Analytical Methods

Differentiation of wines according to grape variety and geographical origin based on volatiles profiling using SPME-MS and SPME-GC/MS methods

Angelika Ziółkowska, Erwin Wąsowicz 1, Henryk H. Jeleń *

Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31, 60-642 Poznań, Poland

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A B S T R A C T

Among methods to detect wine adulteration, profiling volatiles is one with a great potential regarding robustness, analysis time and abundance of information for subsequent data treatment. Volatile fraction fingerprinting by solid-phase microextraction with direct analysis by mass spectrometry without compounds separation (SPME-MS) was used for differentiation of white as well as red wines. The aim was to differentiate between varieties used for wine production and to also differentiate wines by country of origin. The results obtained were compared to SPME-GC/MS analysis in which compounds were resolved by gas chromatography. For both approaches the same type of statistical procedure was used to compare samples: principal component analysis (PCA) followed by linear discriminant analysis (LDA). White wines (38) and red wines (41) representing different grape varieties and various regions of origin were analysed. SPME-MS proved to be advantageous in use due to better discrimination and higher sample throughput.

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1. Introduction

Wine flavour is one of the most complex in food products and is dependent on aroma compounds originating from grapes, fermentation process, ageing and storage. Wine distinct flavour come from specific groups of compounds characteristic of some grapes (i.e. terpenes in Muscat wines), and compounds which originate from fermentation and also maturation processes. However, key odorants form only a relatively small fraction of wine volatile compounds. There are as many as 800 volatiles detected in wine, among them mainly alcohols, esters, aldehydes and ketones. There are also numerous compounds representing other classes (terpenoids, norisoprenoids, thiols, methoxyypyrazines; Ebeler, 2001). The profile of the volatile compounds in wine depends on many factors, such as geographical origin, grape variety, vintage and growing conditions (Jurado et al., 2008; Setkova, Ristichevic, & Pawliszyn, 2007; Arozarena, Caps, Marín, & Navarro, 2000; Cozzolino, Smyth, Cynkar, Dambergs, & Gishen, 2005; Martí, Busto, & Guasch, 2004; Alvarez, Aleixandre, García, Casp, & Zúñica, 2003).

Various methods have been used for the differentiation and classification of wines based on the volatile compounds analysis. They involve gas chromatography and gas chromatography–mass spectrometry (GC–MS) (Díaz, Conde, Méndez, & Pérez Trujillo, 2002; Rebolo et al., 2000; Câmara, Alves, & Carlos Marques, 2007; Falqué, Fernández, & Dubourdieu, 2002). A different approach, the use of electronic noses (e-noses) based on chemical sensors (Buratti, Benedetti, Scampicchio, & Pangerod, 2004; Pilar, Boque, Busto, & Guasch, 2005) or on mass spectrometers (Cozzolino et al., 2005; Cynkar, Dambergs, Smith, & Cozzolino, 2010; Martí et al., 2004), to fingerprint wine flavour has been used.

In the MS-based electronic nose, the mass analyser (spectrometer) is used as an “array of sensors”. Signals acquired by this type of electronic nose are ions of specific m/z and their intensities as registered by MS. These ions originate from the TIC (total ion current) acquired from a single peak after introduction of volatile compounds into the MS without chromatographic separation. To achieve such a “spectrum”, the analytical column is replaced by a restricting capillary with no stationary phase. There is usually no information on particular compounds (as the compounds are not separated) but provides a fingerprint of each sample based on the uniqueness of certain ions (Jeleń, Mildner-Szkudlarz, Jasieńska, & Wąsowicz, 2007). The main advantages of MS-based electronic noses over classic electronic noses (based on metal oxide sensors, conducting polymers or quartz microbalance) are their resistance
to moisture, high sensitivity and selectivity and some qualitative information (Pavón et al., 2006). Extraction methods based on headspace analysis are used to introduce the sample. Methods that include sample pre-concentration step, such as solid-phase microextraction (SPME) or purge-and-trap (P&T) provide better discrimination than these based on static headspace techniques (Lozano, Santos, Gutierrez, & Horillo, 2007). SPME-MS has been already used for the analysis of food products of various types, such as milk, cheeses, adulteration of olive oil and botanical origin of spirits (Jeleń, Ziolkowska, & Kaczmarek, 2010; Majcher, Kaczmarek, Klenzporf-Pawlik, Pikul, & Jelen, 2015; Marsili, 1999; Mildner-Szkudlarz & Jeleń, 2008; Péreș, Viallon, & Berdagüe, 2001).

Linear discriminant analysis (LDA), principal component analysis (PCA), canonical analysis, HJ-biplot, and artificial neural networks (ANN) have already been employed to process data and enabled classification of wines in regard to varieties and regions of origin (Cozzolino, Smyth, Cynkar, Damberg, & Gisen, 2005; Márquez, Castro, Natera, & García-Barroso, 2008; ; Diaz, Conde, Méndez, & Pérez Trujillo, 2003; Römisch et al., 2009; Saurina, 2010; Setkova et al., 2007).

The aim of this study was to evaluate the usefulness of a method based on solid-phase microextraction combined with mass spectrometry (SPME-MS) for the differentiation of both white and red wines. The wines were classified based on their grape varietal origin, which was the main goal and also with regard to country of production. For varietal classification there was an abundant representation of 3 white varieties (38) and 2 red varieties (41 bottles) used for wine production. The wine came from fourteen different countries of origin, making the number of samples from each country low; therefore the aim in this comparison was to indicate the capabilities of the system to classify samples.

The SPME-MS method was compared for the majority of wines with SPME-GC–MS, where compounds were resolved on a chromatographic column prior to multivariate statistical analysis. The same extraction type (SPME) was used for both approaches, however different types of data were collected – averaged mass spectra and peaks of particular compounds for subsequent statistical analysis. The same multivariate techniques (PCA and LDA) were used to process data from both techniques.

2. Experimental

2.1. Wines

In this study a total of 79 monovarietal wines were analysed: 38 white wines obtained from 3 different grape varieties (Chardonnay, Sauvignon Blanc, Muscat) and 41 red wines (Cabernet Sauvignon and Merlot) were studied. For a comparative study in-laboratory mixtures (2) of Cabernet Sauvignon and Merlot were prepared Commercially available white wines were produced in Chile (9), USA (California) (8), France (6), Bulgaria (4), Moldova (5), Spain (3), Argentina (1), Australia (1) and South Africa (1), and red wines in Chile (11), Bulgaria (9), California (8), France (6) and Moldova (7). Wines were purchased in wine shops in Poznań. All the wines were bottled at the place of production and were produced within two years to minimise the influence of vintage on discrimination.

2.2. Analytical equipment

For the SPME-MS analysis a Hewlett-Packard HP5890 series II gas chromatograph coupled to a Hewlett-Packard 5971 quadrupole mass spectrometer was used. Capillary analytical column was replaced with fused silica capillary tubing with no phase coating (5 m × 0.2 mm; Supelco, Bellefonte, PA). For the SPME-GC–MS analysis an Agilent 7890A gas chromatograph with a split/splitless injection port, coupled with Agilent 5975C VL MSD quadrupole mass spectrometer was used. Compounds were resolved on a Supelcowax-10 (30 m × 0.25 mm × 0.25 μm, Supelco, Bellefonte) column. SPME injections were performed manually in both methods.

2.3. Extraction methods of volatile compounds

Analyses of wine samples were performed in two independent experiments: SPME-MS and SPME-GC–MS. Prior to these experiments extraction conditions were optimised for each method independently. Wines (10 mL) were placed in a 20-ml vial and the vial was then tightly capped with PTFE/silicone septum. Different SPME fibres were tested for their extraction efficiency at the same time (20 min.) and temperature (50 °C): polydimethylsiloxane (PDMS), polyacrylate (PA), Carboxen/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS), divinylbenzene/polydimethylsiloxane (DVB/PDMS) and Carboxen/polydimethylsiloxane (CAR/PDMS). All these fibres were 1 cm long except for the CAR/DVB/PDMS, which was 2 cm. PA fibre and CAR/DVB/PDMS fibres were chosen based on the highest total peak areas for SPME-MS and SPME-GC–MS experiments, respectively. In the next step extraction times ranging from 2 to 30 min were compared. Volatile compounds were sampled at 50 °C after 5 min preheating. For SPME-MS experiment the highest total area responses was obtained after 2 min of extraction so this time was selected for the study. For SPME-GC–MS experiment total peak area was highest for 20 min extraction and as total analysis time is influenced mainly by the GC run time 20 min sampling time was selected. Compounds were desorbed for 3 min at 280 °C in splitless mode, using a 0.75 mm dedicated SPME liner. In the SPME-MS experiment GC–MS operating conditions were as follows: helium flow 0.4 mL min⁻¹, oven temperature 250 °C (isothermal). The spectrometer operated in electron impact (EI) mode (70 eV). The ion source was indirectly heated by transfer line set to 280 °C. Detection was carried out in scan mode over a range of m/z 29–289. In the SPME-GC–MS experiment helium flow was 0.8 mL min⁻¹, initial oven temperature was 40 °C (1 min), then 15 °C min⁻¹ to 140 °C and 50 °C min⁻¹ to 260 °C (1.5 min). The spectrometer operated in electron impact mode (EI, 70 eV) at 240 °C. Detection was carried out in full scan mode over a range of m/z 29–289. Each sample was run in 3–5 replicates for SPME-MS and 2–3 replicates for SPME-GC/MS.

2.4. Statistical analysis

Statistical analyses were performed using Statistica 8.0 software (Statsoft, Tulsa, OK) equipped with a multivariate statistics package. Principal component analysis (PCA) and linear discriminant analysis (LDA) were used for sample discrimination and classification. In these studies, external validation method was used, dividing each data set into a training set (used to calculate the classification rules – approximately 80% of samples) and an evaluation set (used to evaluate the prediction ability of rules and models – 20% of samples), or with the leave-one-out method when the number of samples was limited. To indicate clustering in LDA, prediction ellipses were created using Statistica, based on normal distribution with a coefficient of 0.95.

3. Results

3.1. Differentiation of white wines and red wines of different grape varieties using SPME-MS and SPME-GC–MS

White wines (38) of three different grape varieties: Chardonnay, Sauvignon Blanc and Muscat, and red wines (41) produced from...
Cabernet Sauvignon and Merlot grapes, from different countries were analysed, in order to classify them according to grape varieties. SPME-MS analysis produced a single peak for which an averaged spectrum represents mixed spectra of compounds transferred from headspace to mass spectrometer. Although the identification of particular compounds on a basis of an “averaged” mass spectrum is usually not possible, the presence of certain ions can provide some hints on the character of analysed compounds. The most abundant ions in all spectra were the ion of m/z 31, which is base peak and characteristic ion for both methanol and ethanol, and m/z 45, characteristic for ethanol. That is in accordance with the presence of methanol and ethanol in the analysed wines. However, no ion intensities vs amounts of methanol or ethanol in wines correlations can be expected as both ions are of relatively low specificity. Other ions which could be observed on a spectrum were ions m/z 44 which can be related to aldehydes, m/z 41, attributed among other compounds to higher alcohols, m/z 43 which is characteristic for acetates and m/z 88 which is related to ethyl esters. One must bear in mind that low m/z fragments are usually of limited usefulness in drawing conclusions on identities of particular compounds or compound groups.

SPME-MS data were transferred from their tabulated form in Chemstation software to Excel similarly to the above methodology, but the table was built with identified compounds vs. their peak intensities (TIC areas). Then data were processed in the same

Fig. 1. PCA projection of white (A, C) and red (B, D) wines made from different grape varieties based on correlation analysis obtained using SPME-MS (A, B) and SPME-GC–MS (C, D) methods.

As a first step, principal component analysis (PCA) was carried out in order to visualise eventual grouping of samples (Fig. 1). White and red wines analysed by SPME-MS yielded visible clusters on the PCA graph (Fig. 1A and B) with the first two components representing 67.61% and 91.98% variance for white and red wines, respectively. Discriminant ions for white (m/z 31, 32, 35, 42, 43, 60, 72, 73) and red wines (m/z 30, 31, 32, 40, 41, 42, 44, 46) were similar. As can be seen in Fig. 1A Chardonnay formed a uniform cluster (with one Muscat outlier in it); similar grouping was observed for Muscat. Sauvignon Blanc formed two clusters, one of them very close to Muscat. Based on PCA, two clusters for two grape varieties were observed in the case of red wines (Fig. 1B). Cabernet Sauvignon/Merlot mixtures were not included using SPME-MS and PCA.

SPME-GC–MS data were transferred from Agilent MSD Chemstation software to Excel similarly to the above methodology, but 50% of samples such ions were eliminated from the table. Data acquired for all the samples were processed using logarithmic transformation (log(x + 1) because of significant differences in ions intensities and also to compensate for the occurrence of zero values in matrix data. Then all variables were autoscaled (Berrueta, Alonso-Salces, & Heberger, 2007). Afterwards all data were subjected to multivariate analysis.
way as for the SPME-MS approach. For discrimination in PCA isooamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl dodecanoate, 2-phenylethanol and furfural were selected for white wines and isobutanol, 1-hexanol, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate for red wines. In the case of the SPME-GC–MS method the clustering was less pronounced compared to SPME-MS, especially for white wines, where the first two components represented only 45.84% variance (Fig. 1C). For red wines analysed by SPME-GC–MS, apart from Cabernet Sauvignon and Merlot monovarietal wines, wines produced from a mixture of these varieties were also included (Fig. 1D). The first two PCs represented 64.27% of total variance. As indicated in Fig. 1 PCA for SPME-MS data presented better group discrimination than for SPME-GC–MS.

To improve classification of investigated samples, in a next step linear discriminant analysis (LDA) was carried out. LDA is a supervised classification technique and is based on the determination of linear discriminant functions, which maximise the ratio of between-class variance and minimise the ratio of within-class variance. Features were selected by forward stepwise selection, which chooses variables producing the highest F-values and the highest decrease of Wilks’ $\lambda$. To assess the discriminant capacity of variable F-tests and Wilks’ $\lambda$ tests were performed. LDA was performed, not based on ions selected in PCA, but using all ions from the matrix. For SPME-MS method ions with the best discrimination power (highest F-values) were selected for each group of wines. For white wines the following ions were chosen: $m/z$ 30, 31, 32, 33, 35, 42, 43, 47, 57, 60, 72, 73, 74, 75, 90, 99, 120, 123 and 124. For discrimination of red wines the following ions were selected: $m/z$ 30, 32, 36, 39, 40, 43, 48, 56, 60, 64, 72, 77, 79, 82, 85, 92, 93, 102, 107 and 129. Fig. 2 shows the clusters of examined white (Fig. 2A) and red (Fig. 2B) wines after LDA analysis based on SPME-MS data. In both cases the discrimination between sample clusters was very good. To assess the ability of the model to identify wine authenticity and classify wine according to grape variety, all samples were divided randomly into a learning set and a verification (testing) set. Classification and prediction ability for the model built for white and for red wines using SPME-MS-LDA was 100%.

When the same statistical approach was used to classify wine samples based on SPME-GC–MS data the results were not resolved satisfactorily: Fig. 2C and D show classification of white and red wines, respectively, obtained after SPME-GC–MS data analysis. Although it was possible to distinguish separate clusters for red wine, it was not possible for white wines. The compounds with the highest discriminating power for white wines in this method were isoamyl acetate, furfural, ethyl octanoate, ethyl decanoate and ethyl dodecanoate. For red wines 1-hexanol, ethyl decanoate and 2-phenylethanol were discriminant compounds in LDA. The ability of a model to classify and predict red wines of different varieties correctly based on SPME-GC–MS was 95%. The SPME-GC–MS-LDA method was applied also by Câmara et al. (2007) for the differentiation of white wines of different grape...
varieties (Boal, Malvazia, Sercial and Verdelho) using the same SPME fibre but the extraction lasted 120 min.

3.2. Differentiation of white wines of different geographical origin using SPME-MS and SPME-GC–MS

To focus on the most informative approaches in samples discrimination for geographical origin classification only LDA data are presented. Fig. 3 shows data on white wines analysis using SPME-MS (3A, 3C, 3E for Chardonnay, Sauvignon Blanc and Muscat, respectively) and the same varieties analysed using SPME-GC–MS (3B, 3D, 3F, respectively). LDA evaluation of data of analysed samples was carried out for the white wines produced in various countries: Chile, USA (California), Bulgaria, France, Moldova, Spain, Argentina, Australia and South Africa. Looking at data in Fig. 3 shows that differentiation using SPME-MS within white wines for all analysed grape varieties (Chardonnay, Sauvignon Blanc and Muscat) was excellent when geographical regions were compared. Similarly, classification of white wines from different geographical origins was also satisfactory, not for Chardonnay, but for Sauvignon Blanc and Muscat when SPME-GC/MS was used. LDA has been also applied to the differentiation of wines by Falqué et al. (2002), Câmara et al. (2007), and Bevin, Dambergs, Ferguson, and Cozzolino (2008). Falqué used gas chromatography with a flame ioniser detector (GC-FID) and GC–MS for the classification of white wines Loureira, Dona Branca and Treixadura. GC–MS coupled with HS-SPME was also adopted by Câmara to distinguish white wines of different grape varieties. Each of these authors obtained good results using LDA but usually using longer extraction times.

The classification and prediction abilities of the model with regard to grape variety for white wines for SPME-MS were 100%. LDA in the treatment of SPME-MS data is a reliable tool for white wine samples recognition and prediction. SPME-MS has also been used by other authors coupled with other statistical methods like PLS (Marsili, 2000) and SIMCA (Laguerre, Mestres, Davrieux, Ringuet, & Boulanger, 2007) with satisfactory results.

Fig. 3. SPME-MS-LDA (A, C, E) and SPME-GC–MS-LDA (B, D, F) analysis of volatile fraction of white wines of different regional origin; Chardonnay (A, B), Sauvignon Blanc (C, D), Muscat (E, F).
For SPME-GC–MS of wine samples of particular varieties, country of origin classification was also satisfactory in the majority of cases. The only unresolved clusters were for Chardonnay wines from France and Chile (Fig. 3B). The most discriminant variables were isoamyl acetate, furfural, decanoic acid ethyl ester, dodecanoic acid ethyl ester and octanoic acid ethyl ester. These volatiles are mostly the same as in PCA; therefore differences between results obtained using PCA and LDA depend only on the different mathematical analysis used in these techniques. Most discriminating compounds in white wines were simultaneously the most abundant when extracted by SPME. Esters are the main volatile compounds in wines; therefore, they were expected to influence data preparation for multivariate analysis. Moreover, ethyl esters of short chain fatty acids (ethyl caproate, caprylate etc.) are usually very well adsorbed on the SPME fibre as their partition constants favour their adsorption in the fibre.

3.3. Classification of red wines of different geographical origin using SPME-MS and SPME-GC–MS

Linear Discriminant Analysis (LDA) was carried out, in order to obtain suitable classification of red wines of various geographical origin. In this case ions with the best discrimination power were those having the \( m/z \) values 30, 32, 36, 39, 40, 43, 48, 56, 60, 64, 72, 77, 79, 82, 85, 92, 93, 102, 107 and 129. Four of these ions were the same as in PCA but the remaining ones were different. Fig. 4 shows samples groupings based on LDA showing differentiation of red wines according to varieties and regions of origin. Good classification of the samples of Cabernet Sauvignon and Merlot was achieved using SPME-MS, though for Cabernet Sauvignon originating from Chile and Bulgaria their volatile profiles were so different from the remaining countries that the rest of samples formed a single cluster separable only after extraction from the original set of data (Fig. 4A1). For Merlot the cluster separation

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**Fig. 4.** SPME-MS-LDA (A1, A2, B) and SPME-GC–MS-LDA (C, D) analysis of volatile fraction of red wines of different regional origin; Cabernet-Sauvignon (A1, A2, C), Merlot (B, D).
was satisfactory. The classification ability for the model was 93%. Similar results were obtained by Bevin et al. (2008) for differentiation of Cabernet Sauvignon, Merlot and Syrah based on mid-infrared spectroscopy (MIR). Good differentiation of red wines according to geographical origin was achieved by Rebole et al. (2000), who classified Galician Ribera Sacra wines using GC-FID data with pattern recognition analysis coupled also with LDA.

Classification and prediction abilities of the model for SPME-M, with regard to different grape varieties, was 100%. Based on geographical origin, in the case of Cabernet Sauvignon the classification and prediction ability of the model was also 100% (after extracting a subset of samples as in Fig. 4A1). However, in the case of Merlot, the prediction ability was 90%.

When SPME-GC/MS was used, discrimination of volatile compounds using PCA (results not shown) was based on the following compounds: isobutanol, 1-hexanol, hexanoic acid ethyl ester, octanoic acid ethyl ester, decanoic acid ethyl ester, dodecanoic acid ethyl ester. When LDA was carried out on the data, compounds with the best discrimination power were 1-hexanol, decanoic acid ethyl ester, dodecanoic acid ethyl ester and phenylethyl alcohol. As can be observed in Fig. 4B and C, sample discrimination using SPME-GC/MS was much worse than for SPME-M.

4. Conclusions

Comparing obtained results for both approaches, SPME-M provided much better separation of clusters after LDA compared to SPME-GC/MS. As presented here SPME-M can provide reliable data especially for grape origin of wines. SPME-M is a very fast method, due to the lack of chromatographic separation, and as a consequence can yield results in a short time, which is particularly important in analysing large sets of samples and replicates required for statistical treatment. Analysis time using SPME-GC/MS is 10 times shorter than for SPME-GC/MS. Moreover, this method does not require a dedicated instrument, but can be used with any GC/MS system after exchanging analytical column for a restricting capillary.

Conflict of interest statement

Angelika Ziolkowska, Erwin Wąsowicz and Henryk Jeleń declare no conflict of interest.

References


