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Optimisation of SPME Arrow GC/MS method for determination of wine volatile organic compounds

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ABSTRACT

A new technology called solid phase microextraction (SPME) Arrow has recently been used in the analysis of food volatile organic compounds (VOCs). This technology is still not widely used for the analysis of wine VOCs, which play a crucial role in sensory properties. For optimisation of SPME Arrow extraction conditions, Box–Behnken experimental design and response surface methodology were used. The optimised conditions were extraction temperature, incubation time, and exposure time. The most significant factors affecting extraction efficiency were extraction temperature and exposure time. An increase in temperature and exposure time increased the analytical signal of most classes of VOCs extracted from both white and red wine samples. For VOCs in white wines, the optimal conditions are an extraction temperature of 50 °C, an incubation time of 10 min, and an exposure time of 60 min, while for VOCs in red wines, the optimal conditions are an extraction temperature of 60 °C, an incubation time of 17 min, exposure time of 53 min. The new optimised method allows more accurate and time-saving analysis of VOCs from wines, enabling the analysis of a large number of samples.

KEYWORDS: SPME Arrow, method optimisation, wines, VOCs

INTRODUCTION

The volatile profile of food and beverages is composed of thousands of compounds with varying physico-chemical properties, such as molecular structure, polarity, and molecular weight (Pérez-Jiménez *et al*., 2021). Furthermore, the concentrations of volatile compounds in different matrices vary greatly, ranging from a few ng/L to more than hundreds of mg/L (Pérez-Jiménez *et al*., 2021; Roman *et al*., 2020). Wine is a complex matrix with more than 800 volatile organic compounds (VOCs) identified (Roman *et al*., 2020). The complex aromas of the final wine are derived from several sources: a) grape and varietal VOCs, found in grape berries in free or bound form (terpenoids, C_{13}-norisoprenoids, volatile phenols, methoxypyrazines); b) fermentative VOCs derived from alcoholic and malolactic fermentations through yeast and bacterial metabolism (esters, higher alcohols, carbonyls, volatile fatty acids); c) maturation/ageing VOCs, which develop during wine ageing and/or extracted from wood barrels [lactones, furanic compounds, volatile phenols] (Francis and Newton, 2005; Gonzalez-Barreiro *et al*., 2015; Pittari *et al*., 2021).

These numerous compounds define the aroma and flavour of wine and consequently the wine quality and consumer acceptance. Thus, the analysis of wine VOCs is of great importance in the wine industry. The extraction of the analytes from the matrix is one of the main steps in the sample preparation and is essential to determine the volatile composition of wine (Roman *et al*., 2020). Some of the conventional extraction methods, such as liquid–liquid extraction (LLE) or solid phase extraction (SPE), have many drawbacks, for example, low reproducibility, low selectivity, and analytes loss. Furthermore, they require the use of solvents hazardous to the users' health and damaging to the environment (Pérez-Jiménez *et al*., 2021; Polaskova *et al*., 2008; Roman *et al*., 2020). Thus, solvent-free techniques have been developed, such as solid phase microextraction (SPME) or stir bar sorptive extraction (SBSE), with an emphasis on rapid, sensitive, and automated analysis. SPME became a popular and widely used extraction method due to its advantages of being simple, fast, inexpensive, solvent-free, and requiring limited manipulation of the sample (Azzi-Achkouty *et al*., 2017). The performance of SPME largely depends on the selection of appropriate fused silica fibre, which is primarily responsible for the analyte extraction (Azzi-Achkouty *et al*., 2017). There are several commercially available fibre coatings, the most common polydimethylsiloxane (PDMS), polyacrylate (PA), divinylbenzene (DVB), carboxen (CAR), and their combinations (PDMS/DVB, CAR/PDMS, DVB/CAR/PDMS). The PDMS is a non-polar phase and is used for the extraction of non-polar analytes, such as volatile flavour compounds. The more-polar PA fibre is preferred for the extraction of more-polar analytes, while mixed coating fibres increase retention capacity (Kataoka *et al*., 2000).

The SPME technique also has disadvantages, such as operating temperature, the breakage of the fibre, the coating stripping, the expense of the needle, poor inter-device reproducibility, and small extraction phase volume (Olcer *et al*., 2019; Roman *et al*., 2020). A new technique called SPME Arrow was introduced with the advantage of higher sample capacity due to the larger volume of the sorbent phase. This allows better reproducibility and for more volatile compounds to be extracted and analysed (Kim *et al*., 2020). However, like any SPME method, SPME Arrow requires careful optimisation of parameters influencing the extraction efficiency. The most important parameters are the type and volume of the extracting phase, the agitation method, the extraction time and temperature, and sample modifications, like adjusting pH or ionic strength (Pati *et al*., 2021). Other important parameters are the desorption conditions on GC instruments (temperature, time), which also need to be optimised.

Since SPME Arrow is a new technique, it is not widely used in the analysis of wine volatiles. Lisanti *et al*. (2021) optimised SPME Arrow extraction conditions for the analysis of compounds contributing to minty aromas in red wines. However, the optimisation process included only nine compounds as targeted analytes, which disenables the usage of the developed method for untargeted analysis and profiling of wine volatiles. Thus, this study aimed to develop and evaluate the SPME Arrow extraction technique coupled with GC/MS instruments for the analysis of volatile organic compounds (VOCs) from red and white wines.

MATERIALS AND METHODS

1. Wine samples for method optimisation

Wine samples were obtained from commercial wineries. The samples included red and white wines from different grapevine varieties, regions, vintages, and ageing (Table S1). A separate mixture of red and white wines was used for the optimisation process (including experiments for the determination of sample volume and salt content), which was obtained by mixing 100 mL of each wine.

2. Wine samples for method application

For the method application samples of Grk, Pošip, Maraština, and Vugava white wines together with Teran and Plavina red wines, all of them being autochthonous Croatian grape varieties, were used. Grapes used for white wine vinification were produced in the wine region Dalmatia, the wine-growing hill ZadarBiograd. Teran grapes were produced in the wine-growing hill Western Istria (Poreč) while Plavina grapes were produced in Dalmatia. The vinification process was carried on in the experimental cellar of the Department of Viticulture and Enology, Faculty of Agriculture University of Zagreb. In total, 100 kg of grapes of each variety, vintage 2021, were destemmed, crushed, and in the case of white grapes pressed, and distributed into 70 L stainless steel fermenters. For red wine production maceration process lasted for 7 days at 20 °C and during that period, mash aeration and cap management were carried out by mechanical mixing while in the case of white wine samples were racked after 24-hour settlement. In all variants, the addition of sulfur dioxide (SO_2) in the concentration of 50 mg/L and inoculation

by commercial *S. cerevisiae* strains was conducted: for white wines Lalvin EC-1118 (Lallemand) and red UVAFERM BDX (Lallemand) The course of fermentation was monitored by the sugar consumption, and it was considered complete when the residual sugar concentrations were under 4.0 g/L. The sugar content was analysed according to the OIV methods for residual sugars (OIV, 2019). In all grape variety variants fermentation started 24 hours after inoculation and lasted between 12 and 14 days. The final wines were bottled in 750 mL glass bottles with screw caps and transported to the laboratory of the Department of Viticulture and Enology, Faculty of Agriculture University of Zagreb for chemical analysis.

3. SPME Arrow and GC/MS Analysis

The SPME Arrow extraction was performed by the RSH TriPlus autosampler (Thermo Fisher Scientific Inc., Brookfield, USA). The suitable sample volume was put into 20 mL headspace screw-top vials sealed with PTFE/silicone septum-containing caps. The needle depth in the vial was standard, with a desorption time of 7 min.

Sample analysis was conducted on the TRACE™ 1300 Series gas chromatographer coupled to an ISQ 7000 TriPlus quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Brookfield, USA) equipped with a TG-WaXMS A capillary column (60 m × 0.25 mm × 0.25 µm film thickness; Thermo Fisher Scientific, Brookfield, USA). The volatile compounds injected into the inlet were delivered to the column in a splitless mode, and helium was used as a carrier gas at a constant flow rate of 1 mL/min. The split flow was 15 mL/min, while the purge flow was 5 mL/min. The oven temperature program was as follows: an initial temperature of 40 °C was maintained for 5 minutes, followed by an increase of 2 °C/min to 210 °C and held for 10 minutes. The transfer line and ion source temperature were 250 °C. The MS spectra were recorded in the electron impact ionisation mode (EI) at an ionisation energy of 70 eV. The mass spectrometer was performed in full scan mode at 30–300 m/z. The total time of analysis was 100 min. The data obtained was processed using the Chromeleon™ Chromatography Data System (Thermo Fisher Scientific Inc., USA). The volatile compounds were identified by comparing the recorded mass spectrum with the data available in the Wiley Registry 12th Edition/NIST Spectral Library. The retention index (RI) was calculated using alkane standards C8-C20 (Sigma Aldrich, St. Louis, USA) according to the equation described in Song, *et al*. (2019) and compared with previously reported in the literature (Babushok *et al*., 2011; Babushok and Zenkevich, 2009) (Table S2 and Table S3).

4. Optimisation of SPME Arrow method for determination of wine VOCs

4.1. Identification of VOCs present in wine

To identify VOCs present in wine samples, in a volume of 5 mL of wine, 1.5 g of NaCl was added. The sorption conditions were as follows: after incubating the appropriate sample volume at 60 °C for 20 minutes, the DVB/CAR/PDMS (120 µm × 20 mm) SPME Arrow was exposed for 60 minutes. Then, the desorption was done at 250 °C for 7 min. After desorption, the arrow was conditioned for 10 minutes at 250 °C. The SPME Arrow sorption and desorption conditions were chosen based on the previously reported research (Canuti *et al*., 2009; Carpentieri *et al*., 2019; Yang *et al*., 2019). All experiments were conducted in triplicate.

4.2. Determination of sample volume

For determining the suitable sample volume, 3-, 4-, and 5 mL volumes were used. The sorption and desorption conditions were the same as previously described. All experiments were conducted in triplicate. The parameters were evaluated based on the sum of the peak areas for all compounds of each group.

4.3. Determination of salt content

To determine the salt content, 5 mL of wine was treated with 0-, 1-, and 2 g of NaCl. The sorption and desorption conditions were the same as previously described. All experiments were conducted in triplicate. The parameters were evaluated based on the sum of the peak areas for all compounds of each group.

4.5. Experimental design

Box–Behnken experimental design (BBD) and response surface methodology were applied to optimise the SPME Arrow extraction conditions. Sample volume, the amount of NaCl, and SPME Arrow coating were constant: 5 mL of wine sample, 2 g of NaCl, and DVB/CAR/PDMS. Desorption was done at 250 °C for 7 min, while conditioning was done at 250 °C for 10 min. The variables selected for SPME Arrow optimisation were the extraction temperature, incubation time, and exposure time (Table 1). In total, 15 experiments were generated by BBD and were executed in randomised order. For establishing the optimum conditions for individual classes of VOCs, the analysis of variance (ANOVA), regression, and plotting of the response surface plot were conducted. For optimisation, multicriteria methodology

TABLE 1. Independent factors and their levels used in the BBD for optimisation of SPME Arrow extraction for determination of volatile compounds from white and red wines.

Factors	Factor levels		
Coded levels	–1	0	1
A: Extraction temperature (° C)	40	50	60
B: Incubation time (min)	10	20	30
C: Exposure time (min)	30	45	60

(Derringer function or desirability function) was used. The experimental design analysis and calculation of the predicted data were completed using the DesignExpert software (Stat-Ease Inc., Minneapolis, USA).

4.6. Calibration and method performance

Calibration curves were constructed to quantify VOCs using the optimised SPME Arrow conditions. The stock solutions of standard compounds (Table S4) were dissolved in methanol. The linear ranges of the method were analysed by performing calibration curves using five concentration levels diluted in a model synthetic solution comprised of 11 % (v/v) ethanol and 1 mM tartaric acid (pH 3.1). The regression curves and squared determination coefficient were evaluated to determine the linearity of every compound. The performance of the optimised SPME Arrow method for VPCs was evaluated using 30 compounds belonging to the following groups: alcohols (5 compounds), C_{13}-norisoprenoids (5 compounds), acids (5 compounds), esters (5 compounds), lactones (5 compounds) and monoterpenes (5 compounds). The limit of detection (LOD) was defined, according to IUPAC, as the smallest amount of analyte concentration in the sample that can be reliably distinguished from zero, with the acceptance criteria that the signal-to-noise (S/N) ratio is 3. The limit of quantitation (LOQ) is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision (RSD < 20 %) and accuracy (recovery range 80–120 %), with the acceptance criteria that the S/N ratio is 10. The intraday precision was determined from 3 successive injections of the mix of standard compounds prepared in a model synthetic solution at 1000 µg/L. The interday precision was determined by three injections on three days of the week. The accuracy was evaluated from the recovery of intraday precision samples. The precision was

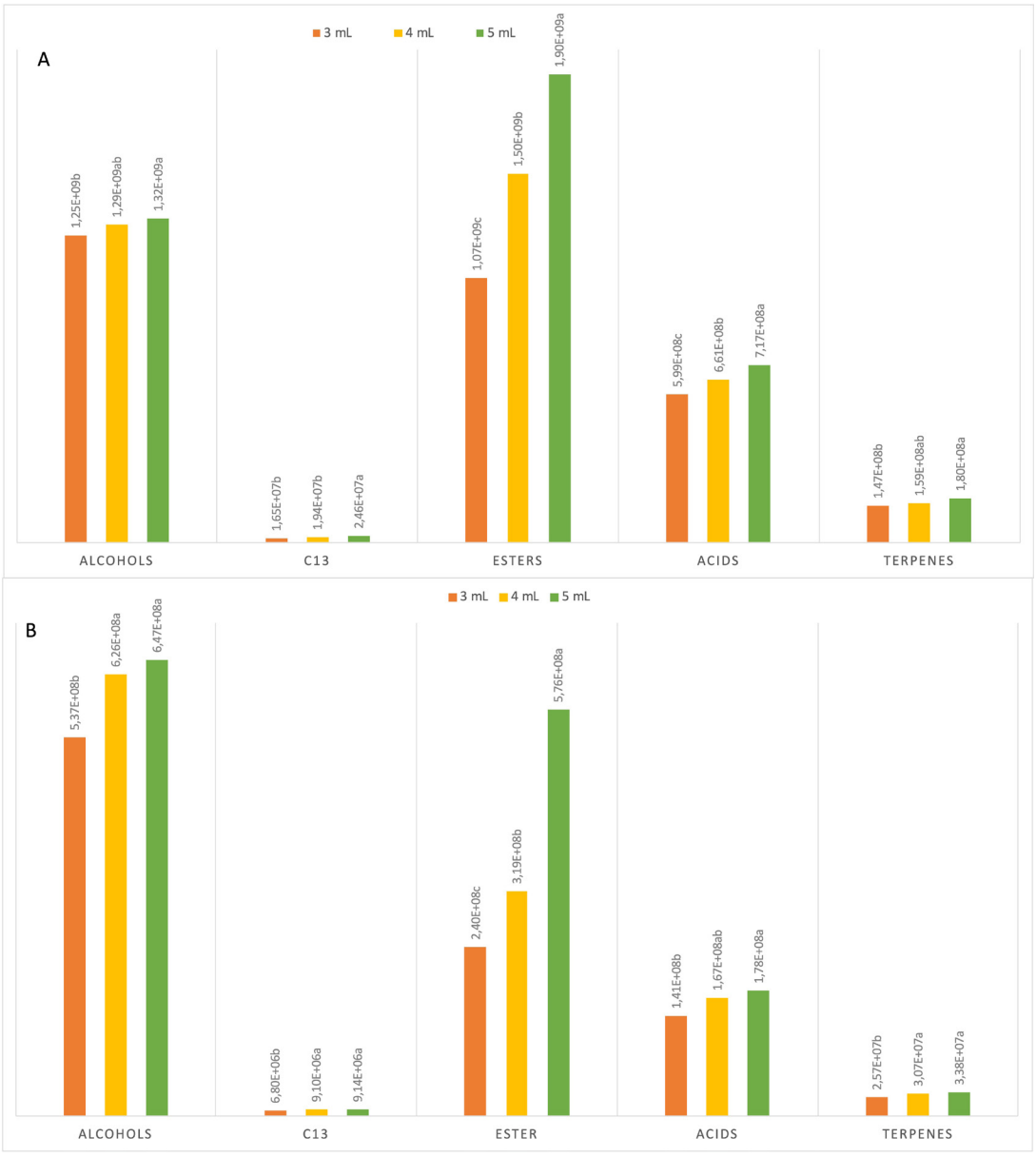


FIGURE 1. The effect of sample volume on absolute peak areas of white wine (A) and red wine (B); n = 3, letters show a statistically significant difference ($p \leq 0.05$).

calculated using the relative standard deviation (RSD %). The reproducibility was determined by five injections of standard solutions at the extremes of calibration curves, *e.g.*, 5 and 5000 µg/L, respectively, or authentic samples, and was calculated using %CV.

4.7. Statistical analysis

An ANOVA was applied to the experimental data. The significant differences were determined by Duncan's *post hoc* test. Principal component analysis (PCA) was performed using VOC profiles (mean value of each compound) of analysed wines. The statistical analysis was performed using XLSTAT (Addinsoft, 2020, New York City, USA).

RESULTS AND DISCUSSION

1. Determination of sample volume

To determine the sample volume, 3-, 4-, and 5 mL of wine samples were chosen. It is well known that sample size is an important factor in any analysis. The results of this one-factor-at-a-time experiment are shown in Figure 1.

The statistically significant difference in peak areas was observed for all analysed groups of compounds for both white and red wine samples. The highest absolute peak areas in both samples were obtained by the largest sample volume of 5 mL. This is not surprising, since the amount of analyte extracted, increases with the sample size (Risticevic *et al.*, 2010).

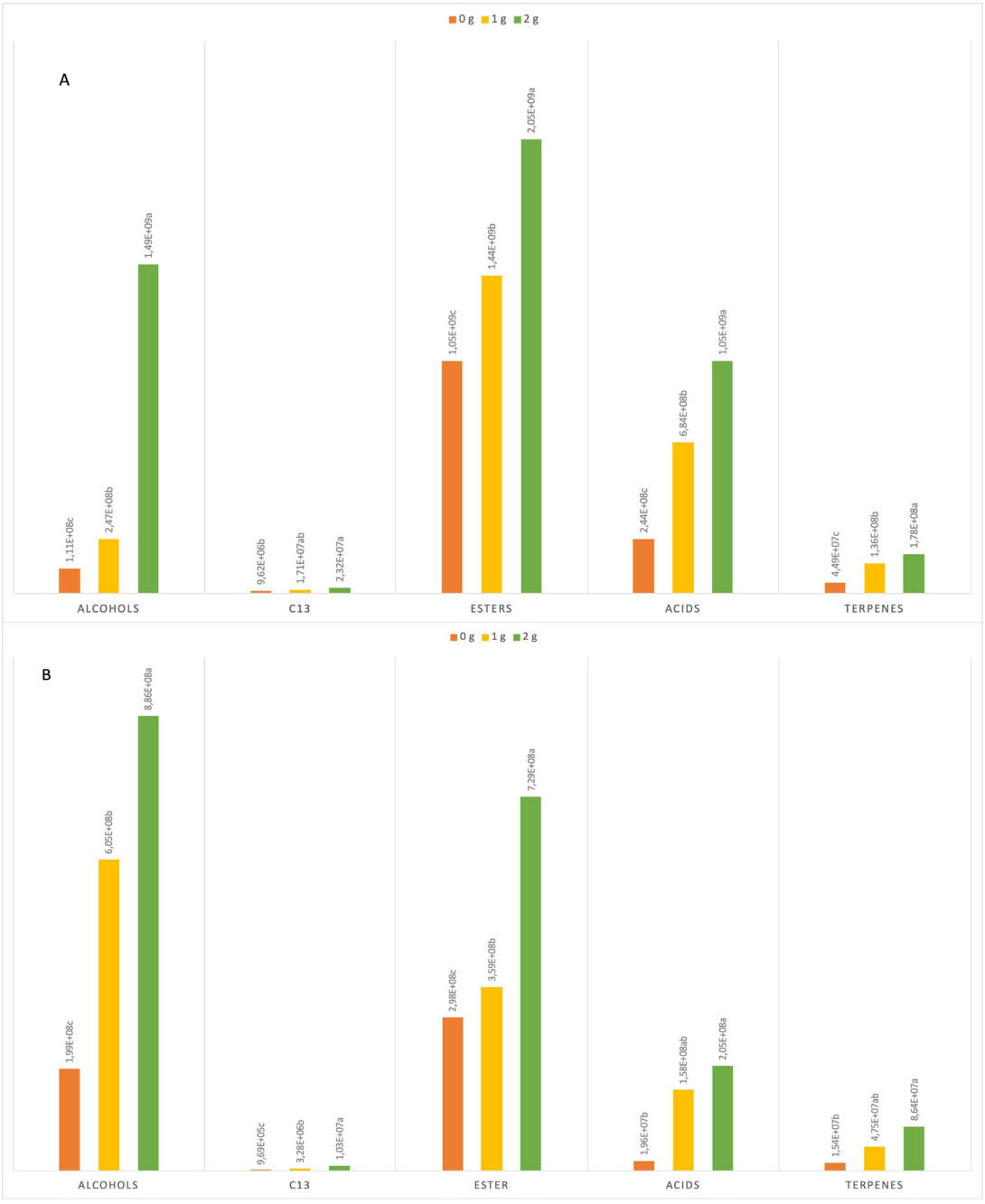


FIGURE 2. The effect of salt content on absolute peak areas of white (A) and red wine (B); n = 3, letters show a statistically significant difference ($p \leq 0.05$).

The obtained results are similar to those obtained for sparkling wine (Tufariello *et al*., 2019a), where a sample volume of 5 mL also provided the best extraction efficiency. Furthermore, wine is an alcoholic beverage containing ethanol, which can also influence the extraction efficiency and sample size. In research by Saha *et al*. (2018), ethanol concentrations and partition coefficient influenced the extraction of other volatile compounds. Ethanol has a relatively high partition coefficient but is present in high concentrations relative to other volatile compounds. This could lead to competition between ethanol and other volatile compounds for SPME fibre sampling sites, resulting in lower sampling sensitivity. Thus, the authors recommend larger sample volumes, that can increase the quantity of some analytes present in the headspace vial relative to ethanol, and consequently increase the sensitivity of SPME sampling for these compounds. The sample volume of 5 mL was used in further optimisation processes for both white and red wine samples.

2. Determination of salt content

In the headspace SPME of aqueous samples, the efficiency of extraction is generally improved by using a salting-out agent able to increase the concentration of the analytes in the headspace, and thus in the SPME fibre coating. The salt increases the ionic strength of the solution and leads to decreased solubility of the analytes in the solution (Fiorini *et al*., 2015). The most commonly used salt to adjust ionic strength is sodium chloride (NaCl). Furthermore, the addition of salts can increase or decrease the amount of analyte extracted, depending on the nature of the targeted analyte and salt concentration (Risticevic *et al*., 2010). To test the effect of salt content on the extraction efficiency 0-, 1-, and 2 g of NaCl were chosen. The results of this one-factor-at-a-time experiment are shown in Figure 2.

The statistically significant difference in peak areas was observed for all analysed groups of compounds for both white and red wine samples. It can be observed that the absolute peak areas increase as the amount of NaCl increases, which is in accordance with other literature reports (Azzi-Achkouty *et al*., 2017; Tufariello *et al*., 2019b). However, the magnitude of the salting-out effect is not the same for all groups of VOCs analysed. The salt addition in the analysed wine samples had the most significant effect on the alcohols and acids due to their high-water solubility (Table S2 and Table S3). The salt addition lowered the partition coefficient and increased their concentration in the headspace.

The amount of salt added should be enough to saturate the sample, which will maintain the same ionic strength and ensure the reproducibility of the analysis. Thus, the salt content of 2 g was chosen for further optimisation as it provided the best extraction efficiency. However, a further increase in the salt content would not positively affect the extraction efficiency, for example (Câmara *et al*., 2006) found that higher salt rates tended to be deleterious for some compounds, like nerol and β-Damascenone. In a study by Rocha *et al*. (2001), it was found that higher content of NaCl had lower reproducibility of the analysis as well as decreasing sensitivity for esters.

3. Optimisation of SPME Arrow conditions: white wines

A total of 103 volatile compounds were extracted and identified in the white wine sample, belonging to the following groups: alcohols (23 compounds), C_{13}-norisoprenoids (9 compounds), esters (28 compounds), acids (12 compounds), terpenes (29 compounds), and other 2 compounds.

For the optimisation process, Box–Behnken experimental design (BBD) was used. It allows the efficient estimation of the mathematical model's first- and second-order coefficients, which is more efficient and economical in comparison to 3^k designs (Bezerra *et al*., 2008). The selection of SPME conditions depends on the nature and complexity of the analysed sample and the properties of the target analytes (Risticevic *et al*., 2010). In this study, the evaluated experimental factors were extraction temperature (40-, 50-, 60 °C), incubation time (10-, 20-, 30 min), and

TABLE 2. Optimal SPME Arrow extraction conditions for individual classes of VOCs in white wine.

Group	Temperature (°C)	Incubation time (min)	Exposure time (min)	Average boiling point (°C)	Vapour Pressure (Pa)	Average log K_{ow}	Water solubility at 25 °C (mg/L)
Acids (n = 12)	54	10	30	239	78	3.01	12807
Alcohols (n = 23)	40	16	41	184	215	1.97	92077
Esters (n = 28)	60	10	56	237	74	3.53	24339
Terpenes (n = 29)	44	30	59	208	108	4.48	225
Norisoprenoids (n = 9)	60	30	60	256	4.39	4.47	78

exposure time (30-, 45, 60 min). The BBD experimental design generated 15 experiments, including three central points. The experiments were randomly performed. The area of the responses based on the sum of the peak areas was applied to evaluate the significance of each studied factor. Fitting the data with various models showed that the contents of acids, alcohols, C_{13}-norisoprenoids, esters, and terpenes were best represented by quadratic polynomial models. The analysis of variance (ANOVA) parameters are shown in Table S5. The determination coefficient (R^2), *p*-value, lack of fit, adapted R^2, and precision are parameters that show the model's significance. To display the model's significance, the *p*-value must be less than 0.05, while the *p*-value of lack of fit must be higher than 0.05. In addition, the difference between the adapted R^2 and predicted R^2 must be less than 0.2. Otherwise, it refers to problems with obtained results and/or models. In the present study, the determination coefficients (R^2) range from 0.9012 to 0.9797, while *p*-values range from < 0.0001 to 0.0135, indicating that models are highly significant. Models also showed a statistically insignificant lack of fit with determined *p*-values higher than 0.2080. All response parameters of quadratic polynomial equations are depicted in Table S5.

SPME Arrow extraction conditions were optimised individually for acids, alcohols, esters, terpenes, and C_{13}-norisoprenoids. The optimal conditions and average physical properties values (boiling point, vapour pressure, water solubility, partition coefficient) are represented in Table 2. The extraction temperature and exposure time are the most significant factors affecting the extraction efficiency of VOCs in white wine. An increase in extraction temperature positively affected the extraction efficiency of acids, esters, and norisoprenoids, with an optimum extraction temperature of 54 °C for acids and 60 °C for esters and norisoprenoids. The optimum extraction temperature for alcohols and terpenes was lower and was 40 °C and 44 °C. These achieved optimum temperatures are in accordance with the physical properties of the analysed VOCs, such as boiling point. The average boiling point for norisoprenoids and esters are 256 °C and 237 °C, respectively, while for the alcohols is 184 °C. As can be seen, the optimum extraction temperature is not the same for all analysed groups of VOCs, which is in accordance with the results presented by Câmara *et al*. (2006), Rochaı *et al*. (2001) and Welke *et al*. (2012). It is expected as the temperature rises, more analytes are released into the headspace. However, due to the decrease in partition coefficients, the absorption of analytes is reduced (Rodrigues *et al*., 2008). Exposure time was another significant factor and is closely related to temperature since an increase in temperature enables shorter exposure time (Welke *et al*., 2012). Furthermore, the volatility of compounds should also be considered as less volatile compounds need a longer exposure time to achieve equilibrium (Câmara *et al*., 2006; Rocha *et al*., 2001). The increase in exposure time of up to 60 min positively influenced the extraction of terpenes and norisoprenoids.

On the other hand, the optimum exposure time for acids and alcohols was significantly lower and was 30 and 40 min, respectively. The incubation time did not significantly influence the extraction efficiency, except in the case of acids and alcohols. The optimum incubation time for acids was 10 min, while for alcohols, it was 16 min.

A surface model for all VOCs was used to visualise the combined effect of extraction temperature and exposure

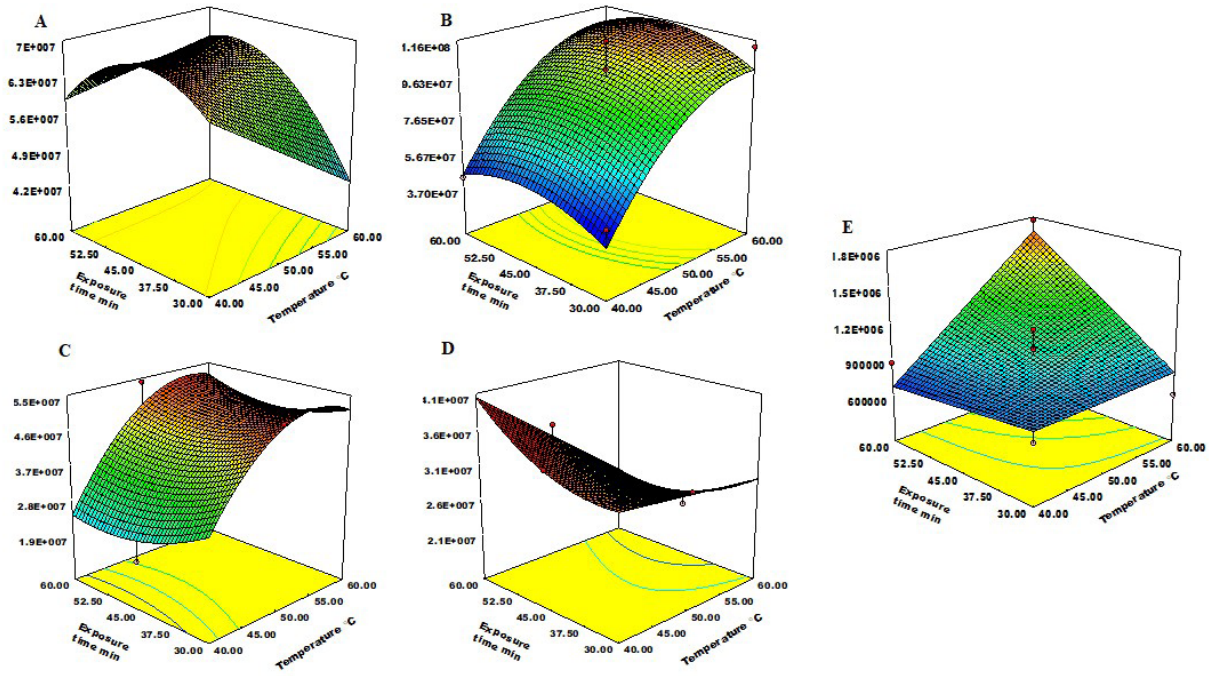


FIGURE 3. Response surface plot for interaction between extraction temperature and exposure time for (A) acids, (B) alcohols, (C) esters, (D) terpenes, and (E) norisoprenoids identified in white wine samples.

time (Figure 3). The analytical signal in the case of acids tends to increase with an increase in extraction temperature and exposure time, with maximum extraction efficiency achieved at 50 °C and 50 min of exposure. A similar tendency showed alcohols, with maximum extraction efficiency also achieved at 50 min of exposure, while the extraction temperature was higher and was 60 °C. Regarding esters, the increase in extraction temperature positively impacted the extraction efficiency, reaching the maximum at 30 min of exposure. However, a further increase in exposure time slightly decreased the analytical signal. On the other hand, terpenes achieved maximum extraction efficiency at lower temperatures (40 °C) and longer exposure time (60 min). Overall, because of the thicker coating on the SPME arrow, a longer extraction duration of 60 minutes was needed to reach equilibrium, compared to the traditional fibre setup. This is in accordance with the previously published results (Xu *et al*., 2021).

The obtained results for individual classes of VOCs relate to their physico-chemical properties, which vary significantly among classes. The Derringer or desirability function was used to obtain the optimal extraction conditions (Table 3). This methodology is used when various responses have to be considered simultaneously, and it is necessary to find optimal compromises between the total number of responses taken into account (Bezerra *et al*., 2008). The estimated and obtained values are similar, indicating the good performance of the developed method for extracting VOCs from white wines.

4. Optimisation of SPME Arrow conditions: red wines

In red wine samples, a total of 96 volatile compounds were extracted and identified, belonging to the following groups: acids (8 compounds), alcohols (34 compounds), C_{13}-norisoprenoids (4 compounds), carbonyls (3 compounds), esters (27 compounds), furanes (8 compounds), and terpenes (11 compounds) and 1 other compound.

For the optimisation process, the Box–Behnken experimental design was again chosen. The experimental factors and their levels were the same as for the white wine optimisation process, that is, extraction temperature (40-, 50-, 60 °C), incubation time (10-, 20-, 30 min), and exposure time (30-, 45-, 60 min). In total, 15 experiments were generated by BBD experimental design, including three central points, which were randomly performed. To evaluate the significance of all studied factors, the area of the responses based on the sum of the peak areas was applied. Fitting the data with various models showed that quadratic polynomial models best represented the content of acids, alcohols, C_{13}-norisoprenoids, esters, furanes, and terpenes. Parameters of analysis of variance (ANOVA) for all studied models are depicted in Table S5. The determination coefficients (R^2) range from 0.9234 to 0.9814, while *p*-values range from 0.003 to 0.0145, indicating that models are highly significant. Models also showed a statistically insignificant lack of fit, with *p*-values higher than 0.1533. All response parameters of quadratic polynomial equations are depicted in Table S5.

TABLE 3. Optimal SPME Arrow extraction conditions predicted and experimentally obtained values for individual classes of VOCs in white wine.

Group	Temperature (°C)	Incubation time (min)	Exposure time (min)	Predicted value (peak area x 10^6)	Obtained value (peak area x 10^6, mean ± SD)
Acids	50	10	60	52.88	54.06 ± 1.30
Alcohols				55.22	57.16 ± 0.81
Esters				121.51	119.21 ± 4.19
Terpenes				22.94	21.74 ± 1.02
Norisoprenoids				1.12	1.28 ± 0.25

TABLE 4. Optimal SPME Arrow extraction conditions for individual classes of VOCs in red wines.

Group	Temperature (°C)	Incubation time (min)	Exposure time (min)	Average boiling point (°C)	Vapour Pressure at 25 °C (Pa)	Average log K_{ow}	Water solubility at 25 °C (mg/L)
Acids (n = 8)	60	30	60	237	60	2.70	13056
Alcohols (n = 34)	60	10	40	191	89	2.04	90078
Norisoprenoids (n = 4)	40	10	46	246	6	4.70	6
Esters (27)	60	10	55	215	57	2.76	35016
Furanes (n = 8)	60	15	40	185	148	1.03	98786
Terpenes (n = 11)	40	20	30	211	62	4.31	180

The extraction conditions were individually optimised for acids, alcohols, C_{13}-norisoprenoids, esters, furanes, and terpenes. The optimal conditions and average values of physical properties are represented in Table 4. Again, the most significant factors were extraction temperature and exposure time, while incubation time did not affect the extraction efficiency. An increase in the extraction temperature up to 60 °C positively influenced the extraction of acids, alcohols, esters, and furanes. Conversely, C_{13}-norisoprenoids and terpenes achieved their maximum extraction efficiency at an extraction temperature of 40 °C. Regarding C_{13}-norisoprenoids, this was the only class of VOCs on which the extraction temperature did not significantly influence the extraction efficiency (p-value 0.8086). Similar results were obtained in the research by Paula Barros *et al*. (2012), where extraction temperature was not a significant factor in the extraction of wine volatiles, including norisoprenoids. Another important factor was exposure time, which varied among classes of VOCs. The lowest exposure time was recorded for terpenes (30 min), while slightly increased time was recorded for alcohols, furanes (40 min), and C_{13}-norisoprenoids (46 min). The maximum extraction efficiency for esters was achieved at 55 min of exposure and 60 min for acids.

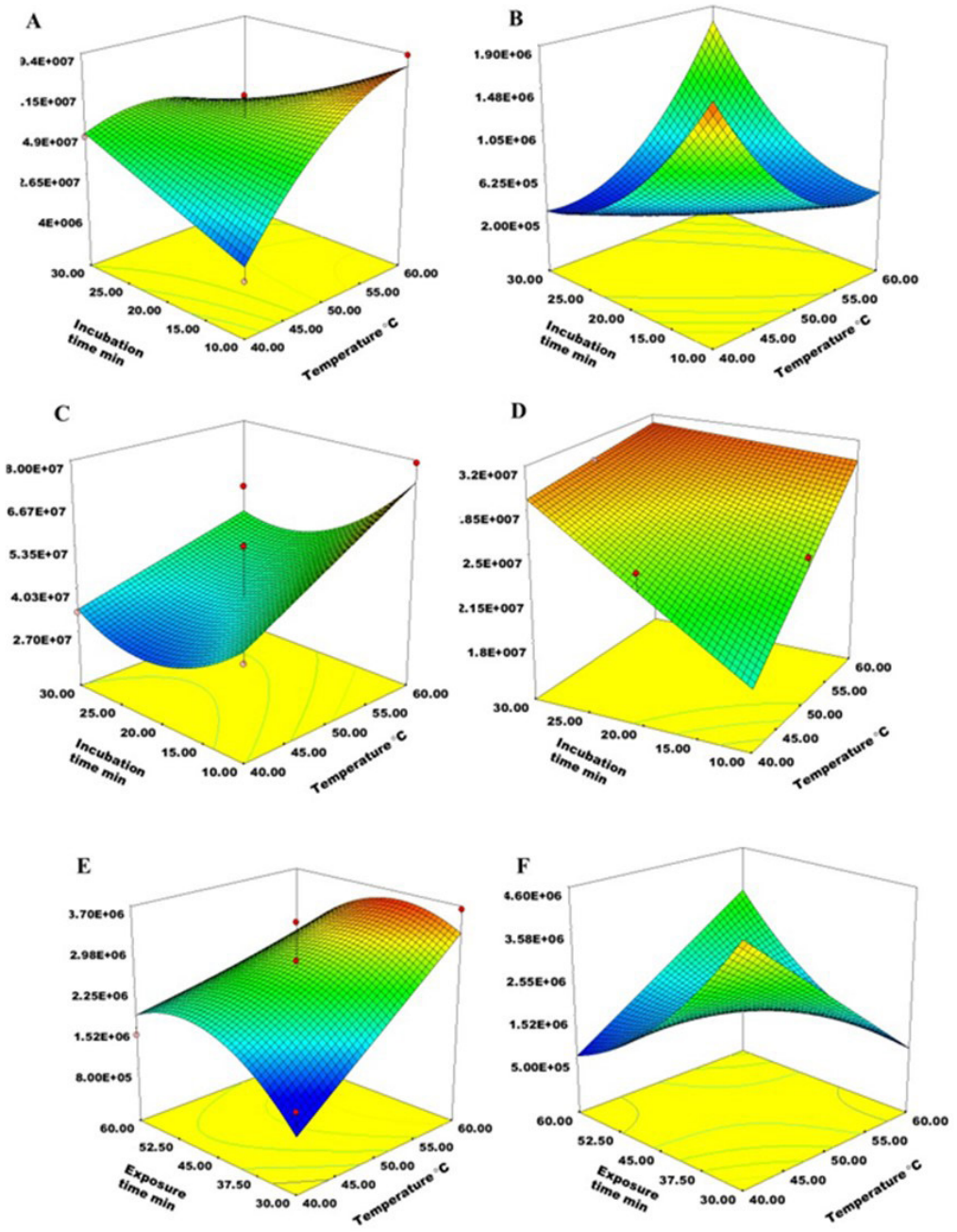


FIGURE 4. Response surface plot for interaction between extraction temperature and incubation time for (A) alcohols, (B) C_{13}-norisoprenoids, (C) esters, (D) acids, and extraction temperature and exposure time for (E) furanes, and (F) terpenes identified in red wine samples.

The incubation time was a significant factor for acids and alcohols, achieving maximum extraction efficiency at 30 and 10 min, respectively. For other classes of VOCs, the optimal incubation time was 10 min. Figure 4 represents 3D surface plots for the effect of extraction temperature and incubation time.

This interaction was significant for acids and alcohols. The analytical signal in the case of alcohols tends to increase with an increase in temperature, reaching maximum extraction efficiency at 10 min of incubation and extraction temperature of 60 °C. For acids, the analytical signal increased with an increase in temperature and incubation time, reaching its maximum at 60 °C and 30 min. C_{13}-norisoprenoids were not affected by increasing temperature or time and got the maximum efficiency at 40 °C and 10 min. The analytical signal for esters increased with an increasing temperature, while an increase in incubation time tends to decrease the analytical signal.

Figure 4E and Figure 4F represent the surface plots for the effect of extraction temperature and exposure time. For furanes, the analytical signal tends to increase with an increase in temperature and exposure time, reaching its maximum at 60 °C and 40 min of exposure. Further increase in exposure time slightly decreased the analytical signal. On the other hand, the analytical signal for terpenes reached its maximum at lower temperatures (40 °C) and shorter exposure time (30 min). Further increases in both temperature and exposure significantly reduced the analytical signal.

The Derringer function or function of desirability was again used to obtain the optimal extraction conditions (Table 5). The estimated and experimentally obtained values presented are similar, indicating the good performance of the method developed for extracting VOCs from red wine.

5. Calibration and method performance

Table 4 depicts the parameters of calibration and method performance obtained by applying the optimal extraction parameters for the synthetic wine. For all analysed compounds, good linearity was obtained with the coefficient of determination in the range between 0.9864 to 1.0000. The determined LOD values were in the range of 1.15 up to 15.14 μg/L for acids, between 0.89 and 4.29 μg/L for alcohols, 0.42 and 1.08 μg/L for norisoprenoids, 0.18 and 8.62 μg/L for esters, 2.09 and 3.37 μg/L for lactones, and 0.81 and 2.42 μg/L for terpenes. These values are in good agreement with the previously published data (Arcari *et al*., 2017; Barros *et al*., 2012). For intraday and interday evaluation, RSD values of precision did not exceed 15 %. The accuracy value for all analysed compounds was within 15 % of the nominal value; thus, the method can be considered accurate. Reproducibility values expressed as %CV at a concentration of 5 μg/L ranged from 2.07 % (βionone) to 4.72 % (isoamyl alcohol), while at a concentration of 5000 μg/L, they ranged from 2.10 % (hexanoic acid) to 4.95 % (cis-Whiskey lactone). In authentic samples, the average values for all detected compounds were 3.42 and 3.96 % for white and red wines, respectively.

6. Method application

To test the applicability of the optimised methodology, the volatile profiles of four white wines and two red wines were established. More than 100 VOCs were identified in both white and red wine samples made from Croatian native grapevine varieties (Table S6).

The most abundant class of VOCs in both white and red wines were alcohols, followed by acids and esters. Among alcohols, the most abundant compound was isoamyl alcohol present in concentrations that are consistent with literature data (Swiegers *et al*., 2005), followed by 1-decanol and 1-propanol, which all together comprised more than 68 % of total alcohols. White wine with the highest content of total alcohol and isoamyl alcohol was Vugava wine, while the smallest content was Pošip wine. Grk and Maraština had similar content of total alcohols, including isoamyl alcohol. Among red wines, Plavina had a higher content of total alcohols and isoamyl alcohol compared to Teran wine. Acids were the second most abundant class of VOCs, with the most abundant compounds hexanoic, octanoic, and isovaleric acid. Although their presence can be linked with the presence of an unpleasant aroma, they are important to the aromatic equilibrium, especially in concentrations from 4 to 10 mg/L (Avram *et al*., 2015). The highest content of total acids had white Pošip and Maraština wines, and red

TABLE 5. Optimal SPME Arrow extraction conditions predicted and experimentally obtained values for individual classes of VOCs in red wine.

Group	Temperature (°C)	Incubation time (min)	Exposure time (min)	Predicted value (peak area x 10^6)	Obtained value (peak area x 10^6, mean ± SD)
Acids	60	17	53	28.51	29.00 ± 0.50
Alcohols				67.43	67.50 ± 0.75
Norisoprenoids				0.51	0.76 ± 0.04
Esters				60.22	58.32 ± 0.19
Furanes				3.06	2.97 ± 0.12
Terpenes				2.40	2.60 ± 0.21

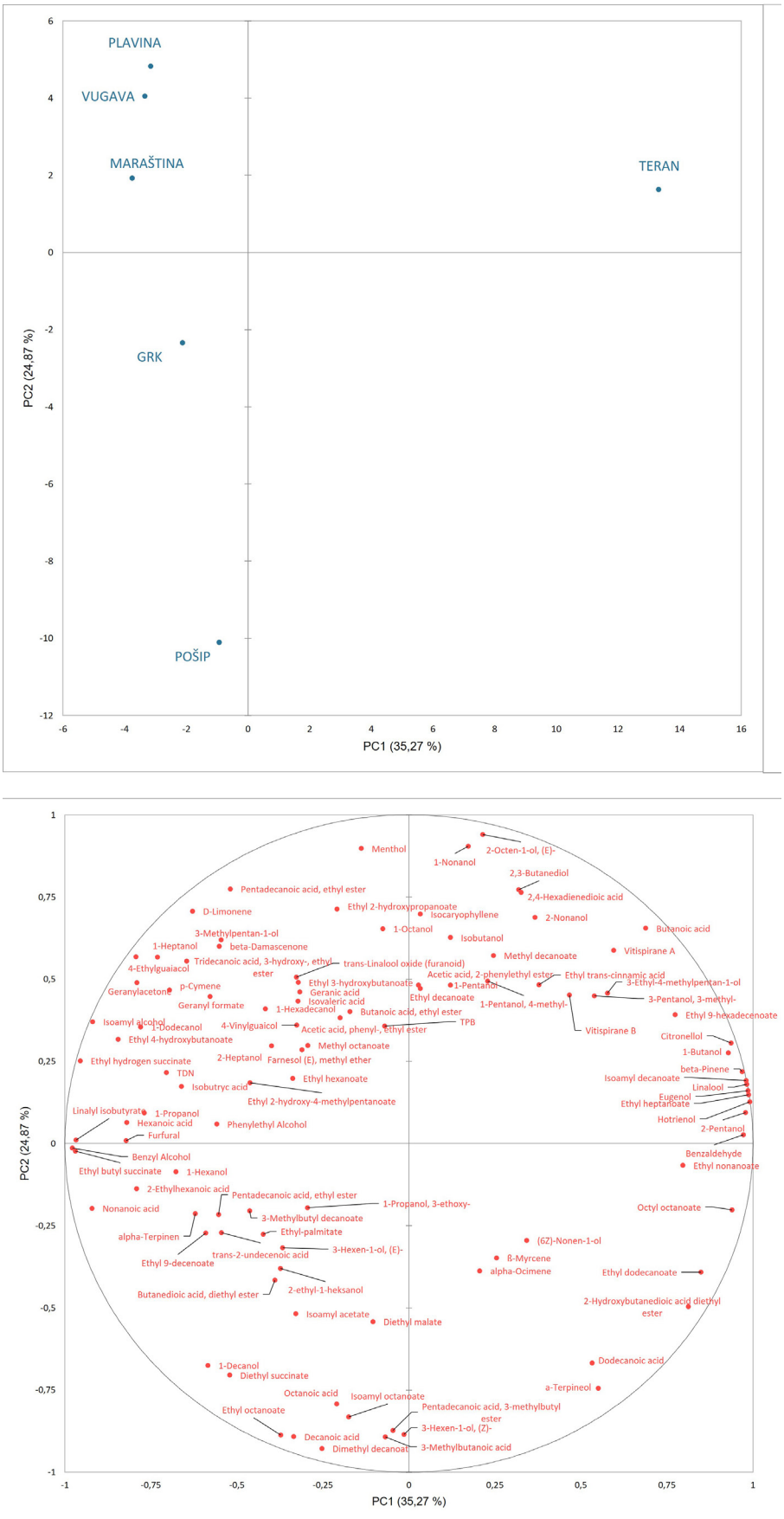


FIGURE 5. The scatter plots represent the distribution of white and red wine samples and the related vector diagram of volatile compounds contributing to the discrimination based on the PCA.

Plavina wine. The most abundant acid, hexanoic acid, was found in the highest content in Vugava wine, followed by Pošip while in red Teran wine was the lowest. Regarding the esters, whose concentrations were in accordance with the results reported in the literature (Benkwitz *et al*., 2012; Sumby *et al*., 2010), the most abundant compounds in white wines were ethyl hexanoate and ethyl octanoate, while in red wines were isoamyl acetate and 2-phenylethyl acetate. The highest content of esters was recorded for Vugava and Pošip wines, while Maraština and Grk had similar content. The Vugava wine was characterised by a higher content of ethyl hexanoate, while Pošip wine was characterised by a higher content of ethyl octanoate. In red wines, Plavina had a significantly higher content of total esters, including the most abundant compound isoamyl acetate. Among C_{13}-norisoprenoid compounds, the most abundant was β-Damascenone whose presence was in accordance with the literature data (Waterhouse *et al*., 2016); Vugava white wine had the highest concentration.

PCA analysis was used to evaluate the VOC profile variability of six wines made from Croatian native cultivars. The first two principal components explained 60.14 % of the variability (PC1—35.27 % and PC2—24.87 %). The six varietal wines are presented in Figure 5A in the space defined by the first two principal component axes, next to (Figure 5B) scatter plot representing correlations of original variables (VOCs) and first two principal components. Wine samples can be clearly separated by their volatile profile. Plavina, Vugava, and Maraština wines were situated closely and separated from other wines. They can be defined by a high content of terpenoid compounds menthol, limonene, geranylacetone, geranyl formate, and other compounds such as 4-ethylguaiacol, *p*-cymene, 1-heptanol, and isoamyl alcohol. Grk and Pošip wines were separated from earlier mentioned wines but also in between based on the variability defined by the second principal component (F2). Grk wine was characterised by the high content of nonanoic acid, *α*-terpinene, isoamyl acetate, and diethyl malate, while Pošip wine was characterised by esters diethyl succinate, ethyl octanoate, isoamyl octanoate. Teran wine was most distinct from other samples mainly based on the high content of alcohols (*E*)-2-octen-1-ol, 1-nonanol, 1-butanol, 2-pentanol, and terpenoids citronellol, hotrienol, linalool, and eugenol.

CONCLUSION

The SPME Arrow method is a new extraction technology that has been employed in the analysis of volatile organic compounds from wines. Though this technology is still not widely used for the analysis of wine VOCs, which play a crucial role in sensory properties, SPME Arrow has proven to be fast and efficient for the analysis of both white and red wine VOCs. Since the SPME Arrow is very sensitive to experimental conditions, the process was optimised by employing the Box–Behnken experimental design. The optimal conditions for VOCs in white wines are extraction temperature of 50 °C, incubation time of 10 min, and exposure time of 60 min, while for VOCs in red wines, are: extraction temperature of 60 °C, incubation time of 17 min, exposure time 53 min. Applying the optimised method provides a powerful tool for establishing the global volatile profile of wines. The new optimised method allows more accurate and time-saving analysis of VOCs from wines, enabling the analysis of many samples. Moreover, the method is automated and can be applied to a large number of samples for expeditious analysis of major classes of volatile organic compounds in wines.

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