

Application of Volatile Compound Analysis for Distinguishing between Red Wines from Poland and from Other European Countries

A. Stój^{1*}, T. Czernecki¹, D. Domagała², Z. Targoński¹

(1) Department of Biotechnology, Human Nutrition and Food Commodity Science, Faculty of Food Sciences and Biotechnology, University of Life Sciences, Skromna 8, 20-704 Lublin, Poland

(2) Department of Applied Mathematics and Computer Science, Faculty of Production Engineering, University of Life Sciences, Głęboka 28, 20-612 Lublin, Poland

Submitted for publication: April 2017

Accepted for publication: July 2017

Key words: Aroma compounds, HS-SPME, GC-MS, Polish red wines, geographical origin, authenticity

Authenticity and the geographical origin of wines are terms of great importance for consumers and producers. This work is focused on distinguishing between red wines from Poland and from other European countries, notably France, Italy and Spain. To achieve this goal, we determined aroma compounds in wines from different countries by headspace solid-phase microextraction/gas chromatography-mass spectrometry. The content of hexan-1-ol in Polish wines was significantly higher (about twice as high) than in French, Italian and Spanish wines. Linear discriminant analysis (LDA) showed that 3-(methylsulfanyl)propane-1-ol, hexan-1-ol, ethyl phenylacetate and ethyl 2-hydroxy-4-methylpentanoate were the most discriminant variables for distinguishing between wines from Poland and from other European countries. Hierarchical cluster analysis (HCA) revealed that Polish wines were separated thoroughly from the other wines based on ethyl phenylacetate, hexan-1-ol, ethyl 2-hydroxy-4-methylpentanoate, (E)-3-hexen-1-ol, 2-phenylethanol and 3-(methylsulfanyl)propan-1-ol, which is important for preventing possible frauds.

INTRODUCTION

A significant increase in viticulture and winemaking has been observed in Poland in recent years, mainly due to numerous changes in the European regulations and in complementary Polish legislation. The most crucial change for Poland was the inclusion of the country in the coldest A zone of viticulture in 2005. Moreover, amendments to the 2008 regulations set smaller producers of wine in Poland (less than 1000 hl per annum) free from warehouse taxation. Despite the cold climate in Poland, it is possible to produce high-quality wines from grape varieties that are more resistant to frost and adapted to the Polish climate. The most commonly grown red grape varieties are *Regent*, *Rondo* and *Pinot Noir* (Wilk, 2011). *Rondo* and *Regent*, both new grape varieties originally from Germany, are known as multispecies hybrids. The hybrids were obtained by crossing two or three species of *Vitis*, *Zarya severa* x *Saint Laurent* and (*Silvaner* x *Mueller Thurgau*) x *Chambourcin*, respectively. *Pinot Noir* is an old French variety (<http://www.vivc.de/>). Grapevine cultivation and winemaking take place in certain regions, mainly in western and south-eastern parts of Poland (Jeleń *et al.*, 2011).

Aroma compounds belong to several chemical classes, such as alcohols, esters, acids, aldehydes, ketones, terpenes

and others. There are hundreds of volatile compounds in wines. Aroma formation is influenced by several factors: grape variety, climate, soil, region, cultivation, yeast, oenological techniques and ageing (Du *et al.*, 2012; Coetzee & Du Toit, 2015).

Solid phase microextraction (SPME) has largely been used for the extraction of aroma compounds in recent years. Compared to traditional techniques, like liquid-liquid extraction (LLE) and solid-phase extraction (SPE), it offers many advantages, such as simplicity, high sensitivity and reproducibility, does not require solvents and needs low sample volumes. Moreover, it is fast, inexpensive and can easily be automated (Welke *et al.*, 2012a). This technique was developed by Pawliszyn and co-workers in 1989 (Marengo *et al.*, 2001). In the SPME, an immobilised stationary phase in the form of fibre is immersed directly in the liquid sample (direct immersion, DI) or is exposed in the headspace above the sample (headspace, HS). The headspace extraction technique (HS-SPME) is used almost exclusively because of the specificity of wine matrices – presence of sugars, proteins, lipids and others compounds (Jeleń *et al.*, 2012). The polydimethylsiloxane (PDMS) and polyacrylate (PA) coatings extract analytes via absorption, while polydimeth-

*Corresponding author: E-mail address: anna.stoj@up.lublin.pl [Tel: 48 81 4623380; Fax: 48 81 462 34 00]

ylsiloxane-divinylbenzene (PDMS/DVB), carboxen-polydimethylsiloxane (CAR/PDMS) and divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) do so via adsorption. Initially, analyte molecules get attached to the fibre surface. Whether they migrate to the bulk of the fibre or remain on its surface depends on the magnitude of the analyte diffusion coefficient in the fibre (Câmara *et al.*, 2006; Metafa & Economou, 2013). The SPME is usually combined with gas chromatography and mass spectrometry (GC-MS) for the analysis of aroma compounds in wines (Goldner *et al.*, 2011; Trani *et al.*, 2016).

Multivariate analysis techniques are employed for data processing and enable the classification of wines according to their geographical origin. Linear discriminant analysis (LDA) and hierarchical cluster analysis (HCA) are frequently applied for this purpose (Marengo *et al.*, 2001; Jurado *et al.*, 2008; Ziółkowska *et al.*, 2016). In LDA, a classification model is built step by step; at each step, all variables are revised and the most discriminant variable is selected. Then the variable is included and the processing re-starts. This technique maximises the variance between categories and minimises the variance within the category. In turn, HCA evaluates similarity between objects based on their distances in n-dimensional space. The objects are aggregated according to similarity. The cluster describes a group of objects that are more similar to each other than to objects outside the group (Cabredo-Pinillos *et al.*, 2008; Jurado *et al.*, 2008).

Authenticity and the geographical origin of wines are very important for consumers and wine producers. The characterisation and classification of wines protect consumers from illegal procedures and eliminate unfair producers (Stój, 2011). The analysis of volatile compounds combined with statistical techniques is often used as a tool for the identification of the geographical origin of wines. Several studies have focused on the discrimination of wines from different regions and grape varieties within one country and among countries based on aroma compounds. Jurado *et al.* (2008) and Sáenz *et al.* (2010) correctly differentiated wines from several Spanish denominations of origin. Römisch *et al.* (2009) found that it was easy to discriminate South African wines from East European wines, but the separation of wines between Hungary, the Czech Republic and Romania was more difficult because of the small geographical distances between these countries. Ziółkowska *et al.* (2016) differentiated white and red wines according to grape variety and geographical origin, and reported that discrimination was much worse when SPME-GC-MS rather than SPME-MS data were used. Perestrelo *et al.* (2014) observed two clearly defined clusters: a group constituted of wines from Madeira Island and another one consisting of wines from the Azores and Canary Island, which are located close to each other. Zhang *et al.* (2013) successfully characterised the differences in aroma and sensory composition of Merlot wines from different geographic origins in China.

To date, no studies have been done on Polish red wines in the context of their authenticity, identification of wine area, and discrimination between Polish wines and those produced in other countries. Each wine-producing country has some defined "wine regions", characterised by a rather

homogeneous climate and soil characteristics, and by the presence of dominant varieties and typical wines (Römisch *et al.*, 2009). This work is focused on distinguishing between red wines from Poland and from other European countries, regardless of the growing region, grape variety and winemaking techniques in the countries. Italy, France and Spain are the largest producers of wine in the world, while Poland is a country with only marginal production on a global scale. The relatively high price of Polish wines can create an environment in which wines originating from other countries will be sold under a Polish brand. In this study, we determined aroma compounds in wines from different countries by means of HS-SPME/GC-MS and processed data using the LDA and HCA techniques.

MATERIALS AND METHODS

Wine samples

The method was applied to 37 commercial red wines originating from different countries: France (eight samples), Italy (10 samples), Spain (eight samples) and Poland (11 samples). The wines of the 2008, 2009 and 2010 vintages were produced in different regions in the countries from various varieties of *Vitis vinifera*. Details of the wines are shown in Table 1. All wines were bottled at the place of production. To protect proprietary interests, winery identities are not reported. The wines were selected in consultation with wine experts with the aim to obtain wine representative of each region. French, Italian and Spanish wines had a denomination of origin or were regional wines. In Poland there were no indications of the wine quality of the 2008, 2009 and 2010 vintages.

Chemicals

All chemicals used were of analytical grade. Sodium chloride and hydrochloric acid were purchased from POCh Company (Gliwice, Poland). Sodium chloride was oven dried overnight at 200°C before use. Hydrochloric acid was previously dissolved in water at a concentration of 78 g/L. The internal standard, 4-hydroxy-4-methyl-2-pentanone, was obtained from Sigma-Aldrich Company (Poznań, Poland). The internal standard was previously prepared in water at a concentration of 7 mg/L. A mixture of n-alkanes (C₇ to C₃₀) for the calculation of the linear retention indices (LTPRI) was supplied by Supelco (Bellefonte PA, USA).

HS-SPME

A fibre holder and four types of fibres were used: 85 µm polyacrylate (PA), 85 µm carboxen-polydimethylsiloxane (CAR/PDMS), 65 µm polydimethylsiloxane-divinylbenzene (PDMS/DVB) and 50/30 divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte PA, USA). The fibres were preconditioned according to the manufacturer's specifications. The following were placed in a 7 mL glass vial: 0.9 g of NaCl, 3 mL of wine, 50 µL of HCl, 100 µL of 4-hydroxy-4-methyl-2-pentanone (as internal standard) and a magnetic stirring bar. The vial was tightly capped with a PTFE-silicone septum (Supelco, Bellefonte PA, USA). The wine sample was incubated at 29°C for 10 min under continuous stirring at 400 rpm prior to extraction. The fibres were exposed to the headspace (HS)

TABLE 1
Description of red wine samples

Wine sample code	Country	Production area	Vintage	Grape variety	Quality
F_1	France	Languedoc	2009	Merlot	Vin de Pays
F_2	France	Languedoc	2009	Syrah	Vin de Pays
F_3	France	Bordeaux	2009	Merlot	AOC
F_4	France	Loire Valley	2008	Pinot Noir	Vin de Pays
F_5	France	Rhone Valley	2009	Grenache, Syrah	Vin de Pays
F_6	France	Bordeaux	2009	Merlot, Cabernet Sauvignon	AOC
F_7	France	Bordeaux	2010	Merlot	AOC
F_8	France	Rhone Valley	2010	Grenache, Mourvedre, Syrah	AOC
I_1	Italy	Abruzzo	2010	Montepulciano	DOC
I_2	Italy	Apulia	2008	Negroamaro	IGT
I_3	Italy	Sicily	2009	Nero d'Avola	IGT
I_4	Italy	Abruzzo	2009	Montepulciano	DOC
I_5	Italy	Trentino	2008	Cabernet Sauvignon	DOC
I_6	Italy	Veneto	2009	Cabernet Sauvignon	IGT
I_7	Italy	Veneto	2009	Merlot	IGT
I_8	Italy	Marche	2010	Montepulciano	DOC
I_9	Italy	Marche	2010	Montepulciano, Sangiovese	DOC
I_10	Italy	Sicily	2010	Syrah	IGT
S_1	Spain	Castilla-Leon	2009	Tempranillo	DO
S_2	Spain	Rioja	2008	Tempranillo, Garnacha	DOC
S_3	Spain	Castilla-La Mancha	2008	Tempranillo	Vino de la Tierra
S_4	Spain	Castilla-La Mancha	2008	Tempranillo	Vino de la Tierra
S_5	Spain	Rioja	2010	Tempranillo	DO
S_6	Spain	Aragon	2009	Garnacha	DO
S_7	Spain	Castilla-La Mancha	2010	Tempranillo	DO
S_8	Spain	Castilla-La Mancha	2010	Tempranillo	DO
P_1	Poland	Mazowieckie Province	2010	Rondo	
P_2	Poland	Lower Silesia	2008	Regent	
P_3	Poland	Mazowieckie Province	2008	Rondo	
P_4	Poland	Lubuskie Province	2009	Rondo	
P_5	Poland	Lower Silesia	2008	Pinot noir	
P_6	Poland	Podkarpackie Province	2008	Regent	
P_7	Poland	Malopolskie Province	2009	Regent	
P_8	Poland	Swietokrzyskie Province	2008	Rondo, Zweigelt	
P_9	Poland	Lublin Province	2010	Regent	
P_10	Poland	Lublin Province	2010	Rondo	
P_11	Poland	Lublin Province	2010	Rondo	

at 29°C for 30 min under continuous stirring. All wines were extracted in triplicate. After extraction, the fibres were removed from the vial and thermally desorbed in the GC injection port for 2 min at 200°C, in splitless mode. Prior to each analysis, the fibres were cleaned by inserting them into the auxiliary GC injection port for 5 min at an appropriate temperature for each fibre. All wines were injected three times using one injection per vial.

GC/MS

The samples were analysed using a GCMS-QP2010 gas chromatograph coupled to a quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Chromatographic separations were done by using a CP-WAX 57CB capillary column with the following characteristics: 25 m, 0.32 mm ID x 0.2 µm film thickness, 100% polyethylene glycol (Agilent, Santa Clara CA, USA). The carrier gas was helium at a flow rate of 1.8 mL/min. The column oven temperature program was: initial temperature 38°C for 6 min, 38°C to 102°C at a rate of 3°C/min, 102°C to 200°C at a rate of 7°C/min, then held for 3 min, and then 200°C to 210°C at a rate of 6°C/min, held for 1 min. The total run time was 47 min. An electron ionisation source was used, with a source temperature of 200°C and electron energy of 70 eV. Mass spectral data were collected over a range of 30 to 300 m/z in full scan mode (scan time 0.4 s). Data were acquired by using GCMSsolution software v. 2. The identification of volatile compounds was achieved on the basis of their mass spectra and linear retention index. Mass spectrometric information of each chromatographic peak was compared to the NIST 05 mass spectral library. A mixture of n-alkanes (C₇ to C₃₀) diluted in hexane (Supelco, Bellefonte PA, USA) was loaded onto the SPME fibre and injected under the above temperature program to calculate the linear temperature-programmed retention indices (LTPRI) of each extracted compound. Experimental LTPRI were compared to retention indices reported in the literature. Semi-quantitative data on the aroma compounds were calculated by relating the peak area of volatiles to that of the internal standard. The concentration of the volatiles was expressed as µg/L.

Statistical analysis

The data analysis was conducted using the Statistica 10.0 software package (StatSoft, Kraków, Poland). Analysis of variance (ANOVA) was applied to test if there were any statistically significant differences between French, Italian, Spanish and Polish wines. The non-parametric Kruskal-Wallis test was applied for the compounds for which assumptions of analysis of variance were not fulfilled. Multiple comparisons were made in order to determine between which countries the differences were statistically significant. Linear discriminant analysis was conducted based on Wilk's lambda criterion to find volatile compounds that may be used to discriminate the four wine groups based on country of origin. Subsequently, the wines were classified based on the results of the discriminant analysis. In order to verify that the test wines could be divided into groups by taking into account the compounds selected in the discriminant analysis, hierarchical cluster analysis was performed as well. The wines were grouped using the

Ward distance matrix formed on the basis of the Euclidean distance.

RESULTS AND DISCUSSION

Volatile substances belong to several groups of chemical compounds differing mainly in terms of polarity. Four commercial SPME fibres differing in the polarity and thickness of the stationary phase were tested to find which fibre extracted as many volatile compounds as possible. The fibres were: 85 µm polyacrylate (PA), 85 µm carboxen-polydimethylsiloxane (CAR/PDMS), 65 µm polydimethylsiloxane-divinylbenzene (PDMS/DVB) and 50/30 divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS). The results of the coating comparison under the same experimental conditions obtained for Polish wine of the *Rondo* variety (P_1) are shown in Fig. 1. Using CAR/PDMS coating, it was possible to achieve the largest number of identified chromatographic peaks (a total of 34 peaks: 16 alcohols, 12 esters, two ketones, one terpene, one volatile phenol, one furan compound and one sulphur compound) and their total chromatographic area. The PA coating extracted a large number of volatile compounds (31 volatile compounds), although the total peak area of these compounds presented the lowest values. Thus CAR/PDMS fibre was chosen for the analysis of aroma in the examined wines. Similarly, Jurado *et al.* (2008) and Burin *et al.* (2013) reported that CAR/PDMS fibre was the best option for the analysis of volatile compounds in wines. Furthermore, Rodrigues *et al.* (2008), Dziadas and Jeleń (2010) and Welke *et al.* (2012a) found that CAR/PDMS fibre showed high extraction efficiency compared to others fibres.

Volatile compounds identified in the wines on the basis of their mass spectra and linear retention index are listed in Table 2. Many researchers have used such tools for the identification of volatiles (Weldegergis *et al.*, 2011; Sagratini *et al.*, 2012; Dugo *et al.*, 2014). Experimental mass spectra were compared with those stored in mass spectra libraries (e.g. that of the National Institute of Standards and Technologies, NIST), taking into account similarity. However, structurally related compounds give similar spectra, thus ambiguous identifications can be obtained. The retention indices provide complementary information to identify volatile compounds and are very useful in inter-laboratory comparisons because of their independence on operational conditions, except for the polarity of the stationary phase. This approach avoids the use of expensive and time-consuming procedures, in which identification is based on pure compounds. Moreover, complex samples like wines contain hundreds of volatiles, for which not all corresponding standards may be commercially available (Bianchi *et al.*, 2007; Yan *et al.*, 2015). In our study we considered a minimum similarity value of 80% when comparing experimental mass spectra with those from mass spectra library. Weldegergis *et al.* (2011), Dugo *et al.* (2014) and Sagratini *et al.* (2012) have assumed minimum similarity of 70%, 75% and 90% respectively. Dugo *et al.* (2014) reported that a similarity filter value of 75% was used to avoid the omission of low-concentration aroma compounds, which notably are characterised by reduced similarity matches. In our study, peaks were automatically deleted if differences among experimental retention indices

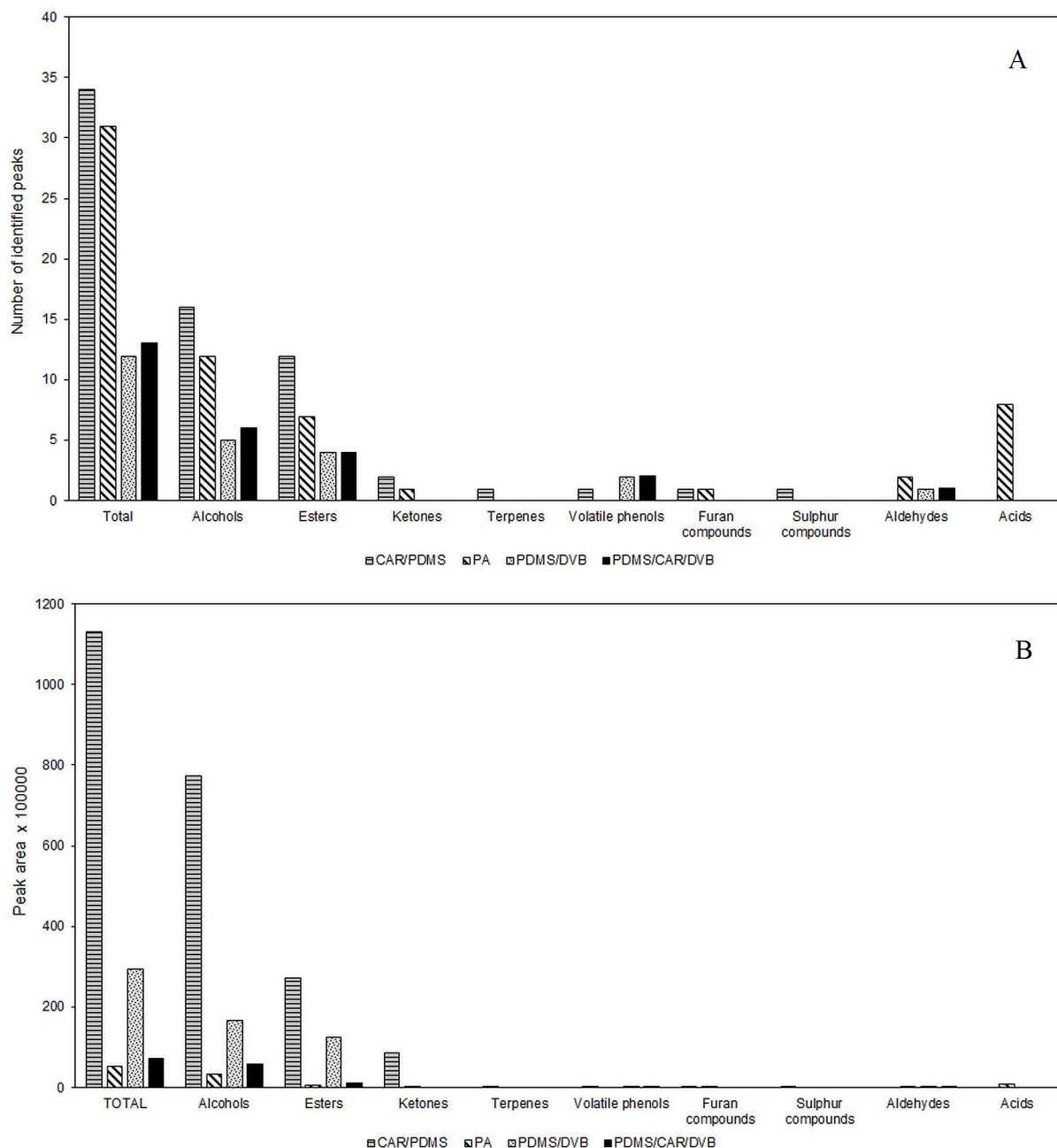


FIGURE 1

Comparison of the extraction efficiency of volatile compounds in Polish wine by HS-SPME with different fibres. The extraction was done for 30 min at 29°C and the results are expressed by: (A) number of identified peaks and (B) peak area.

and retention indices reported in the literature for CP-Wax columns or equivalent stationary phases exceeded ± 20 units. Bianchi *et al.* (2007) comment that differences in retention indices for volatiles in equivalent stationary phases may vary between 5 and 20 units when compounds with retention indices from 500 to 2 000 were considered; however, larger differences have been observed in the literature for the same aroma compounds.

The relative concentrations of aroma compounds extracted using CAR/PDMS and determined by GC-MS in the French, Italian, Spanish and Polish wines are shown in Tables 3, 4, 5 and 6. The data have been arranged into seven

chemical classes, with a total of 41 compounds identified and quantified (19 alcohols, 15 esters, two ketones, one terpene, one volatile phenol, two furan compounds, and one sulphur compound). The major volatiles were alcohols and esters in terms of number and concentration, while ketones, terpenes, volatile phenols, furan and sulphur compounds were the minor ones.

The most abundant alcohols identified in the wines from France were 3-methylbutan-1-ol (592.82 to 1434.81 $\mu\text{g/L}$), 2-phenylethanol (117.98 to 232.90 $\mu\text{g/L}$) and hexan-1-ol (65.44 to 266.51 $\mu\text{g/L}$) (Table 3). The lowest levels were showed by pentan-1-ol, 3-ethoxypropan-1-ol, 2-ethylhexan-

1-ol, 3-ethyl-4-methylpentan-1-ol and octan-1-ol. Among the alcohols, propane-1,2-diol was not detected in any of the French wines. The ester fraction was composed mainly of two ethyl esters – ethyl 2-hydroxypropanoate (273.01 to 388.89 µg/L) and ethyl octanoate (37.39 to 202.81 µg/L), and one acetate ester – 3-methylbutyl acetate (“not detected” to 642.83 µg/L). The minor esters were: ethyl 2-hexenoate, ethyl (2E,4E)-2,4-hexadienoate, ethyl 3-hydroxybutanoate and ethyl phenylacetate. 4-Methyl-3-penten-2-one was the most abundant compound in the two detected ketones, and furan-2-carbaldehyde was the most abundant in the two detected furan compounds. In our work, three of the seven wines from France were of the Merlot variety (F_1, F_3 and F_7). Gamero *et al.* (2013) identified some the same volatile compounds in French red wines of the Merlot variety: (Z)-3-hexen-1-ol (cis-3-hexenol), phenylmethanol (benzyl alcohol) and furfural (furan-2-carbaldehyde), but did not report their concentrations. These authors focused on a comparison of the sensitivity of different extraction methods. Antalick *et al.* (2014) reported that ethyl octanoate was the most abundant ethyl ester in red wines from France, and ethyl hexanoate the second one. Similarly, 3-methylbutyl acetate had the highest concentration among the acetate esters. In contrast, the concentration of ethyl decanoate was one of the highest among the volatile compounds.

The alcohols found in appreciable concentrations in the wines from Italy were 3-methylbutan-1-ol (257.34 to 1578.58 µg/L), 2-phenylethanol (104.97 to 212.45 µg/L) and hexan-1-ol (62.02 to 189.17 µg/L) (Table 4). The

lowest concentrations characterised were pentan-1-ol, (E)-3-hexen-1-ol, 3-ethoxypropan-1-ol, 2-ethylhexan-1-ol, octan-1-ol and propane-1,2-diol. The major esters were ethyl 2-hydroxypropanoate (239.34 to 508.78 µg/L), 3-methylbutyl acetate (“not detected” to 383.54 µg/L) and ethyl octanoate (22.97 to 217.99 µg/L), while minor ones were ethyl 2-hexenoate and ethyl (2E,4E)-2,4-hexadienoate. Ethyl 3-hydroxybutanoate was not detected in any of the wine from Italy. The 4-methyl-3-penten-2-one and furan-2-carbaldehyde showed higher contents in the ketones and furan compounds respectively. In this study, we did not find 2-methylpropanol in wine of the Negroamaro variety (I_2), whereas Tufariello *et al.* (2012) reported that 2-methylpropanol was present in Negroamaro wines at higher concentrations among the alcohols, besides 2+3-methylbutan-1-ol, 2-phenylethanol and hexan-1-ol. Otherwise, the above research determined monoethyl butanedioate, ethyl 2-hydroxypropanoate and diethyl butanedioate as major esters, while ethyl octanoate was not detected or detected in negligible amounts. The 3-methylbutyl acetate was not present in wine I_2, but was detected in the Negroamaro wines examined by Tufariello *et al.* (2012). The only sulphur compound found in wine I_2 was 3-(methylsulfanyl)propan-1-ol (methional), which is in agreement with the results reported by Tufariello *et al.* (2012). Montepulciano wines from Abruzzo (I_1, I_4) and Montepulciano wine from Marches (I_8) contained 1-hexanol at higher concentrations, while Sagratini *et al.* (2012) found low concentrations of 1-hexanol in Montepulciano wines from the Marches region and trace concentrations of this

TABLE 2
Aroma compounds identified in red wines

Peak no.	Compounds	Similarity (%)	Similarity			References
			RT (min) ^a	LTPRI ^b	LTPRI ^c	
<i>Alcohols</i>						
4	2-Methylpropanol	93	4.304	1113	1100	Mendes <i>et al.</i> (2012)
6	Butan-1-ol	99	5.764	1154	1153	Mallouchos <i>et al.</i> (2007)
7	3-Methylbutan-1-ol	99	8.429	1222	1216	King <i>et al.</i> (2014)
10	Pentan-1-ol	97	10.08	1259	1259	Mallouchos <i>et al.</i> (2007)
12	4-Methylpentan-1-ol	98	12.841	1321	1309	Duarte <i>et al.</i> (2010)
14	3-Methylpentan-1-ol	97	13.361	1333	1335	Mallouchos <i>et al.</i> (2007)
16	Hexan-1-ol	99	14.545	1360	1361	Mallouchos <i>et al.</i> (2007)
17	(E)-3-Hexen-1-ol	96	14.779	1366	1358	Duarte <i>et al.</i> (2010)
18	3-Ethoxypropan-1-ol	96	15.186	1375	1371	Welke <i>et al.</i> (2012b)
19	(Z)-3-Hexen-1-ol	98	15.547	1383	1379	Duarte <i>et al.</i> (2010)
22	Octen-3-ol	99	18.514	1455	1451	Song <i>et al.</i> (2014)
23	Heptan-1-ol	98	18.806	1462	1470	Welke <i>et al.</i> (2012b)
25	2-Ethylhexan-1-ol	96	20.162	1495	1486	Duarte <i>et al.</i> (2010)
27	3-Ethyl-4-methylpentan-1-ol	99	20.838	1513	1509	Welke <i>et al.</i> (2012b)
29	Octan-1-ol	98	22.846	1565	1567	Mallouchos <i>et al.</i> (2007)
30	Butane-2,3-diol	98	23.99	1595	1582	Kallio (1989)
31	Propane-1,2-diol	97	24.5	1609	1599	Welke <i>et al.</i> (2012b)
39	Phenylmethanol	99	31.871	1876	1879	King <i>et al.</i> (2014)
40	2-Phenylethanol	99	32.502	1910	1900	Welke <i>et al.</i> (2012b)

TABLE 2 (CONTINUED)

Peak no.	Compounds	Similarity			References	
		(%)	RT (min) ^a	LTPRI ^b		LTPRI ^c
<i>Esters</i>						
1	Ethyl butanoate	99	2.683	1029	1032	Duarte <i>et al.</i> (2010)
2	Ethyl 3-methylbutanoate	97	3.164	1059	1066	Duarte <i>et al.</i> (2010)
3	3-Methylbutyl acetate	99	4.17	1100	1100	Mallouchos <i>et al.</i> (2007)
8	Ethyl hexanoate	93	8.455	1223	1229	Mendes <i>et al.</i> (2012)
9	Hexyl acetate	98	9.995	1257	1272	Mallouchos <i>et al.</i> (2007)
13	Ethyl 2-hexenoate	84	13.049	1326	1344	Mallouchos <i>et al.</i> (2007)
15	Ethyl 2-hydroxypropanoate	98	13.534	1337	1339	Welke <i>et al.</i> (2012b)
20	Ethyl octanoate	99	17.195	1423	1429	Welke <i>et al.</i> (2012b)
24	Ethyl (2E,4E)-2,4-hexadienoate	93	19.762	1486	1484	Mendes <i>et al.</i> (2012)
26	Ethyl 3-hydroxybutanoate	90	20.735	1510	1514	Welke <i>et al.</i> (2012b)
28	Ethyl 2-hydroxy-4-methylpentanoate	99	21.767	1537	1538	Welke <i>et al.</i> (2012b)
32	Ethyl decanoate	99	25.236	1629	1643	Welke <i>et al.</i> (2012b)
34	Diethyl butanedioate	99	26.648	1669	1672	Duarte <i>et al.</i> (2010)
37	Ethyl phenylacetate	96	29.564	1768	1787	Mallouchos <i>et al.</i> (2007)
38	2-Phenylethyl acetate	99	30.252	1795	1810	Duarte <i>et al.</i> (2010)
<i>Ketones</i>						
5	4-Methyl-3-penten-2-one	98	4.488	1119	1139	Jørgensen <i>et al.</i> (2000)
11	3-Hydroxybutan-2-one	98	10.721	1273	1289	Mallouchos <i>et al.</i> (2007)
<i>Terpenes</i>						
35	2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol	97	27.573	1694	1691	Duarte <i>et al.</i> (2010)
<i>Volatile phenols</i>						
41	2,4-Di-tert-butylphenol	97	38.402	2334	2346	Pereira <i>et al.</i> (2014)
<i>Furan compounds</i>						
21	Furan-2-carbaldehyde	98	17.871	1439	1432	Goodner (2008)
33	Furan-2-methanol	97	26.367	1661	1669	Mallouchos <i>et al.</i> (2007)
<i>Sulphur compounds</i>						
36	3-(Methylsulfanyl)propan-1-ol	98	28.211	1717	1715	Duarte <i>et al.</i> (2010)

^a RT – retention time

^b LTPRI – linear-temperature-programmed retention index experimentally determined

^c LTPRI – linear-temperature-programmed retention index reported in the literature for a CP-Wax columns or equivalent stationary phase

alcohol in Montepulciano wines from the Abruzzo region. We did not detect ethyl hexanoate in the Montepulciano wines from the two regions, although Sagratini *et al.* (2012) reported that ethyl hexanoate was one of the major ester after ethyl octanoate and ethyl decanoate.

The dominating alcohols in the wines from Spain were 3-methylbutan-1-ol (794.89 to 2143.45 µg/L), hexan-1-ol (112.24 to 165.34 µg/L) and 2-phenylethanol (56.15 to 123.44 µg/L) (Table 5). The lowest levels of alcohols were pentan-1-ol, (E)-3-hexen-1-ol, 3-ethoxypropan-1-ol, octen-3-ol, 2-ethylhexan-1-ol, 3-ethyl-4-methylpentan-1-ol, octan-1-ol and phenylmethanol. Ethyl 2-hydroxypropanoate, 3-methylbutyl acetate and ethyl octanoate (272.41 to 547.28 µg/L, “not detected” to 663.69 µg/L and 8.18 to 409.46 µg/L, respectively) were present at the highest concentrations compared to other esters, and ethyl 2-hexenoate, ethyl 3-hydroxybutanoate and ethyl phenylacetate at the lowest concentrations. The 4-methyl-

3-penten-2-one was the most abundant ketone, and furan-2-carbaldehyde the most abundant furan compound. In our study, most of the wines from Spain were of the Tempranillo variety: S_1, S_3, S_4, S_5, S_7 and S_8. As in our examinations, Noguero-Pato *et al.* (2014) found that isoamyl alcohols, 2-phenylethanol and 1-hexanol were the main alcohols in Tempranillo wines (aliphatic, aromatic and C₆ alcohols respectively). Also, ethyl 2-hydroxypropanoate (ethyl lactate) was the most abundant ethyl ester. According to Vilanova *et al.* (2013) and Noguero-Pato *et al.* (2014), 3-methylbutyl acetate (isopentyl acetate) was the most abundant acetate ester, which is consistent with our results. We detected low concentrations of 2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol (α -terpineol) in Tempranillo wines, as did Noguero-Pato *et al.* (2014).

Among the identified alcohols in wines from Poland, 3-methylbutan-1-ol had the highest concentrations (529.19 to 2 306.11 µg/L), followed by hexan-1-ol (122.39 to

TABLE 3
Relative concentrations of aroma compounds ($\mu\text{g/L}$) in red wines from France

Compounds	Wine								Mean $\mu\text{g/L} \pm \Delta\text{S}$
	F_1 $\mu\text{g/L}$	F_2 $\mu\text{g/L}$	F_3 $\mu\text{g/L}$	F_4 $\mu\text{g/L}$	F_5 $\mu\text{g/L}$	F_6 $\mu\text{g/L}$	F_7 $\mu\text{g/L}$	F_8 $\mu\text{g/L}$	
Alcohols									
2-Methylpropanol	136.47	nd	60.70	nd	nd	237.42	80.59	216.05	91.41 \pm 96.37
Butan-1-ol	10.60	6.47	6.05	6.81	11.30	8.71	7.74	5.50	7.90 \pm 2.14
3-Methylbutan-1-ol	898.27	1 273.68	1 434.81	1 219.40	1 224.44	908.46	592.82	1 328.66	1 110.07ab \pm 282.36
Pentan-1-ol	nd	0.91	nd	1.05	nd	nd	nd	nd	0.25 \pm 0.46
4-Methylpentan-1-ol	2.58	2.12	2.79	1.88	4.86	3.24	2.68	2.18	2.79 \pm 0.94
3-Methylpentan-1-ol	4.52	3.06	4.37	3.88	11.52	5.06	3.98	2.56	4.87 \pm 2.80
Hexan-1-ol	266.51	117.08	65.44	96.37	106.91	117.14	90.45	133.30	124.15a \pm 61.06
(E)-3-Hexen-1-ol	2.22	nd	nd	2.00	2.60	1.88	2.66	1.56	1.62 \pm 1.06
3-Ethoxypropan-1-ol	1.60	nd	nd	0.18	nd	nd	0.24	nd	0.25ab \pm 0.55
(Z)-3-Hexen-1-ol	5.85	3.08	0.75	2.51	0.87	1.18	0.85	2.09	2.15b \pm 1.73
Octen-3-ol	1.81	1.13	1.15	1.64	0.63	2.23	1.18	2.40	1.52 \pm 0.61
Heptan-1-ol	3.08	1.12	1.17	1.68	0.91	1.76	1.25	1.54	1.56 \pm 0.68
2-Ethylhexan-1-ol	1.99	nd	1.13	nd	0.79	nd	nd	nd	0.49 \pm 0.75
3-Ethyl-4-methylpentan-1-ol	0.10	0.18	0.26	0.51	nd	0.89	0.31	0.12	0.30 \pm 0.28
Octan-1-ol	0.92	0.42	1.27	nd	0.63	1.28	1.17	0.88	0.82 \pm 0.45
Butane-2,3-diol	22.02	9.08	15.23	8.92	15.76	17.96	17.78	15.82	15.32a \pm 4.44
Propane-1,2-diol	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phenylmethanol	0.94	0.55	1.94	0.85	1.71	1.68	1.63	2.40	1.46b \pm 0.62
2-Phenylethanol	133.54	121.54	183.41	117.98	232.90	199.93	193.35	155.84	167.31c \pm 41.58
Esters									
Ethyl butanoate	37.44	33.79	43.97	43.08	21.10	44.06	42.83	nd	33.28 \pm 15.54
Ethyl 3-methylbutanoate	nd	11.98	16.74	13.80	nd	nd	nd	14.18	7.09 \pm 7.68
3-Methylbutyl acetate	642.83	281.33	307.51	305.73	nd	nd	399.38	311.27	281.01 \pm 208.77
Ethyl hexanoate	nd	nd	nd	213.96	nd	nd	226.39	107.64	68.50 \pm 100.77
Hexyl acetate	nd	nd	nd	7.93	nd	nd	nd	2.06	1.25 \pm 2.79
Ethyl 2-hexenoate	nd	nd	nd	nd	nd	nd	0.46	nd	0.06 \pm 0.16
Ethyl 2-hydroxypropanoate	388.89	273.01	298.52	343.45	302.11	303.50	281.95	334.02	315.68 \pm 37.90
Ethyl octanoate	75.45	37.39	119.15	173.86	46.49	162.64	202.81	168.34	123.27 \pm 63.29
Ethyl (2E,4E)-2,4-hexadienoate	1.58	3.60	nd	nd	nd	nd	nd	nd	0.65 \pm 1.31
Ethyl 3-hydroxybutanoate	nd	nd	nd	nd	0.17	nd	nd	nd	0.02 \pm 0.06

TABLE 3 (CONTINUED)

Compounds	Wine								Mean µg/L ± ΔS
	F_1 µg/L	F_2 µg/L	F_3 µg/L	F_4 µg/L	F_5 µg/L	F_6 µg/L	F_7 µg/L	F_8 µg/L	
Ethyl 2-hydroxy-4-methylpentanoate	1.68	3.20	3.17	3.15	3.38	4.07	3.54	4.44	3.33b ± 0.81
Ethyl decanoate	2.44	2.03	5.17	7.84	3.01	5.33	8.09	8.29	5.28 ± 2.60
Diethyl butanedioate	34.01	46.96	70.35	61.10	73.20	74.50	79.96	71.49	63.95 ± 15.78
Ethyl phenylacetate	0.22	0.29	0.37	0.46	0.41	0.47	0.39	0.39	0.37a ± 0.08
2-Phenylethyl acetate	2.86	1.39	2.33	2.93	2.11	1.67	2.53	1.67	2.19ab ± 0.58
Ketones									
4-Methyl-3-penten-2-one	93.96	285.66	255.62	301.27	336.88	284.01	418.55	315.72	286.46 ± 91.87
3-Hydroxybutan-2-one	0.76	nd	0.63	nd	1.49	nd	nd	nd	0.36 ± 0.55
Terpenes									
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol	0.12	0.15	0.21	0.19	0.21	0.22	0.20	0.32	0.20 ± 0.06
Volatile phenols									
2,4-Di-tert-butylphenol	0.85	2.67	0.56	0.61	0.46	0.41	0.32	0.14	0.75 ± 0.80
Furan compounds									
Furan-2-carbaldehyde	0.73	1.23	1.21	1.09	2.76	0.93	0.37	0.91	1.15 ± 0.71
Furan-2-methanol	0.31	0.08	0.45	0.08	nd	0.20	0.49	nd	0.20 ± 0.19
Sulphur compounds									
3-(Methylsulfonyl)propan-1-ol	1.20	0.75	1.22	0.84	1.79	1.61	1.18	1.10	1.21b ± 0.35

Nd - not detected

Bold and different letters in the same row indicate means significantly different among the wines from the four selected countries.

TABLE 4
Relative concentrations of aroma compounds ($\mu\text{g/L}$) in red wines from Italy

Compounds	Wine											Mean $\mu\text{g/L} \pm \Delta\text{S}$
	I_1 $\mu\text{g/L}$	I_2 $\mu\text{g/L}$	I_3 $\mu\text{g/L}$	I_4 $\mu\text{g/L}$	I_5 $\mu\text{g/L}$	I_6 $\mu\text{g/L}$	I_7 $\mu\text{g/L}$	I_8 $\mu\text{g/L}$	I_9 $\mu\text{g/L}$	I_10 $\mu\text{g/L}$		
Alcohols												
2-Methylpropanol	70.05	nd	nd	nd	nd	nd	71.02	nd	nd	nd	nd	14.11 \pm 29.74
Butan-1-ol	10.22	10.62	8.84	8.59	5.51	10.24	9.88	6.32	4.90	7.53	7.53	8.27 \pm 2.10
3-Methylbutan-1-ol	465.16	387.53	888.87	786.21	1578.58	661.16	621.77	589.27	540.21	257.34	257.34	677.61a \pm 365.78
Pentan-1-ol	nd	nd	nd	1.58	nd	nd	1.50	nd	nd	nd	nd	0.31 \pm 0.65
4-Methylpentan-1-ol	2.99	1.89	1.97	1.85	2.38	2.63	3.06	1.88	2.03	1.45	1.45	2.21 \pm 0.53
3-Methylpentan-1-ol	5.18	3.18	3.32	2.42	3.66	5.30	5.23	1.99	4.00	2.21	2.21	3.65 \pm 1.26
Hexan-1-ol	85.92	72.76	80.79	100.89	160.86	166.53	189.17	62.02	79.93	112.58	112.58	111.15a \pm 44.91
(E)-3-Hexen-1-ol	nd	nd	nd	nd	nd	1.94	2.24	nd	nd	nd	nd	0.42a \pm 0.88
3-Ethoxypropan-1-ol	nd	0.30	0.32	0.10	0.23	0.17	0.16	nd	0.37	0.22	0.22	0.19a \pm 0.13
(Z)-3-Hexen-1-ol	4.98	1.34	1.56	2.32	2.58	1.46	1.85	2.82	1.77	1.70	1.70	2.24 \pm 1.08
Octen-3-ol	2.73	4.58	1.77	3.28	nd	2.06	2.53	1.11	2.07	2.86	2.86	2.30 \pm 1.24
Heptan-1-ol	2.31	3.17	2.23	2.22	1.11	2.34	2.57	0.84	1.81	3.49	3.49	2.21 \pm 0.81
2-Ethylhexan-1-ol	nd	nd	nd	nd	0.81	4.74	nd	nd	nd	nd	nd	0.55 \pm 1.49
3-Ethyl-4-methylpentan-1-ol	0.16	1.45	1.55	0.30	1.35	2.03	2.85	0.14	0.25	2.88	2.88	1.3 \pm 1.070
Octan-1-ol	1.28	1.12	0.93	0.95	nd	0.94	0.95	0.59	nd	1.58	1.58	0.83 \pm 0.51
Butane-2,3-diol	11.61	8.88	35.38	10.16	13.99	12.07	8.26	20.24	14.82	10.50	10.50	14.59a \pm 8.09
Propane-1,2-diol	nd	nd	nd	3.05	nd	nd	nd	nd	nd	nd	nd	0.31 \pm 0.97
Phenylmethanol	0.80	0.69	0.71	0.94	0.96	0.98	1.06	0.75	2.04	0.71	0.71	0.96b \pm 0.40
2-Phenylethanol	212.45	117.57	119.04	113.89	132.41	182.81	188.50	179.48	138.50	104.97	104.97	148.96bc \pm 38.15
Esters												
Ethyl butanoate	23.03	45.64	32.92	31.48	nd	27.61	38.42	22.43	22.27	42.63	42.63	28.64 \pm 13.07
Ethyl 3-methylbutanoate	nd	8.74	11.93	nd	12.18	nd	nd	nd	nd	nd	nd	3.29 \pm 5.37
3-Methylbutyl acetate	nd	nd	342.40	317.05	249.19	278.48	212.30	325.87	296.97	383.54	383.54	240.58 \pm 135.47
Ethyl hexanoate	nd	nd	nd	nd	nd	nd	160.93	nd	nd	nd	nd	16.09 \pm 50.89
Hexyl acetate	nd	14.80	5.51	nd	1.19	nd	nd	nd	3.88	10.01	10.01	3.54 \pm 5.17
Ethyl 2-hexenoate	nd	nd	nd	nd	nd	0.49	1.01	nd	nd	0.64	0.64	0.21 \pm 0.37
Ethyl 2-hydroxypropanoate	293.72	339.78	254.62	246.79	508.78	397.62	418.29	239.34	343.15	314.96	314.96	335.71 \pm 86.05
Ethyl octanoate	125.60	179.86	102.15	71.80	22.97	91.32	133.82	93.27	113.63	217.99	217.99	115.24 \pm 54.59
Ethyl (2E,4E)-2,4-hexadienoate	nd	2.41	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.24 \pm 0.76

TABLE 4 (CONTINUED)

Compounds	Wine										Mean µg/L ± ΔS	
	I_1 µg/L	I_2 µg/L	I_3 µg/L	I_4 µg/L	I_5 µg/L	I_6 µg/L	I_7 µg/L	I_8 µg/L	I_9 µg/L	I_10 µg/L		
Ethyl 3-hydroxybutanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl 2-hydroxy-4-methylpentanoate	3.51	2.44	nd	2.47	2.37	3.03	3.59	2.93	2.51	2.96	2.58ab ± 1.01	6.24 ± 2.88
Ethyl decanoate	5.18	9.44	7.59	4.04	1.70	4.69	5.37	5.91	6.54	11.94	90.58	78.19 ± 18.48
Diethyl butanedioate	77.81	69.99	75.51	54.39	50.55	96.54	112.32	74.65	79.55	90.58	1.03b ± 0.50	3.01b ± 2.53
Ethyl phenylacetate	2.13	1.06	0.52	1.23	0.46	0.97	1.55	0.87	0.70	0.86	2.94	323.82 ± 204.31
2-Phenylethyl acetate	2.10	10.07	2.59	1.87	1.48	2.74	1.94	2.56	1.83	2.94	1.42 ± 3.11	
Ketones												
4-Methyl-3-penten-2-one	601.94	nd	408.23	340.20	223.59	366.74	671.23	216.14	122.32	287.82	287.82	1.42 ± 3.11
3-Hydroxybutan-2-one	nd	nd	8.91	nd	nd	nd	nd	5.28	nd	nd	nd	
Terpenes												
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol	0.33	0.29	0.25	0.17	0.14	0.20	0.15	0.26	1.36	0.27	0.34 ± 0.36	
Volatile phenols												
2,4-Di-tert-butylphenol	0.55	0.21	2.22	0.64	1.67	0.39	1.29	0.38	0.48	0.59	0.84 ± 0.66	
Furan compounds												
Furan-2-carbaldehyde	1.14	2.91	0.83	1.08	1.94	2.25	1.78	1.19	1.31	2.39	1.68 ± 0.68	
Furan-2-methanol	nd	0.37	0.18	0.03	0.14	0.07	nd	0.06	0.19	0.06	0.11 ± 0.11	
Sulphur compounds												
3-(Methylsulfanyl)propan-1-ol	1.91	0.73	0.81	0.80	0.95	1.62	1.95	0.94	1.00	0.82	1.15ab ± 0.48	

Nd - not detected

Bold and different letters in the same row indicate means significantly different among the wines from the four selected countries

TABLE 5
Relative concentrations of aroma compounds ($\mu\text{g/L}$) in red wines from Spain

Compounds	Wine								Mean $\mu\text{g/L} \pm \Delta\text{S}$
	S_1 $\mu\text{g/L}$	S_2 $\mu\text{g/L}$	S_3 $\mu\text{g/L}$	S_4 $\mu\text{g/L}$	S_5 $\mu\text{g/L}$	S_6 $\mu\text{g/L}$	S_7 $\mu\text{g/L}$	S_8 $\mu\text{g/L}$	
Alcohols									
2-Methylpropanol	nd	144.77	nd	nd	129.74	nd	nd	nd	34.31 \pm 63.66
Butan-1-ol	7.66	9.83	10.47	6.73	10.67	nd	nd	nd	5.67 \pm 4.88
3-Methylbutan-1-ol	854.77	794.89	1 493.52	1 181.59	865.23	1 580.29	1 615.03	2 143.45	1 316.10b \pm 475.24
Pentan-1-ol	nd	nd	1.24	nd	nd	nd	nd	nd	0.15 \pm 0.44
4-Methylpentan-1-ol	1.69	1.65	2.27	1.96	2.37	1.84	1.77	2.65	2.03 \pm 0.36
3-Methylpentan-1-ol	3.21	2.24	6.29	4.05	4.05	2.48	4.54	6.72	4.20 \pm 1.63
Hexan-1-ol	154.70	165.34	152.87	112.24	161.05	114.43	148.52	117.23	140.80a \pm 22.29
(E)-3-Hexen-1-ol	nd	nd	nd	1.67	nd	nd	nd	nd	0.21a \pm 0.59
3-Ethoxypropan-1-ol	0.30	0.50	0.59	0.36	0.34	0.34	1.26	0.21	0.49ab \pm 0.33
(Z)-3-Hexen-1-ol	3.74	5.24	4.51	2.08	2.50	0.86	3.42	3.05	3.17 \pm 1.39
Octen-3-ol	nd	1.81	0.93	0.84	0.77	1.24	nd	1.35	0.87 \pm 0.63
Heptan-1-ol	1.01	1.39	1.99	1.74	0.95	0.92	0.84	1.11	1.24 \pm 0.42
2-Ethylhexan-1-ol	nd	nd	nd	nd	0.33	0.52	0.55	nd	0.18 \pm 0.25
3-Ethyl-4-methylpentan-1-ol	0.27	0.35	0.25	0.25	0.13	0.43	0.15	0.16	0.25 \pm 0.10
Octan-1-ol	1.65	0.88	0.68	nd	0.72	0.38	nd	nd	0.54 \pm 0.57
Butane-2,3-diol	30.32	19.96	27.74	24.58	34.40	48.91	90.42	19.96	37.04b \pm 23.51
Propane-1,2-diol	nd	nd	nd	nd	nd	26.37	39.55	nd	8.24 \pm 15.66
Phenylmethanol	0.30	0.83	0.65	0.50	0.33	0.63	0.35	0.36	0.49a \pm 0.19
2-Phenylethanol	108.01	68.94	70.09	91.78	123.44	73.91	56.15	87.00	84.92a \pm 22.34
Esters									
Ethyl butanoate	57.91	25.42	39.23	32.15	40.48	30.06	38.22	66.58	41.26 \pm 14.10
Ethyl 3-methylbutanoate	14.74	7.94	9.33	10.66	5.90	10.98	nd	8.72	8.53 \pm 4.31
3-Methylbutyl acetate	663.69	nd	310.38	219.54	407.36	nd	nd	nd	200.12 \pm 248.01
Ethyl hexanoate	347.86	nd	nd	nd	nd	nd	nd	nd	43.48 \pm 122.99
Hexyl acetate	13.50	nd	nd	nd	nd	nd	4.94	2.47	2.61 \pm 4.75
Ethyl 2-hexenoate	0.87	nd	0.11	0.12	nd	nd	0.25	nd	0.17 \pm 0.30
Ethyl 2-hydroxypropanoate	440.85	547.28	334.74	326.64	287.41	272.41	308.75	480.72	374.85 \pm 101.23
Ethyl octanoate	409.46	8.18	13.41	32.01	39.34	12.46	20.42	60.99	74.54 \pm 136.45
Ethyl (2E,4E)-2,4-hexadienoate	nd	nd	nd	10.36	nd	nd	nd	nd	1.30 \pm 3.66
Ethyl 3-hydroxybutanoate	0.55	nd	0.19	0.23	0.22	nd	nd	0.27	0.18 \pm 0.19

TABLE 5 (CONTINUED)

Compounds	Wine								Mean µg/L ± ΔS
	S_1 µg/L	S_2 µg/L	S_3 µg/L	S_4 µg/L	S_5 µg/L	S_6 µg/L	S_7 µg/L	S_8 µg/L	
Ethyl 2-hydroxy-4-methylpentanoate	2.35	1.90	1.72	2.17	1.47	1.65	1.42	1.98	1.83a ± 0.33
Ethyl decanoate	26.38	0.24	0.32	1.82	1.87	0.70	0.33	1.65	4.16 ± 9.00
Diethyl butanedioate	174.73	42.75	66.35	74.17	54.67	32.28	47.78	44.01	67.09 ± 45.51
Ethyl phenylacetate	0.42	0.21	0.17	0.27	0.16	0.16	nd	0.12	0.19a ± 0.12
2-Phenylethyl acetate	2.73	0.43	1.14	1.24	0.88	0.51	1.45	2.70	1.39ab ± 0.89
Ketones									
4-Methyl-3-penten-2-one	259.44	280.31	250.75	198.03	187.34	205.95	nd	nd	172.73 ± 111.36
3-Hydroxybutan-2-one	15.84	nd	7.98	27.85	nd	11.01	5.26	4.44	9.05 ± 9.29
Terpenes									
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol	0.12	0.10	0.12	0.14	0.07	0.12	0.04	0.04	0.09 ± 0.04
Volatile phenols									
2,4-Di-tert-butylphenol	0.21	0.27	0.38	0.18	0.12	0.52	1.35	1.38	0.55 ± 0.52
Furan compounds									
Furan-2-carbaldehyde	2.38	3.60	2.49	3.39	0.96	3.09	1.81	2.04	2.47 ± 0.88
Furan-2-methanol	0.06	0.31	0.31	0.18	nd	0.07	nd	0.15	0.14 ± 0.12
Sulphur compounds									
3-(Methylsulfonyl)propan-1-ol	0.73	0.64	0.57	0.88	0.94	0.60	0.46	0.70	0.69a ± 0.16

Nd - not detected

Bold and different letters in the same row indicate means significantly different among the wines from the four selected countries

TABLE 6
Relative concentrations of aroma compounds ($\mu\text{g/L}$) in red wines from Poland

Compounds	Wine											Mean $\mu\text{g/L} \pm \Delta\text{S}$
	P_1 $\mu\text{g/L}$	P_2 $\mu\text{g/L}$	P_3 $\mu\text{g/L}$	P_4 $\mu\text{g/L}$	P_5 $\mu\text{g/L}$	P_6 $\mu\text{g/L}$	P_7 $\mu\text{g/L}$	P_8 $\mu\text{g/L}$	P_9 $\mu\text{g/L}$	P_10 $\mu\text{g/L}$	P_11 $\mu\text{g/L}$	
Alcohols												
2-Methylpropanol	nd	217.14	nd	nd	180.37	207.09	230.96	114.96	218.74	nd	nd	106.30 \pm 106.16
Butan-1-ol	12.04	10.54	8.76	6.17	13.85	14.79	5.79	7.75	17.00	11.29	nd	9.82 \pm 4.81
3-Methylbutan-1-ol	1 348.28	864.92	1 069.21	1 410.69	895.89	534.97	714.82	529.19	722.79	1 460.86	2 306.11	1 077.97ab \pm 526.44
Pentan-1-ol	nd	nd	nd	nd	nd	nd	1.88	nd	nd	1.42	nd	0.30 \pm 0.68
4-Methylpentan-1-ol	2.50	2.58	2.53	1.86	3.71	1.22	2.85	2.28	4.13	3.47	3.59	2.79 \pm 0.87
3-Methylpentan-1-ol	4.18	4.62	8.23	2.67	5.01	nd	4.47	3.05	16.72	11.68	5.59	6.02 \pm 4.65
Hexan-1-ol	432.09	448.36	217.67	122.39	325.92	143.27	217.70	347.25	266.04	301.92	190.30	273.90b \pm 108.50
(E)-3-Hexen-1-ol	nd	nd	nd	1.66	nd	nd	nd	nd	nd	nd	nd	0.15a \pm 0.50
3-Ethoxypropan-1-ol	0.59	2.60	1.81	0.93	0.41	nd	0.33	1.05	0.40	1.15	nd	0.84b \pm 0.79
(Z)-3-Hexen-1-ol	8.98	13.35	1.40	1.22	2.18	14.92	6.17	1.17	9.14	2.38	0.44	5.58 \pm 5.26
Octen-3-ol	1.87	4.10	0.74	nd	1.09	nd	4.68	0.95	nd	nd	nd	1.22 \pm 1.69
Heptan-1-ol	5.12	7.08	3.09	1.42	1.87	0.64	nd	3.12	9.66	8.96	1.03	3.82 \pm 3.40
2-Ethylhexan-1-ol	3.75	nd	2.05	nd	2.20	nd	nd	13.41	72.38	77.12	18.15	17.19 \pm 29.10
3-Ethyl-4-methylpentan-1-ol	0.10	0.14	0.53	0.39	0.58	0.06	0.70	0.48	0.06	0.37	0.44	0.35 \pm 0.23
Octan-1-ol	2.46	1.72	1.69	nd	0.69	nd	3.55	2.20	1.69	2.51	0.83	1.58 \pm 1.11
Butane-2,3-diol	22.46	11.11	27.40	8.42	13.36	6.87	10.53	6.91	13.91	10.30	17.55	13.53a \pm 6.54
Propane-1,2-diol	nd	nd	13.80	nd	nd	nd	nd	nd	nd	nd	nd	1.25 \pm 4.16
Phenylmethanol	0.51	0.99	0.58	0.26	1.40	0.72	2.28	0.25	0.40	0.63	0.26	0.75ab \pm 0.61
2-Phenylethanol	92.60	77.46	199.76	28.09	77.42	50.88	158.38	80.77	182.83	135.92	123.95	109.82ab \pm 54.82
Esters												
Ethyl butanoate	48.96	35.02	58.27	87.72	nd	nd	nd	nd	39.60	nd	25.98	26.87 \pm 30.03
Ethyl 3-methylbutanoate	7.09	9.62	17.46	8.04	28.87	nd	nd	nd	nd	nd	nd	6.46 \pm 9.41
3-Methylbutyl acetate	342.69	279.52	1 498.94	412.02	nd	nd	518.42	nd	459.59	412.86	nd	356.73 \pm 431.60
Ethyl hexanoate	nd	nd	nd	nd	nd	nd	nd	110.33	nd	nd	26.05	12.40 \pm 33.41
Hexyl acetate	nd	nd	6.59	3.87	nd	nd	nd	nd	4.8	5.07	nd	1.85 \pm 2.64
Ethyl 2-hexenoate	0.52	1.80	nd	1.10	0.44	nd	0.55	nd	nd	nd	nd	0.40 \pm 0.59
Ethyl 2-hydroxypropanoate	10.65	579.47	56.46	554.27	93.79	552.05	540.88	221.58	311.50	269.94	474.16	333.16 \pm 218.01
Ethyl octanoate	240.26	149.37	290.11	316.22	37.51	48.95	55.28	106.43	143.62	266.72	69.74	156.75 \pm 104.28
Ethyl (2E,4E)-2,4-hexadienoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl 3-hydroxybutanoate	0.42	nd	0.57	nd	0.2	nd	nd	nd	0.78	0.74	nd	0.25 \pm 0.32

TABLE 6 (CONTINUED)

Compounds	Wine											Mean µg/L ± ΔS
	P_1 µg/L	P_2 µg/L	P_3 µg/L	P_4 µg/L	P_5 µg/L	P_6 µg/L	P_7 µg/L	P_8 µg/L	P_9 µg/L	P_10 µg/L	P_11 µg/L	
Ethyl 2-hydroxy-4-methylpentanoate	2.05	2.72	2.43	3.96	3.92	6.26	4.31	2.22	4.89	5.20	3.10	3.73b ± 1.36
Ethyl decanoate	9.97	4.12	21.19	6.27	1.60	2.11	3.31	3.15	7.00	12.84	2.55	6.74 ± 5.95
Diethyl butanedioate	26.30	38.73	77.62	55.54	32.98	23.67	62.95	77.14	13.03	38.35	56.45	45.70 ± 21.70
Ethyl phenylacetate	0.25	0.20	0.45	0.20	0.24	0.15	0.43	0.44	0.27	0.32	0.28	0.29a ± 0.11
2-Phenylethyl acetate	0.86	1.14	4.75	0.36	0.46	0.36	1.32	nd	nd	nd	0.86	0.92a ± 1.35
Ketones												
4-Methyl-3-penten-2-one	220.37	217.03	436.42	nd	447.58	322.06	215.44	879.08	329.24	169.40	270.45	318.82 ± 223.94
3-Hydroxybutan-2-one	1.05	nd	nd	2.28	nd	nd	nd	nd	nd	4.05	nd	0.67 ± 1.33
Terpenes												
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol	0.12	0.14	0.15	0.13	0.08	0.13	0.49	nd	0.19	0.28	0.11	0.17 ± 0.13
Volatile phenols												
2,4-Di-tert-butylphenol	0.15	0.32	0.09	0.21	0.07	0.12	0.93	0.87	0.24	0.12	0.08	0.29 ± 0.31
Furan compounds												
Furan-2-carbaldehyde	nd	0.47	3.69	6.70	1.50	4.41	0.37	1.09	0.30	0.28	0.27	1.73 ± 2.21
Furan-2-methanol	0.02	0.50	nd	0.07	0.21	nd	0.39	nd	0.09	0.10	0.31	0.15 ± 0.17
Sulphur compounds												
3-(Methylsulfanyl)propan-1-ol	0.73	0.81	3.00	0.24	1.42	1.72	2.10	0.95	1.67	1.08	1.30	1.37b ± 0.75

Nd - not detected

Bold and different letters in the same row indicate means significantly different among the wines from the four selected countries

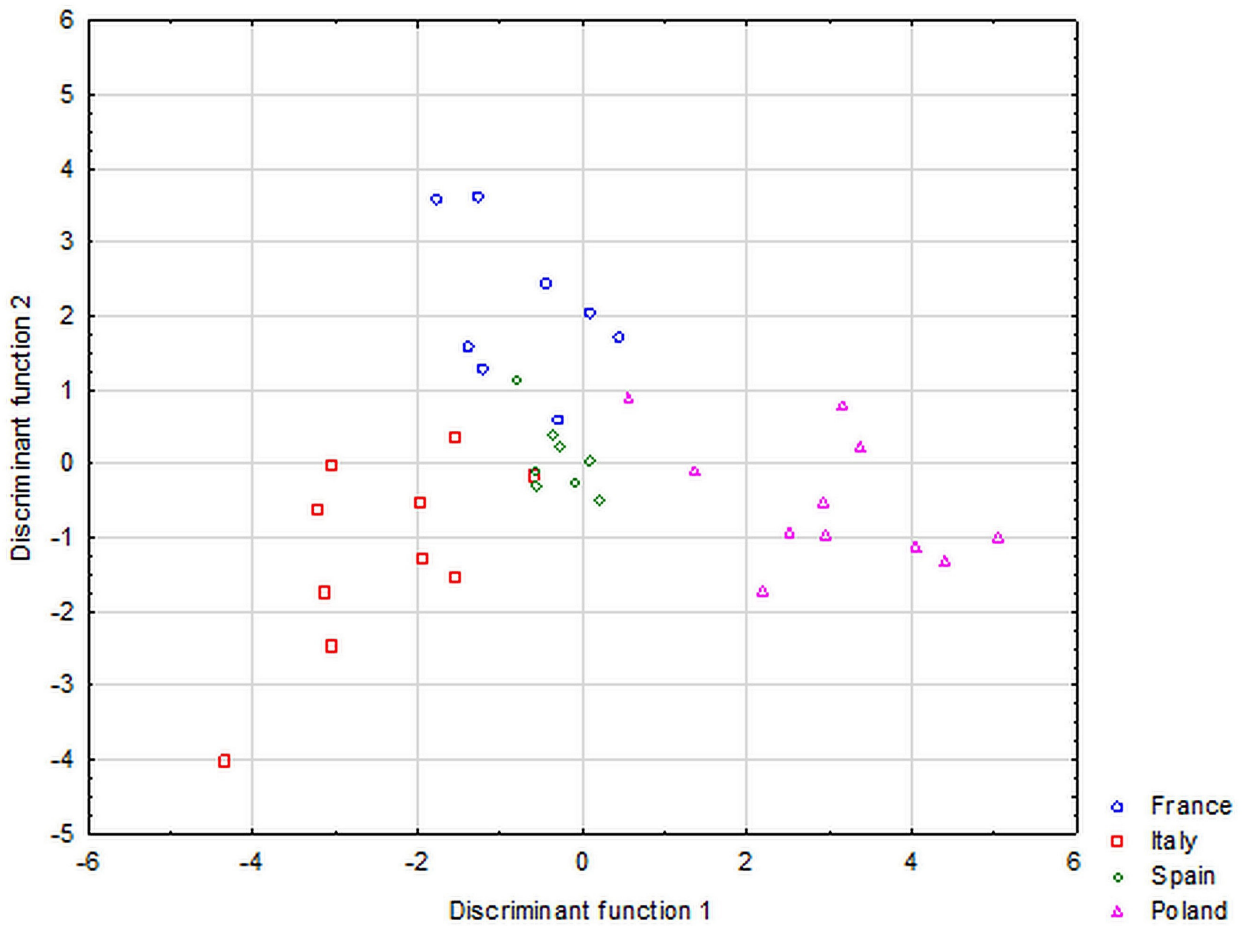


FIGURE 2

Plot on the plane of wines from France, Italy, Spain and Poland defined by the first two canonical discriminant functions

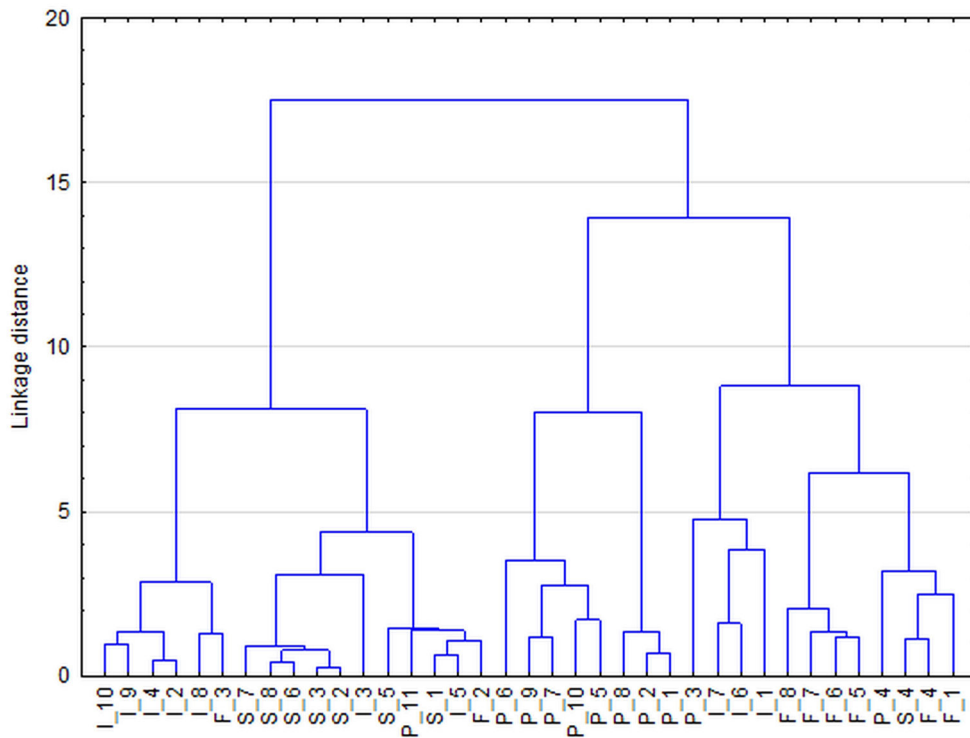


FIGURE 3

Dendrogram constructed with Ward's method for wines from France, Italy, Spain and Poland (see Table 1 for wine sample codes)

448.36 µg/L) and 2-phenylethanol (28.09 to 199.76 µg/L) (Table 6). The lowest levels were shown by pentan-1-ol, (E)-3-hexen-1-ol, 3-ethoxypropan-1-ol, 3-ethyl-4-methylpentan-1-ol and phenylmethanol. The ester fraction was composed mainly of 3-methylbutyl acetate ("not detected" to 1 498.94 µg/L), ethyl 2-hydroxypropanoate (10.65 to 579.47 µg/L) and ethyl octanoate (37.51 to 316.22 µg/L). The minor esters were ethyl 2-hexenoate, ethyl 3-hydroxybutanoate, ethyl phenylacetate, and 2-phenylethyl acetate. Ethyl (2E,4E)-2,4-hexadienoate was not present in any of the wines from Poland. Major volatile compounds within classes were: 4-methyl-3-penten-2-one and furan-2-carbaldehyde. In our work, most of the wines from Poland were produced from the Rondo and Regent varieties. Several researchers have analysed volatile compounds in white and red wines produced in Poland (Dziadas *et al.*, 2010; Tarko *et al.*, 2010; Jeleń *et al.*, 2011), but these wines were from other grape varieties.

Analysis of variance (ANOVA) and the Kruskal-Wallis test showed statistically significant differences between French, Italian, Spanish and Polish wines for the following compounds: 3-methylbutan-1-ol, hexan-1-ol, (E)-3-hexen-1-ol, 3-ethoxypropan-1-ol, butane-2,3-diol, phenylmethanol, 2-phenylethanol, ethyl 2-hydroxy-4-methylpentanoate, ethyl phenylacetate, 2-phenylethyl acetate and 3-(methylsulfanyl)propan-1-ol. The multiple comparisons showed that hexan-1-ol distinguished Polish wines from the others. Its content in Polish wines was significantly higher than in the wines from other countries. Hexan-1-ol, one of the C₆ alcohols, is produced during the pre-fermentative steps (harvesting, transport, crushing and pressing of grapes), when linolenic and linoleic acids are released from the grape skin and react with lipoxygenase. Hexan-1-ol has a "grassy" flavour (Tufariello *et al.*, 2012; Noguerol-Pato *et al.*, 2014).

Linear discriminant analysis (LDA) showed the six most discriminant variables: ethyl phenylacetate, hexan-1-ol, ethyl 2-hydroxy-4-methylpentanoate, (E)-3-hexen-1-ol, 2-phenylethanol and 3-(methylsulfanyl)propan-1-ol. The three designated linear discriminant functions that separated the four groups of wines were statistically significant. The first discriminant function (F1) accounted for 66.73% of the explained variance, the second (F2) for 22.02% and the third (F3) for 11.25%. The canonical correlation coefficients indicated a strong relationship between the first discriminant function and the groups (0.911), while the weakest relationship was found between the third function and the groups. Analysis of standardised coefficients of the canonical discriminant functions showed that 3-(methylsulfanyl)propane-1-ol, hexan-1-ol, ethyl phenylacetate and ethyl 2-hydroxy-4-methylpentanoate had the greatest impact on the first discriminant function. The second discriminant function was mainly determined by 2-phenylethanol, ethyl phenylacetate and (E)-3-hexen-1-ol. Ethyl 2-hydroxy-4-methylpentanoate and 2-phenylethanol had the strongest effect on the third discriminant function. The first discriminant function mainly differentiated Polish wines from the others. The second function discriminated French wines from the others, but the discrimination was not as sharp as for the first function. The third function may have discriminated Spanish wines from the others.

The graphical representation of wines on the plane defined by the first two discriminant functions confirmed that the first discriminant function has the ability to distinguish Polish wines from the others (Fig. 2). The value of Wilk's coefficient $\lambda=0.0359$ shows very good discriminatory power of the model. The results of the wine classification revealed that 91.89% of the wines were classified correctly. The Polish wines were properly classified in 90.91% of cases. One Polish wine (P_4) was classified into the group of Spanish wines. Similarly, Ziółkowska *et al.* (2016) applied LDA for the differentiation of white and red wines according to grape variety and geographical origin based on volatile composition, and reported that discrimination was much worse when SPME-GC-MS rather than SPME-MS data were used. When LDA was carried out on SPME-GC-MS data, compounds with the best discrimination power were hexan-1-ol and 2-phenylethanol, as in our study, as well as ethyl decanoate and ethyl dodecanoate.

Hierarchical cluster analysis (HCA) showed that the tested wines can be divided into three groups when taking into account the compounds selected in the discriminant analysis (Fig. 3). The first group contained most Italian wines (I_2, I_3, I_4, I_5, I_8, I_9, I_10), nearly all Spanish wines (S_1, S_2, S_3, S_5, S_6, S_7, S_8), two French wines (F_2, F_3) and one Polish wine (P_11). Almost all Polish wines (except samples No. 3, 4 and 11) constituted the second group (P_1, P_2, P_5, P_6, P_7, P_8, P_9, P_10). The third group was formed by three Italian wines (I_1, I_6, I_7), nearly all French wines (F_1, F_4, F_5, F_6, F_7, F_8), one Spanish wine (S_4) and two Polish wines (P_3 and P_4). Clearly almost all the Polish wines (8 of 11) were assigned to one group. Two Polish wines (P_3 and P_4) were classified into a separate group containing nearly all French wines and the other one into the group mainly formed by Italian and Spanish wines. The results showed that Polish wines were well separated from other wines, except for samples No. 3, 4 and 11.

The incorrect classification, or clustering, of some wines in LDA and HCA may be due to data obtained by the proposed method of volatile compound analysis (e.g. condition of extraction and technical parameters of GC-MS). Results obtained for LDA and HCA are different since the classification, clustering and mathematical procedures used were different.

CONCLUSIONS

The analysis of aroma compounds has been successfully applied for distinguishing between wines from Poland and wines from France, Italy and Spain. Aroma compounds were determined by HS-SPME-GC/MS. Four commercially available SPME fibres were tested in the same experimental conditions in order to find the fibre that extracted as many volatile compounds as possible. The CAR/PDMS coating extracted the largest number of identified chromatographic peaks with the largest total chromatographic area.

Statistical analysis showed that the content of hexan-1-ol in Polish wines was significantly higher than in the French, Italian and Spanish wines. Hexan-1-ol is produced during pre-fermentative steps, when linolenic and linoleic acids are released from the grape skin and react with lipoxygenase.

The data were processed using linear discriminant analysis and hierarchical cluster analysis. LDA showed that 3-(methylsulfonyl)propane-1-ol, hexan-1-ol, ethyl phenylacetate and ethyl 2-hydroxy-4-methylpentanoate had the highest discrimination power for distinguishing between wines from Poland and those from other European countries. Polish wines were properly classified in 90.91% of the cases. The HCA revealed that 72.73% of the Polish wines were grouped into one cluster. Aroma analysis by means of HS-SPME/GC-MS combined with LDA and HCA is a useful tool for the identification of wines originating from Poland.

LITERATURE CITED

- Antalick, G., Perello, M.C. & De Revel, G., 2014. Esters in wines: New insight through the establishment of a database of French wines. *Am. J. Enol. Vitic.* 63, 293-304.
- Bianchi, F., Careri, M., Mangia, A. & Musci, M., 2007. Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: Database creation and evaluation of precision and robustness. *J. Sep. Sci.* 30, 563-572.
- Burin, V.M., Marchand, S., De Revel, G. & Bordignon-Luiz, M.T., 2013. Development and validation of method for heterocyclic compounds in wine: Optimization of HS-SPME conditions applying a response surface methodology. *Talanta* 117, 87-93.
- Cabredo-Pinillos, S., Cedrón-Fernández, T. & Sáenz-Barrio, C., 2008. Differentiation of "Claret", rosé, red and blend wines based on the content of volatile compounds by headspace solid-phase microextraction and gas chromatography. *Eur. Food Res. Technol.* 226, 1317-1323.
- Câmara, J.S., Alves, M.A. & Marques, J.C., 2006. Development of headspace solid-phase microextraction-gas chromatography-mass spectrometry methodology for analysis of terpenoids in Madeira wines. *Anal. Chim. Acta.* 555, 191-200.
- Coetzee, C. & Du Toit, W.J., 2015. Sauvignon blanc wine: Contribution of ageing and oxygen on aromatic and non-aromatic compounds and sensory composition – A review. *S. Afr. J. Enol. Vitic.* 36, 347-365.
- Du, G., Zhan, J., Li, J., You, Y., Zhao, Y. & Huang, W., 2012. Effect of grapevine age on the aroma compounds in 'Beihong' wine. *S. Afr. J. Enol. Vitic.* 33, 7-13.
- Duarte, W.F., Dias, D.R., Oliveira, J.M., Teixeira, J.A., De Almeida e Silva, J.B. & Schwan, R.F., 2010. Characterization of different fruit wines made from cacao, cupuassu, gabirola, jaboticaba and umbu. *LWT-Food Sci. Technol.* 43, 1564-1572.
- Dugo, G., Franchina, F.A., Scandinaro, M.R., Bonaccorsi, I., Cicero, N., Tranchida, P.Q. & Mondello, L., 2014. Elucidation of the volatile composition of Marsala wines by using comprehensive two-dimensional gas chromatography. *Food Chem.* 142, 262-268.
- Dziadas, M. & Jeleń, H.H., 2010. Analysis of terpenes in white wines using SPE-SPME-GC/MS approach. *Anal. Chim. Acta* 677, 43-49.
- Gamero, A., Wesselink, W. & De Jong, C., 2013. Comparison of the sensitivity of different aroma extraction techniques in combination with gas chromatography-mass spectrometry to detect minor aroma compounds in wine. *J. Chromatogr. A.* 1272, 1-7.
- Goldner, M.C., Di Leo Lira, P., Van Baren, C. & Bandoni, A., 2011. Influence of polyphenol levels on the perception of aroma in *Vitis vinifera* cv. Malbec wines. *S. Afr. J. Enol. Vitic.* 32, 21-27.
- Goodner, K.L., 2008. Practical retention index models of OV-101, DB-1, DB-5, and DB-Wax for flavor and fragrance compounds. *LWT-Food Sci. Technol.* 41, 951-958.
- Jeleń, H.H., Majcher, M., Dziadas, M., Zawirska-Wojtasiak, R., Czaczyk, K. & Wąsowicz, E., 2011. Volatile compounds responsible for aroma of Jutrzenka liqueur wine. *J. Chromatogr. A.* 1218, 7566-7573.
- Jeleń, H.H., Majcher, M. & Dziadas, M., 2012. Microextraction techniques in the analysis of food flavor compounds: A review. *Anal. Chim. Acta.* 738, 13-26.
- Jørgensen, U., Hansen, M., Christensen, L.P., Jensen, K. & Kaack, K., 2000. Olfactory and quantitative analysis of aroma compounds in elder flower (*Sambucus nigra* L.) drink processed from five cultivars. *J. Agric. Food Chem.* 48, 2376-2383.
- Jurado, J.M., Ballesteros, O., Alcázar, A., Pablos, F., Martín, M.J., Vilchez, J.L. & Navalón, A., 2008. Differentiation of certified brands of origins of Spanish white wines by HS-SPME-GC and chemometrics. *Anal. Bioanal. Chem.* 390, 961-970.
- Kallio, H., 1989. Aroma of birch syrup. *J. Agric. Food Chem.* 37, 1367-1371.
- King, E.S., Stoumen, M., Buscema, F., Hjelmeland, A.K., Ebeler, S.E., Heymann, H. & Boulton, R.B., 2014. Regional sensory and chemical characteristics of Malbec wines from Mendoza and California. *Food Chem.* 143, 256-267.
- Mallouchos, A., Loukatos, P., Bekatorou, A., Koutinas, A. & Komaitis, M., 2007. Ambient and low temperature winemaking by immobilized cells on brewer's spent grains: Effect on volatile composition. *Food Chem.* 104, 918-927.
- Marengo, E., Aceto, M. & Maurino, V., 2001. Classification of Nebbiolo-based wines from Piedmont (Italy) by means of solid-phase microextraction-gas chromatography-mass spectrometry of volatile compounds. *J. Chromatogr. A.* 943, 123-137.
- Mendes, B., Gonçalves, J. & Câmara, J.S., 2012. Effectiveness of high-throughput miniaturized sorbent- and solid phase microextraction techniques combined with gas chromatography-mass spectrometry analysis for a rapid screening of volatile and semi-volatile composition of wines – a comparative study. *Talanta* 88, 79-94.
- Metafa, M. & Economou, A., 2013. Chemometrical development and comprehensive validation of a solid phase microextraction/gas chromatography-mass spectrometry methodology for the determination of important free and bound primary aromatics in Greek wines. *J. Chromatogr. A.* 1305, 244-258.
- Noguerol-Pato, R., Sieiro-Sampredro, T., González-Barreiro, C., Cancho-Grande, B. & Simal-Gándara, J., 2014. Effect on the aroma profile of Graciano and Tempranillo red wines of the application of two antifungal treatments onto vines. *Molecules* 19, 12173-12193.
- Pereira, V., Cacho, J. & Marques, J.C., 2014. Volatile profile of Madeira wines submitted to traditional accelerated ageing. *Food Chem.* 162, 122-134.
- Perestrelo, R., Silva, C. & Câmara, J.S., 2014. A useful approach for the differentiation of wines according to geographical origin based on global volatile patterns. *J. Sep. Sci.* 37, 1974-1981.
- Rodrigues, F., Caldeira, M. & Câmara, J.S., 2008. Development of a dynamic headspace solid-phase microextraction procedure coupled to GC-qMSD for evaluation of the chemical profile in alcoholic beverages. *Anal. Chim. Acta* 609, 82-104.
- Römisch, U., Jäger, H., Capron, X., Lanteri, S., Forina, M. & Smeyers-Verbeke, J., 2009. Characterization and determination of the geographical origin of wines. Part III: multivariate discrimination and classification methods. *Eur. Food Res. Technol.* 230, 31-45.
- Sáenz, C., Cedrón, T. & Cabredo, S., 2010. Classification of wines from five Spanish origin denominations by aromatic compound analysis. *J. AOAC Int.* 93, 1916-1922.

- Sagratini, G., Maggi, F., Caprioli, G., Cristalli, G., Ricciutelli, M., Torregiani, E. & Vittori, S., 2012. Comparative study of aroma profile and phenolic content of Montepulciano monovarietal red wines from the Marche and Abruzzo regions of Italy using HS-SPME-GC-MS and HPLC-MS. *Food Chem.* 132, 1592-1599.
- Song, S., Tanga, Q., Hayat, K., Karangwa, E., Zhang, X. & Xiao, Z., 2014. Effect of enzymatic hydrolysis with subsequent mild thermal oxidation of tallow on precursor formation and sensory profiles of beef flavours assessed by partial least squares regression. *Meat Sci.* 96, 1191-1200.
- Stój, A., 2011. Metody wykrywania zafałszowań win. *Żywność. Nauka. Technologia. Jakość.* 75(2), 17-26.
- Tarko, T., Duda-Chodak, A., Sroka, P., Satora P. & Jurasz, E., 2010. Polish wines: Characteristics of cool-climate wines. *J. Food Comp. Anal.* 23, 463-468.
- Trani, A., Verrastro, V., Punzi, R., Faccia, M. & Gambacorta, G., 2016. Phenols, volatiles and sensory properties of Primitivo wines from the "Gioia Del Colle" PDO Area. *S. Afr. J. Enol. Vitic.* 37, 139-148.
- Tufariello, M., Capone, S. & Siciliano, P., 2012. Volatile components of *Negroamaro* red wines produced in Apulian *Salento* area. *Food Chem.* 132, 2155-2164.
- Vilanova, M., Genisheva, Z., Graña, M. & Oliveira, J.M., 2013. Determination of odorants in varietal wines from international grape cultivars (*Vitis vinifera*) grown in NW Spain. *S. Afr. J. Enol. Vitic.* 34, 212-222.
- Vitis International Variety Catalogue, <http://www.vivc.de/>, Accessed June 26, 2017.
- Weldegergis, B.T., De Villiers, A., McNeish, Ch., Seethapathy, S., Mostafa, A., Górecki, T. & Crouch, A.M., 2011. Characterisation of volatile components of Pinotage wines using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC x GC-TOFMS). *Food Chem.* 129, 188-199.
- Welke, J.E., Manfroi, V., Zanus, M., Lazzarotto, M. & Zini, C.A., 2012b. Characterization of the volatile profile of Brazilian Merlot wines through comprehensive two dimensional gas chromatography time-of-flight mass spectrometric detection. *J. Chromatogr. A.* 1226, 124-139.
- Welke, J.E., Zanus, M., Lazzarotto, M., Schmitt, K.G. & Zini, C.A., 2012a. Volatile characterization by multivariate optimization of headspace-solid phase microextraction and sensorial evaluation of Chardonnay base wines. *J. Brazil. Chem. Soc.* 23, 678-687.
- Wilk, K., 2011. Polski rynek win w świetle zmian w krajowych i wspólnotowych uregulowaniach prawnych. *Studia i Prace Wydziału Nauk Ekonomicznych i Zarządzania.* 22, 135-148.
- Yan, J., Liu, X.B., Zhu, W.W., Zhong, X., Sun, Q. & Liang, Y.Z., 2015. Retention indices for identification of aroma compounds by GC: Development and application of a retention index database. *Chromatographia* 78, 89-108.
- Zhang, L., Tao, Y.S., Wen, Y. & Wang, H., 2013. Aroma evaluation of young Chinese Merlot wines with denomination of origin. *S. Afr. J. Enol. Vitic.* 34, 46-53.
- Ziółkowska A., Wąsowicz E. & Jeleń H.H., 2016. Differentiation of wines according to grape variety and geographical origin based on volatiles profiling using SPME-MS and SPME-GC/MS methods. *Food Chem.* 213, 714-720.