

## **SOLID PHASE MICROEXTRACTION FOR PROFILING VOLATILE COMPOUNDS IN LIQUERED WHITE WINES\***

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**Background.** Profile of volatile compounds is a distinct feature of wine, which is dependent on the type of wine, grapes, fermentation and ageing processes. Profiling volatile compounds in wine using fast method provides information on major groups of compounds and can be used for classification/differentiation purposes. Solid phase microextraction (SPME) was used for the profiling of volatile compounds in liquered white wines in this study.

**Material and methods.** Different fibers were tested for this purpose: PDMS, Carboxene/PDMS, Carboxene/DVB/PDMS, Polyacrylate, Divinylbenzene/PDMS. Different times were compared to optimize extraction process. Profile and amount of volatile compounds extracted by SPME fiber was compared for eight liquered white wines.

**Results.** Carboxene/DVB/PDMS showed the highest efficiency in extracting higher alcohols, esters, carbonyls and terpenes. Of tested extraction times ranging from 5 to 30 min. 20 minutes was chosen providing sufficient peak responses. Using SPME total amount of volatile compounds in eight liquered wines was compared – Riversaltes, Offley Porto and Jutzenka having the highest amount of adsorbed volatiles. Profiles of volatiles of analysed wines revealed that dominating compounds in 6 wines were esters, followed by higher alcohols, two analysed Muscat wines had high terpene contents compared to remaining wines.

**Conclusion.** SPME can be used for relatively fast profiling of wine volatiles, that can be used for wines classification.

**Key words:** liquered wines, profiling, volatile compounds, SPME

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## INTRODUCTION

Wine is one of the most complex food products in terms of volatile compounds composition. Several hundred volatiles have been identified in wine, belonging to different chemical classes. Dominating classes of volatile compounds in wines are higher alcohols and esters, also carbonyls are present, acids, terpenes, norisoprenoids, sulfur compounds and pyrazines. Some groups, as terpenes or pyrazines are related to a specific wine/grape types [Mateo and Jimenez 2000, Sala et al. 2002]. Volatile compounds in wines are derived from grapes and transferred from must, formed in the fermentation process and during ageing, as a result of interactions between wine constituents, or extracted from the oak barrels used for ageing [Ebeler 2001]. Volatile compounds in wine are usually present in a concentrations ranging from mg/L down to a few ng/L. In the analysis of key odorants by gas chromatography – mass spectrometry and gas chromatography – olfactometry, extraction and preconcentration of these compounds, often present in trace concentrations pose a serious analytical challenge, both for the extraction and preconcentration step and for the detection and quantification process [Guth 1997, Siebert et al. 2005]. For the isolation of wine and grapes volatile compounds different sampling techniques are used, usually liquid/liquid extraction, static headspace [Ortega-Heras et al. 2002], solid phase extraction (SPE) – especially for fractionation of free and bound volatile compounds [Piñeiro et al. 2004], stir bar sorptive extraction (SBSE) [Zalacain et al. 2007], and solid phase microextraction (SPME) [Sánchez-Palomo et al. 2005, Peña et al. 2005]. Apart from target analysis of selected odorants, volatile compounds in wine are analysed also for the wines of different origin, variety and ageing comparison. For this purpose profiles of volatile compounds are usually compared and subjected to multivariate analysis methods to visualize groupings related to grape variety, region, country of origin etc. [Pozo-Bayón et al. 2001, Setkova et al. 2007 a]. For this purpose a fast method of extraction, which enables a full profile of extracted compounds, which would be sensitive, robust and fast is required. Solid phase microextraction (SPME) developed in late eighties by Pawliszyn and coworkers is a method that proved its suitability in the analysis of volatile compounds in food matrices and can be used for volatiles profiling. It offers uncomplicated manual sampling and thermal desorption in one syringe-like device, which can be used with all GC and GC/MS systems. Its advantage is selective extraction based on adsorption processes in polymer based fibers that provides high sensitivity and is routinely used in the target analysis of compounds responsible also for wine flavours and off-flavours [Jeleń 2006], and for profiling volatile compounds in wine [Setkova et al. 2007 a].

The goal of this study was a comparison of different SPME fibers in terms of their usefulness in extraction of main classes of volatile compounds in liquered white wines, with the contents of ethanol ranging from 15-19% and compare different wines in terms of their main groups of volatiles using SPME.

## **MATERIAL AND METHODS**

### **Wines**

Jutrzenka fortified white wine was used for SPME fiber and extraction times selection. The wine was produced in 2004 at Golesz Winery in Jasło. Jutrzenka wine is produced from the grapes of variety of the same name. Fortified (liquered) Jutrzenka has a 17% of ethanol. The desired contents of ethanol comes from the addition of distillate to wine. The distillate is produced from the same grape variety in a previous year. For volatile compounds profiles comparison seven other fortified wines were purchased in wine shops in Poznań. They were: Massandra Krymwejn (17% alc.), Moscatel de Setubal (17% alc.), Riversaltes (16% alc.), Leyenda Sherry Cream (18% alc.), Moscatel Oro (15% alc.), Marsala Superiore Riserva (18% alc.), and Offley White Porto (19% alc.).

### **SPME fibers**

SPME fibers were purchased from Supelco in a version for manual sample introduction. Manual holder was also purchased from Supelco (Poznań, Poland). The following fiber coatings were tested for the study: Polydimethylsiloxane (PDMS), Polyacrylate (PA), Carboxene/Divinylbenzene/Polydimethylsiloxane (C/D/PDMS), Divinylbenzene/polydimethylsiloxane (D/PDMS), and Carboxene/polydimethylsiloxane (C/PDMS). All fibers were preconditioned according to producers manual.

### **Gas chromatograph/mass spectrometer (GC/MS)**

All the analyses were performed on GC×GC/MS system Pegasus 4 (LECO, St., Joseph, MI). Gas chromatograph (Agilent 6890N, Agilent Technologies, Wilmington, DE) equipped with a split/splitless injection port, was coupled to time-of-flight (ToF) mass spectrometer (Pegasus 4). The MS was a fast time-of-flight system with a unit resolution designed for comprehensive (GC×GC) gas chromatography-mass spectrometry. The gas chromatograph was equipped with cryogenic modulator, secondary column and oven, but was run as a single dimensional system, where the secondary column served only as a transfer line and was kept at 30°C above the first oven temperature. Compounds were separated using DB-5MS column (25 m × 0.200 mm × 0.33 μm, Agilent Technologies). The secondary column was a midpolar BPX-50 (1.090 m × 0.100 mm × 0.1 μm, SGE, Australia). Injection port temperature was 260°C, and the same for all tested SPME fibers. Oven temperature was initially 40°C (kept for one minute), then increased at 10°C to 250°C. Temperature of secondary oven was programmed at 30°C more than the primary oven temperature (from 70°C to 280°C). GC/MS transfer line was kept at 280°C. Carrier gas (He) flow was 0.8 ml/min and was kept constant during the whole analysis. The spectra collection rate was set at 50 spectra/sec. in a range 33-433 Da. Temperature of the ion source was 240°C. Acquired spectra were processed using Chromatof 3.21 software (LECO). Coeluting peaks were identified after deconvolution process being the integral part of the software. Fiber comparison was based on the unique ions comparison for analysed compounds. Compounds used for comparison of fibers were listed in Table 1. All were identified tentatively using NIST 02 Mass Spectral Library.

Table 1. Compounds used for fibers and wines comparison

Compound classes	Compounds
Alcohols	1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-pentanol, 2-ethyl-1-hexanol, 1-octanol, phenylethanol
Esters	ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate, 2-methylpropyl acetate, ethyl butyrate, 2-pentenyl butyrate, ethyl 2-butenolate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, pentyl acetate, ethyl hexanoate, methyl octanoate, ethyl octanoate, isopentenyl hexanoate, ethyl nonanoate, methyl nonanoate, ethyl 9-decenoate, ethyl decanoate
Carbonyls	Furfural, benzaldehyde
Terpenes	p-cymene, 1-methyl-4-(1-methylethenyl)-cyclohexene, 3,7-dimethyl 1,3,6-octatriene, 1-methyl-4-(1-methylethylidene)-cyclohexene, 3,7-dimethyl 1,6-octadienol, 3,7-dimethyl 1,5,7-octatrien-3-ol, 3,7-dimethyl-1,6-octadien-3-ol 2-aminobenzoate, a,a-4-trimethyl 3-cyclohexene-1-methanol, 3,7-dimethyl 6-octen-1-ol, 1,2,3,4-tetrahydro-1,1,6-trimethyl naphthalene, 1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene, 3,7-dimethyl-2,6-octadien-1-ol, 1,2-dihydro-1,5,8-trimethyl-naphthalene, limonene, cis- $\alpha$ -ocimene, linalol, hotrienol, cis rose oxide, trans rose oxide, terpineol, geranyl vinyl ether, trans- $\alpha$ -bergamotene, cis- $\alpha$ -farnesene

### Sample preparation

Wine sample (20 ml) was placed in a 40 ml screw-top vial capped with a cap with a silicon rubber/teflon membrane and a hole enabling SPME sampling. The sample was preheated at 50°C for 15 minutes using IKA heater/stirrer prior to SPME sampling, after placing vials in an aluminum block. Immediately after sampling the fiber was desorbed in the injection port of GC/MS system working in a splitless mode (purge valve time – 1 min).

## RESULTS AND DISCUSSION

### Fiber comparison

Different fibers were tested for their efficiency in extracting different groups of compounds in fortified wine at the same conditions. Figure 1 shows a comparison of peak areas of main groups of compounds extracted from the Jutrzenka wine using tested 5 different fibers. By comparing all four graphs it is evident that C/D/PDMS fiber has the ability to extract the highest amounts of analysed compounds belonging to all classes. Its suitability for the extraction of alcohols is proved by an over twofold higher amount of adsorbed compounds compared to D/PDMS fiber. The lowest total area was noted for Polyacrylate fiber. In the analysis of esters also the C/D/PDMS fiber had the highest ability to extract these compounds from Jutrzenka wine, however PDMS fiber followed with almost the same recovery. For the extraction of aldehydes in this study (furfural and benzaldehyde) C/D/PDMS fiber was the most efficient. Moreover, it can be observed that fibers containing Carboxene in their structure performed much better than the remaining ones. It leads to a conclusion that carboxene (at least for these two analysed compounds) is the best adsorbent for aldehydes extraction. For terpenes

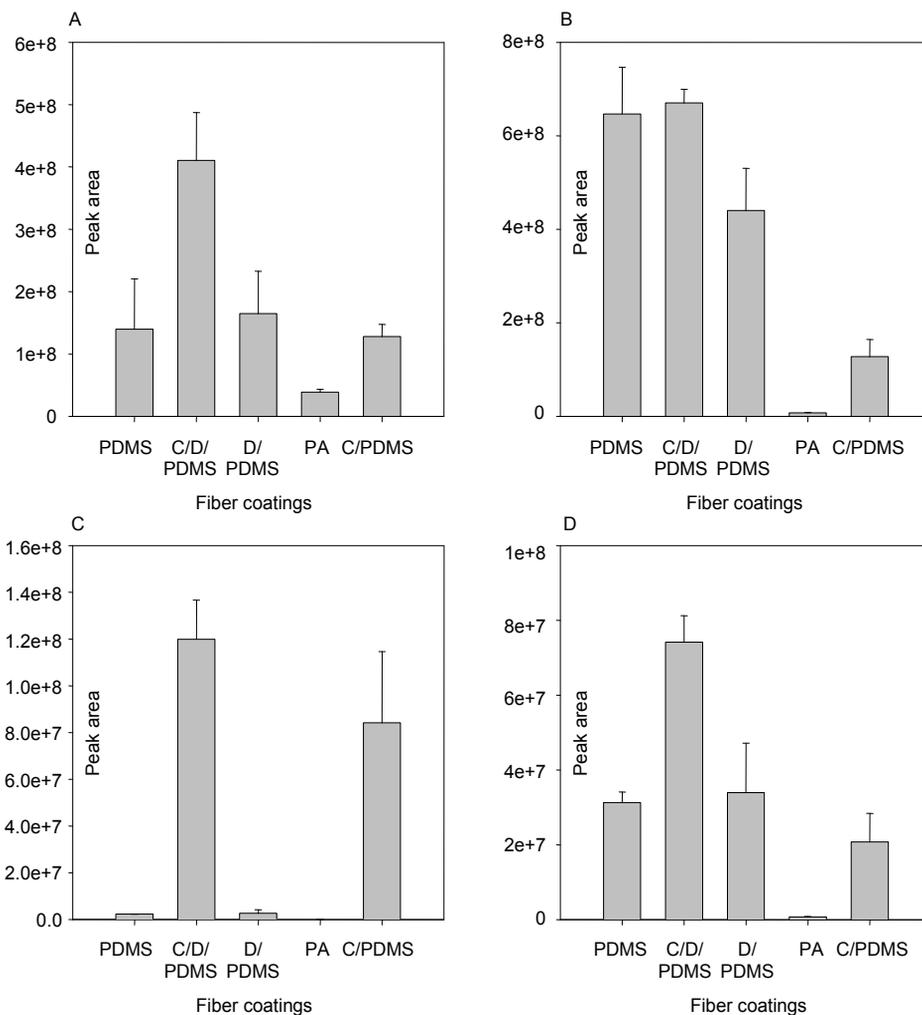


Fig. 1. Different volatile compounds groups extracted from liquered white wines using different SPME fiber coatings: PDMS – Polydimethylsiloxane, C/D/PDMS – Carboxene/Divinylbenzene/Polydimethylsiloxane, D/PDMS – Divinylbenzene/Polydimethylsiloxane, PA – Polyacrylate, C/PDMS – Carboxene/Polydimethylsiloxane. Extracted compounds groups: A – alcohols, B – esters, C – carbonyls, D – terpenes

extraction also Carboxene/Divinylbenzene/Polydimethylsiloxane fiber was the best, compared to other tested ones with the adsorption pattern similar to that obtained for alcohols extraction – it was followed by D/PDMS, PDMS and C/PDMS. In the study on method optimization for the isolation of terpenoids in Madeira wine polyacrylate was the most suitable, better than PDMS, C/PDMS and D/PDMS [Camara et al. 2006]. For these compounds, due to a possible artifact formation 40°C was recommended (among 28, 40 and 60°C tested). Terpenes could not be extracted using Carbowax/PDMS and polyacrylate fibers as found out by Cabredo-Pinillos [2004]. For fermentation products

analysed by SPME using stable isotope dilution analysis authors observed the best overall extraction efficiency for the Carbowax/DVB fiber, observing also a better efficiency for PDMS for ethyl esters but not so selective for higher alcohols and carboxylic acids [Siebert et al. 2005].

From the data presented in Figure 1 it can be concluded that Carboxene/Divinylbenzene/Polydimethylsiloxane is the most efficient fiber for extraction of volatile compounds regardless of their character (among those classes tested). It can be used either for profiling volatile compounds – responding well to all tested compounds classes, or for target analysis of compounds belonging to these groups – providing the highest peak areas, therefore the perspective for the best sensitivity. C/D/PDMS fiber offered also a relatively good reproducibility for all tested compounds classes. The same observations for the fiber choice in the analysis of profiles of flavor compounds of ice wines were published by Setkova et al. [2007 b]. Fiber selection is a first step in the development of SPME method. The adsorption process that undergoes on the fiber is related to fiber coating and also to matrix, where its main constituents (sugars, ethanol) present in various concentrations can influence the absorption/adsorption process. Rodriguez-Bencomo [2003] examined several fibers (PDMS, Polyacrylate, DVB/PDMS and Carbowax/Divinylbenzene (CW/DVB) using sweet wine as a matrix. Different extraction profiles were obtained using different fibers: the most effective one in extraction of methanol and higher alcohols was Carbowax-Divinylbenzene fiber – for extraction of ethyl acetate PDMS was the most effective. Using CW/DVB fiber for their studies authors did not notice a significant influence of sugar contents and alcohol contents on the amount of compounds extracted into the fiber from the wine.

### Extraction time

For the optimization of SPME procedure different extraction times are usually checked to provide the highest amount of compounds extracted onto the fiber. It is of a special importance, when sensitivity is an issue. Volatile compounds can be sampled after equilibrium is reached, or alternatively in a shorter time, when equilibrium time is long or impossible to attain in a reasonable time. For the present study sampling times ranging from 5 to 30 minutes were tested for main classes of analysed compounds. Figure 2 shows extraction curves for four groups of analytes. For all groups an increase was noted between 5 and 30 minutes, the lowest for terpenes, and the highest in case of alcohols (ethanol was excluded from all the comparisons in present study). Extraction time of 20 minutes was chosen for wines comparison. Though it did not provide the highest responses, mainly for alcohols and esters the amount of extracted compounds was high enough to facilitate the comparison. Because samples were preheated for 15 minutes prior to sampling, so the whole extraction process lasted 35 minutes – approximately as long as the whole GC-MS run time and was not the limiting step in the analysis.

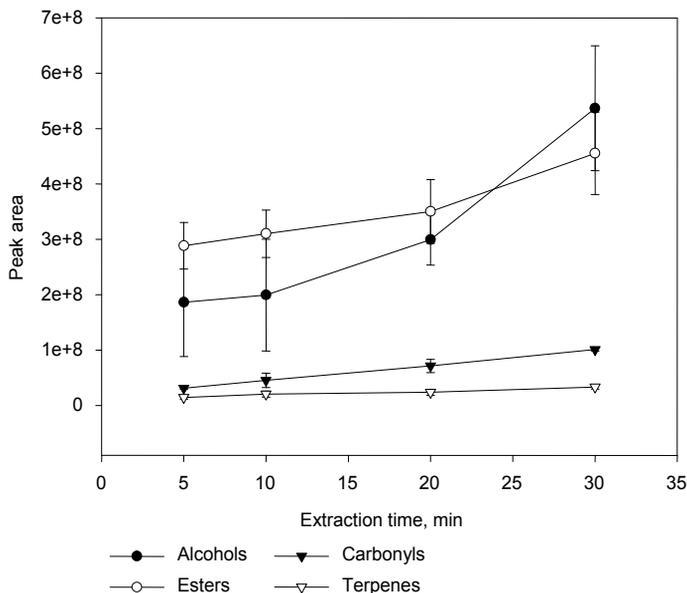


Fig. 2. Influence of extraction time on the amount of volatile compounds adsorbed on a Carboxene/Divinylbenzene/PDMS fiber at 50°C

### Comparison of volatile compounds profiles in liquered white wines

All analysed wines contained different amount of total volatile compounds. The highest amount of volatiles (in total) was noted for Riversaltes, followed by Offley Porto and Jutrzenka wines. It shows that Jutrzenka, being a variety with a strong flowery/fruity notes and a rich aroma yields also wine rich in volatile compounds. Much less volatiles was noted for the remaining wines – Muscat Oro being the one with the lowest amounts of total volatile compounds (Fig. 3). When main groups of volatile compounds are compared (Fig. 4) it can be seen that 5 wines have profiles resembling each other, with the dominating fraction of esters followed by alcohols. These wines are Jutrzenka, Krymwejn, Leyenda, Marsala and Riversaltes. They have a comparable amount of carbonyl compounds (5-10%) and terpenes contents not exceeding 10%. Two Muscat type wines have higher amounts of terpene compounds compared to remaining ones, which is a characteristic feature of these wines. However both Muscat wines have a totally different profile of volatiles: in Muscat Oro about 60% of volatile compounds were terpenes, and only about 5% alcohols – the lowest level of all analysed wines. Muscat Setubal had over four times less terpenes than Muscat Oro. Offley Porto was a wine with the highest amount of isolated alcohols. It had also the highest contents of ethanol, and the measured higher alcohols are the by-products of fermentation processes.

In conclusion, solid phase microextraction (SPME) using Carboxene/Divinylbenzene/Polydimethylsiloxane enables relatively fast extraction of volatile compounds in white wines. that can be used for their profiling and wines comparison.

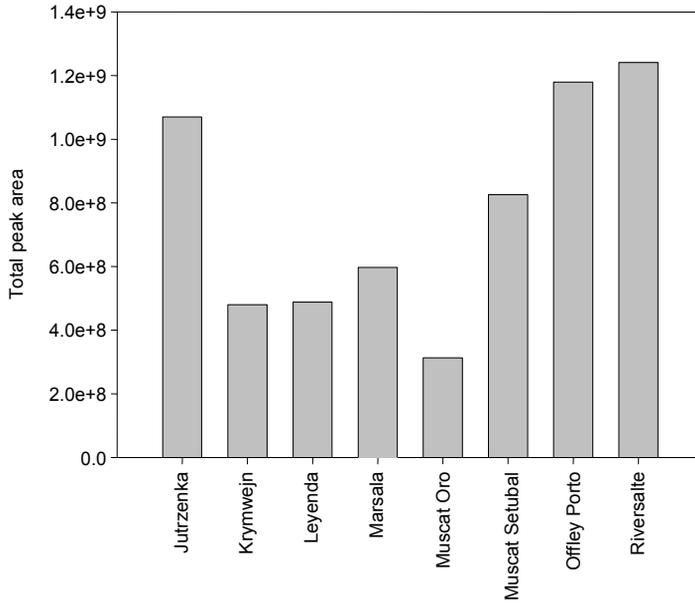


Fig. 3. Comparison of total volatile compounds extracted from different liquered white wines using SPME and Carboxen/Divinylbenzene/PDMS fiber after 20 min extraction

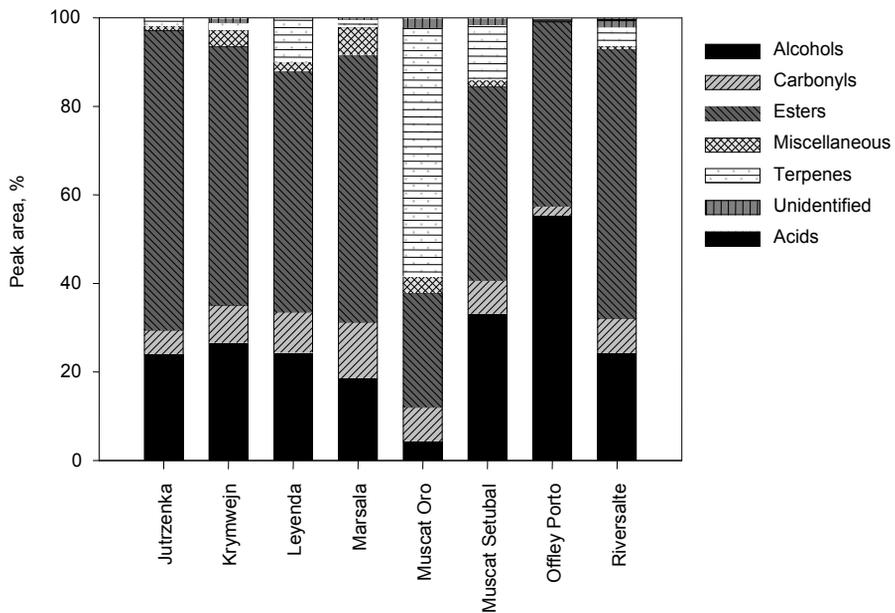


Fig. 4. Comparison of flavour compounds profiles extracted from analysed liquered white wines using SPME (Carboxene/Divinylbenzene/PDMS fiber)

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## PROFILOWANIE ZWIĄZKÓW LOTNYCH BIAŁYCH WIN LIKIEROWYCH ZA POMOCĄ MIKROEKSTRAKЦИИ DO FAZY STACJONARNEJ

**Wstęp.** Profil związków zapachowych jest charakterystyczną cechą wina, zależną od jego rodzaju, użytych winogron, procesu fermentacji i dojrzewania. Profilowanie związków lotnych wina za pomocą szybkich metod dostarcza informacji o głównych grupach związków i może być używane do klasyfikowania/różnicowania win. Mikroekstrakcja do fazy stacjonarnej (SPME) została wykorzystana do profilowania związków lotnych białych win likierowych.

**Materiał i metody.** Przetestowano przydatność różnych włókien/faz SPME: PDMS, Carboxen/PDMS, Carboxen/DVB/PDMS, Poliakrylan, Divinylobenzen/PDMS. Porównano różne czasy ekstrakcji w celu zoptymalizowania procesu. Porównano ilość związków lotnych i profil związków wyekstrahowanych za pomocą włókna SPME dla ośmiu win likierowych.

**Wyniki.** Spośród testowanych włókien Carboxen/DVB/PDMS cechowała największa zdolność do ekstrakcji wyższych alkoholi, estrów, związków karbonylowych oraz terpenowych. Spośród testowanych czasów ekstrakcji (od 5 do 30 min) wybrano 20 minut jako czas zapewniający wystarczająco duże powierzchnie pików. Wykorzystując SPME, porównano całkowitą ilość związków lotnych w analizowanych ośmiu winach likierowych – największe ilości zaadsorbowanych związków odnotowano w Riversaltes, Offley Porto i winie Jutrzenka. Porównanie profili analizowanych win wykazało, że w sześciu analizowanych winach dominującymi grupami związków były estry, a następnie wyższe alkohole, dwa analizowane wina Muscat cechowała duża zawartość terpenów w porównaniu z winami pozostałymi.

**Wnioski.** SPME może być wykorzystana do relatywnie szybkiego profilowania związków lotnych wina, co może być pomocne w ich klasyfikacji.

**Słowa kluczowe:** wina likierowe, profilowanie, związki lotne, SPME

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