Ajda OTA[*], Emil ZLATIĆ[*], Mojca KOROŠEC[*], Sanja RADONJIĆ[**], Vesna MARAŠ[**], Rajko VIDRIH[*], Tatjana KOŠMERL[*]

CLONES DIFFERENTIATION OF AUTOCHTHONOUS GRAPEVINE VRANAC USING AROMA COMPOUNDS DETECTION BY GC — MS AND SENSORY ANALYSIS

Abstract: The aim of this study was to investigate presence and stability of wine specific aroma-active compounds, which can be used to establish the main differences among clones of Vranac (*Vitis vinifera* L.), Montenegrin autochthonous grapevine variety. Aroma-active compounds were determined by gas chromatography — mass spectrometry (GC — MS).

From the total of 16 identified aromatic compounds (expressed as ethyl-nonanoate equivalent), the characteristic of vintage 2013 is a lower variability range of their total concentration (927–1229 μg/L) as compared to the vintage 2015 (573–1019 μg/L). Statistically significant differences in the content of wine aroma compounds were found among all seven clones as well as between both vintages (2013 and 2015) studied. The average content of wine aroma compounds is 73 ± 180 μg/L in the vintage 2013 and 55 ± 147 μg/L in the vintage 2015.

The majority of certain aromatic substances represent the desired fruity esters (ethyl butanoate, ethyl lactate, ethyl 2- and 3-methyl butanoate, 3-methylbuthyl acetate, diethyl succinate, 2-phenylethyl acetate). The floral and/or buttery aromas that are well sensory perceived are presented by 2,3-butanediol, ethyl lactate, hexanol, 2-phenylethanol and ethyl decanoate.

Among the identified aromas, a typical blueberry aroma for Vranac wine would be highlighted, for which the compound ethyl 2-hydroxy-4-methyl pentanoate is responsible. This character-impact compound was present at significantly higher concentrations in the vintage 2013 (on average 3.01 ± 0.47 μg/L) and the three clones (5, 6 and 7) contained it more than a control wine (3.05 μg/L). In the vintage 2015 (on average 1.19 ± 0.29 μg/L), however, concentration was higher in all clones in comparison to the control wine (0.87 μg/L).

Key words: *aroma compounds, wine, Vranac, clone, gas chromatography — mass spectrometry, sensory analysis*

[*] Biotechnical Faculty, University of Ljubljana, Slovenia
[**] "13. jul — Plantaže", Podgorica, Montenegro

INTRODUCTION

Genetic improvement of autochthonous grapevine varieties is most commonly carried out through individual clonal selection. Even though aroma substances of wine are responsible for much of the complexity of wine, aroma profile is checked in later selection stages. Several hundred aroma-active compounds such as alcohols, esters, ethers, aldehydes, ketones, acids, terpenes etc., present in very variable concentration levels, have been identified in wines [1].

Several groups of odorants present within the grapes, originate from specific odorless precursors, especially as glycosidic forms and can be released during winemaking through the action of glycosidase enzymes [2, 3], the action of yeasts and lactic bacteria [4–6]. The balance between the aromatic compounds deriving from glycoside hydrolysis can determine specific aroma nuances, which contribute to the varietal character of wines [7].

Grape aroma plays a determining role in wine aroma that is directly linked to specific varietal aroma. Clonal variation, through somatic mutations, can modify the aromatic profile of grapes, as was reported in a study by Genovese et al. [7], that showed different aroma profile of free and bound volatile compounds in Aglianica and Uva di Troia grapes. The aim of this study was to investigate presence and stability of wine specific aroma-active compounds, which can be used to establish the main differences among clones of Vranac (*Vitis vinifera* L.), Montenegrin autochthonous grapevine variety.

MATERIALS AND METHODS

PLANT MATERIAL AND VINIFICATION

Seven clones of Vranac (*Vitis vinifera* L.) grown at micro locality Nikolj Crkva, Ćemovsko field, sub-region Podgorica were studied during the 2013 and 2015 vintage. The space between vines was 2.6×1.0 m and double horizontal cordon training system was formed. Short winter pruning with 10–12 buds per vine was applied. All plants have been subjected to identical cultivation practices and had received identical protection treatments.

Grapes of vintage 2013 were harvested between 8th and 12th of September, while grapes of vintage 2015 were harvested on the 15th of September. At harvest, grapes from all seven clones were harvested manually and transported to the experimental cellar. Microvinification was carried out in the experimental cellar of the winery "13. jul — Plantaže". Alcoholic fermentations of all trials were performed in Ganimede fermenters (Italy) of 300 L capacity. For the vinification, an average of 200–250 kg grapes of all clones was used. Potassium

metabisulfite, purchased from Agroterm KFT, Hungary, was added; 8 g 100 kg^{-1} of grapes from all clones. All enzymes, wine yeasts, lactic acid bacteria and yeast nutrients were obtained from Lallemand, France. Enoferm BDX commercial yeasts was used (30 g /hL), Lalvin EX-V for maceration (2 g 100 kg^{-1}) and yeast nutrient Go-ferm protect (30 g/hL) were added at the beginning of fermentation, while yeast nutrient Fermaid E (25 g/hL) was added at the stage when alcoholic fermentation proceeded to 1/3. During the first two days of alcoholic fermentation the frequency of pumping over was set on 8 h, while to the end of alcoholic fermentation the frequency was on 6 h. After alcoholic fermentation wines were racked and commercial lactic acid bacteria Lalvin VP41 was added to perform malolactic fermentation. After completion of malolactic fermentation wines were racked, potassium metabisulfite was added in amount depending on free SO_2 in analysed wine samples and cold stabilization was conducted (4 weeks at T of — 5 °C). All wines then aged for a period of three months prior bottling. Bottled wines were stored in cellar at ~ 15 °C.

CLIMATE EVALUATION

Weather conditions during vegetative period (April — September) of both vintages were monitored by meteorological station located in the vineyard. Mean temperature, total rainfall, total insolation and sum of active temperatures were reported monthly.

HEAD-SPACE SPME EXTRACTION

The extraction of wine volatiles was carried out using a solid-phase microextraction (SPME) fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) sorbent (1 cm long, 50/30 μm thickness, StableFlex™, Supelco, USA). A 3 mL of wine was placed in 10 mL headspace vial, each sample vial contained 1.2 g NaCl, 3 mL of model wine solution (12 % (v/v) ethanol, 7.0 g/L tartaric acid, pH 3.3) and 50 μL of ethyl nonanoate solution (internal standard, 0,175 mg/L solution in ethanol). The sample vials were conditioned in a temperature-controlled heating module at 40 °C for 30 min. After extraction, the fiber was removed from the sample and the analytes were thermally desorbed in the injector port of the GC.

DETERMINATION OF AROMA-ACTIVE COMPOUNDS BY GAS CHROMATOGRAPHY — MASS SPECTROMETRY (GC — MS)

Analysis was conducted on a GC 7890A gas chromatograph (Agilent Technologies, CA, USA) equipped with a MPS2 Multipurpose autosampler (Gerstel GmbH, Mülheim an der Ruhr, Germany) and 5975C mass

spectrometer (Agilent Technologies). Volatile compounds were desorbed into a GC injector port at 250 °C in splitless mode for 2 min. The gas chromatograph was fitted with a ZB-WAX capillary column, 60 m × 0.32 mm i. d. with 1 μm film thickness. Helium was the carrier gas at a flow rate of 1.2 mL/min at 40 °C. Oven temperature was programmed as follows: initial temperature 40 °C held for 5 min, then 4 °C/min to 230 °C. The volatile compounds were identified with a mass selective detector (5975C, Agilent Technologies, CA, USA). The detector operated in the m/z range between 30 and 250, ion source and quadrupole temperature were maintained at 250 and 150 °C, respectively. Identification of compounds was performed by comparison of their mass spectra with those of available commercial standards; all compounds were also confirmed by matching of their mass spectra with NIST 2.0 mass spectral database (National Institute of Standards and Technology, USA). All volatiles were quantified in equivalents of ethyl nonanoate.

SENSORY ANALYSIS

All wines were also sensorially assessed. A descriptive sensory analysis was carried out with a panel of seven trained assessors, according to sensory analysis guidelines [8].

RESULTS AND DISCUSSION

METEOROLOGICAL DATA FOR VEGETATIVE PERIOD DURING 2013 AND 2015

During the vegetative season of 2013 (from the 1st April to 30th of September), in the subregion Podgorica, next meteorological parameters were registered: 147 summer days (Tmax ≥ 25 °C), 85 tropical days (Tmax ≥ 30 °C), 67 tropical nights (T ≥ 20 °C) and 53 rainy days. There were 11 days with the heavy rain intensity (≥ 20 L/m^2 of rain per 24 h). An average daily maximum temperature was 2 °C higher than climate normal, while average mean temperature was 3.3 °C higher than climate normal and it can be concluded that 2013 vegetative season was evidently warmer than the climate average.

Mean temperature during vegetative period was 22.3 °C, and mean relative humidity was ~ 62%. Some tropical waves were registered and the strongest was noticed in the period from 03rd — 10th August, when the temperature was above the 30 °C for more than 16 h per day. Total rainfall during this period was 451 L/m^2 which represent about 28% of total annual rainfall. Total insolation hours were 1694 h and June and July had the highest insolation hours, 318 and 345 respectively, while less insolation was evident

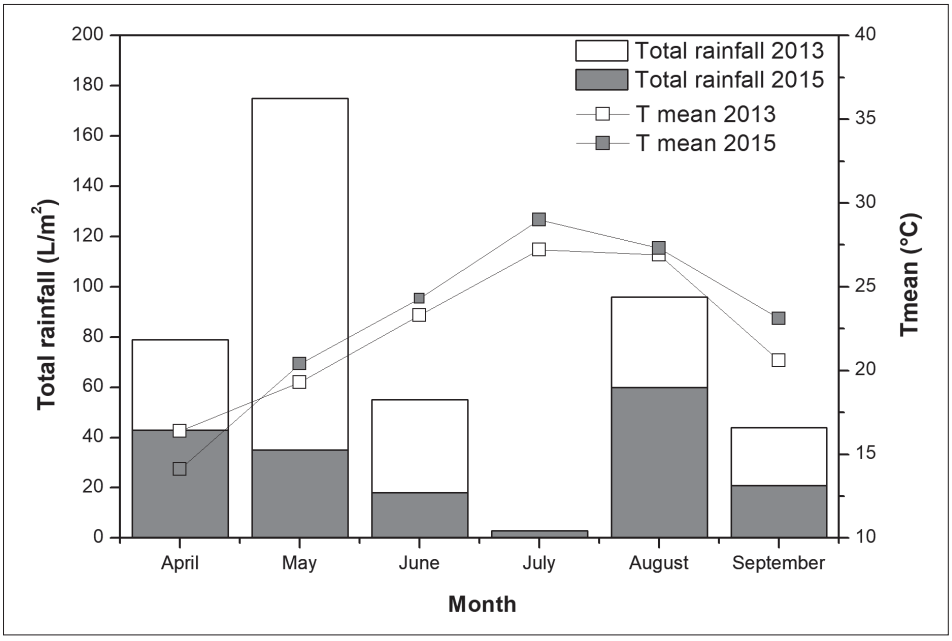

Figure 1. Basic meteorological parameters per month, during vegetative seasons 2013 and 2015

Table 1. Total insolation and sum of active temperatures during vegetative seasons 2013 and 2015

Year	Month	April	May	June	July	August	September	April — Sept.
2013	Total insolation (h)	253	271	318	345	268	239	1694
	ΣT_{active} (°C)	481	599	699	837	754	621	3991
2015	Total insolation (h)	246	311	312	331	296	234	1730
	ΣT_{active} (°C)	423	631	729	901	847	692	4223

during August (Table 1). Besides, a fairly frequent stronger north wind, at high temperatures was noticed. This phenomenon is very unfavourable, followed by extreme values of potential evapotranspiration.

Generally, the meteorological conditions were relatively favourable, except in some short periods when they significantly deviated from the climate normal. The oscillations within the rainfall regime were registered and insolation was quite favourable except in the third decade of August which was followed by higher rainfall frequency.

The vegetative period during 2015 year was quite favourable. During the vegetative season of 2015 following meteorological parameters were registered: 140 summer days (Tmax ≥ 25 °C), 93 tropical days (Tmax ≥ 30 °C),

and 62 tropical nights (T ≥ 20 °C) and 37 rainy days. There were no days with the heavy rain intensity (more than 20 L/m^2 per 24 h).

An average temperature during vegetative season was 23.0 °C, which is 1.6 °C above average and the mean relative humidity was 57.1%. The sum of active temperatures was 4223 °C, the highest sum was registered in July (901 °C) and August (847 °C). Total rainfall during vegetative season was 180 L/m^2, which is 337 L/m^2 less than the long-term average. The total duration of insolation was 1730 h, which was 104 h higher than average (Figure 1, Table 1).

During the 2015 vegetative period frequent clime extremes were noticed. Beside the rainfall deficit, air temperatures were exceptionally high. Regarding to tropical days and nights, 26 days above average were registered and even 62 tropic nights, which is 31 nights more than year before. Tropical days and nights, followed by low relative humidity, particularly during the first and third decade of July and in the first decade of August, with a strong wind increased the evapotranspiration.

THE AROMA PROFILE OF WINES MADE FROM CLONES

Analysis of aroma-active compounds was carried out by gas chromatography-mass spectrometry (GC — MS). Statistically significant differences in the content of wine aroma compounds were observed between all seven clones and between both vintages (2013 and 2015) studied. The characteristic of vintage 2013 was lower variability of their total concentration (927–1229 mg/L) as compared to the vintage 2015 (573–1019 mg/L). The average content of aroma compounds for the vintage 2013 was 73 ± 180 mg/L and for the vintage 2015 it amounted to 55 ± 147 mg/L (Figure 1). Since water stress must be avoided in the spring and early summer (up to véraison) [9], the differences are most probably a result of different meteorological conditions, where significantly lower rainfall and higher Tmean were determined during this period in 2013, compared to 2015.

Significant differences were also observed, when concentration variability of aroma compounds (expressed as cv, %) in clones between vintages 2013 and 2015 was compared (Figure 3). Most variable were the concentrations of acetal (1,1-diethoxyethane) (cv2013 = 30.5%; cv2015 = 66.8%) and 2,3-butanediol (cv2013 = 19.4%; cv2015 = 56.5%). Concentration of 2,3-butanediol in wine can range from about 0.2 g/L to 3 g/L, with a mean value of about 0.57 g/L [10], although our samples contained much lower concentrations. Higher concentrations of 2,3-butanediol can have some effect on the wine bouquet because of its slightly bitter taste and on the wine body because of its viscosity [10].

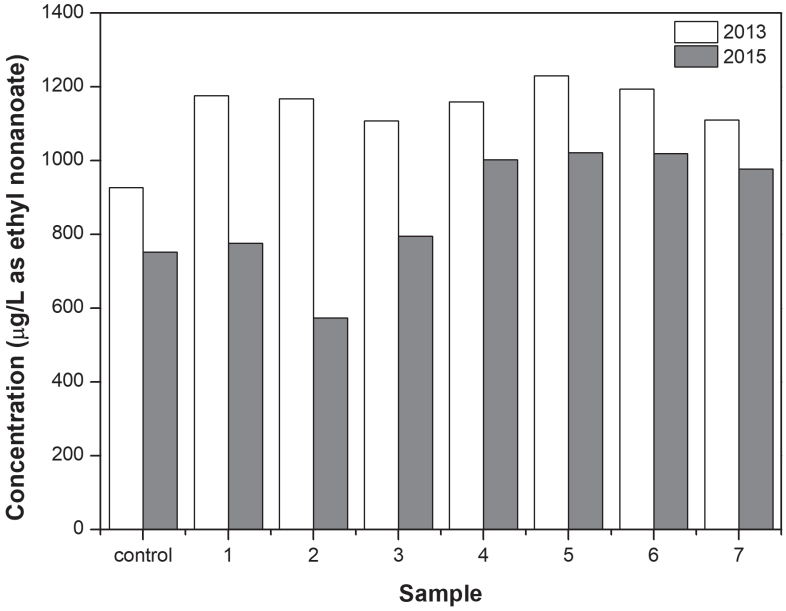

Figure 2. Total concentration of all analysed aroma compounds given as ethyl nonanoate equivalent

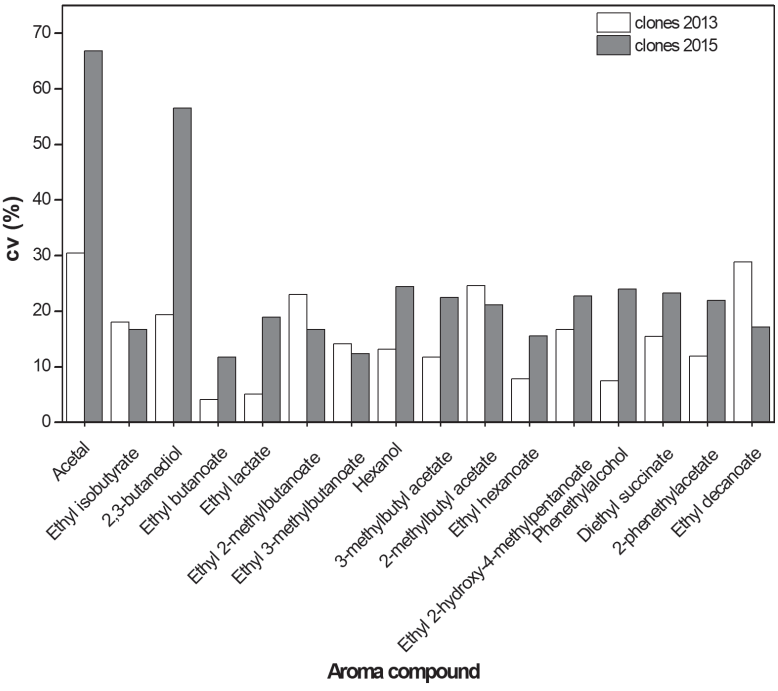

Figure 3. Variability of concentration of aroma compounds in clones between vintages 2013 and 2015

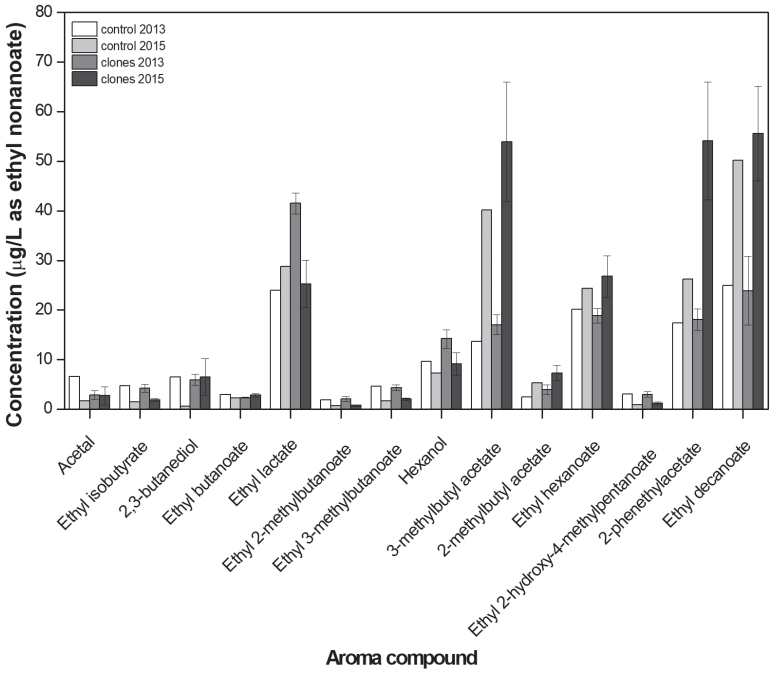

Figure 4. Specific aroma compounds present in clones of vintages 2013 and 2015 at lower concentrations

The majority of identified aromatic compounds represent the desired fruity esters (ethyl butanoate, ethyl lactate, ethyl 2- and 3-methyl butanoate, 3-methylbuthyl acetate, diethyl succinate, 2-phenylethyl acetate). The floral and/or buttery aromas that were well sensory perceived are represented by 2,3-butanediol, ethyl lactate, hexanol, 2-phenylethanol, ethyl nonanoate and ethyl decanoate.

Vintage effects influenced the concentration ranges of the majority of aroma compounds. The concentration ranges of the two compounds in red wines, diethyl succinate and ethyl lactate, were, contrary to the results reported in the study by Louw et al. [11], also influenced by vintage effects (Figure 5).

Among the identified aromas, ethyl 2-hydroxy-4-methyl pentanoate resembles blueberry aroma and is character-impact compound for Vranac wine (Figure 6). Ethyl 2-hydroxy-4-methylpentanoate that has been identified in fresh fruits [12] and more recently, in freshly distilled Calvados and Cognac [13], was first identified as a compound exhibiting blackberry notes in red and white table wines in a study by Falcao et al. [14]. Ethyl leucate was also one of the compounds thought to be strongly influenced by the lactic bacteria strain, regardless of matrix composition or the yeasts used for alcoholic

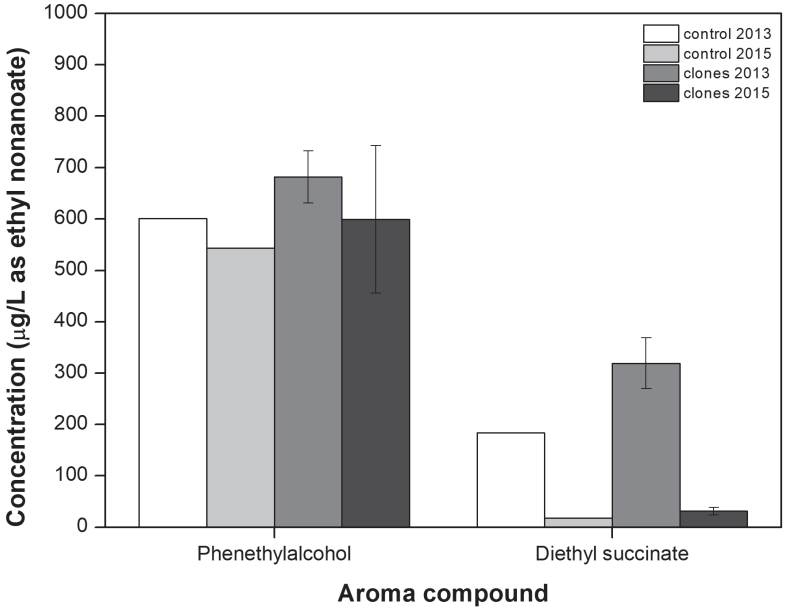

Figure 5. Specific aroma compounds present in clones of vintages 2013 and 2015 at higher concentrations

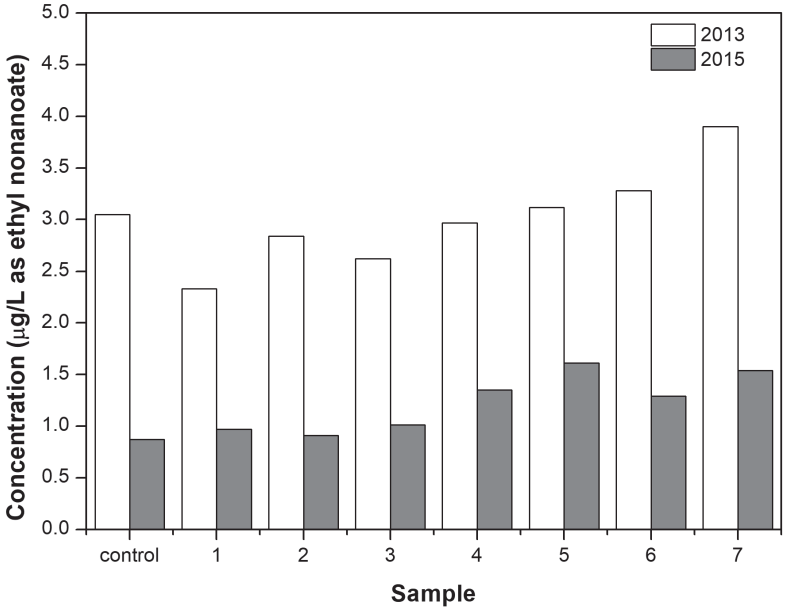

Figure 6. Concentration of ethyl 2-hydroxy-4-methyl pentanoate in different Vranac clones

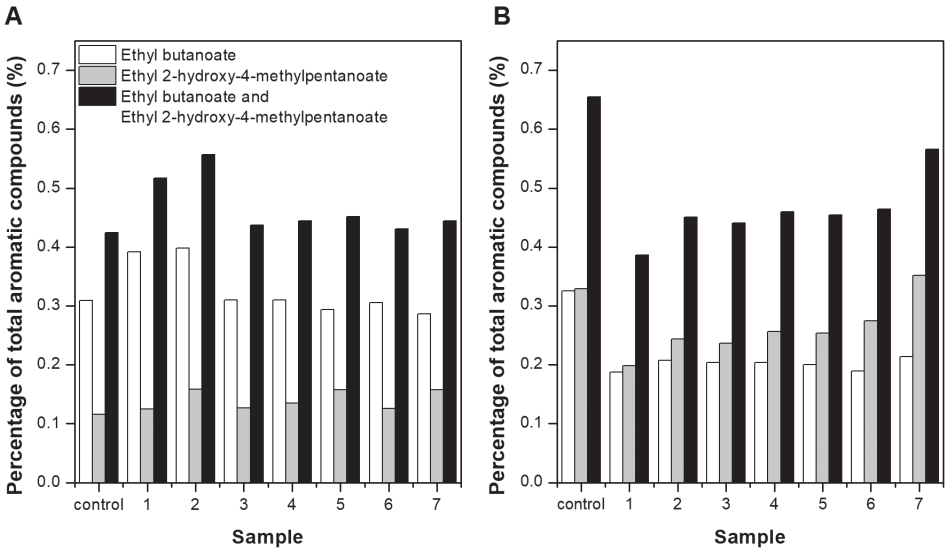

Figure 7. Percentage of ethyl butanoate, ethyl 2-hydroxy-4-methyl pentanoate and their share in the sum of total aromatic compounds

fermentation [15]. The concentration of ethyl leucate in clone samples was much lower (3.01 ± 0.50 μg/L in clones of vintage 2013; 1.24 ± 0.28 μg/L in clones of vintage 2015) than average concentration in the various wines (~ 400 μg/L) as reported in the study of Falcao et al. [14].

Despite low concentrations of this aromatic compound, the blueberry aroma was detected by sensory panel in clone 1 of vintage 2013 that also contained one of the highest ratio (%) of ethyl butanoate. Since even low variations in the concentrations of one or more esters may have a significant effect on the perception of fruity aroma [15] and previously identified perceptive interaction of ethyl leucate and ethyl butanoate and reported their synergistic effect [14]. This could be the reason for detection of blueberry aroma in this sample. When ratio of ethyl butanoate and ethyl leucate in total aromatic compounds was examined, significantly higher level of ethyl butanoate compared to ethyl leucate in wine from clones of 2013 vintage was detected in the contrary to the samples of vintage 2015, where ethyl leucate was the dominant of the two. Interestingly, even though the highest ratio of ethyl leucite, the blueberry aroma was not detected in clone 7 of vintage 2015.

SENSORY ANALYSIS

All wines were also sensorially assessed. Results of a descriptive sensory analysis, carried out with a panel of seven trained assessors, according to sensory analysis guidelines are given in Table 2.

Table 2. Results of a descriptive sensory analysis, carried out with a panel of seven trained assessors

Wines	Vintage 2013	Vintage 2015
Control	More pronounced odour on the undergrowth and dry leaf	Pharmacy and green note, discrete fruitiness, pleasant and intense smell
Clone 1	Blackberry and caramel aroma; the disturbed presence of diacetyl (too much buttery flavour)	Pronounced cherry (Marasca)
Clone 2	More intense fruitiness, with note of garlic and smoke	Very closed, hidden fruitiness; it seems very single-layer
Clone 3	Very closed, fruitiness covered by almonds as well as oxidative aroma	Pleasant fruitiness, cherry (Marasca) and buttery scent; multi-layer aroma
Clone 4	Very mature aroma, multi-layer, fruity and rubber	Pronounced fruitiness and grassy notes, distracting perception of acetic acid
Clone 5	Significantly the most beautiful and best fruitiness, recognizable mature forest fruits with mint and citrus scent	Less intense aroma, more volatile sulphur compounds (garlic)
Clone 6	Very closed, single-layer aroma, untypical fruitiness	The best aroma of all 2013 samples; very matured and multi-layer, plum compote and cocoa beans
Clone 7	Very similar to control sample (medicine, pharmacy, ether, acetaldehyde); fruitiness is completely covered with acetaldehyde	Aroma of raw Japanese persimmon; no buttery scent

CONCLUSIONS

Among the identified aromas, a typical blueberry aroma for Vranac wine can be emphasized here, for which the compound ethyl 2-hydroxy-4-methyl pentanoate is responsible. The above mentioned compound was present at significantly higher concentrations in the vintage 2013 (on average 3.01 ± 0.47 mg/L) and the three clones (5, 6 and 7) contained it more than a control wine (3.05 mg/L). In the vintage 2015 on average 1.19 ± 0.29 mg/L of ethyl 2-hydroxy-4-methyl pentanoate was present, where its concentration was higher in all clones than in the control wine (0.87 mg/L).

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Ajda OTA, Emil ZLATIĆ, Mojca KOROŠEC, Sanja RADONJIĆ,
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DIFERENCIJACIJA KLONOVA AUTOHTONE SORTE VINOVE LOZE VRANAC DETEKCIJOM AROMATSKIH JEDINJENJA POMOĆU GC — MS I SENZORSKE ANALIZE

Sažetak

Cilj ovog rada bio je da se ispita prisustvo i stabilnost specifičnih aroma-aktivnih jedinjenja vina, koja se mogu koristiti za utvrđivanje glavnih razlika između klonova sorte vranac (*Vitis vinifera* L.), crnogorske autohtone sorte vinove loze. Aroma-aktivna jedinjenja su određena gasnom hromatografijom — masenom spektrometrijom (GC — MS).

Od ukupno 16 identifikovanih aromatskih jedinjenja (izraženih kao etil-nonanoatni ekvivalent), karakteristika berbe 2013. je niži opseg varijabilnosti njihove ukupne koncentracije (927–1229 μg/L) u poređenju sa berbom 2015 (573–1019 μg/L). Statistički značajne razlike u sadržaju aromatičnog jedinjenja vina pronađene su među svih sedam klonova, kao i u obje ispitivane berbe (2013. i 2015). Prosječan sadržaj aromatičnog jedinjenja vina je 73 ± 180 μg/L u berbi 2013. i 55 ± 147 μg/L u berbi 2015. godine.

Većina određenih aromatičnih supstanci predstavlja željene voćne estere (etil butoanat, etil laktat, etil 2- i 3-metil butoanat, 3-metilbutil acetat, dietil sukcinat, 2-feniletil acetat). Cvjetne i/ili arome maslaca, koje se dobro opažaju, su predstavljene sa 2,3-butandiolom, etil-laktatom, heksanolom, 2-feniletanolom i etil-dekanoatom.

Između identifikovanih aroma, istaknuta je tipična aroma borovnice za vranac vino, za koju je odgovorno jedinjenje etil 2-hidroksi-4-metil pentanoat. Ovo jedinjenje, koje utiče na karakter vina, bilo je prisutno u značajno višim koncentracijama u berbi 2013 (prosječno 3,01 ± 0,47 μg/L), a tri klona (5, 6 i 7) su sadržala više nego kontrola (3,05 μg/L). U berbi 2015 (u prosjeku 1,19 ± 0,29 μg/L), koncentracija je bila viša u svim klonovima u odnosu na kontrolu (0,87 μg/L).

Ključne riječi: aromatična jedinjenja, vino, vranac, klon, gasna hromatografija — masena spektrometrija, senzorska analiza