EFFECT OF ENZYME TREATMENT ON VOLATILE PROFILE OF WHITE AND RED WINES FROM MACEDONIA BY USING HS-SPME-GC/MS

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1 INTRODUCTION

The volatile compounds of white and red wines have distinct physicochemical properties including polarity, volatility, and odor impact as a result of their functional groups (alcohol, aldehyde, acid, etc.) These compounds have three different origins: (i) from the grape (pre-fermentative aroma); (ii) from the yeast during the first or second fermentation (fermentative aroma); and (iii) from the aging process (post fermentative aroma).[1] Wine volatiles have an exceptional (huge) range of aromas, from citrus, lemon-like in young white wines to woody notes in older wines, and honey or waxy nuances in wines subjected to oxidative aging.[1]

Solid-phase microextraction (SPME) is a suitable technique for extraction of volatile compounds from a complex matrix such as wine. In the work of Tat *et al.* [2] the CRB-DVB-PDMS fiber appeared to work best for the extraction of wine volatiles. The results showed that Carboxen/PDMS coating had good sensitivity for volatile compounds, but bad resolution of chromatographic peaks. Vaz Freire *et al.*[3] found PDMS coating as the most suitable for the analysis of esters and polyacrylate coating as the most suitable for alcohols and terpenes in wines. Furthermore, 32 wine esters in 19 French wines were identified and quantified using HS-SPME in quantities less than ng/L.[4] Volatile profiles of sparkling wines determined by three extraction techniques showed that the SPME technique is sensitive enough compared to solid phase extraction (SPE) and liquid-liquid extraction.[5] The effect of the sample matrix on the volatile compounds in wines was studied by Whinton *et al.*[6] who explained the influence of an alcohol matrix on the extraction of volatiles using HS-SPME. Validation of a method for HS-SPME of wine volatiles, and their identification and quantification using several internal standards was the object of a study by Howard *et al.*[7] According to their results, the most appropriate fiber for extracting wine volatiles was CRB-DVB-PDMS coating in combination with GC-MS.[7] Galvan *et al.*[8] found the CAR/PDMS fiber to be the most appropriate for the extraction of 3-alkyl-2-methoxypyrazines in Frontenac and Leon Millot wines. The determination of esters in dry and sweet white wines was studied by Rodríguez-Bencomo.[9] Quantification of volatile compounds of 27 Greek wines was obtained using SPME in the head space mode with a 85 μm Polyacrylate coated fiber.[10]

Extraction of free and liberation of bound volatiles from sweet Fiano wines using liquid-liquid and C18 reverse phase extraction was done by Genovese *et al.*[11] According to

his findings, 42 of 57 compounds responsible for the aroma of Fiano wines were identified by Gas Chromatography-Olfactometry (GC-O).[11] The impact of "*Trepat"* and "*Monastrell"* red grape varieties during the manufacture of rosé sparkling Cava wines has been investigated by Bayón *et al*.[12] Also, volatile profiles of red wines from Azores Island and Portugal, aroma profile from "*Tanat"* red wines from Uruguay, white Albarín and red aged wines from Roja and Canary Island (Spain) as well as wines from Changli County (China) have been investigated.[13-18] Optimization of a method for flavor analysis of Greek "*Boutari"* white wines was studied by Demyttenaere *et al.*[19]

High concentrations of volatile terpenes were also found in grapes and wines in bound form as non-volatile glycosides. Liberation of glycosidically bound compounds from Riesling leaves and wines has been done by Winterhalter *et al*.[20,21] Noble *et al*.[22] found that the release of glycoside bound terpenes increases the bitter taste of "*Muscat of Alexandria*" wine. β-Glucosidase, α-arabinosidase and α-rhamnosidase activities of three enological yeast strains during winemaking did not result in a significant difference in the sensory analysis of the wines produced.[23] Also, the stability of free and glycosidically-bound fractions of some components of Muscat grape aroma (terpenols and aromatic alcohols) was investigated during alcoholic fermentation and in wines of different ages. The results showed that concentrations of free linalool and α-terpineol increased while geraniol and nerol decreased in older wines.[24] The distribution of free and glycosidically-bound monoterpenes among skin, juice, and pulp fractions in "*Muscat of Alexandria*", white "*Frontignac*", and "*Traminer*" grapes was evaluated.[25] In the work of Tate *et al*.[26] wines produced with *S. bayanus* strain EC1118 and *S. cerevisiae* strain VL1 had increased melon and "muscat" aromas. The effect of carbonic anaerobiosis on the concentrations of free and bound volatile compounds after nine days in "*Muscat de Frontignan*" wines was studied by Bitteuer *et al*.[27] Liberation and detection of glycosidically bound volatiles from white and red wines and the effect of "AR 2000" enzyme has been studied many authors.[28,33]

To the best of our knowledge this is the first report of the volatile profiles of Macedonian white and red wines using HS-SPME/GC. The next goal of this study was the determination of free and liberation of glycosidically-bound volatile compounds in the most popular Macedonian white and red wines. The last objective of this study is a comparison of the influences of two enzymes, "Endozym Aromatic" and "AR 2000," applied during and after the wine-making process respectively, in order to improve the volatile profile of the Macedonian wines.

2. MATERIALS AND METHODS

2.1 Regents and Standards

2-Nonanal used for quantification was purchased from Merck, Germany. SPME cartridge 65μm PDMS/DVB, 50/30 μm CRB-DVB-PDMS, 85 μm Carboxen/PDMS and 85 μm Polyacrylate fibers were purchased from Sigma Aldrich, Belgium.

2.2 Wine Samples

12 commercial white and 3 commercial red wines were obtained from the biggest wineries in Macedonia: Tikveš, Bovin, Popova Kula, Popov, Fonko and Stobi from the Povardarije wine-growing region and Skovin from the Skopje wine-growing region. The labels of the wines, variety of grapes, vintage year of production and the winery is presented in Table 1.

Table 1 *Labels of wine samples*

	Wine	*Name of wine*	*Variety*	*Vintage*	*Winery*
V1.	White wine	Temjanika	Muscat de Frontignane	2008	Popov
V2.	White wine	Temjanika	Muscat de Frontignane	2008	Popova Kula
V3.	White wine	Muscat	Muscat de Frontignane	2007	Skovin
V4.	White wine	Muscat	Muscat de Frontignane	2008	Bovin
V5.	White wine	Muscat	Muscat de Frontignane	2007	Bovin
V6.	White wine	Muscat	Muscat de Frontignane	2006	Skovin
V7.	White wine	Temjanika	Muscat de Frontignane	2007	Tikveš
V8.	White wine	Temjanika	Muscat de Frontignane	2008	Tikveš
V9.	White wine	Temjanika	Muscat de Frontignane	2009	Tikveš
V10.	White wine	Alexandria Riesling	Riesling	2008	Tikveš
V11	White wine	Bistro Bianco	Chardonney	2008	Fonko
V12.	Red wine	T'ga za Jug	Vranec	2008	Tikveš
V13.	Red wine	Makedonsko Crveno	Merlot	2008	Skovin
V14.	Red wine	Alexandria Cabernet	Cabernet Sauvignone	2008	Tikveš
V15.	White wine	Muscat Ottonel	Muscat Ottonel	2009	Stobi

2.3 Optimization of the Procedure by Using Model Wine

For optimization of the method, red and white model wine was used. The following parameters were optimized: fiber type, contact time between fiber and headspace, sample temperature and addition of salt.

2.3.1 Type of Fiber. Volatile compounds were extracted from white and red wines using different fibers. The total chromatographic peak area of the extracted volatiles was compared for each fiber type. The total peak area from "Temjanika" wine (V7) was higher using 50/30 μm CRB-DVB-PDMS than with the 85 μm polyacrylate fiber. The other two fibers, 65 μm PDMS/DVB and 85 μm Carboxen/PDMS, showed lower adsorption of extracted components.

2.3.2 Contact Time Between Fiber and Headspace. Different contact times of the fiber and sample head-space (20, 30 and 40 min.) were evaluated. 20 min was not enough for complete extraction of polar semivolatile compounds even using the 85 μm polyacrylate fiber. With a 40 min contact time, we found that the peak areas of some highly volatile compounds were lower than using a 30 min contact time, indicating desorption of some highly volatile compounds. In conclusion, the optimal time for extraction of both volatile and semivolatile components from the headspace was 30 min.

2.3.3 Temperature. The effect of temperature on the extraction of volatile compounds was studied in white and red wine samples at 20ºC, 30ºC and 50ºC. The most appropriate extraction temperature was 30ºC. All analyses were performed in duplicate.

2.3.4 Salt Addition. The effect of salt addition on the extraction of wine volatiles was found to depend on the type of fiber. Extraction using 50/30 μm CRB-DVB-PDMS or 65 μm PDMS/DVB coated fibers was better with the addition of 0.5 g of NaCl in order to increase the concentration of the volatile compounds in the headspace. The addition of salt did not increase the volatiles extracted when either the 65 μm PDMS/DVB or the 85 μm polyacrylate fiber were used.

2.4 Isolation of Free Volatile Compounds

Isolation of free wine volatiles was performed with a 50/30 μm CRB-DVB-PDMS (Carbowax-Divinylbenzene-Polydimethylsiloxan) coated fiber. Optimal conditions for wine sample preparation were the following: 30 min. continuous stirring of 2 ml wine mixed with 50 μL of internal standard solution in a 5 ml vial at 30°C and addition of 0.5 g of NaCl. Before each exposure, the fiber was cleaned in the injection port at 260°C for 5 min. All analyses were performed in duplicate.

2.5 Liberation of Bound Volatile Compounds by Using Solid Phase Extraction and Enzyme Treatment with AR 2000

Isolation of bound volatile compounds from wines was performed using a glass column packed (45 x 5 cm) with XAD-2 resin. The free volatile components of 150 mL of each wine sample were eluted with 500 mL of nanopure water and 500 mL of pentane/diethyl ether (1:1) solution. The bound volatile extract of wine was eluted with 500 mL of methanol. After evaporation at 40ºC, extracts were lyophilized. For incubation of "AR 2000" enzyme, a citric acid - disodiumhydrogenphosphate dihydrate buffer was prepared. Solvent A was prepared by dilution of 1.921 g of anhydrous citric acid with nanopure water till 100 mL. Solvent B was prepared by dilution of 3.560 g of disodiumhydrogenphosphate dihydrate with nanopure water till 100 mL. Mixing 48.5 mL of solvent A and 51.5 mL of solvent B yielded Mcilvaine buffer with pH 5.0.

The extract of each wine sample obtained using solid phase extraction was diluted in 10 mL of buffer solution. 3 mg of "AR 2000" enzyme was added to each, and they were incubated at 40ºC for 18 hours.

2 mL of wine extract obtained by enzymatic breakage of bound volatiles was inserted into an SPME vial, and 50 μL of internal standard solution was added. The extraction of liberated volatiles obtained by enzymatic breakage was performed for 30 min at 30ºC using a 50/30 μm CRB-DVB-PDMS fiber in the headspace mode with addition of 0.5 mg NaCl.

2.6 Qualitative Analysis of Volatile Compounds

Qualitative analysis of volatile compounds was performed using Gas Chromatography - Mass Spectrometry (GC-MS) HP 6890 with an HP5973 mass selective detector. For identification purposes, NIST and Wiley mass spectra databases as well as a homemade database were used. Separation of the compounds was performed using an HP Carbowax column (60 m x 0.25 mm x 0.25 μm) and identification was based on comparison of retention time and mass spectra with those from the libraries. The temperature gradient was 45°C to 180°C at 3°C/min and then 180°C to 260°C at 20°C/min. Injector temperature was 260ºC; injection mode split with split ratio 1:5; Helium was the carrier gas, at 35 kPa (32 cm/s); interface temperature was 280ºC; acquisition mass range, 40-400 m/z; solvent cut, 2 min.

2.7 Quantitative Analysis of Volatile Compounds

For quantification of volatile compounds, 2-heptanol at 4 μg/L was used as the internal standard. The semiquantitative analyses were carried out assuming a response factor equal to one. For this purpose 50 μL of 2-heptanol was diluted in 100 ml of diethyl ether. 50 μL of internal standard solution was added to the SPME vials containing 2 mL of wine. All analyses were performed in duplicate, and the results were expressed as mg and μg of

compound per liter of wine. The same temperature gradient used for MS identification was applied for the quantification of volatiles.

2.8 Wine Making Technology

2.8.1 Wine-Making Procedure for Temjanika and Muscat Wines (V1-V9). In brief, Muscat de Frontignan grapes were harvested at optimal maturity. After crushing the grapes, an aqueous solution of potassium metabisulfite was added (50 mg/L total SO_2.) The must was obtained by pressing using a vacuum press and liquid CO_2 to prevent oxidation. Extraction of polyphenolic compounds was done after 4 hours maceration of the skin and seed from the grapes. "Endozym Aromatic" (AEB, Bresica, Italy) was added, 2 g/hL of must. The double role of this enzyme was better extraction of free aromatic compounds due to its pectolitic activity, and its action on aroma precursors by its β-glycosidasic activity. Sedimentation of the must was accomplished at 8-10ºC over a 48 h period. Two yeasts, Fermol Arôme Plus and Fermol Charmat (*Saccharomyces cerevisiae*) obtained from the same Italian company were used in dosages of 20 mg/100 kg crashed grapes. The selection of those yeasts was due to their ability to generate aroma precursors and to produce esters and acetates during low temperature fermentation. Cold maceration (12-14ºC) was done during alcoholic fermentation. Wines were aged in the bottles for 2 years.

2.8.2 Wine-Making Procedure for Muscat Ottonel Wine (V15). Grapes of the Muscat Ottonel variety were harvested at optimal maturity of pH 3.4, 230 g/L sugar and total acidity of 4.9 g/L. After crushing the grapes with the addition of dry ice and nitrogen to prevent oxidation, cold maceration (10ºC) was performed for 9 hours. The grape mash was pressed. The grape juice obtained had a turbidity of 85 NTU, and it was inoculated with *S. cerevisiae* ph.r. *cerevisiae* yeast at a concentration of 25 g/hL. Alcoholic fermentation at 12-15 ºC was carried out for 2 weeks. The resulting young wine contained around 1 g/L of sugar residue. After filtration, cold stabilization of the wine was done at –6 ºC. During cold stabilization the PVPP agent was added to remove polyphenols extracted from seed and skins of the grape during cold maceration. Finally, the wine obtained was filtered (first with a 60 μm filter and then with a 40 μm filter) and bottled. The wines were aged in the bottles for 2 years.

3. RESULTS AND DISCUSSION

The volatile profile of Temjanika and Muscat wines (V1-V9) produced from Muscat de Frontignane grapes by application of "Endozym Aromatic" is presented in Table 2. The volatile compounds of control and treated wines (V10-V15) produced from different grape varieties (in Table 1) with enzyme "AR 2000" are shown in Table 3.

Table 2 *Mean concentrations of free varietal compounds (mg/L) and relative standard deviations (n = 2) of Temjanika and Muscat wines (V1-V9) from Macedonia*

Components	*V1*	*V2*	*V3*	*V4*	*V5*	*V6*	*V7*	*V8*	*V9*
Terpenes									
Limonene	0.21±0.05	1.35±0.33	1.12±0.16	1.38±0.42	0.22±0.03	2.17±0.41	0.79±0.63	1.28±0.36	2.16±0.45
γ-Terpinene	n.d.	0.35±0.02	n.d.	n.d.	n.d.	1.25±0.03	0.22±0.01	n.d.	n.d.
p-Cymene	n.d.	0.10±0.02	n.d.	n.d.	n.d.	0.30±0.06	n.d.	n.d.	n.d.
α-terpineol	n.d.	0.11±0.03	0.62±0.04	n.d.	n.d.	0.54±0.00	0.45±0.03	1.79±0.13	0.16±0.02
β-citronellol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.39±0.03	n.d.
α-terpinolene	0.71±0.02	0.72±0.23	n.d.	n.d.	n.d.	0.43±0.02	n.d.	n.d.	0.58±0.23
Linalool	n.d.	n.d.	1.50±0.16	0.57±0.02	3.56±0.29	n.d.	0.42±0.03	6.60±1.24	n.d.
Geraniol	n.d.	n.d.	0.16±0.02	n.d.	0.37±0.01	n.d.	n.d.	0.98±0.05	n.d.
Hotrienol	n.d.	n.d.	n.d.	n.d.	0.35±0.01	n.d.	n.d.	0.51±0.05	n.d.
Total terpenes	**0.92**	**2.63**	**3.40**	**1.95**	**4.50**	**4.69**	**1.88**	**11.55**	**2.90**
Alcohols									
3-Methyl butanol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.75±0.63	n.d.	n.d.
Octanol	n.d.	n.d.	0.13±0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nonanol	n.d.	0.18±0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenylethyl alcohol	7.52±0.24	2.75±0.33	2.78±0.04	n.d.	11.22±0.69	2.36±0.62	3.03±0.34	13.60±1.34	1.19±0.24
Total alcohols	**7.52**	**2.93**	**2.91**	**0.00**	**11.22**	**2.36**	**8.78**	**13.60**	**1.19**
Aldehydes									
Octanal	0.34±015	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furfural	n.d.	n.d.	n.d.	n.d.	0.43±0.01	n.d.	n.d.	0.23±0.03	0.53±0.29
2-Nonenal	n.d.	n.d.	n.d.	0.03±0.00	n.d.	n.d.	n.d.	n.d.	n.d.
Furfuraldehyde	n.d.	n.d.	n.d.	0.98±0.07	n.d.	n.d.	n.d.	n.d.	n.d.
5-methyl-2-furaldehyde	n.d.	n.d.	n.d.	0.29±0.00	n.d.	n.d.	n.d.	n.d.	n.d.
Total aldehydes	**0.34**	**0.00**	**0.00**	**1.30**	**0.43**	**0.00**	**0.00**	**0.23**	**0.53**
Esters									
Ethyl hexanoate	0.98±0.63	0.96±0.09	0.78±0.31	3.90±0.10	1.83±0.96	0.76±0.25	0.63±0.05	1.86±0.21	0.92±0.56
Hexyl acetate	1.46±0.08	0.68±0.09	0.29±0.07	n.d.	0.34±0.01	n.d.	n.d.	1.41±0.05	2.46±0.09
Ethyl octanoate	2.49±0.66	2.65±1.81	8.57±0.51	1.65±0.27	3.73±1.18	2.49±0.60	1.03±0.07	4.08±1.87	5.71±1.02
Ethyl decanoate	6.39±0.45	n.d.	1.62±0.24	2.49±0.06	7.46±0.45	n.d.	1.17±0.02	7.31±1.02	1.05±0.45
Diethyl succinate	n.d.	n.d.	0.70±0.29	n.d.	3.67±0.28	1.22±0.02	1.56±0.11	1.15±0.23	n.d.
Phenylethyl acetate	n.d.	n.d.	n.d.	n.d.	0.43±0.05	n.d.	n.d.	1.48±0.04	n.d.
Total esters	**11.32**	**4.29**	**11.96**	**8.04**	**17.46**	**4.47**	**4.39**	**17.29**	**10.14**
Ketones									
2,3 Butadienon	0.53±0.02	n.d.	0.59±0.03	0.57±0.00	n.d.	0.49±0.04	0.42±0.08	n.d.	n.d.
Acids									
Hexanoic acid	n.d.	0.73±0.02	0.61±0.00	n.d.	1.50±0.07	0.51±0.03	0.54±0.01	1.50±0.16	0.77±0.05
Octanoic acid	2.21±0.24	1.58±0.24	1.55±0.69	n.d.	2.35±1.45	2.26±1.07	1.07±0.23	3.15±0.93	1.06±0.69
Decanoic acid	2.47±0.36	0.75±0.09	0.63±0.05	n.d.	0.81±0.09	0.58±0.22	2.58±0.24	2.35±0.67	5.58±0.39
Total acids	**4.68**	**3.06**	**2.79**	**0.00**	**4.66**	**3.35**	**4.19**	**7.00**	**7.41**

Table 3 *Mean concentrations of bond varietal compounds and relative standard deviations (n = 2) of white and red wines (V10-V15) from Macedonia*

Components	*V10 control wine (mg/L)*	*V10 treated wine (μg/L)*	*V11 control wine (mg/L)*	*V11 treated wine (μg/L)*	*V12 control wine (mg/L)*	*V12 treated wine (μg/L)*
Terpenes						
Geraniol	n.d.	n.d.	n.d.	19.21±0.02	n.d.	n.d.
Hotrienol	3.25±1.43	312.23±1.54	n.d.	n.d.	n.d.	n.d.
Total terpenes	**3.25**	**31.23**	**0.00**	**19.21**	**0.00**	**0.00**
Alcohols						
2-Methyl propanol	1.28±0.04	n.d.	1.85±0.63	n.d.	12.71±5.06	n.d.
3-Methylbutanol	23.46±2.05	25740.2±70.2	48.23±9.11	126.32±24.71	10.17±1.29	n.d.
Isoamyl alcohol	n.d.	n.d.	n.d.	n.d.	12.11±0.52	n.d.
2-Propanol	0.81±0.12	n.d.	1.28±0.18	n.d.	0.80±0.26	n.d.
Butanol	n.d.	n.d.	n.d.	n.d.	n.d.	7.29±1.01
Hexanol	1.45±0.09	3512.8±23.72	0.54±0.02	647.41±13.23	2.03±0.26	64.53±5.39
Octanol	n.d.	n.d.	n.d.	23.75±4.12	n.d.	n.d.
Farnesol	n.d.	n.d.	n.d.	23.43±9.07	n.d.	n.d.
2,6-nonadien-1-ol	n.d.	411.72±12.78	n.d.	n.d.	n.d.	n.d.
Benzyl alcohol	n.d.	691.39±43.78	n.d.	160.75±30.02	n.d.	n.d.
Phenylethyl alcohol	4.68±1.00	918.91±12.58	1.89±0.22	518.21±16.61	2.96±6.46	2622.1±34.92
Total alcohols	**31.68**	**31275.02**	**53.54**	**1499.87**	**40.78**	**2693.92**
Esters						
Ethyl acetate	n.d.	n.d.	n.d.	n.d.	28.45±3.23	277.12±26.43
Ethyl Butyrate	0.70±0.02	n.d.	0.705±0.363	n.d.	n.d.	15.76±1.00
2-Methyl butylacetate	n.d.	n.d.	n.d.	n.d.	2.96±0.34	n.d.
Isoamyl acetate	5.74±0.97	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamyl acetoacetate	1.14±0.04	1811.0±150.7	1.21±0.31	n.d.	2.53±0.87	1202.2±20.49
Ethyl hexanoate	1.98±0.12	n.d.	1.12±0.57	n.d.	4.93±1.57	n.d.
Hexyl acetate	2.21±0.87	n.d.	0.32±0.09	n.d.	n.d.	n.d.
Methyl heptanoate	n.d.	n.d.	n.d.	2.07±0.01	n.d.	n.d.
Ethyl octanoate	8.47±0.91	n.d.	1.37±0.27	15.23±0.79	1.45±0.43	n.d.
Ethyl decanoate	3.52±0.80	n.d.	1.51±0.51	n.d.	n.d.	n.d.
Diethyl succinate	0.54±0.07	n.d.	n.d.	51.23±0.06	5.80±0.87	n.d.
Total esters	**24.30**	**1811**	**6.23**	**68.53**	**46.12**	**1495.08**
Aldehydes						
Furfural	0.98±0.02	n.d.	n.d.	n.d.	n.d.	n.d.
Benzaldehyde	n.d.	n.d.	n.d.	37.23±0.02	n.d.	n.d.
Total aldehydes	**0.98**	**0.00**	**0.00**	**37.23**	**0.00**	**0.00**
Acids						
Acetic acid	0.57±0.02	43327±172.3	n.d.	n.d.	2.75±0.45	635.67±0.18
Lactic acid	0.59±0.17	n.d.	1.08±0.09	n.d.	7.09±1.13	171.09±0.02
Butanoic acid	n.d.	n.d.	n.d.	n.d.	0.74±0.17	n.d.
Hexanoic acid	0.56±0.17	n.d.	n.d.	45.12±0.03	n.d.	n.d.
Octanoic acid	4.61±0.66	1216.1±24.74	2.11±0.09	n.d.	0.92±0.08	n.d.
Decanoic acid	n.d.	207.81±14.71	n.d.	588.24±0.04	n.d.	n.d.

Geranic acid	n.d.	374.32±13.01	n.d.	n.d.	n.d.	144.27±3.24
Total acids	**6.33**	**45125.23**	**3.19**	**633.36**	**11.50**	**951.03**
Others						
Dihydro-3-methylen-2,5 -furandione	n.d.	n.d.	n.d.	n.d.	n.d.	7971.1±2.40
trans-β-ionon-5,6-epoxide	n.d.	n.d.	n.d.	n.d.	n.d.	45.23±0.01

Components	*V13 control wine (mg/L)*	*V13 treated wine(μg/L)*	*V14 control wine (mg/L)*	*V14 treated wine (μg/L)*	*V15 control wine (mg/L)*	*V15 treated wine (μg/L)*
Terpenes						
α-terpineol	n.d.	n.d.	n.d.	n.d.	1.27±0.13	230.91±23.62
β-citronellol	n.d	n.d	n.d	n.d	n.d.	56.48±15.43
Nerol	n.d.	n.d.	n.d.	n.d.	n.d.	1688.2±46.49
Geraniol	n.d.	n.d.	n.d.	n.d.	n.d.	1526.4±65.23
Hotrienol	n.d	n.d	n.d	n.d	1.63±0.72	350.12±29.3
Linalool	n.d.	n.d.	n.d.	n.d.	1.80±0.04	90.23±17.84
Total terpenes	**0.00**	**0.00**	**0.00**	**0.00**	**4.70**	**3942.34**
Alcohols						
2-Methyl propanol	4.32±1.86	n.d.	4.61±0.43	n.d.	0.83±0.27	n.d.
3-Methylbutanol	43.42±2.92	n.d.	68.27±1.79	342.92±13.98	12.89±1.94	n.d.
Isobutyl alcohol	n.d	n.d	4.49±0.25	n.d.	n.d.	n.d.
Propanol	0.31±0.02	n.d.	0.55±0.35	n.d.	n.d.	n.d.
2-Propanol	n.d	n.d	0.35±0.07	n.d.	n.d.	n.d.
Hexanol	1.09±0.10	53.57±0.31	1.47±0.72	91.23±21.02	n.d.	52.87±13.07
Heptanol	n.d	n.d	2.10±0.46	n.d.	n.d	n.d
Octanol	n.d.	n.d.	n.d.	n.d.	n.d.	24.97±.2.15
Guaiacol	n.d.	4.89±0.02	n.d.	n.d.	n.d.	n.d.
Benzyl alcohol	n.d.	143.23±13.98	n.d.	79.29±27.35	n.d.	244.23±91.43
Phenylethyl alcohol	6.93±0.23	1113.2±71.61	11.92±1.43	1521.2±15.3	6.35±0.13	384.91±11.64
Total alcohols	**56.07**	**1314.89**	**93.76**	**2034.64**	**20.07**	**706.98**
Esters						
Ethyl Acetate	3.81±0.02	n.d.	n.d.	n.d.	7.06±0.09	n.d.
Isobutyl acetoacetate	4.19±0.08	n.d.	n.d.	n.d.	3.72±1.78	143.21±13.79
Isoamyl acetoacetate	n.d.	61.27±0.42	n.d.	n.d.	n.d.	n.d.
Ethyl hexanoate	1.07±0.08	n.d.	0.15±0.07	n.d.	3.94±0.52	n.d.
Hexyl acetate	n.d.	n.d.	n.d.	n.d.	0.48±0.03	n.d.
Ethyl octanoate	1.83±0.31	n.d.	4.97±0.21	n.d.	3.62±1.09	n.d.
Geranyl propionate	n.d.	n.d.	n.d.	n.d.	0.57±0.06	n.d.
Ethyl decanoate	7.80±2.20	n.d.	n.d.	n.d.	3.02±0.65	n.d.
Diethyl succinate	3.26±0.27	55.13±0.07	n.d.	62.98±27.24	2.82±0.31	28.57±0.31
Total esters	**21.96**	**116.4**	**5.12**	**62.98**	**25.23**	**171.78**
Aldehydes						
Benzylaldehyde	n.d.	n.d.	n.d.	n.d.	n.d.	47.24±13.92
Furfural	n.d.	n.d.	n.d.	n.d.	1.33±0.06	n.d.

Total aldehydes	**0.00**	**0.00**	**0.00**	**0.00**	**1.33**	**47.24**
Ketones						
2-Butanone	n.d.	n.d.	n.d.	n.d.	0.17±0.01	n.d.
Oxides						
trans-Linalool oxide	n.d.	n.d.	n.d.	n.d.	n.d.	26.98±7.29
Nerol oxide	n.d.	n.d.	n.d.	n.d.	1.70±0.13	74.39±39.12
Total oxides	**0.00**	**0.00**	**0.00**	**0.00**	**1.70**	**101.37**
Acids						
Hexanoic acid	n.d.	n.d.	0.07±0.03	n.d.	n.d.	30.93±3.85
Lactic acid	4.30±0.23	n.d.	3.14±0.57	n.d.	0.38±0.01	n.d.
Acetic acid	1.78±0.29	n.d.	7.25±1.29	n.d.	0.96±0.06	26.39±11.95
Octanoic acid	n.d.	213.64±19.27	0.10±0.07	1040.2±65.23	2.13±0.12	635.71±24.97
Decanoic acid	n.d.	50.34±7.59	n.d.	83.29±16.97	n.d.	423.93±51.98
Geranoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	651.95±20.49
Total acids	**6.08**	**263.98**	**10.56**	**1123.49**	**3.47**	**1737.98**
Phenols						
4-vinyl phenol	n.d.	n.d.	n.d.	347.99±31.82	n.d.	24.79±7.28
Others						
Dihydro-3-methylen-2,5-furandione	3.94±0.10	59.37±10.75	n.d.	276.17±31.98	n.d.	n.d.
Vinylfuran	n.d.	5.98±0.09	n.d.	n.d.	4.14±0.08	n.d.

Esters were the most predominant volatile compounds in most of the analyzed wines. The highest concentrations of esters were observed for wine V5 from the 2008 vintage, and the highest concentrations of terpenes and alcohols were found in wine V8 from the 2007 vintage. The largest abundance of acids was observed in wine V9 from the 2006 vintage. The lowest concentrations of total volatiles were extracted from wines V6 and V7 from 2007 and 2006 vintage.

One sample of white wine was produced from “Muscat Ottonel”- the least abundant grape variety in Macedonia. The vinification procedure of this wine included cold maceration at 10°C for 9 h, but it had not been enzymatically treated. This wine sample together with the other selected wines from Riesling, Chardonnay, Vranec, Merlot and Cabernet Sauvignon grape varieties were enzymatically treated with “AR 2000” in order to liberate the glycosidically bound volatile compounds responsible for the aromatic profile of the wines.

The volatile compounds of control and treated wines (V10-V15) produced from white and red grape varieties with enzyme “AR 2000” are depicted in Table 3.

The most abundant volatiles were alcohols with the exception of wine V12 from the “Vranec” variety where the most abundant compounds were esters. The largest concentration of alcohols (106.20 mg/L) was extracted from wine produced from Chardonnay grapes. This level was still less than the critical level of 400 mg/L when hexanol can produce an unpleasant flavor.[32]

Table 3 shows the concentrations of volatiles liberated after application of enzyme “AR 2000”. It is notable that the effect of this enzyme is greatest on the release of alcohols in all samples except for “Muscat Ottonel” where more acids were released.

Results presented in Tables 2 and 3 indicate a strong dependence of the volatile compounds on the enzymatic treatment and variety of grapes. From the results described

below, it can be concluded that enzymatic treatment with "Endozyme Aromatic" affects most of the terpenes in the Muscat variety. On the other hand, application of "AR 2000" affects most of the alcohols and acids in the other treated wines.

3.1. Terpenes

Terpenes are among the most important contributors to the overall aroma of Muscat wines.[33] Limonene is one of the most important monoterpenes, responsible for the characteristic "citrus-like" taste of "Temjanika" wines, and formed by cyclisation of geranyl pyrophosphate. Limonene was the most abundant monoterpene in wine samples produced from "Muscat de Frontignan" grapes with concentrations from 0.22 to 2.17 mg/L (Table 2).

Apart from the large quantity of limonene, significant concentrations of other terpenes such as γ-terpinene, *p*-cymene, α-terpinolene, α-phellandrene and α-terpineol were found in wines V6 and V9. Their concentrations, estimated by head space solid phase microextraction and gas chromatography mass spectrometry (HS-SPME/GC-MS), were in good agreement with the published results for Muscat grapes by Kang *et al*.[33]

Furthermore, the concentration of *α*-terpineol in Muscat wines from Macedonia was in good agreement with its abundance in white wines from Poland extracted similarly using SPE-SPME-GC/MS.[34] Cyclisation reactions of other terpenes such as nerol, geraniol and linalool in acid conditions can produce α-terpineol. The relatively high concentration in Temjanika wines (0.11-0.62 mg/L) could be the result of overripe grapes. A similar concentration of α-terpineol (196 μg/L) in sweet Fiano wines was observed as result of overripe grapes by Genovese *et al*.[11]

Citronellal, detected in some wines, can either be a product of the metabolic activity of yeast or it can be synthesized from nerol and geraniol.[35]

Hotrienol is the most abundant terpene in "Muscat" grapes and wines. This terpene alcohol is formed by the elimination of water from 2,6-dimethyl-3,7-octadiene-2,6-diol.[28] Significant effects of "AR 2000" on the concentration of hotrienol are found in wines produced from "Riesling" and "Muscat Ottonel" (Figure1).

The histogram depicted in Figure 1 shows the concentrations of free and bound hotrienol in wines produced from "Muscat de Frontignan", "Riesling" and "Muscat Ottonel" grape varieties. More precisely, wines V5 and V8 produced from "Muscat de Fronatignan" grapes and treated with "Endozyme Aromatic" during winemaking contained 350 μg/L and 510 μg/L of total hotrienol (Table 2). On the other hand, enzymatically treated wines V10 and V15 produced from "Riesling" and "Muscat Ottonel" grape varieties released 312.23 μg/L and 350.12 μg/L only from bound forms (Table 3). This difference can be the due to the different varieties or the different enzymes.

Geraniol and nerol are the most important terpenes responsible for the fruity flavor of white wines. Geraniol was quantified in bound form in wine V11 produced from Chardonnay grapes in the lowest concentration of 19.21 μg/L. The concentration of geraniol in some wines produced from "Muscat de Frontignan" grapes was in the range of 160 to 980 μg/L. However, the highest concentrations of the monoterpenes geraniol and nerol were detected in bound form in "Muscat Ottonel" wine V15. Their concentrations in treated wine V15 were 1526 μg/L and 1688 μg/L, respectively. From these results we can conclude that high levels of geraniol and nerol are present as glycosides which were cleaved after treatment with "AR 2000" since no detectable quantities of the two monoterpenes were found in control V15 wine. The result for the liberated level of geraniol was in the same range (1622 μg/L) in Muscat grapes treated with the same enzyme, "AR 2000", as reported by Kang *et al*. [33] β-citronellol was found only in the

sample of "Muscat Ottonel" in bound form at a concentration of 56.48 μg/L. As Tables 2 and 3 show, linalool was found only in wines produced from "Muscat" grapes.

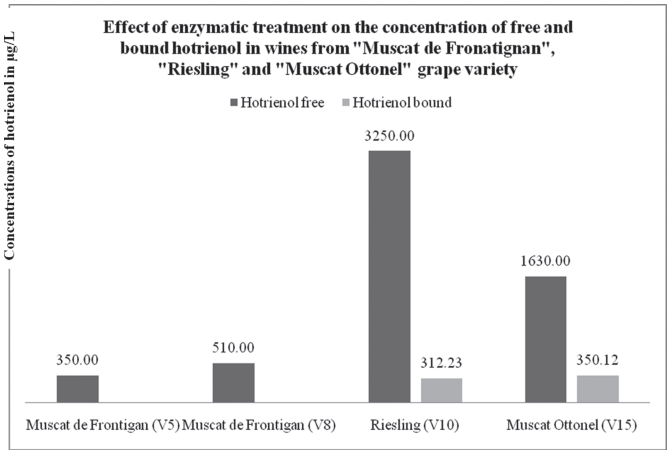

Figure 1 *Histogram of the concentration of free and bound hotrienol in wines from "Muscat de Frontignan", "Riesling" and "Muscat Ottonel" grape variety*

From Tables 2 and 3 it is obvious that the relatively high abundance of terpenes in wines V1-V9 (produced from "Muscat de Frontignan") and wine V15 (from "Muscat Ottonel"), compared to the other wines, is characteristic for "Muscat" cultivars.

3.2. Alcohols

Alcohols are the largest group of compounds formed by the metabolic activity of yeasts during alcoholic fermentation.[32] If we compare the quantities of total alcohols in wines produced from "Muscat de Frontignan" by "Endozyme Aromatic" and wines from Riesling, Muscat Ottonel, Merlot, Vranec and Cabernet Sauvignon by "AR 2000", we see that those treated with "AR 2000" had a higher abundance of alcohols. Apart from 3-methylbutanol, the major alcohols were 2-methylpropanol, hexanol and phenylethyl alcohol. Their quantities were in good correlation with the concentration of alcohols in free and glycosidically bound forms in Muscat "á petit grains" wines, in red wine from "*Vitis vinifera* cv. Castañal" grown in Galicia, "Muscadine grape juices" and red wines of "*Vitis Vinifera* L. cv. Őkűzgőzű and Bogazkere" grown in Turkey.[28,30,36,37]

Most of the alcohols are potent odorants, and they can have a negative effect on wine flavor if their concentration exceeds 400 mg/L.[32] Hexanol, found in relatively higher concentration in free form in some red and white wines, might have a negative effect on the overall flavor, giving a typical unpleasant "vegetal" or "herbaceous" flavor.[32] The high level of benzyl alcohol, detected only in the bound form with a maximal concentration of 631.39 μg/L, can be explained as a product of the metabolic activity of yeasts or as a product of benzaldehyde reduction.[11]

3.3 Volatile Phenols

Volatile phenols are formed via decarboxylation of hydroxycinnamic acids, naturally present in grapes. The most dominant volatile phenol, 4-vinylguaiacol, is a derivative of ferulic acid. Its maximal concentration of 4.14 mg/L, found in "Muscat Ottonel" wine, was in good agreement with its reported concentration in Muscat grapes.[33]

3.4. Aldehydes

The most abundant aldehyde was furfural at 1.33 mg/L. It is formed via the degradation of carbohydrates during aging.[11]

Benzaldehyde is a very potent aldehyde with an "almond-like" aroma. This aldehyde was only found in its bound form with concentrations from 21.89 μg/L to 47.24 μg/L. The quantity of benzaldehyde was the same in wines from Muscat "á petits grains" grapes in the work of Castro Vázquez *et al*.[31] Benzaldehyde is formed by the enzymatic oxidation of benzyl alcohol. Its higher quantities in some wines is related to infection of the grapes with *Botrytis cinerea*.

3.5. Esters and Acetates

Two types of esters are present in wines: acetates formed by reaction of acetyl CoA and higher alcohols and the esters of fatty acids and ethanol which are synthesized from acyl CoA and alcohols. Esters occur in all studied samples as the major volatile constituents. Their odor has been described in the literature as "fruity".[32]

Ethyl acetate was quantified in Macedonian wines in concentrations of 28.45 mg/L. This concentration is less than that at which it can generate a "sour vinegar" odor. Other esters such as hexyl acetate and benzyl acetate have powerful fruity flavors. They are produced during cold maceration at temperature below 15 °C. Esters such as ethyl hexanoate, ethyl octanoate and ethyl decanoate are responsible for the flavor of red wines. Usually their concentrations are markers for the quality of red wines. Some esters such as ethyl lactate and diethyl succinate do not contribute significantly to the overall flavor of the wines. The high concentrations of esters in all samples analyzed are in good agreement with those found in wines from "Chardonnay" and "Pinot Gris" extracted using a Divinylbenzene-Carboxen-Polydimethylsiloxane (DVB/Carboxen/PDMS) fiber and HS-SPME in the study of Howard *et al*.[7] Their concentrations are independent from the activity of "AR 2000" (Table 3).

3.6. Acids

Acetic acid can be produced from acetic bacteria or as a product of ethanol oxidation during alcoholic and malolactic fermentation. Its concentration is an indicator of lactic and acetic bacterial activity in the wines. In higher concentration, acetic acid gives rise to an undesirable aroma and taste. The wine can be very "sour" with an unpleasant "vinegar" scent.

Fatty acids are products of yeast activity during winemaking. Their production is governed by the initial composition of the must and the fermentation conditions.[32] High concentrations of hexanoic, octanoic and decanoic acids can be produced by lipid oxidation. The concentrations of fatty acids found in white and red wines from Macedonia

were in good agreement with those estimated using two extraction procedures, HS-SPME and XAD-2 resin, in the work of Bohlscheid *et al*.[38]

3.7. Effects of "Endozym Aromatic" and "AR 2000" Enzymes on Wine Volatiles

The enzyme "Endozym Aromatic" is known to act as a pectolytic enzyme for better extraction of free volatile compounds while β-glycosidase allows the glycosidic cleavage of bound volatile compounds.

White wines (V1-V9) from "Muscat de Frontignan" grapes were produced with "Endozym Aromatic" (as described in the winemaking procedure). If we compare the results from analyses of wines V1-V9 presented in Table 2, we see that terpenes have a significant impact on the flavor of Temjanika and Muscat wines. Limonene is the terpene present in largest amounts in this grape variety and might have the highest impact on Temjanika wines due to its "citrus-like" flavor. However, the concentrations of volatile compounds, especially terpenes, differ from wine to wine depending on the maturity of the grapes, the wine-making conditions in different wineries, the aging in the bottles and conditions of storage.

Pectinase AR 2000 has multiple glycosidase activities. It is known to possess all glycosidase activities required for the release of bound monoterpenyl aglycons.[36]

Wines (V10-V15) were treated with "AR 2000" in order to release the bound volatile compounds. As shown in Table 3, the highest influence of Pectinase "AR 2000" was on the levels of alcohols. The most significant effect of pectinase "AR 2000" was on benzyl alcohol (wines V13 and V14). On the other hand, it was noted that benzaldehyde was absent in the same wines. According to Castro Vázquez *et al*.[31] significant quantities of benzaldehyde were only present in must of grapes before fermentation.

If we compare the volatile profile of wines produced from "Muscat de Frontignan" with the volatile profile of enzymatically treated "Muscat Ottonel" wine a similarity between these two varieties is obvious. When making from "Muscat" variety wines, it is necessary to include enzymatic treatment in order to release the bound volatiles to increase the overall flavor.

Principal component analysis was used to determine the impact of enzymatic treatment (Figures 2 and 3). The results indicated that the first principal component explained 90 % of the total variability. In Figure 2, the wines are separated in two groups based on the enzymatic treatment. Temjanika wines (V1-V9) produced from "Muscat de Frontignan" grapes treated with "Endozym Aromatic" and wines (V11-V15) treated with "AR 2000" belong to the same group. The second group in the PCA plot were wines without enzyme treatment. Only wine V10 produced from Riesling grapes was classified in the same group as non-treated wines since the volatile profile of this wine was not significantly changed after enzymatic treatment. The non-treated wine V12 from Vranec grapes did not belong to either group since its volatile profile was significantly different from all of the other wines. As shown in Table 3, only Vranec wine had alcohols as the major class of volatile compounds. The other wines presented on the same histogram all had esters as the most predominant group of volatiles.

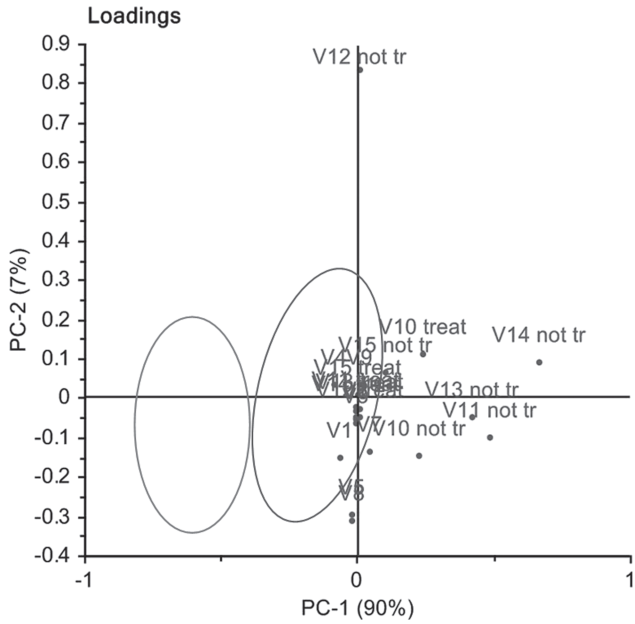

Figure 2 *PCA scater plot of Temjanika, Chardonnay, Vranec, Merlot, Cabernet Sauvignon and M uscat Ottonel wines with and without ezymatic treatment*

Figure 3 shows the components most responsible for the classification of the wines in the PCA score plot in Figure 2. We can conclude that *p*-cymene, hexanol and phenylethyl alcohol were mainly responsible for the separation of the wines into two groups. More precisely, the most significant components for the separation of the wines into two groups were those whose concentrations were highly affected by enzymatic treatments. Statistical analyses confirmed that the effect of enzymatic treatment was more significant than the varieties of grapes from which the wines were produced. The type of enzyme was not significant since the wines treated with "Endozym Aromatic" and "AR 2000" were classified in the same group.

4. CONCLUSION

The volatile profiles of Macedonian white and red wines were studied using head space solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS). Terpenes, alcohols, esters and fatty acids were identified as the most important volatiles contributing to the overall flavor of the wines. White wines produced from Muscat varieties "Muscat de Frontignan" and "Muscat Ottonel" had significant quantities of limonene, linalool, geraniol, nerol, β-citronellol, α-terpineol and α-terpinolene. The flavors of these wines are strongly dependent on the concentration of monoterpenes. Wines produced from Riesling, Chardonnay, Merlot, Vranec and Cabernet Sauvignon contained alcohols and esters as the most abundant group of volatile compounds.

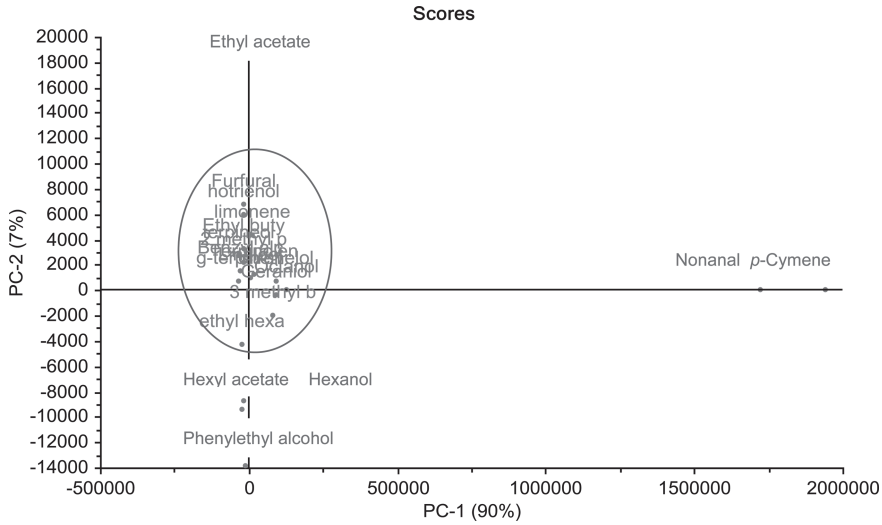

Figure 3 *PCA scater plot of the volatile components from Temjanika, Chardonnay, Vranec, Merlot, Cabernet Sauvignon and Muscat Ottonel wines with and without enzymatic treatment*

The effect of the enzymes "Endozym Aromatic" and "AR 2000" on the volatile profile of wines was studied. Enzyme treatment with "AR 2000" had the most significant effect on alcohols and fatty acids in wines from Riesling, Chardonnay, Merlot, Vranec and Cabernet Sauvignon. The volatile profile of enzymatically treated wine from "Muscat Ottonel" grapes was very similar to that of the treated wines from "Muscat de Frontignan." Terpenes were one of the major volatile classes responsible for the smell and the taste of the wine. Geraniol and nerol liberated in concentrations of 1526.4 μg/L and 1688.2 μg/L as well as nerol in at 350.12 μg/L indicated that enzymatic treatment is necessary for improving the volatile profile of Muscat wines.

The results from principal component analyses (PCA) showed that the type of enzyme was not significant since the wines treated with "Endozym Aromatic" and "AR 2000" were classified in the same group.

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References:

1 E. Sarrazin, D. Dubourdieu, and P. Darriet, *Food Chemistry,* 2007, **103,** 536–545.
2 L. Tat, P. Comuzzo, I. Stolfo, and F. Battistutta, *Food Chemistry*, 2005, **93**, 361–369.
3 L. M. T. Vaz Freire, A. M. Costa Freitas, and A. M. Relva, *Journal of Microcolumn Separations*, 2001, **13**, 236-242.
4 G. Antalick, M. C. Perello, and G. de Revel, *Food Chemistry*, 2010, **121**, 1236-1245.

5 J. Bosch-Fuster, M. Riu-Aumatell, J. M. Guadayol, J. Caixach, E. Lopéz-Tamames, and S. Buxaderas, *Food Chemistry,* 2007, **105**, 428–435.
6 R. S. Whiton, and B. W. Zoecklein, *American Journal of Enology and Viticulture*, 2000, **51**, 379-382.
7 K. L. Howard, J. H. Mike, and R. Riesen, *American Journal of Enology and Viticulture*, 2005, **56**, 37-45.
8 T. L. Galvan, S. Kells, and W. D. Hutchison, *Journal of Agricultural and Food Chemistry,* 2008, **56**, 1065–1071.
9 J. J. Rodríguez-Bencomo, J. E. Conde, M. A. Rodríguez-Delgado, F. García-Montelongo, and J. P. Pérez-Trujillo, *Journal of Chromatography A*, 2002, **963**, 213–223.
10 E. Koussissi, A. Paterson, and J. R. Piggott, *European Food Research Technology*, 2007, **225**, 749–756.
11 A. Genovese, A. Gambuti, P. Piombino, and L. Moio, *Food Chemistry*, 2007, **103**, 1228–1236.
12 M. A. Pozo-Bayón, P. J. Martín-Álvarez, M. V. Moreno-Arribas, I. Andujar-Ortiz, and E. Pueyo, *LWT - Food Science and Technology*, 2010, **43**, 1526-1532.
13 J. A. B. Baptista, J. F. da P. Tavares, and R. C. B. Carvalho, *Food Research International*, 2001, **34**, 345-355.
14 E. Boido, A. Loret, K. Medina, L. Farin, F. Carrau, G. Versini, and E. Dellacassa, *Journal of Agricultural and Food Chemistry,* 2003, **51**, 5408–5413.
15 A. Masa, and M. Vilanova, *Food Chemistry*, 2008, **107**, 273–281.
16 M. Aznar, R. López, J. F. Cacho, and V. Ferreira, *Journal of Agricultural and Food Chemistry,* 2001, **49**, 2924–2929.
17 R. Lopéz, N. Ortín, J. P. Pérez-Trijillo**,** J. Cacho, and V. Ferreira, *Journal of Agricultural and Food Chemistry,* 2003, **51**, 3419–3425.
18 Y. Tao, and L. Zhang, *LWT - Food Science and Technology*, 2010, **43**, 1550-1556.
19 J. C. R. Demyttenaere, C. Dagher, P. Sandra, S. Kallithraka, R. Verhé, and N. De Kimpe, *Journal of Chromatography A*, 2003, **985**, 233–246.
20 P. Winterhalter, *Journal of Agriculture and Food Chemistry,* 1991, **39**, 1825-1829.
21 P. Winterhalter, G. Full, M. Herderich, and P. Schreier, *Phytochemical Analysis*, 1991, **2**, 93-96.
22 A. C. Noble, C. R. Strauss, P. J. Williams, and B. Wilson, *American Journal of Enolology and Viticulture*, 1988, **39**, 129-131.
23 A. Delcroix, Z. Gunata, J. C. Sapis, J. M. Salmon, and C. Bayonove, *American Journal of Enolology and Viticulture*, 1994, **45**, 291-296.
24 Y. Z. Gunata, C. L. Bayonove, R. L. Baumes, and R. E. Cordonnier, *American Journal of Enolology and Viticulture*, 1986, **37**, 112-114.
25 B. Wilson, C. R. Strauss, and P. J. Williams, *American Journal of Enolology and Viticulture*, 1986, **37**, 107-111.
26 D. Tate, and A. G. Reynolds, *American Journal of Enolology and Viticulture*, 1986, **57**, 60-68.
27 S. Bitteur, C. Tesniere, J. Sarris, R. Baumes, C. Bayonove, and C. Flanzy, *American Journal of Enolology and Viticulture*, 1992, **43**, 41-48.
28 E. Sánchez Palomo, M. S. Pérez-Coello, M. C. Díaz-Maroto, M. A. González Viñas, and M. D. Cabezudo, *Food Chemistry*, 2006, **95**, 279–289.
29 M. Ugliano, and L. Moio, *Journal of the Science of Food and Agriculture*, 2006, **86**, 2468–2476.
30 M. Vilanova, and C. Martínez, *European Food Research Technology*, 2007, **224**, 431–436.

31 L. Castro Vázquez, M. S. Pérez-Coello, and M. D. Cabezudo, *Analytica Chimica Acta*, 2002, **458**, 39–44.
32 E. Gómez García-Carpintero, E. Sánchez-Palomo, and M. A. González-Viñas, *Food Research International*, 2011, **44**, 61-70.
33 W. Kang, Y. Xu, L. Qin, and Y. Wang, *Journal of the Institute of Brewing*, 2010, **116**, 70-77.
34 M. Dziadas, and H. H. Jeleń, *Analytica Chimica Acta*, 2010, **677**, 43-49.
35 E. Sánchez Palomo, M. A. González-Viñas, M. C. Díaz-Maroto, A. Soriano-Pérez, and M. S. Pérez-Coello, *Food Chemistry*, 2007, **103**, 631–640.
36 H. H. Baek, and K. R. Cadwallader, *Journal of Food Science*, 1999, **64**, 441-444.
37 T. Cabaroglu, A. Canbas, J. P. Lepoutre, and Z. Gunata, *American Journal of Enology and Viticulture*, 2002, **53**, 64-68.
38 J. C. Bohlscheid, X. D. Wang, D. S. Mattinson, and C. G. Edwards, *Journal of Food Quality*, 2006, **29,** 1–15.