefore chosen for further experiments. Next, the sample temperature during headspace sampling with SPME was optimised. The samples were placed in headspace vials, sealed and left in a water bath for 15 min to allow for equilibration at the chosen temperature. The septum was pierced with a SPME needle and the analytes were extracted from the headspace. We tested sampling at temperatures in the range 25–50 °C. At 50 ºC, the amount of analytes on the fibre was still increasing except for linalool, but we decided not to increase the temperature any further for safety reasons. The last step of the optimisation was to choose the optimal extraction time. The equilibrium amount of compounds on the fibre (at 50 °C) was reached at 25–35 min, so we chose 35 min as the optimal extraction time.

However, for SPME of the two aliphatic aldehydes, the chosen temperature (50 °C) was found to be too high: because of the higher volatility of these analytes and their relatively high concentration in the grape aroma, the headspace concentration was very high and the fibre phase was saturated already at lower concentration, leading to a sub-optimal linear range of the method for these compounds. The optimal conditions for SPME of hexanal and (E)-2-hexenal were found to be the extraction at room temperature (25 °C) for 15 min.

At the optimal sampling conditions, a part of the method evaluation was performed using the synthetic aqueous solutions of the analytes. The results are shown in Table 1. It is evident that the method shows good repeatability and excellent linearity over a wide linear range, making it suitable for determination of concentrations of aroma compounds both in aromatic and non-aromatic grape varieties.

3.2. Optimisation of SBSE Conditions

Due to the lack of suitable thermal desorption unit (TDU) for the sorptive stir-bar thermal desorption, we conducted a series of improvised experiments in SB sorptive extraction, the results of which hint at the method's real potential.

The sorption time was 1 h, after which the stir-bar was transferred into 2 mL of *n*-hexane and placed in an ultrasonic bath to desorb the analytes into the organic solvent. Although different variations to this procedure were tried, the best extraction recoveries obtained were 18–40% for the analysed terpenes, 17% for hexanal and an inexplicably high value of 208% for benzaldehyde (possible carry-over effect). The extraction recoveries for the same terpenes using TDU are reported to be 72–78%, but only 6% for benzaldehyde and 7% for hexanal.[15] In another study, no extraction recoveries for wine aroma compounds using SBSE are reported, although the authors conclude it is more efficient then SPME.[21]

3.3. Determination of Free Aroma Compounds in Grapes

In the preliminary experiments done on synthetic solutions, we established the optimal conditions for HS-SPME of aroma compounds. Grape berries (cca. 20 g)

Table 1: Parameters of the HS–SPME–GC–MS method for the quantified compounds.

compound	lin. range/ μ L^{-1}	r^2	t_R / min	RSD (t_R) / %	RSD (area) / %
α-terpineol	1.3–630	0.9962	24.0	0.07	2.3
nerol	1.6–790	0.9993	24.3	0.08	2.8
geraniol	2.7–530	0.9993	24.9	0.07	2.9
linalool	1.2–600	0.9940	20.6	0.07	3.1
benzaldehyde	1.0–970	0.9957	19.2	0.09	6.3
hexanal	19.4–1940	0.9987	14.1	0.05	7.1
(E)–2–hexen–1–al	5.1–5080	0.9980	15.7	0.04	6.9

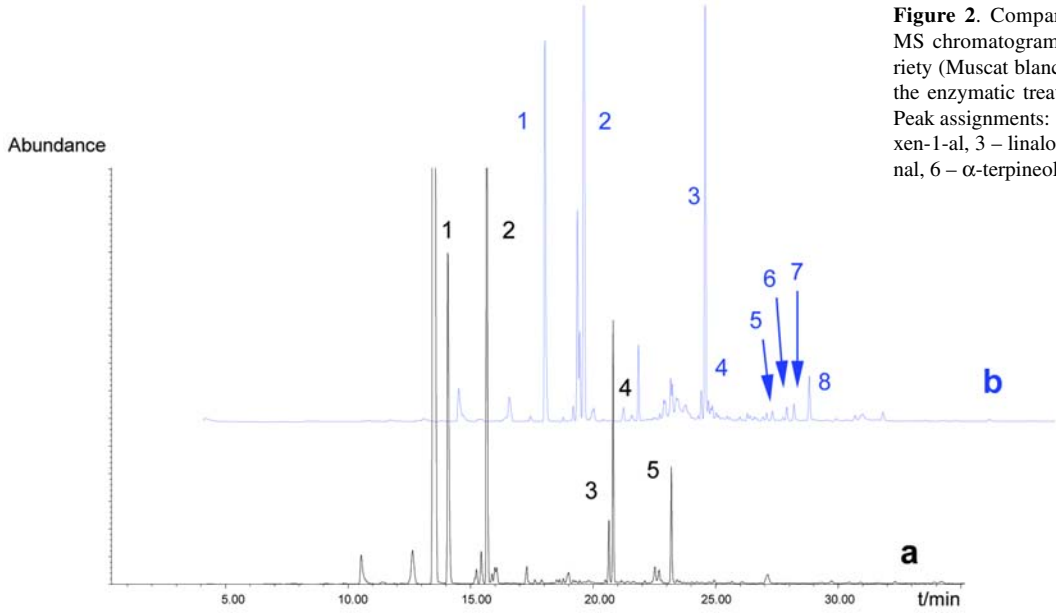

Figure 2. Comparison of HS-SPME-GC-MS chromatograms of the same grape variety (Muscat blanc) before (**a**) and after (**b**) the enzymatic treatment (Lallzyme BETA). Peak assignments: 1 – hexanal, 2 – (E)-2-hexen-1-al, 3 – linalool, 4 – nonanal, 5 – decanal, 6 – α-terpineol, 7 – nerol, 8 – geraniol.

were homogenised with water to add up to 100 mL of the final macerate. An aliquot (5 mL) was sealed in a headspace vial and extracted under the same conditions as synthetic solutions used for the evaluation of the method.

In Figure 2, track **a**, a HS-SPME-GC-MS chromatogram of a "Muscat blanc" grape variety is shown. The highest peaks in this chromatogram belong to the aliphatic aldehydes hexanal, (E)-2-hexen-1-al, nonanal and decanal, which were the prevailing compounds also in all other analysed grape varieties. In the subsequent experiments we separately analysed the pulp and the skins of grape berries. The results revealed that aliphatic aldehydes were present mainly in the skin: 55–65% more than in the pulp.

A small amount of terpenes (mainly linalool and geraniol) was present only in the most aromatic "Muscat blanc" and "Aurora" varieties. A part of the quantitative results for some compounds in three grape varieties is shown in Table 2, column 3 ("before hydrolysis").

3.4. Different Hydrolytic Approaches for the Release of Bound Aroma Compounds in Grapes

The glycosidically bound terpenes can be released by enzymatic treatment (glycosidase)[2,10,16,20,22,24,25] or by acidic hydrolysis.[2,10] We optimised and tested both.

Enzymatic hydrolysis of terpene diglycosides is usually a two-step process, involving two different enzymes, although one-step hydrolysis has also been described.[2,24] The glycosidases can be of plant or microbial origin, but are not readily available. A frequently used practical approach is to apply pectinase enzymatic preparations with substantial glycosidase side-activity.[2,10,16,25] We tested two such preparations: Rohavin MX from AB Enzymes and Lallzyme BETA from Lallemand. The later has, according to the producer, a strong glucosidase activity, and may be used to enhance the intensity of wine aroma.

Table 2: Concentration (in μg g^{-1} of grape berries; RSD 2–7%) of some aroma compounds in different grape varieties before and after hydrolytic treatment. ND – not detected.

grape variety	compound	before hydrolysis	Rohavin MX hydrolysis	Lallzyme BETA hydrolysis	acid hydrolysis, pH 3	acid hydrolysis, pH 1
Muscat blanc	linalool	0.15	4.40	5.27	2.79	0.02
	geraniol	0.11	0.33	1.00	0.31	ND
	benzaldehyde	0.04	ND	ND	ND	ND
	hexanal	34.4	15.9	20.9	23.2	4.31
Danijela	linalool	ND	ND	ND	ND	ND
	geraniol	ND	ND	ND	ND	ND
	benzaldehyde	0.03	0.06	0.03	0.03	0.04
	hexanal	6.87	1.08	11.6	4.45	1.68
Pinot noir	linalool	ND	ND	ND	ND	ND
	geraniol	ND	0.01	0.03	ND	ND
	benzaldehyde	0.02	0.05	0.02	0.02	0.01
	hexanal	43.9	8.39	19.0	13.2	1.72

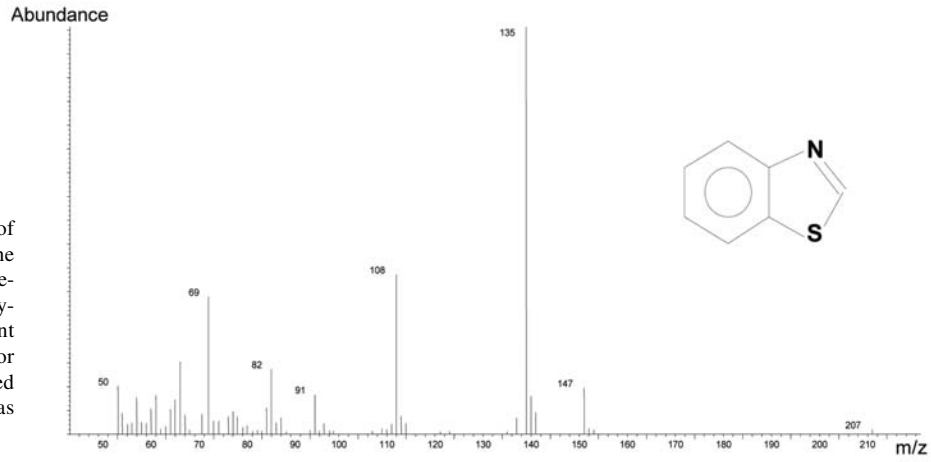

Figure 3. Mass spectrum of the compound present in the aroma of several grape varieties (see Table 3) after enzymatic hydrolytic treatment with either Rohavin MX or Lallzyme BETA (identified from its mass spectrum as benzothiazol).

We first conducted the hydrolysis with an amount of enzymes specified by the producers (5 mg/100 mL); however, no change in the aroma profile was observed after such a treatment. Glycosidases in general are inhibited by pH below 4[24] and presence of high levels of phenolic compounds,[25] while glucosidase is strongly inhibited by glucose.[24] Most of the research on the enzymatic hydrolysis of the terpene disaccharides was focused on the more-or-less glucose-free wine as sample,[24] therefore no clear guidelines are available about the amount of enzymes that should be used in the treatment of non-fermented musts. In the available literature on musts, enzymes were applied mostly on extracted glycosides,[10,22,25] although one study reports good results on a heat-treated must sample, using approximately the above quantity of the enzyme.[16]

Having in mind these difficulties, we used a ten-fold recommended amount of enzyme, incubating the macerate for 24 h at 40 °C. This approach was successful in releasing a substantial part of terpenes, as can be seen from Figure 2, track **b**, for the "Muscat blanc" grape variety. Linalool content was substantially increased, while at least three new terpenes appeared in the GC-MS chromatogram: nerol, geraniol and α-terpineol. A part of the quantitative results is shown in Table 2. Results obtained with Lallzyme BETA were slightly better than those for Rohavin MX, which is not marketed for the purpose of aroma-enhancement. As is also obvious from Table 2 and Figure 2, the enzymatic treatment had practically no effect on the benzaldehyde content, while the content of the aliphatic aldehydes was significantly decreased. The later fact is also favourable, as these compounds contribute to the unpleasant, "herbaceous" smell of wines.[17]

Another observation was that several new peaks appeared in the GC-MS chromatogram after hydrolysis. The compounds yielding these peaks were preliminary identified by the aid of the library of mass spectra, an example for seldom identified benzothiazole[16] is shown in Fig. 3. Table 3 lists some compounds that were most often observed only after the enzymatic treatment. Some of them are reported to appear in the aroma profile of musts and wi-

Table 3: Some compounds identified in the grape aroma only after hydrolytic treatment (identification from mass spectra).

Compound	Hydrolytic treatment	Grape variety
benzothiazole	EH–L, EH–R	Beograjska rana, Muscat blanc, Pinot noir, Perlette, Danijela
furfural	EH–L	Perlette
	AH3	Aurora
thymine	EH–L	Perlette
2–phenylethyl acetate	EH–R	Perlette
linalool oxide, pyranic form	EH–L, EH–R, AH1	Muscat blanc
	AH1	Aurora
2,2–dimethyl–5–(1–methyl–1–propenyl)– tetrahydrofuran	AH1	Muscat blanc, Aurora, Danijela
3,6–dihydro–4–methyl–2–(2–methyl–1–propenyl)–pyran	AH1, AH3	Muscat blanc
eucalyptol / 1,8–cineol	AH1	Aurora, Muscat blanc

Abbreviations: EH–R = enzymatic hydrolysis with Rohavin MX, EH–L = enzymatic hydrolysis with Lallzyme BETA, AH3 = acid hydrolysis at pH 3, AH1 = acid hydrolysis at pH 1.

nes, e.g. 2-phenylethyl acetate,[3,9,12,17] linalool oxide[7,13,16] and furfural.[16–17] These compounds contribute to sweet and fruity aroma of wines.[17]

Acidic hydrolysis was also tested as the means to release the bound terpenes. This procedure is thought to simulate the processes taking place during the storage and ageing of wines.[2,10] The hydrolysis was conducted at two different pH values: pH 3, resembling pH conditions in wine,[10] and pH 1, chosen as at these more drastic conditions rearrangement reactions of terpenes are probably induced.[2]

A part of the results is shown in Table 2. Acidic hydrolysis at pH 3 obviously results in a release of terpenes from glycosides, although to a lesser extent than enzymatic hydrolysis. Benzaldehyde concentration is not significantly affected, while the concentration of aliphatic aldehydes is decreased. After hydrolysis at pH 1, the content of monitored terpenes is even lower than before hydrolysis, which is in agreement with the literature data.[2] Aliphatic aldehydes are also significantly decomposed at these conditions. However, it is interesting to observe the release (or formation) of several compounds not noticed after enzymatic hydrolysis (examples in Table 3), e.g. eucalyptol (or 1,8-cineol), a typical product of too harsh hydrolytic conditions.[2]

3.4. LC-MS Analysis of Terpene Glycosides

We conducted some LC-MS analyses (electrospray interface) of non-hydrolysed terpene glycosides extracted from the grape berries. The results of these preliminary experiments were quite promising. An extracted LC-MS chromatogram (*m/z* 310-314) of the grape extract is shown in Figure 4. The ions at *m/z* 310-314 interval were chosen as possible fragments of the disaccharide part of terpene glycosides. At least two compounds eluting at t_R 25,0 min and t_R 30,9 min could be identified as terpene glycosides based on their mass spectra (example for the compound eluting at t_R 30,9 min shown as an insertion in Figure 4). Typical ions featuring in these spectra are at *m/z* 156, 174, which could be attributed to the aglycone (terpene) part of the terpene glycosides.[26] Ions at *m/z* 115 and 133 could be fragments of the monosaccharide moieties.[26] However, in spite of these interesting results, the content of terpene glycosides in the extract was obviously quite low and their peaks were not clearly visible in the chromatogram. Therefore, the extraction procedure seems to be less then satisfactory and has to be further optimised.

4. Conclusions

We developed an effective and rather simple extraction procedure for the aroma compounds from musts and wines, using solid-phase microextraction from the headspace of heated samples. Combined with GC-MS analysis, the overall method proved to be applicable to different aroma compounds (aliphatic, aromatic aldehydes, terpenes) in a broad concentration range (1–5000 μg L^{-1}). The extraction method was also compared with a stir-bar sorptive extraction procedure for the same samples, however, SBSE was not very effective due to the lack of a suitable desorption device.

The method was applied to the analysis of free aroma compounds in must samples of different grape varieties and to follow their release after enzymatic or acidic hydrolysis of bound aroma compounds. Different hydrolytic approaches were tried and the most successful in releasing terpene compounds was the enzymatic hydrolysis (with two different enzymes) and acidic hydrolysis at pH 3.

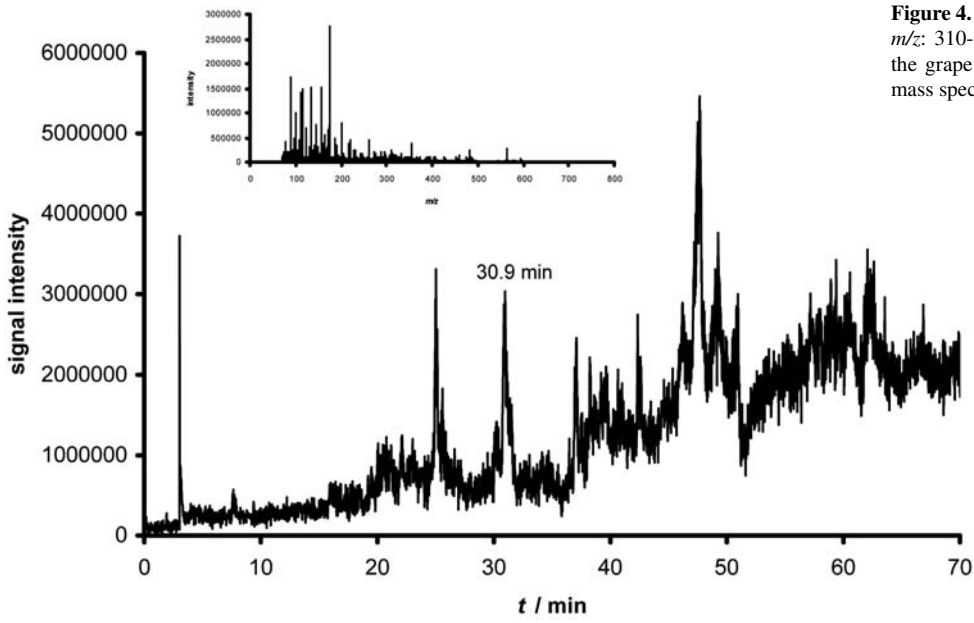

Figure 4. LC-MS chromatogram (extracted *m/z*: 310-314) of a glycoside extract from the grape variety Muscat blanc. Insertion: mass spectrum of peak at 30.9 min.

Acidic hydrolysis at pH 1 resulted in substantial decomposition and re-arrangement reactions of terpenes.

Non-hydrolysed terpene glycosides were extracted from the musts using solid-phase extraction and the extract was analysed with LC-MS (ESI interface). The preliminary results are quite promising, but the extraction procedure has to be further optimised.

5. Acknowledgements

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Povzetek

Razvili smo ekstrakcijski postopek za sestavine arome mošta in vina, kjer smo uporabili mikroekstrakcijo na trdno fazo (DVB/CAR/PDMS vlakno). Ekstrahirali smo iz plinske faze nad ogretim vzorcem (50 °C). Analiza je potekala s plinsko kromatografijo z masno spektrometrično detekcijo (GC-MS). Metodo smo uporabili za analizo različnih sestavin arome (alifatski in aromatski aldehidi, terpeni) v širokem koncentracijskem območju (1–5000 μg L^{-1}). Preizkusili smo tudi ekstrakcijo na mešalo s sorbentom (SBSE), vendar učinkovitost postopka ni bila zadovoljiva, saj nismo imeli na voljo primerne naprave za desorpcijo. V moštu različnih grozdnih sort smo analizirali proste sestavine arome, sledili pa smo tudi njihovemu sproščanju med encimsko ali kislinsko hidrolizo. Preizkusili smo različne postopke hidrolize. Najbolj učinkovita je bila encimska hidroliza (dvoje različnih encimov) in kislinska hidroliza pri pH 3. Med kislinsko hidrolizo pri pH 1 je prišlo do znatnih razgradenj in premestitev terpenskih spojin. Nehidrolizirane terpenske glikozide smo ekstrahirali iz mošta s pomočjo ekstrakcije na trdno fazo, ekstrakt pa smo analizirali s tekočinsko kromatografijo in masno spektrometrijo (LC-MS, ESI vmesnik). Nekatere spojine smo lahko identificirali kot terpenske glikozide.